

CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

**The Effects of Different Drying Techniques on Phenolic Compounds,
Aroma-Active Compounds and Antioxidant Capacity of cvs. Forastero,
Criollo and Nacional Cocoa (*Theobroma cacao*) Bean Varieties**

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ABSTRACT

In this research, the effect of different drying processes (sun, convective, microwave and freeze-drying methods) on aroma profile and phenolic compounds of fermented cocoa beans obtained from three key varieties, Forastero, Criollo, and Nacional, among the most important types of cocoa cultivated in Cote d'Ivoire were investigated through instrumental, sensory and chemometric methods. The aroma profiles of fermented and dried cocoa beans obtained from the three cocoa cultivars, along with the aroma-active compounds were determined for the first time using GC-MS-O techniques. The extraction of aroma compounds was carried out using the purge and trap method, which was applied to cocoa beans for the first time. Additionally, phenolic compounds by LC-MS-MS technique, and other quality parameters of fermented and dried cocoa beans were thoroughly investigated. With respect to the aroma profiles, differences were observed in aroma compositions and content among the drying techniques of all cocoa bean cultivars. In the results of analysis based on sun dried cocoa beans, the number of aroma profiles, aroma-active compounds and phenolic compounds of Forastero, Criollo and Nacional cultivar exhibited significant differences. Among the cocoa cultivars, Nacional cocoa type presented the highest content of aroma and phenolic compounds. On the other hand, all the dried cocoa bean samples obtained from the three cocoa cultivars were evaluated with sensory analysis and the results were measured with basic clustering analysis. As a result of sensory assesement among the sun-dried cocoa bean samples obtained from the three cultivars, the most appreciated was Nacional cocoa cultivar.

Keywords: Cocoa bean, Aroma profile, Aroma-active Compounds, Olfactometry, Phenolic Compounds

**Farklı Kurutma Yöntemlerinin Forastero, Criollo ve Nacional
Kakao (*Theobroma cacao*) Çeşitlerine Ait Fenolik Bileşikler,
Aroma Aktif Bileşiler ve Antioksidan Kapasitesi Üzerine
Etkileri**

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ÖZ

Bu çalışmada, Fildişi Sahili’nde yetiştirilen ekonomik değeri yüksek Forastero, Criollo ve Nacional olmak üzere üç temel kakao çeşidinden elde edilen fermente kakao çekirdeklerinin aroma, aroma-aktif profili ve fenolik bileşenleri üzerinde farklı kurutma işlemlerinin (güneş, konvektif, mikrodalga ve dondurarak kurutma yöntemleri) etkisi enstrümantal, duyuşal ve kemometrik yöntemlerle incelenmiştir. Üç kakao çeşidinden elde edilen fermente ve kurutulmuş kakao çekirdeklerinin aroma profilleri ve aroma-aktif bileşikleri GC-MS-O teknikleri kullanılarak ilk kez belirlenmiştir. Aroma bileşiklerinin ekstraksiyonupurge ve trap yöntemi ile gerçekleştirilmiştir. Ayrıca, örneklerin fenolik bileşikleri LC-MS-MS yöntemi ile ayrıntılı bir şekilde incelenmiştir. Aroma profilleri açısından, tüm kakao çekirdeklerinin kurutma yöntemleri arasında aroma bileşikleri ve içeriklerinde fark gözlemlenmiştir. Güneş altında kurutulmuş kakao çekirdeklerine dayalı analiz sonuçlarına göre, Forastero, Criollo ve Nacional çeşitlerinin aroma, aroma-aktif bileşenler ve fenolik bileşikleri sayısı arasında önemli farklılıklar ortaya çıkmıştır. Kakao çeşitleri arasında en yüksek aroma ve fenolik bileşik içeriği Nacional kakao çeşidinde tespit edilmiştir. Öte yandan, üç kakao çeşidinden elde edilen tüm kurutulmuş kakao çekirdeği örnekleri duyuşal analizlerle değerlendirilmiş ve sonuçlar temel kümelenme analizi ile ölçülmüştür. Üç çeşit kakao çekirdeğinden elde edilen güneş altında kurutulmuş kakao çekirdeği örnekleri arasında yapılan duyuşal değerlendirme sonucunda en çok beğenilen çeşit Nacional kakao türü olmuştur.

Anahtar Kelimeler: Kakao çekirdeği, Aroma profili, Aroma-aktif bileşikler, Olfaktometri, Fenolik bileşikler.

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LIST OF ABBREVIATIONS

SDFB	: Sun dried Forastero bean
CDFB	: Convective dried Forastero bean
MDFB	: Microwave dried Forastero bean
FDFB	: Freeze dried Forastero bean
SDCB	: Sun dried Criollo bean
CDCB	: Convective dried Criollo bean
MDCB	: Microwave dried Criollo bean
FDCB	: Freeze dried Criollo bean
SDNB	: Sun dried Nacional bean
CDNB	: Convective Nacional bean
MDNB	: Microwave Nacional bean
FDNB	: Freeze dried Nacional bean
LAB	: Lactic acid bacteria
AAB	: Acetic acid bacteria
GC	: Gas chromatography
MS	: Mass spectrometry
GC-MS-O	: Gas chromatography-mass spectrometry-olfactometry
FID	: Flame Ionization Detector
PTE	: Purge and Trap Extraction
AEDA	: Aroma Extract Dilution Analysis
FD	: Flavor dilution
OAV	: Odor activity value
CHARM	: Combined hedonic response measurement
LRI	: Linear Retention Index
CIE	: Commission International de l'Eclairage
Std	: Standart
Tent	: Tentative
ANOVA	: Analysis of variance
kg	: Kilogram
g	: Gram
mg	: Miligram
µg	: Microgram
°C	: Celsius degree or Centigrade degree
L	: Litre
mL	: Millilitre

μL	: Microlitre
m	: Metre
cm	: Centimeter
mm	: Millimetre
μmol	: Micromol
mM	: Millimol
s	: Second
hr	: Hour
min	: Minute
N ₂	: Nitrogen
m ²	: Square meter
v/v	: Volume/volume
MHz	: Megahertz
GHz	: Gigahertz
W	: Watt
kW	: Kilowatt
C	: Carbon
DPPH	: 2,2- Diphenyl-1-picrylhydrazyl
ABTS	: 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ORAC	: Oxygen radical absorbance capacity
HS-SPME	: Head Space-Solid Phase Micro-Extraction
DW	: Dry weight
FI	: Fermentation Index
UPLC-MS/MS	: Ultra Performance Liquid Chromatography-Mass Spectrometry
RH	: Relative humidity
<i>p</i>	: Para
(E)	: Trans isomer
(Z)	: Cis isomer
TE	: Trolox equivalent
CE	: Catechin equivalent
GAE	: Gallic acid equivalent
LC-DAD-ESI-MS/MS	: Liquid chromatography combined with a diode array detector and ion spray mass spectrometry
m/z	: Mass/charge ratio
HPLC	: High-Performance Liquid Chromatography
DAD	: Diode Array Detector
ESI	: Electrospray ionization

ECE : Epicatechin equivalent
CGE : Cyanidin-3-glucoside equivalent
OH : Hydroxyl group
H : Hydrogen



1. INTRODUCTION

Nature regroups large number of plants that play a major role in the life of humans and animals by constituting a food source. These plants are composed of medicinal, aromatic plants and food-based plants are rich in flavors, aroma, colors and bioactive compounds and are widely used in many industries such as herbal medicine, chemical and biotechnology, food and additives, pharmacy, cosmetics and perfumery industries. Nowadays, consumers are more concerned with healthy and quality food, and they still accept standard flavor and taste given that the product provides health benefits. The compounds found in those plants that have a positive relationship to health and sensory properties are bioactive and volatiles compounds, which are naturally found in many vegetables, fruits, cereals, and beverages. Among these plants, tobacco, tea, coffee, sugar cane, cacao and spices have been widely distributed all over the world, in which cocoa bean is one of the most traded foodstuffs worldwide.

Cocoa (*Theobroma cacao L.*) is a highly valuable cash crop of huge economic importance in the world and serves as the primary raw material for chocolate production (Krähmer et al., 2015; Zhao & Fleet, 2015). It represents a significant agricultural export commodity for numerous countries in West and Central Africa, including Cote d'Ivoire, Ghana, Nigeria, and Cameroon (Afoakwa et al., 2011). Cacao tree is an important tropical, neotropical, perennial crop belonging to the malvacea family (made up of 243 genera, and 4225 species), to theobroma genus (including 22-26 species and subspecies), and to the theobroma cacao family- the only one with edible fruits. The cultivation of cacao tree is widely spread in Africa, America, Asia and Oceania. Cacao tree is cultivated for its fruit, which contained beans, and used for the production of chocolate bar, cake, liquor, butter, and soft drink. The cocoa beans are also used in folk medicine to produce cocoa powder and drink due to their high antioxidant capacity, which are beneficial in human nutrition and medicine (Bonvehí & Coll, 1997; Drewnowski & Gomez-Carneros, 2000).

Today, more than fifty million people around the globe depend on cocoa for their livelihood with a total production capacity of 68% for Africa, 17% for Asia, and 15% for America (Rusconi & Conti, 2010; Tardzenyuy et al., 2020) (ICCO, 2014). Cocoa is one of the most important agricultural export commodities in the world and forms the backbone of the economies of some countries in West Africa, such as Cote d'Ivoire and Ghana. According to Cocoa beans account for 0.051% of the overall world trade, which represent 8.54 billion U.S dollars of the total trade (OECD World, 2023). Growing cacao tree is a widespread activity in south, east central and west region of Cote d'Ivoire and is a substantial issue for the economic activity of these regions. According to some reports and data released, Cote d'Ivoire is the leading producer with a production estimated at 1900 million MT/years (40% of the total production) of cocoa beans, followed by Ghana 850 MT (18.1 % of the total production), Indonesia (11%), Nigeria (6%), and Brazil (5%) of overall supply in the cocoa market (PHAMA, 2018; ICCO 2013), where as Indonesia is the biggest cocoa beans exporter worldwide.

Different subspecies and genotype of cacao three, including Criollo, Forastero, Trinitario and Nacional are botanically cultivated by several countries throughout different regions (Lima et al., 2011; Rusconi & Conti, 2010). The most cultivated subspecies in Cote d'Ivoire are Forastero, Criollo and Nacional, among which Forastero is the most prevailing and the western region of Cote d'Ivoire remains the most dominant area in cocoa cultivation.

Overall, cocoa bean obtained from the fruit of cacao tree (*Theobroma cacao*) through different processes can be separated from the point of view of chemical composition into major and minor fraction. The major compounds include glycerols (triglyceride and fatty acids) (Ziegler & Biehl, 2003). Several chemical substances, including phenolics (catechins, anthocyanins, flavonol glycosides and proanthocyanidins), alkaloids (caffeines, theobromine and theophylline), sterols, hydrocarbons are the minor compounds with small amount in cocoa beans (Afoakwa et al., 2013; Bordiga et al., 2015; Wollgast & Anklam, 2000a). Some of these minor compounds (alkaloids, phenolics, sterols) in cocoa beans have substantial biological activities.

Numerous indicators including bean size and count, bean colour and acidity are employed to evaluate the quality of cocoa beans. However, the most substantial quality indicator of cocoa beans is the quantity of volatile flavor components release (Krähmer et al., 2015; Magi et al., 2012). Flavor is a crucial aspect contributing to the acceptability of cocoa beans and products like chocolate (Afoakwa et al., 2008), and consequently participates to determining their quality (Owusu, 2010). The characteristic flavors of cocoa beans are attributed to a mixture of aroma fraction composed of about hundreds compounds (Magi et al., 2012). Up to date, more than 600 aroma components have been detected from cocoa beans and cocoa products (Ziegler, 2017), which include aldehydes, ketones, esters, alcohols, hydrocarbons, pyrazines, furans, thiazoles, pyrones, volatile acids, phenols, pyrroles, oxazoles and others (Counet et al., 2006; Frauendorfer & Schieberle, 2008; Granvogl et al., 2006; Jinap et al., 1998; Schnermann & Schieberle, 1997). Furthermore, the chemical composition of cocoa beans is greatly affected by cacao cultivars, cultivation practices, regions, climatic conditions. It has been also reported to be strongly influenced by two main post-harvest processes, including fermentation and drying techniques, which directly or indirectly affect the quality of the cocoa beans and subsequent products. Both post-harvest processes have interdependent and significant influence on bean quality, since chemical change (release of color, flavor, aroma and taste) generated during fermentation continue along the drying processes (Dina et al., 2015; Guehi et al., 2010).

Fermentation is generally conducted naturally in boxes through the inoculation of microorganisms, namely yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and Bacillus, and are indispensable in achieving a successful fermentation of cocoa and they constitute a key factor that strongly affect the quality of final product (Ouattara et al., 2017; Schwan & Wheals, 2004; Soumahoro et al., 2020; Yao et al., 2017). After the fermentation, the beans are removed from the heap or box for drying.

Drying is one of the oldest and most efficient technique employed for centuries for food preservation and food shelf-life prolongation, including cocoa beans. During cocoa bean drying process, the moisture content is reduced from ca. 80-60% to 8-5%, a moisture content that can be regarded safe for expanded storage. The shelf life of food products, including cocoa bean through drying process is of extreme importance, because the product must remain stable, keeping its original functional, nutritional, biological activity and sensory properties during storage. However, it is well known that a number of food matrices develop off-flavors and off aromas due to chemical and microbiological reactions, such as enzymatic and non-enzymatic browning, fermentation, molds and toxins contamination, lipid and protein oxidation, and the hydrolysis of food constituents (in't Veld, 1996). In particular, for fatty food products, chemical lipid auto-oxidation is one of the most prevalent reactions leading to off flavor development (Vercellotti et al., 1992). Cocoa bean and product's characteristic aroma, taste, color, nutritive properties and stability distinguish them from other edible vegetable grains.

Several drying methods are used in cocoa farming. These methods can be classified into two categories including solar drying and artificial drying (Hii et al., 2013). However, the choice of drying method is an important concern, which is strongly linked to different factors like regional weather conditions, cocoa cultivar, and the economic conditions of each farmer (Dzelagha et al., 2020). It is also important to consider that each type of technique has associated characteristics that can influence the quality of the final cocoa beans and products. In this regard, in term of suitability and possible utilization for large-scale drying by farmers (smallholder), the conventional (natural) or sun open drying technique is the oldest passive mechanism. This type of drying technique is the most popular in tropical and subtropical countries where the solar radiation is intense during harvest season (Bala & Hossain, 2012). It is a non-polluting technique (environmentally friendly), freely available and most preferred due to its simplicity (using rudimentary procedures) and low construction and running costs. In addition, this type of intermittent drying technique enables a slow migration of moisture all through the beans, which carries the aroma precursors formed during fermentation. However, the main drawbacks of this drying technique are the fact that, it is a labor intensive and weather dependent technique, which limit its application throughout the year and lead to cacao beans spoilage.

The process of drying is problematic in Côte d'Ivoire, it is particularly difficult where the rainfall and humidity are extremely high like in east and western part of the country, and often coincide with peak harvest seasons. In this country, sun open drying remains the most common and most used method throughout cocoa culture regions (Lainé, 2001). However, the poor environmental conditions associated with these localities make sun open drying unpredictable in terms of quality. The exposition of the beans lasts about seven days when the weather conditions are good and can sometimes last 15 to 22 days when these are not favorable (Barel, 2013; Lainé, 2001). This constraint promotes, on the one hand, the risks of moisture reabsorption with the corollary of the growth of molds with the production of mycotoxins (ochratoxin A) and toxins, the increase in the free fatty acid

content and the development of undesirable tastes and off-flavor, on the other hand, it leads farmers to use non-recommended drying devices such as bitumen, sheet metal or even a wood fire to dry the cocoa beans, which increase the risk of obtaining poor quality beans with lower price and beans losses. As such, cocoa beans of commercial grade should always satisfy to certain criteria among which are its moisture content, acidity and astringency, slatiness, polyphenols, mouldiness, and mycotoxins release before delivering to cocoa bean product manufacturing industries (Aroyeun et al., 2009). In order to provide a better solutions to these shortcomings and bypass uncontrollable environmental factors, numerous research have been carried out on various artificial drying systems, which employ a combination of heat and airflow to dry cocoa beans (Barel, 2013; Hii et al., 2013; Lass & Wood, 1985).

The artificial drying processes are essential in many countries such as Cote d Ivoire, and some other countries where the harvesting season coincide with frequent rainfall weather periods in order to mitigate cocoa beans poor quality, losses and contaminations (Dzelagha et al., 2020). The artificial drying techniques developed include oven dryers, dehydrators, wood-fired dryers, microwave and freeze dryers. Of all the artificial techniques, firewood oven dryers are the most popular applied owing to the easy usage and relatively low cost. However, their main drawbacks lies in the difficulty to control the smoke, which transfer undesirable aromas and flavors to the dried cocoa beans, and to maintain a constant temperature (30-65) and distribution of heat throughout the beans (Dzelagha et al., 2020; Füllemann et al., 2022). Other artificial dryers less commonly used are electric dehydrators, oven drying (laboratory setting type of low-temperature convection or forced air oven, where the moisture is removed evenly and slowly), microwave drying (produces a rapid energy coupling into the moisture and leads to fast heating and drying), and freeze drying (frozen water in the food turns to vapor by sublimation and is eliminated by a strong vacuum). The advantages of artificial drying include more control over drying rate, which can result in a quicker turnover of marketable products. Despite their great economy of time and general effectiveness in eliminating moisture and producing a homogeneous and controlled drying process, most of these dryers produce quick-dried or even over-dried cocoa, which leads to the hardening of the shell, causing the entrapment or locking of the volatile acids inside the bean, obtaining a high-acid and low-chocolate-flavored cocoa character, off-flavors, and the degradation of bioactive volatiles compounds (Jinap et al., 1994; Urquhart, 1961).

The exploration of cleaner, sustainable, environmentally friendly, less expensive, and more efficient technologies is a great challenge for farmers in order to counter the less efficient characteristics of conventional techniques. Therefore, the development and implementation of sustainable and alternative drying processing techniques with slight variations in conventional techniques that will increase the quality of cocoa beans, tackle the raining season in cocoa growing regions, and induced low changes of the volatile and bioactive compounds in cocoa beans could be

a good option in cocoa grown regions, including Cote d'Ivoire to maintain its reputation in global cocoa market.

To date numerous research related to the influence of drying techniques, particularly open sun drying and conditions on chemical composition and the quality of cocoa beans have been reported in the literature (Guehi et al., 2010; Hii et al., 2006; Lasisi et al., 2014). However, there are few detailed studies on the effect of artificial drying on the chemical composition and the quality of cocoa beans. In the perspective to reduce the cocoa beans drying time and enhance the cocoa beans quality, we investigated in this research for three cacao cultivars (Forastero, Criollo and Nacional) grown in the western part of Cote d'Ivoire (the region of Ladjiboua), the impact of microwave, convective air oven, and freeze-drying techniques on the chemical composition, organoleptic characteristic and quality of cocoa beans. For such purpose, the cocoa fruits were harvested and processed, and the fresh beans obtained were spontaneously fermented in the banana heap. The fermented cocoa beans were dried through microwave, convective air oven, and freeze-drying techniques, and sun drying. All fermented and dried cocoa beans were subjected to (i) physico chemical analysis, (ii) total phenolic contents and antioxidant capacity analysis, (iii) aroma and aroma active compounds analysis by GC-MS-Olfactometric techniques, (iv) phenolic compounds analysis by LC-MS-MS technique, (v) fatty acid compositions and sensory properties analysis. The results obtained bring out the characteristics of the cocoa beans in terms of aroma, phenolic, and aroma-active compounds for these cocoa beans to introduce the most appropriate drying technique of cocoa beans.

2. LITERATURE REVIEW

2.1 Cacao tree and origin

Theobroma cacao Linnaeus 1753 is a food plant that was first discovered by Olmec population in 1500-400 BC. Cacao trees are originated from neotropical rainforests, its fruits are called “Pod” and contain “cocoa beans “with a muscillagineous sweet pulp that surround the beans. Moreover, cocoa is known as the drink of God (Theo=God, Broma=drink) and belong to *Theobroma cacao* L. Plant species. According to the literature, cocoa tree was cultivated and domesticated by Mesoamericans (600 BC) and Aztecs (400 AC), then disseminated to Europe in 1502 (Lachenaud & Oliver, 2005; Verna, 2013). Cocoa three is thereafter introduced to tropical and equatorial regions (with hot and humid climates) (South-East Asia and West Africa, South America) where it has been intensively grown (Owusu, 2010). A domesticated cocoa tree can produce approximately 80 pods a year (Birlouez, 2013).

2.2 History of cocoa culture in Côte D'Ivoire

During the era of colonialism, particularly under the influence of French missionaries, cocoa cultivation began to emerge sporadically across the forest regions of Côte d'Ivoire. However, by 1908, cocoa production remained confined to isolated Island, with an approximate annual production of two tons. The south, east and west parts of Côte d'Ivoire, characterized by its dense forests provided the ideal natural conditions for the cultivation cocoa trees. These favorable conditions were found within the region spanning between 5° and 8° north with a subequatorial climate. The cocoa tree, which is very demanding from the point of view of the physical properties of the soil, adapts very easily to chemical conditions such as lateritic clays and loose laterites of the forest (Planhol, 1947). The eastern region of the lower coast, adjacent to the Gold Coast (Indénié), experienced a remarkable surge in cocoa production characterized by rapid growth. Notably, the circle of Abengourou alone contributed to 2/5 of Côte d'Ivoire's total cocoa production. The cultivation of cocoa gradually spread from this influential region to the Center and Center-West regions of the country. Thus, the exports of cocoa beans from Côte d'Ivoire reached 1.68 million tons in 2021 (FAOSTAT, 2021).

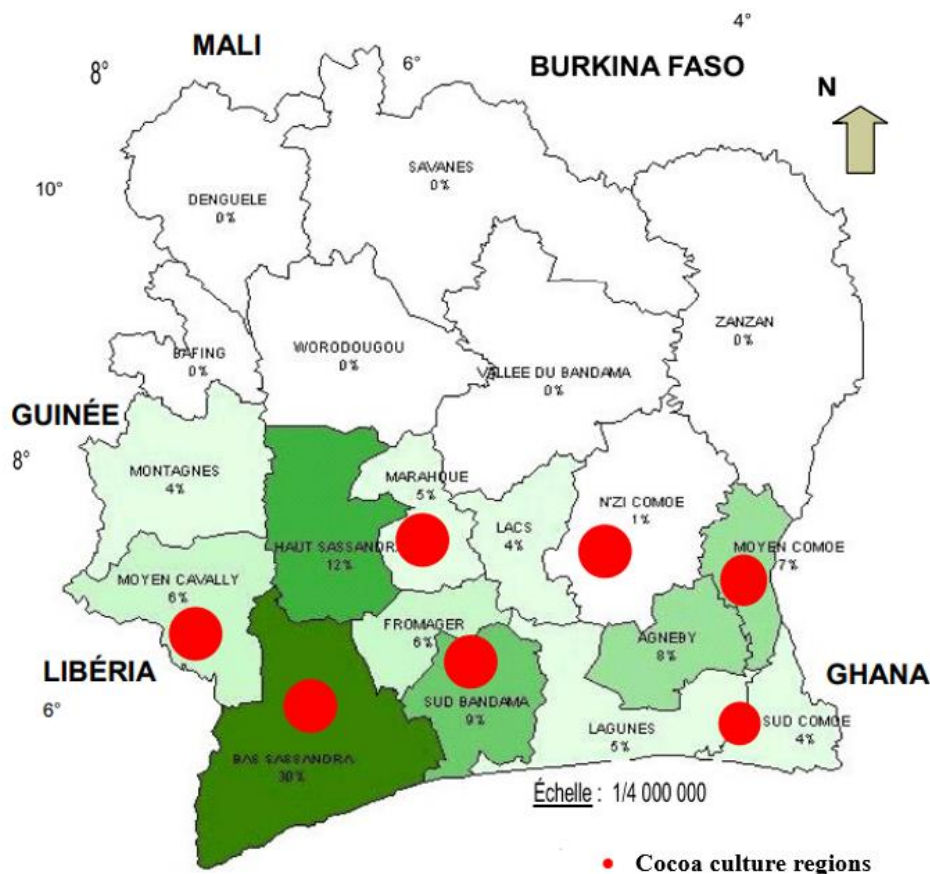


Figure 2.1. Map of the cocoa growing areas of Cote d'Ivoire (Abu et al., 2021; Läderach et al., 2013)

2.3 Taxonomy and botanical characteristics of cacao tree

As aforementioned, cacao tree belong to the genus *Theobroma*, to Malvales order and Malvaceae family (Loor Solórzano, 2007). The genus *Theobroma* is separated into 6 major classes including *Theobroma bicolor*, *Theobroma cacao*, *Theobroma herrania*, *Theobroma grandiflora*, *Theobroma microcarpa* and *Theobroma speciosa* (Kennedy, 1985) with 22 species and subspecies (Cuatrecasas, 1964; Motamayor et al., 2008). The beans of these species can be employed in chocolate industries, however, *Theobroma cacao* is the only specie widely for the production of beans, which are used for the production of chocolate and cocoa butter and related products (Gálvez et al., 2007). Based on the botanical parameters, cocoa was classified into two subspecies, namely Criollo and Forastero (Morris, 1882). Criollo is considered as the first domesticated cocoa and was largely cultivated in central, North and South America (Sounigo et al., 2003). Forastero, native to the lower Amazonia is grown in Brazil and Venezuela, but a special morphological species named Amelonado was introduced and cultivated in Africa (Sounigo et al., 2003). Years later, cocoa underwent three morphological classifications of subspecies, including Criollo, Forastero and Trinitario. Indeed, the later is a spontaneous hybridization between Forastero and Criollo, mostly cultivated in Trinidad (Lanaud et al., 2003; Umaharan, 2018).

2.3.1. Ecology of cacao tree

Temperature, rainfall and soil composition play a substantial role in cocoa cultivation. Indeed, the cocoa tree shows good growth and produces better at optimal temperature range of 24 and 26°C. However, it presents difficulties in the growth of cocoa trees when the temperature is below 18°C, and above 32°C the growth of cocoa tree is reduced. Thus, maintaining suitable temperature conditions is vital for ensuring the successful growth and productivity of cocoa trees. Moreover, the cocoa tree requires an annual rainfall greater than 1700 mm and soil humidity close to 80%. Therefore, the dry season in the production area should not exceed four months (Zuidema et al., 2005). The cocoa tree requires shade as it is very sensitive to sun and wind as well as well-drained, aerated soils with a pH of 6.5 and rich in mineral elements (phosphorus, potassium) and organic matter (Kumah et al., 2018; Zuidema et al., 2005). Temporary shade is essential during the first years of cultivation of the young cocoa tree. This shade must be relatively dense, allowing only 50% of the total light to pass through for at least two years after planting. This shading should be progressively reduced as the development of the cocoa tree, but not before well-established crown formation (Mossu, 1990).

2.3.2. Cultivated varieties of cacao tree

Advances in genetic engineering have intensified knowledge of cocoa genome and varieties, among which some dissimilarities based uniquely on morpho-geographical criteria are supported (Jean-Marie et al., 2022). For instance, a genetic discrimination was detected between High Amazonians and Low Amazonians Forasteros, Criollos and Trinitarios by through ribosomal DNA probes or RAPD markers (Laurent et al., 1994; N'Goran et al., 1994). Moreover, Marranon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Perus, Nacional and Guianas are the distinct and specific genetic groups recently classified (Motamayor et al., 2008). Today, the botanical classification of cocoa is still based on the both types, comprising the old morphological classification "Criollo, Forastero and Trinitario" as well as the genetic classification proposed by Motamayor and collaborators (Jean-Marie et al., 2022). Cocoa tree are often depicted based on the hybridizations from which they are derived and listed in genteic banks (Johnson et al., 2004).

Forastero. Forastero variety is the most commonly used, compromising 95% of the world production of cocoa, but it is reported with poor quality in term of flavor (Lima et al., 2011; Rusconi & Conti, 2010). Brazil and some West Africa countries cultivate Forastero in large areas, among which West Africa alone produces more than 70% of the world total production (ICCO, 2019). Forastero cocoa tree has smooth, sometimes warty, elongated and rounded pod (Amelonado) a color varying from green to yellow (Tixier, 2013). Forastero type cacao comprises two subgroups, including Amelonado and Amazon, which are themselves subdivided into lower Amazone and Upper Amazon, depending on their origin. However, there are no significant chemical composition differences between these Forastero subgroups (Tomlins et al., 1993).

Criollo. Criollo is known for its sensitivity to pests and diseases (Schmitz & Shapiro, 2012), associated with a fragile plant structure and poor seed productivity (Guerrero et al., 2017). Criollo possesses an elongated pod, pointed, warty and reddish in color. The pods are red before maturity and turned orange when mature. The fresh beans are plump, round and white in colors with a slight bitterness. Criollo is known as the finest, and the most aromatic, with sweet and fruity notes. It is historically the first cultivated cacao, indigenous to southern Central America and northern South America (Ferraio, 2002; Fowler & Coutel, 2017).

Nacional. Nacional cocoa tree is mostly cultivated in Ecuador (Solorzano), and it possesses a large rough pod, which color vary from slightly green to red and orange. Nacional cacao beans are purple in color and quite large (Tixier, 2013). The Nacional constitutes only a small portion of the world cocoa beans total production. Its cocoa bean offers good aroma, which makes it a highly appreciated fine and aromatic cocoa. Criollo comes from a local Amazonian population that has undergone some hybridization with the Trinitario variety. This hybrid is considered as a third variety of cocoa beans (Caligiani et al., 2010; Counet et al., 2004). Over time, this variety has been classified in a different way. Thus, some authors consider that it belongs to the Forastero type, while others list it as being of Criollo type (Enriquez, 1992; Nosti Nava, 1953). According to other authors, it belongs to the Trinitario and Criollo owing to its fine and pronounced flavor (Lass & Wood, 1985). More recently, researchers have demonstrated applying new molecular technologies that Nacional cocoa is genetically closer to Forastero than Criollo (Lerceteau et al., 1997).

Trinitario. Trinitario is a hybrid of Criollo and Forastero, it has higher yield and is more resistant to diseases than the others, and it is known for its higher quality (Aoyama et al., 1995). It is very polymorphic and possesses all the intermediate proprieties of Criollo and Forastero (Tixier, 2013). The trinitario cultivar is known to have chocolate sensory and a certain vinous type of aroma absent in other varieties (Afoakwa et al., 2008).

The International Cocoa Organization defined cocoa in two main categories according to their flavor properties on the market, known as fine or flavor and bulk cocoa. Flavor cocoa is exclusively produced from Criollo, Trinitario, and Nacional types, which are characterized by their extraordinary flavor properties, especially for their pronounced fruity, floral aroma attributes. Bulk cocoa, a cocoa with average flavor properties, is mainly produced from Forastero cocoa (Lima et al., 2011; Rusconi & Conti, 2010). By comparison of the market shares of bulk and flavor cocoa on the world cocoa market, bulk cocoa, which is used to produce cocoa products such as cocoa mass, cocoa powder, and chocolate, is, with its market share of 90%, substantially higher than flavor cocoa, with an estimated market share of only 5–10%, which is mainly employed for the production of high-quality chocolate (Afoakwa, Paterson, Fowler, & Vieira, 2009; Rusconi & Conti, 2010).

2.3.3. Cocoa Fruits and Beans

The cocoa fruit is a lengthened berry, named pod, measuring 10-32 cm long and whose structure and appearance play a substantial role in the identification of varieties and populations. The pod surface characterized by ten ridges can be smooth to warty, and its bottle neck, shoulder and apex can be more intense. The oval-shaped pod possess different characteristics depending on the variety, Its color vary from light green to yellow or orange, from yellow-red or orange to purple (Chingandu, 2016; Toxopeus, 2001). A pod contains approximately 30 to 60 fresh cocoa beans surrounded by abundant mucilage (Chingandu, 2016; Lachenaud & Oliver, 2005). Cocoa beans possess different shapes and sizes, whih between 10-25 mm long and 8 mm thick flattened aspect. Cocoa bean is described by an almond that contains two cotyledons and a small embryo linked to a shell which surrounds the bean (Fowler & Coutel, 2017).

2.4 Postharvest Processing of Cocoa Beans

Before cocoa beans can be traded and transformed into final industrial products and derivatives, they must go through post harvest processing on the farm and plantation. These processes include opening the fresh mature cocoa pods and removing the beans, which will be thereafter fermented by placing the beans in fermentation boxes or heaps for a specific periods and dried (Castro-Alayo et al., 2019; da Veiga Moreira et al., 2018; Kumari et al., 2018). Fermentation and drying are the two major steps in the post harvest processing of cocoa beans. These steps play a substantial role in the formation of flavor and taste, which should be treated properly in order to enhance the quality of cocoa beans, which significantly contribute to the quality of the final product (Dina et al., 2015).

2.4.1. Cocoa Beans Fermentation

Cocoa beans fermentation techniques vary according to producing countries and farmers (Afoakwa et al., 2013; Guehi et al., 2010; Romero-Cortes et al., 2012). Regardless of the technique used, the fermentation of cocoa beans is mainly alcoholic, then lactic and finally acetic (Guehi et al., 2010; Romero-Cortes et al., 2012). The fermentation of cocoa beans is the first stage in the cocoa beans and cocoa products production processes.

2.4.1.1. Spontaneous Fermentation of Cocoa Beans

In cocoa growing regions, traditional fermentation, which is conducted naturally on the farms and plantations is the commonly applied cocoa bean fermentation process. In this step, microorganisms are indispensable in achieving a successful fermentation of cocoa beans and they constitute a key factor that strongly influences the quality of the final product (Ouattara et al., 2017; Schwan & Wheals, 2004; Soumahoro et al., 2020; Yao et al., 2017). The pulp is an excellent medium for the growth of micro-organisms since it contains 10–15% of sugars. When the beans are removed

from the pods, the pulp is inoculated naturally with a variety of micro-organisms from the surrounded environment, mainly from the soils, air, the surface of cacao pods, banana and plantain leaves used to build the heaps and cover the boxes, farmers' hands and the materials used in the harvest and postharvest process (Camu et al., 2007; Jespersen et al., 2005; Nielsen et al., 2007). The bean pulp mass is then usually heaped up or filled in wooden fermentation boxes, heaps, baskets, trays, or on platforms, the mass is inoculated with ubiquitous microbial flora including yeasts, lactic acid, and acetic acid strains, leading to a spontaneous fermentation which is not easy to control. The fermentation is usually performed for 2 to 10 days depending on the countries and farmers practices (Rusconi & Conti, 2010). In this first stage of bioconversion, the sugars and associated polysaccharides are mainly digested by yeasts in anaerobic condition into ethanol. Successful cocoa bean fermentation involves a succession of specific microbial activities, namely those indigenous yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB).

2.4.1.2. Fermentation of Cocoa Beans using Starter Culture

Cocoa fermentation using starter cultures, also known as controlled or inoculated fermentation has recently gained interest in cocoa growing countries and scientific community. Biotechnological studies have increased data on the functional role of yeasts, Lactic acid bacteria (LAB) and Acetic acid bacteria (AAB) strains implied in cocoa bean fermentation (Adler et al., 2014; Illegheems et al., 2013; Moens et al., 2014). For instance, (Lefeber, Gobert, et al., 2011) and (Moens et al., 2014) successfully developed cocoa pulp simulation media for LAB and AAB to mimic the spontaneous cocoa bean fermentation process in laboratory scale bioreactor. The fermentation using starter culture involves the deliberate addition of selected microorganisms to the cocoa fermentation process. The goal is to enhance and standardize the fermentation process, ensuring consistent flavor development and reducing the risk of undesirable microbial contamination (De Vuyst & Weckx, 2016; Lefeber, Gobert, et al., 2011). Cocoa fermentation using starter cultures provides greater control over the fermentation process, resulting in more consistent flavor profiles and reduced batch-to-batch variation. It is commonly practiced in the chocolate industry to achieve specific flavor characteristics and meet quality standards (Craack et al., 2013; Meersman et al., 2016; Ramos et al., 2014).

(Lefeber et al., 2012) performed an investigation on different cocoa beans fermentation using *Saccharomyces cerevisiae* H5S5K23 *Lactobacillus fermentum* 222, and *Acetobacter pasteurianus* 386B as starter culture to ferment the beans in three heaps and one box. In addition, another group of starter culture composed of *Lactobacillus fermentum* 222 and *Acetobacter pasteurianus* 386B (seven heaps and one box) were employed. The results obtained showed that employing both sets of starter cultures led to the fermentation of dry cocoa beans that resulted in consistent milk and dark chocolates with a reliable flavour, independent of cocoa-producing region or fermentation method applied.

In another study investigated (Lefeber, Janssens, et al., 2011), specific strains of lactic acid bacteria and acetic acid bacteria were isolated from Ghanaian cocoa bean heap fermentations in order to develop a suitable starter culture. The results successfully demonstrated that a mixture of *Lactobacillus plantarum* 80, *Lactobacillus fermentum* 222, and *Acetobacter pasteurianus* 386B can be employed as mixed-strain starter culture for better controlled, reliable and more effective cocoa beans fermentation processes.

2.4.1.3. Microorganisms Involved in Cocoa Beans Fermentation

Numerous studies (Hamdouche et al., 2015; Tchotch et al., 2017) have focused on the succession of microbial population during the fermentation of cocoa beans. It has been demonstrated that yeasts are the first types of microorganisms to colonize the medium, then come lactic acid bacteria, followed by acetic acid bacteria which are replaced by aerobic sporulated bacilli such as *Bacillus* species. The microbial succession during the fermentation of cocoa beans is the result of the biochemical composition of the bean pulp and the aeration conditions of the cocoa mass. In fact, microbial relays only take place if the aeration of the environment is sufficient, leading to a temperature and pH profile (Schwan et al., 2014).

Yeasts Populations

As aforementioned, yeasts are the first microorganisms to metabolize the cocoa beans pulp and rapidly become the majority and dominant. They transform the sugars present in the pulp (sucrose, glucose and/or fructose) into ethyl alcohol with the release of carbon dioxide. Alcoholic fermentation takes place during the first 48 hours of fermentation. It takes place under anaerobic conditions due to the density of the pulp which prevents the aeration of the beans and to the acidity (pH = 3.5) due to the citric acid in the pulp. The enzymes in the yeasts have good pectinolytic activity. These enzymes hydrolyse the mucilage of the beans and lead to the liquefaction of the pulp (Crafack et al., 2013). During this stage, the maximum yeast load varies from 6 to 8 log CFU/g of cocoa bean (Gálvez et al., 2007). The alcoholic fermentation is moderately exothermic (93.3 kJ with a rise in temperature to 35-40°C after 48 h) and is accompanied by a slight increase in pH resulting from the degradation of citric acid by the yeasts. Some studies have shown that yeasts of the genus *Hanseniaspora* are most frequently isolated during the initial phase of fermentation (de Melo Pereira et al., 2016). For instance, *Hanseniaspora guilliermondii* in particular is the most frequently isolated species from fermented cocoa in Ghana and Ivory Coast along with *Hanseniaspora opuntiae* (Papalexandratou et al., 2013). *Saccharomyces cerevisiae* is also involved in the fermentation of cocoa from various cocoa producing regions such as Ivory Coast, Indonesia (Ardhana & Fleet, 2003), Brazil (de Melo Pereira et al., 2016), the Dominican Republic (Gálvez et al., 2007). Other species have been implicated in cocoa fermentation. (Hamdouche et al., 2015) and (de Melo Pereira et al., 2016) performed different studies on yeast population during cocoa beans fermentation process. They

observed that *Kloeckera apis*, *Candida tropicalis*, *Candida krusei*, *Candida zemplinina*, *Saccharomyces cerevisiae*, *Candida diversa*, *Issatchenkia orientalis*, *Pichia membranifaciens* were detected as the prevailing yeasts in fermented cocoa beans. (Koné et al., 2016) successfully identified six main yeast strains involved in the fermentation of Ivorian cocoa in different regions, these include *Saccharomyces cerevisiae*, *Candida tropicalis*, *Pichia kudriavzevii*, *Pichia galeiformes*, *Galactomyces geotrichum* and *Wickerhamomyces anomalus*.

Lactic Acid Bacteria (LAB) Populations

Following the alcoholic fermentation, the micro-aerophilic conditions, the acidic environment (pH = 4) and the availability of substrates, LAB strains gradually increased after the decline of yeast population in the medium due to the large reduction of liquid by drainage of the pulp, the elevated temperature and oxygen levels. In a certain way, the yeast strains foster the growth of LAB species. Moreover, the growth LAB communities range from 7 to 7.8 log CFU.g⁻¹ of cocoa beans (Gálvez et al., 2007). *Lactobacillus fermentum*, *Lactobacillus plantarum* followed by *Leuconostoc pseudomesenteroides* are the LAB species commonly isolated from fermenting cocoa beans (Lefeber, Gobert, et al., 2011; Papalexandratou et al., 2013; Papalexandratou et al., 2011). Other species of the genus *Fructobacillus*, *Enterococcus* and *Weissella* have also been detected in fermented cocoa beans from different origins (Hamdouche et al., 2015; Lefeber, Gobert, et al., 2011; Papalexandratou et al., 2011). LAB mainly transforms citric acid and pulp sugars into lactic acid, with small amount acetic acid and ethanol in the case of heterolactic fermentation. The lactic acid produced penetrates inside the beans, which increase the acidity of the cotyledons and thus induce cocoa beans with low commercial quality and unpleasant aroma (Barel, 2013; Koné et al., 2016). Assimilation of citric acid by lactic acid bacteria generates a slight rise in the pH and temperature of the pulp. The presence of ethanol and the increase in oxygen pressure due to the degradation of the pulp allow the start of the second phase of cocoa bean fermentation, which is led by acetic acid bacteria.

Acetic Acid Bacteria (AAB) Populations

The excessive increase of oxygen, temperature, and metabolic products (ethanol, lactic acid, and mannitol) during the first two phases of cacao bean fermentation with the substantial reduction of yeast and bacteria populations promote the growth of AAB species. Ethanol, lactic acid and mannitol are converted into acetic acid by AAB through oxidation for 48 to 96 hours (Guehi et al., 2010b). The acetic acid penetrates into the cotyledon increasing the interior acidity of the beans (Ardhana & Fleet, 2003; Soumahoro et al., 2020). The maximum microbial load of these microorganisms on cocoa beans ranges from 7.8 to 8 log CFU.g⁻¹ (Gálvez et al., 2007). The acetic acid produced is a key metabolite in the fermentation process of cocoa beans. It penetrates inside the beans where it causes, with the elevation of the temperature due to oxidation of ethanol, destruction

of internal cells and death of the embryo. This is accompanied by biochemical changes in the beans, leading to the formation of the precursor compounds of the characteristic aromas of cocoa (Afoakwa & Paterson, 2010; Koné et al., 2016). This process of formation of aroma precursor compounds (Ketoamine, amino acids and reducing sugars) continues during the drying and roasting of the beans. The common AAB isolated from fermented cocoa beans include genus *Acetobacter* of which *Acetobacter pasteurianus* has been identified as the most predominant species, and *Acetobacter syzygii*, *Acetobacter ghanensis*, *Acetobacter senegalensis*, *Acetobacter tropicalis*. The growth period of acetic acid bacteria during fermentation is still uncertain. Indeed, (Lefeber, Gobert, et al., 2011) showed that they are present only at the end of fermentation whereas (Papalexandratou et al., 2011) noticed the presence of *Acetobacter senegalensis* at the beginning of fermentation.

Other Microorganisms Populations

When fermentation lasts longer than 168 hours, the microbial substrates in the medium are exhausted and aeration becomes important. New microbial populations classified as spore-forming bacteria significantly appear in the later stages of fermentation with the increase of aeration, pH value (3.5 to 5.0), and elevated temperature to about 45–50°C (Binh et al., 2017). *Bacillus* species are the main bacteria found at this stage of fermentation. These microorganisms produce a number of chemical compounds influencing the aromatic properties of cocoa beans. Moreover, some isolated *Bacillus* species have the capacity to bio-convert all metabolic products (ethanol, lactic acid, mannitol, and acetic acid) into unwanted chemical compounds. Indeed, they have been reported to contribute to the generation of off-flavor components in fermented cacao beans. The *Bacillus* species mostly identified in cacao beans are *Bacillus brevis*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus licheniformis*, and *Bacillus subtilis* in Brazil (Schwan, 1998); *Bacillus cereus*, *Bacillus coagulans*, *Bacillus licheniformis*, and *Bacillus subtilis* in Trinidad (Ostovar & Keeney, 1973); *Bacillus licheniformis* and *Bacillus subtilis* in Ghana and Malaysia, while *Bacillus cereus* and *Bacillus subtilis* are the most dominant species in Ivory Coast (Ouattara et al., 2011). The recent mapping of main microbial strains implied in Ivorian cacao bean fermentation indicated the presence of *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus fusiformis*, *Bacillus thuringensis*, and *Bacillus pumilus* in different cacao-producing regions, which may differ from season to season (Hamdouche et al., 2015).

2.4.1.4. Products Release During Cocoa Beans Fermentation

During the fermentation, ethanol, organic acid, and free amino acids are produced inducing a reduction of the pH level. The endogenous enzymes (invertases, glucosidases, proteases, and polyphenol oxidases) in cacao pulps play a remarkable role during fermentation and they ensure the characteristic cocoa flavor and color (Afoakwa, Paterson, Fowler, & Ryan, 2009). In addition, pectinolytic enzymes present in the pulps degrade the pectin into aroma precursors such as fatty

acids, alcohols, and fatty esters (Illeghems et al., 2013). After 1–2 days, the alcohol concentration and temperature drastically increase, which consequently inhibit the yeast population activity. Then, the medium containing residual sugars, citric acid, and oxygen becomes suitable for the growth of lactic acid bacteria (LAB) by bio-converting these substrates into lactic acid, mannitol, and low concentration of acetic acid. The increased amount of oxygen in the medium consisting mainly of lactic acid and high quantity of ethanol used as substrates by acetic acid bacteria (AAB) are converted into acetic acid, carbon dioxide, and water. All the compounds liberated at the different stages of fermentation are very important in the development of complex aroma compounds. During fermentation, the phytochemical substances including phenolic compounds, mainly phenolic acids and anthocyanidins decrease as some permeate out of the beans while others are oxidized and polymerized into insoluble and high molecular-weight compounds like tannins (Afoakwa et al., 2013; Castro-Alayo et al., 2019). Moreover, the accumulation of organic acids during fermentation reduces the pH, which is important for the inactivation of the embryo, initiating enzymatic reaction that generate flavour precursors. This induces changes in the internal colour of the dried cocoa beans, from dark grey (slaty) of the unfermented bean through the deep purple colour of fermented beans to the brown colour of fully fermented cocoa beans (Castro-Alayo et al., 2019). In addition, the changes in cocoa beans colour during fermentation is observed using simple method through the determination of the degree of fermentation, called cut-test. The cut-test is the simplest and the commonly applied method to evaluate the quality of a random sample beans from a batch by visual inspection (Kongor et al., 2013).

2.5 Cocoa Beans Drying Processes

A drying system that minimizes the food's exposure to light (specifically ultraviolet (UV) light), oxidation, and heat plays a crucial role in preserving the essential bioactive and volatile compounds is needed for maintaining high-quality products (Ahmed et al., 2013). The primary objective of drying is to reduce the moisture content of cocoa bean from 60% to less than 10-7% so that it can be readily stored without any microbial growth and suitable for marketing (Nair, 2010). However, drying process can also enhance the shelf life and the quality of dried cocoa beans. The fermented dried cocoa beans triggers major polyphenol oxidation reactions catalyzed by polyphenol oxidase, which strongly contributes to the reduction of bitterness and astringency of cocoa beans as well as to the production of acetic acid (Afoakwa & Paterson, 2010; Nair, 2010). Numerous studies have demonstrated that volatile compounds such as pyrazines, furans, lactones are mostly formed during drying process (Watson et al., 2013). Drying is a crucial process for the final quality of the cocoa beans and should be slow in order to promote the evaporation of volatile acids such as acetic acid (Guehi et al., 2010; Saltini et al., 2013). In fact, a rapid drying of fermented cocoa beans leads to an accumulation of acidity in the beans (Kongor et al., 2013). However, slower drying allow a rapid reabsorption of moisture which induces the proliferation of molds in the beans (Guehi et al.,

2010; Kongor et al., 2013). Up to date several drying techniques have been used in cocoa growing regions, which can be classified in two main types, including natural or solar drying techniques and the artificial or industrial drying techniques. These drying techniques serve as the foundation for various research efforts aimed at enhancing drying efficiency and improving the flavours and the quality of dried cocoa beans (Guda et al., 2017; Lasisi et al., 2014; Ofori et al., 2014).

2.5.1. Open Sun Drying

Sun drying is the oldest, most affordable, highly popular, and readily accessible technique that can be implemented using both basic and advanced techniques, with a particular advantage in regions abundant in solar radiation such as the tropical and subtropical countries (Bala & Hossain, 2012; Bala & Janjai, 2012). A significant portion, more than 60% of African and Asian cocoa beans are locally dried using the open sun approach (Tardzenyuy et al., 2020), which contributes to the relatively higher moisture content observed when farmers sell their products. Solar energy stands out among other renewable energy resources as an indispensable source for the future due to its cost-effectiveness, abundant availability, unlimited potential, environmental friendly, and non-pollutant (Sahdev et al., 2017). Open sun drying is a widely practiced method that involves spreading cocoa beans on raised wooden mats, plastic sheets, or concrete floors to expose them to direct sunlight, and it is generally employed by small farmers. During this process, the beans are manually turned at regular intervals, requiring constant supervision to prevent biotic contaminants and to relocate the beans to a shaded area in case of rain. While this method is freely accessible, it is heavily reliant on weather conditions, labor-intensive, time-consuming (typically taking 7-22 days), and exposes the beans to potential vermin and environmental pollutants (Lasisi et al., 2014). Consequently, open sun drying carries an increased risk of producing inferior quality beans compared to more controlled drying methods. Extended drying periods can lead to mold growth, further compromising the quality (Misnawi, 2012). It is worth noting that some uninformed farmers resort to drying their cocoa beans directly on bare ground, increasing the risk of contamination from stones, soil, and surface organisms. Additionally, drying beans on tarred road sides exposes them to potential contamination by carcinogenic compounds (Tardzenyuy et al., 2020). Although open sun drying is the most cost-effective and accessible option for every farmer, its practical feasibility is limited during periods of heavy rain and high humidity.

Jinap et al. (1994) tested and evaluated the open sun drying process using bamboo planks lined with gunny sacks which were raised approximately 2 m above the ground. The cocoa beans were dried for 120 hours under the sun from 8 am to 6 pm, and the quality parameters of cocoa bean were thereafter conducted when the moisture content reached 8%.

Hii et al. (2008) investigated the drying behavior of cocoa beans applying open sun drying process. The beans were thinly exposed on a meshed wooden tray with area about 30 cm x 90 cm and raised 1 m above ground. The drying was carried out from 9 am to 6 pm daily and beans were

turned every 1 h to assure uniformity. The ambient temperature and relative humidity were recorded, and a theoretical design was applied on the drying kinetics using Fick's law of diffusion to determine the efficient diffusivity value.

In their work, Sahdev et al. (2017) tried to determine the mass effect on the convective heat transfer coefficient of sun drying peanuts. Experiments were carried out in the natural climatic conditions of Rohtak, India, in May 2016. Under open sun drying (OSD) conditions, 130 g and 198 g peanut samples were dried until almost no change in mass was recorded. Data on evaporated mass, peanut temperature, relative humidity, and ambient temperature were collected hourly. The experimental data obtained were used to determine the constants "C" and "n" in the expression of Nusselt numbers using the linear regression method. The convective heat transfer coefficient increased as the peanuts' mass increased. The experimental errors ranged from 44.29 to 48.77 %.

2.5.2. Artificial Drying

Artificial drying is a widely employed method for drying cocoa beans, offering controlled and efficient drying conditions compared to natural or sun drying methods (Guda et al., 2017). The primary purpose of artificial drying is to reduce the moisture content of cocoa beans in a controlled environment, ensuring uniform drying and preserving their quality. By using specialized drying equipment and techniques, artificial drying of cocoa beans provides several advantages, including time efficiency, quality preservation and flexibility. Several artificial drying techniques using various equipment have been developed for cocoa beans drying in different cocoa growing regions (Guehi et al., 2010; Hii et al., 2019).

2.5.3. Microwave Drying

Microwave drying operates on the same principle as heating food in a microwave oven, where the drying process stops when the microwave machine or oven is switched off. Feng et al. (2012) explain that microwave drying relies on electromagnetic radiation at frequencies of 915 or 2,450 MHz, which enables a unique volumetric heating mode. The lousy nature of food products leads to rapid energy absorption by moisture, resulting in fast heating and drying. This technology has shown promising results by significantly reducing drying time and improving product quality. To enhance microwave drying processes, there is a need for advancements in engineering design and process optimization. Computer simulation techniques have been developed to predict temperature and moisture distribution within the drying product. However, despite the experimental successes achieved in drying cocoa beans using microwaves, this method is limited to small-scale applications in rural areas and large-scale cocoa production. Microwave drying relies on the supply of additional energy that is preferentially absorbed by the solvents, promoting evaporation. Microwaves belong to the electromagnetic energy spectrum, ranging from 300 MHz to 300 GHz, and are generated by magnetrons through perpendicular electric and magnetic fields. Microwave heating is a direct

method, wherein polar materials within the rapidly alternating electric field align and reorient themselves in response to the field's direction. The rapid changes at 2,450 MHz induce molecular reorientation, leading to friction and the generation of heat. The properties of materials exposed to microwaves vary depending on their ability to absorb energy, which is characterized by the loss factor (Bossche & Griet, 2014).

Firihu and Sudiana (2016) conducted a study using cocoa beans to observe the experimental system performance of microwave drying process. Cocoa beans drying process was carried out by applying three power levels, calculated as 600 W for high power, 300 W for medium power and 150 W for low power at 2.5 GHz with a power consumption of 1.43 kW. The results showed that the humidity content and drying rate continuously reduced with drying. The authors stated that the research provides a succinct description effort made to obtain basic drying parameters under various microwave conditions from experimental results.

In their comparative study on cocoa beans drying process, Guda et al. (2017) tried to evaluate the effect of different drying methods including microwave, tray dryer, solar cabinet and air oven dryer on moisture content, drying time and quality. The objective was to determine the most effective drying method for proposition to the farmers. The experimental results were analyzed to identify the optimal drying method. The findings revealed significant variations in the time required for drying cocoa beans from an initial moisture content of 49.35% (w.b) to a final moisture content of 6.36% (w.b). Microwave drying exhibited the shortest drying time of 0.4 hours. The solar cabinet dryer and tray dryer recorded drying times of 16 hours and 8 hours, respectively. The tray dryer offered a time-saving of approximately 22 hours, while the solar cabinet dryer saved around 14 hours. Additionally, the tray dryer produced a higher quality cocoa bean powder, as indicated by the cut test score. The average drying rates were determined to be 0.0046 kg/kg-h for the tray dryer, 0.0032 kg/kg-h for the solar dryer, the cut test scores for the tray dryer, solar cabinet dryer, and microwave oven, were 985, 970, 935, respectively. The authors concluded that microwave and tray dryers proved to be the most efficient drying method, offering significant time savings and producing higher-quality cocoa beans.

Boldor et al. (2005) examined the influence of microwave energy level on temperature profiles and moisture reduction in free range peanuts during continuous microwave drying with a flat applicator. The peanuts' moisture content and the microwaves' power level affect temperature profiles determined with fiber optic probes. While the power level primarily determined the optimum temperature in the microwave applicator, the rate at which this maximum was achieved was determined by the peanuts' dielectric properties and moisture content.

Schirack et al. (2007) investigated the blanching of peanuts using a continuous microwave method to describe the effective processing parameters. The microwave exposure time increased airflow in the microwave applicator during processing. Factors investigated when the treatments were based on consumed energy were mean and maximum internal temperature, moisture loss, and bleach ability. It was discovered that high bleach ability resulted from higher temperatures and lower

moisture loss. It was also stated that treatments above 110 °C, resulting in final moisture content of 5.5 % or less, resulted in bleach percentages higher than the industry standard of 85 %. These authors concluded that microwave technology substantially reduces the time needed to produce adequate heat to dry the peanuts for suitable bleach-ability.

John and Otten (1989) investigated the drying behaviors of single layers of peanut pods and kernels exposed to various microwave power levels. For this purpose, freshly dug and partially dried peanuts were exposed to adequate power levels ranging from 250 to 1000 W. The airflow, temperature, and relative humidity are maintained at constant levels. Drying rates for pods have varied from 10 to 94 times the normal traditional railcar (wagon) drying period, and grain drying rates have ranged from 8 to 32 times.

Lewis (2011) developed a feedback controller to regulate the temperature of the drying air as well as the drying time. Inputs such as inlet air temperature, ambient air temperature, relative humidity, and kernel moisture content are processed by the device. The microwave drying process has been used to determine the kernel moisture content in unshelled peanuts in a non-destructive and rapid manner using measurements of their diverse permittivity in free space independent of bulk density

2.5.4. Oven Drying

A oven drying is a specialized type of oven commonly utilized in laboratory settings, designed to facilitate the gradual and uniform removal of moisture from food matrices, tools, and temperature-sensitive chemicals. It operates through low-temperature convection or forced air, creating an environment within a drying chamber that promotes efficient moisture evaporation. In contrast, an electric dehydrator is a self-contained appliance equipped with a heat source, a circulation fan, and multiple drying trays, enabling the simultaneous drying of various food items. Higher-quality dehydrators often incorporate thermostats and double-walled construction to optimize energy usage. These devices allow researchers to dry different types of produce at desired temperatures for experimental purposes, offering flexibility and control (Guehi et al., 2010; Waheed & Komolafe, 2019).

Ndukwu (2009) attempted to assess the effect of various drying parameters on cocoa bean drying using three temperature ranges of 55 °C, 70 °C, and 81 °C) and three air velocity levels corresponding to 1.3 m/sec, 2.51 m/sec, and 3.7 m/sec, respectively). The dryer required a thin layer of cocoa bean 5 cm deep to dry from 79.6 % (d.b.) to 6 % (d.b.) in 4 to 6 h of continuous drying. The drying kinetics increased with temperature and air velocity but decreased over time. It was estimated that at 55 to 81 °C and an air velocity of 1.3 m/sec, the dryer could remove an average of 4.66 kg of water per day, while at 2.51 m/sec, it could remove an average of 5.3 kg of water per day under the same drying conditions.

Hii et al. (2008) studied the effect on thin layer kinetics of cocoa beans using natural sun light and hot air-ventilated oven. For this purpose, cocoa beans were artificially dried employing an hot air-ventilated oven at 60°C, 70°C, and 80°C, in which the beans were exposed in single layer (about 1.05 cm thick) and the relative humidity were found to be 6%, 4.7%, and 2.9% at drying temperature of 60°C, 70°C, and 80°C. Drying was conducted for 8 h and the beans were turned every 2 hours to assure uniformity. Theoretical design was conducted on the drying kinetics using fick's law of diffusion to determined the efficient diffusivity value. The authors observed that the coefficient of determination (R^2) between the experimental and predicted moisture ratio data yielded favorable values within the range of 0.9845 to 0.9976.

Hii et al. (2009) attempted to evaluate the effect of drying technique using artificial and natural drying process of cocoa beans. The authors demonstrated that the obtained drying data were fitted to various existing thin layer drying models. In addition, The selection of the most suitable model was determined by comparing the coefficient of determination (R), reduced chi-square (χ), and root mean square error (RMSE) between the experimental and predicted values. The results indicated that the new model provided the best description of the drying kinetics for both artificial and natural drying of cocoa beans under the tested conditions.

In an investigation, Guehi et al. (2010) evaluated and compared the effect of different drying technique (sun drying, oven drying, and sun-oven mixed drying) on the chemical quality traits of raw cocoa beans. The drying was carried out using an air-ventilated oven at temperature of 60°C until the moisture content attained 7%. The obtained results showed that the sun dried cocoa beans presented better quality compared to oven dried beans and sun-oven mixed dried beans.

In another study, Jinap et al. (1994) attempted to evaluate the impact of different drying methods, oven drying (60°C for 20 hours) and air blow drying (60°C for 10 hour) on acidity and volatile fatty acids such as acetic, propionic, butyric, isobutyric and isovaleric acids content of cocoa beans, and on the flavor of the resultant chocolate. Based on the results, there were no significant differences between cocoa beans that underwent sun-drying and air-blow drying in the acidity and C3-C5 volatile fatty acid content; both were evaluated favourably by taste panellists. It was also observed that oven-dried beans had high concentration of volatile fatty acid content and produced chocolates with a high intensity of off-flavour.

In the study conducted by Tardzenyuy et al. (2020) in Fako/Cameroon, a locally constructed firewood oven dryer was established using bricks and cement plaster. The dryer featured a tilting platform designed to support the suspended heating and combustion chamber above the ground. To address the issue of smoke, a 2-meter heat/air conveying duct (the kiln) was implemented, effectively separating the combustion chamber from the drying chamber. Inside the kiln, dry wood was burned to generate heat. The drying chamber included a perforated drying mat to ensure unrestricted airflow. The rotation rates of the cocoa beans during drying and the rate of exit for saturated air matched the rate of entry for hot and dry air into the drying chamber. While this approach yielded promising

outcomes, achieving smoke control and maintaining consistent drying temperatures between 35-40°C, as well as ensuring uniform heat distribution, posed challenges during the experimental process. Notably, a drying efficiency of 7% was achieved within 96 hours of continuous drying. The authors stated that further studies should be performed on the cocoa bean quality such polycyclic aromatic hydrocarbons (PAHs), acidity, mould content, and ochratoxin A (OTA).

Nwakuba (2017) carried out a study to predict the drying rate of thin-layer cocoa beans in a convective hot air dryer using dimensional analysis. The experiment involved drying 600 g of fermented cocoa beans in an air-ventilated oven dryer. Three different air temperatures (60°C, 70°C, and 80°C) were utilized, with a natural convective air flow rate of 0.01 m/s. The relative humidity of the drying air was measured at each temperature, resulting in readings of 6%, 4.7%, and 2.9%, respectively. Drying was considered complete when the desired moisture content of approximately 7.5% (dry basis) was reached. The study demonstrated high coefficients of determination, namely 94.7%, 99.8%, and 99.5% at 60°C, 70°C, and 80°C, respectively, indicating the reliability of the predictive method. The authors concluded that drying cocoa beans at higher temperatures, lower moisture contents, and a constant air velocity led to a decrease in the drying rate.

2.5.5. Freeze-Drying

Freeze drying, initially employed in 1949, is a distinctive drying technique that effectively eliminates all moisture from food and minimizes the impact on its taste, bioactive and volatile compounds compared to conventional dehydration methods. This process involves freezing the food and subjecting it to a powerful vacuum environment. As a result, the frozen water within the food undergoes sublimation, directly transforming from solid to vapor state (Banboye et al., 2020). Freeze drying has demonstrated its suitability for experimental drying of cocoa beans in chocolate industries.

2.5.6. Solar Drying

Solar dryers, as described by (Fagunwa et al., 2009), are devices designed to utilize solar energy for the purpose of drying various substances, particularly food items, including cocoa beans. These dryers operate by heating the air within a controlled chamber to a consistent temperature, harnessing the generated heat to facilitate the drying process of the produce. The air employed for drying is directed through a collector surface, where it undergoes heating before being circulated to effectively dry the food items enclosed within the dryer. Unlike open sun drying, solar dryers provide a protected environment for the produce, shielding it from direct wind exposure. Ventilation in these dryers is achieved through regulated air inlets and outlets, small solar ventilators, or temperature differentials resulting from factors such as solar exposure or vertical height variations. While solar dryers are commonly used for experimental and research purposes in drying cocoa beans, a select number of farmer cooperatives in Asia have successfully employed greenhouse dryers on a larger scale to dry cocoa beans (Bala & Hossain, 2012; Bala & Janjai, 2012). Solar dryers have been

extensively studied by researchers like Sivakumar and Rajesh (Sivakumar & Rajesh, 2016), who, along with other studies have identified four main types of solar dryers, including direct solar dryer, indirect solar dryer, hybrid solar dryers.

Bonaparte et al. (1998) studied the effect solar drying process on the quality of cocoa beans by applying four method of drying (indirect solar dryer, direct solar dryer, open air/perforated steel surface and open air/ non-perforated wooden surface. These methods were conducted at three loading rates: 13.7, 26.9 and 40.4 kg/m². The solar driers were more effective than sun drying units in terms of moisture removal efficiency at loading rates of 26.9 and 40.4 kg/m², particularly in the initial stages at 13.5 kg. Moreover, external mould development were significantly mitigated. However, during the final stages at 13.5 kg/m², open air sun drying on wooden surfaces proved to be more effective. Despite the faster drying process in the driers, the dried beans exhibited similar internal quality. Of all methods applied, the authors concluded that the beans from the indirect dryer demonstrated the highest quality.

Hii et al. (2013) conducted an investigation to analyze the kinetics of heat pump drying for cocoa beans under stepwise drying conditions. The analysis included a detailed examination of heat and mass transfer using advanced 3-D computer simulation. The drying experiments involved fermented cocoa beans subjected to three drying profiles: constant temperature (56°C), step-up temperature (30.7°C- 43.6°C- 56.9°C), and step-down temperature (54.9 °C- 43.9 °C). In order to accurately capture the drying process, the heat and mass transfer models incorporated a shrinkage factor to account for bean shrinkage. The simulation results exhibited favorable outcomes, with mean relative errors ranging from 3.1% to 12.1% for predicted moisture ratio profiles in both models (with and without the shrinkage factor). Additionally, the predicted bean temperature profiles demonstrated excellent agreement with experimental data, with mean relative errors below 5%. The obtained results showed that shrinkage played a minor role in the analysis due to the limited shrinkage ratio observed before and after drying. Consequently, the incorporation of shrinkage resulted in precise predictions of moisture ratio and temperature profiles during heat pump drying of cocoa beans. The autheors stated that highlights the successful application of advanced modeling techniques and heat pump drying analysis for cocoa beans.

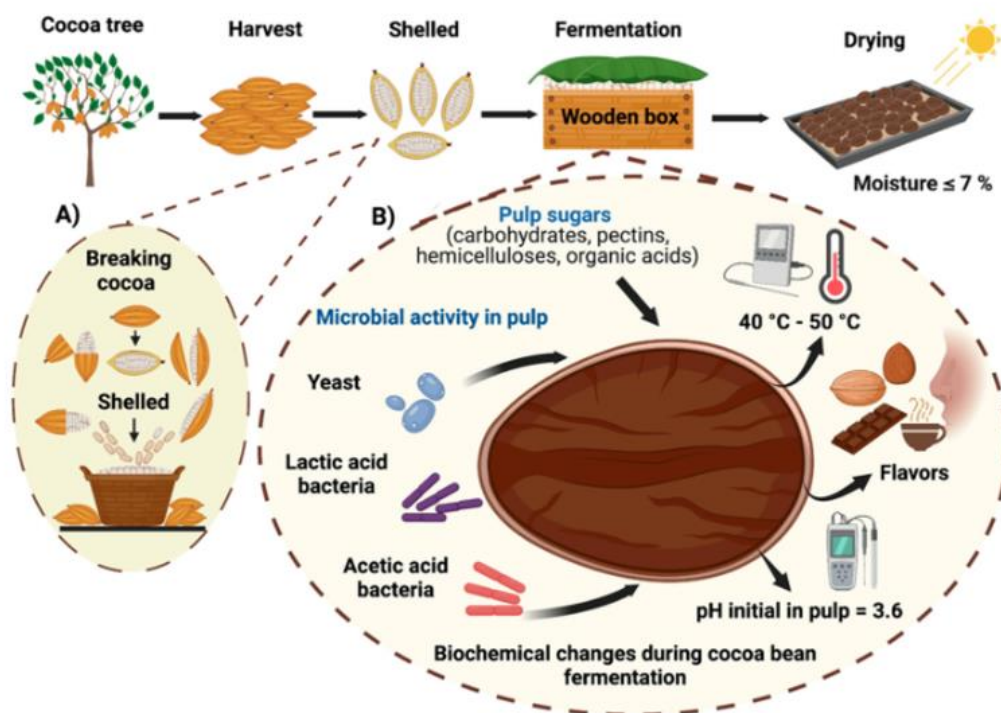


Figure 2.2. Cocoa bean post-harvest stages (adapted from Gutiérrez-Ríos et al., 2022).

2.6 Physicochemical Composition of Cocoa Beans

Depending geographical regions, where cultivated cocoa varieties are the same, each location exhibits unique characteristics that distinguish them from one another. These distinguishing factors include variations in bean size, chemical composition, organoleptic characteristics, and the degree of fermentation (Clapperton et al., 1994; Counet et al., 2004; Jalil & Ismail, 2008). These differences arise from the genetic variations among individual cocoa bean varieties (Caligiani et al., 2010; del Rosario Brunetto et al., 2007; Rodriguez-Campos et al., 2011). Cocoa bean is made up of two primary components namely, the testa (seed coat or the shell) and the embryo (Wollgast & Anklam, 2000a). Attached to the testa is the sugary, white mucilaginous pulp that enfolds it, while the embryo is contained within the seed coat or testa. The main parts of the embryo are two folded cotyledons linked by a small embryonic axis (Ziegler & Biehl, 2003). The dry weight of cocoa bean considerably differ, but is around 1.0–1.2 g (Ziegler & Biehl, 2003).

The chemical composition of cocoa beans have been intensively investigated by several workers (Afoakwa & Paterson, 2010; Bertazzo et al., 2011; Wollgast & Anklam, 2000a; Ziegler & Biehl, 2003). Unfermented fresh cocoa bean contains approximately 40-60% water, carbohydrate including 2-3% sucrose, 1-1.5% pectin and 1% acid. Proteins, amino acids, 5-6% polyphenols, 1-2% theobromine, 1% caffeine, vitamin C and mineral salts are also present in the pulp surrounding the beans of the mature cocoa pods (Bertazzo et al., 2011; Jinap et al., 1995; Kongor et al., 2016). The chemical composition of the pulp and the bean changes significantly during the pot-harvest

processing of cocoa and in particular during the fermentation. After fermentation and drying, the average contents of cocoa bean is approximately 5 to 8 % water, 17.25% protein, 52.41% fat, 6.20% fiber, 4.26% ash, and 15.69% carbohydrates (Liendo et al., 1997; Sotelo & Alvarez, 1991). The composition of cocoa beans greatly vary depending on the specific variety, growing region, practices and processing methods employed during fermentation and drying. Moreover, cocoa beans contains various aroma precursors, including sugars, amino acids, phenolic compounds, lipids (fats) that contribute to the complex flavor and aroma profile of cocoa beans and chocolate. These aroma precursors are converted into aroma compounds through different processes comprising fermentation, maillard or caramerization reaction (drying and roasting process) and oxidation reaction.

2.6.1. Carbohydrate in Cocoa Beans

Cocoa beans contain an important number of carbohydrates, although their composition can vary depending on factors such as the cocoa variety and the stage of ripeness. Carbohydrates (10% to 24% of the total weight) in cocoa beans include various types of sugars, fiber, and starch. Cocoa beans is a mixture of different types of sugars, including sucrose, glucose, fructose, sorbose, mannitol and inositol, which have been reported to be predominant in the beans with sucrose made up of approximately 90% of the total sugars (Afoakwa & Paterson, 2010; Afoakwa et al., 2013; Kongor et al., 2016; Ziegleder & Biehl, 2003). Cocoa fibers are composed of pentosans, galactans, mucins containing galacturonic acid, and cellulose, also contain dietary fiber, which is primarily composed of non-digestible carbohydrates. The fiber content in cocoa beans can range from around 2% to 4% of the total weight. In addition, starch which is consider to be the most abundant and complex carbohydrate in plant kingdom has been reported in cocoa beans. The starch content in cocoa beans can vary, typically ranging from 4% to 6% of the total weight (Bertazzo et al., 2011).

2.6.2. Lipids in Cocoa Beans

Cocoa butter consisting the fat of the beans is a mixture of triglycerides (Watson et al., 2013). It is presented in different aspects: crystallized, more or less unctuous depending on the storage temperature and the proportions of liquid and solid triglycerides. Cocoa butter is distinguished from other vegetable fats by a limited yield of unsaturated fatty acids. Cocoa fat contains approximately 95% triacylglycerols, 2% diacylglycerols, <1% monoacylglycerols, 1% polar lipids, and 1% free fatty acids as percentage of lipids (Ziegleder & Biehl, 2003). The characterization of the fatty acid composition employing gas chromatographic analysis revealed mainly oleic, palmitic and stearic acids (Torres-Moreno et al., 2015). Cocoa butter represents 13.69 mg/g of cocoa powder. The principal fatty acids in cocoa fat are saturated (35% of stearic acid (18:0) and 25% of palmitic acid (16:0) and monounsaturated, mainly dominated by oleic acid (18:1, 35%), with the remaining fat

being polyunsaturated linoleic acid composed of 3% of total fatty acids in cocoa butter (Bracco, 1994).

2.6.3. Proteins and Amino Acids in Cocoa Beans

Proteins are among the main aroma and taste precursors of cocoa beans products, comprising chocolate and related products, which represents about 16-17% of fermented cocoa beans. The free amino acids constitute the non-protein nitrogen fraction of which 0.02% is in the form of ammonia formed during the fermentation of the beans. In their work, (Zak & Keeney, 1976) identified four major fractions of protein in cocoa beans accounting for 95% (w/w) of the total seed proteins, and these include globulins (salt-soluble), prolamins (alcohol-soluble), albumins (water-soluble) and glutelins (soluble in dilute acids and alkali). Of all the proteins found in cocoa beans, albumin is detected as the predominant polypeptide representing about 25% of the total bean protein and contains a molecular weight of 21 kDa (Dodo & Furtek, 1994; Lerceteau et al., 1999; Voigt et al., 1993).

Different type of enzymes are found in beans, namely α -amylase, β -fructosidase, β -glucosidase, β galactosidase, pectinesterase, polygalacturonase, proteinases, alkaline and acid phosphatases, lipase, catalase. The activities of these enzymes along with peroxidase and polyphenol oxidase were detected in fresh cocoa beans. These enzymes are inactivated to a large extent during post-harvest treatments (Hamdouche, 2015). The vicilin-class globulins are quantitatively digested by some enzymes during fermentation (88–90% of the initial content) into aroma and taste precursors such as peptides and amino acids, which are important precursors for the formation of cocoa flavour through Maillard reactions during drying and roasting processes (Hue et al., 2016; Voigt et al., 1993). Moreover, proteins in cacao pulp mass and within the beans are metabolized into peptides and free amino acids, namely, internal precursors (tyrosine, alanine, and leucine) and external precursor (phenylalanine) by proteolysis reaction induced by carboxypeptidase and certain *Bacillus* and yeasts strains. These are the main aroma precursors and they significantly contribute to the aroma of the fermented cocoa and its related products (Beckett, 2009; Janek et al., 2016; Kratzer et al., 2009).

2.6.4. Volatile and Aroma-Active Compounds in Cocoa Beans

Volatile compounds are low molecular weight compounds (less than 300 Da) with a high volatility vaporizing easily at room temperature which comprise an appropriate number of molecules that can reach the olfactory epithelium as the molecular dispersion transported by the air streams because of inhalation and expiration, adequate hydrosolubility distributing into the mucus that covers the sensitive olfactory cells, delicate liposolubility dissolving in membrane lipids connected to proteins of receptors and chemical characteristics binding to specific proteins (Angerosa et al., 2000) (Angerosa, 2002). In fact, some of volatile compounds reach the olfactory epithelium, dissolve into

the mucus and may bond with olfactory receptors to give an aroma sensation (Angerosa et al., 2000; Kalua et al., 2007).

Cocoa volatiles are derived from aroma precursors generated during fermentation and beans drying. In cocoa beans and products, volatile compounds are responsible for aroma, while phenolic compounds are associated to taste. Food flavor is straightly referred to quality and strictly effects the distinction, choice, and admissibility of the food, impacting consumers' preferences. Therefore, food flavor plays a significant role in human nutrition and reveals, to a particular level, the life manner and quality of consumers. Flavor gathers the complex conception of aroma and taste relating to the somatosensory information. Different cocoa types may demonstrate different and specific flavors since the concentration and sensory character of these compounds vary significantly (Tournier et al., 2007; Yin et al., 2017; Ziegler, 2017).

The unique flavor of cocoa beans and products distinguished it from other edible seeds owing to its complex mixture of volatile and some non-volatile compounds and is characterized by a series of positive sensory features that generate a correct balance of green, fruity, bitter, and pungent sensory characteristics. The distinctive flavors of cocoa beans and associated products are attributed to the complex volatile fraction consisting of a diverse mixture of compounds (Magi et al., 2012). To date, more than 600 flavor compounds have been detected from cocoa beans and cocoa products (Crafack et al., 2013). These compounds are made up of various chemical classes such as nitrogen and oxygen heterocyclic compounds, aldehydes and ketones, esters, alcohols, hydrocarbons, nitriles and sulphides, pyrazines, ethers, furans, lactones, thiazoles, pyrones, acids, phenols, imines, amines, oxazoles, and pyrroles (Afoakwa et al., 2008; Frauendorfer & Schieberle, 2008; Granvogl et al., 2006; Jinap et al., 1995; Schnermann & Schieberle, 1997). Table shows a summary of some main volatile compounds identified in different cocoa beans varieties from several scholars (Afoakwa et al., 2012; Ramos et al., 2014; Rodriguez-Campos et al., 2012). Most of these volatile compounds possess particular flavor characteristics. Thus, while most esters confer a fruity/flowery attribute to flavor, pyrazines usually give earthy/roasted flavor to cocoa beans and products (Owusu, 2010).

Different sensory descriptions of cocoa beans and products flavor, include green, sweet, chocolate and caramel like, fruity, bitter, and pungent being the most prominent sensory perceptions of consumers.

Table 2.1. Some main volatiles compounds identified in fermented and dried cocoa beans

Esters	Pyrazines	Alcohols	Aldehydes	Furans	Acids
Ethyl acetate	2-Methylpyrazine	2-Methyl-1-butanol	2-Phenyl acetaldehyde	2-furfural	Acetic acid
Isobutyl acetate	2-Ethylpyrazine	2,3-Butanediol	2-Methylpropanal	5-Methyl-2-furfural	Hexanoic acid
Isoamyl acetate	2,5-Dimethylpyrazine	2-Pentanol	2-Phenylpropanal	2-Furfuryl acetate	Nonanoic acid
Benzyl acetate	2,6-Dimethylpyrazine	1-Hexanol	2-Methylbutanal	2-Acetylfuran	Cinnamic acid
Ethyl phenyl acetate	2-Ethyl-5-methylpyrazine	2-Hexanol	3-Methylbutanal	2-Furfuryl propionate	
2-phenylethyl acetate	2,3-Diethylpyrazine	E-3-Hexen-1-ol	2-Phenyl-2-butanal	Furaneol	
Ethyl lactate	2,3-Dimethylpyrazine	1-Phenylethanol		Maltol	
Ethyl 2-methylbutanoate	Pyrrole	2-Phenylethanol			
Ethyl 3-methylbutanoate	2-Acetylpyrrole	Benzyl alcohol			
Isoamyl benzoate	Pyrrole-2-carboxaldehyde				
Ethyl decanoate					
Methyl cinnamate					
Ethyl cinnamate					

Although volatile compounds are present at very low concentrations, their amounts in cocoa beans quantitatively and qualitatively are variable and belong to various factors such as variety, geographical origin, pedoclimatic conditions, fermentation index of cocoa beans, processing and storage conditions. The organoleptic quality of cocoa beans is linked to the fermentation, drying and roasting stage of cocoa beans (Kongor et al., 2016; Ramli et al., 2006). Over the fermentation, drying and roasting process, several metabolic procedures occur in cocoa beans with the generation or alteration on profiles of some compounds. These variations have the effect on the quality degree, sensorial properties, oxidative stability and/or nutritional value of the cocoa beans and products (Delgado-Ospina et al., 2020). Cocoa beans and related products have higher content of total aroma compounds being formed from the fermentation, drying and roasting process, and they are direct metabolites generated in plant organs by intracellular biogenic pathways, oxidative procedures, Maillard reaction and the Strecker degradation of flavor precursors and their intermediates (Afoakwa et al., 2008). All of them are responsible for sensory features valued by consumers (Morales et al., 2005). Moreover, metabolized products, derived from over fermentations, drying and roasting, conversion of some amino acids, enzymatic activities of molds, bacteria (*Bacillus spp*) or oxidative procedures, are closely associated with some off-flavor compounds of cocoa beans and products. As

exhibited in figure, all the volatile compounds determined in cocoa beans have been associated to three major biosynthesis pathways, with main precursors compounds being amino acids, fatty acid and sugars (Colonges, Jimenez, et al., 2022; Colonges, Seguine, et al., 2022; Dzialo et al., 2017).

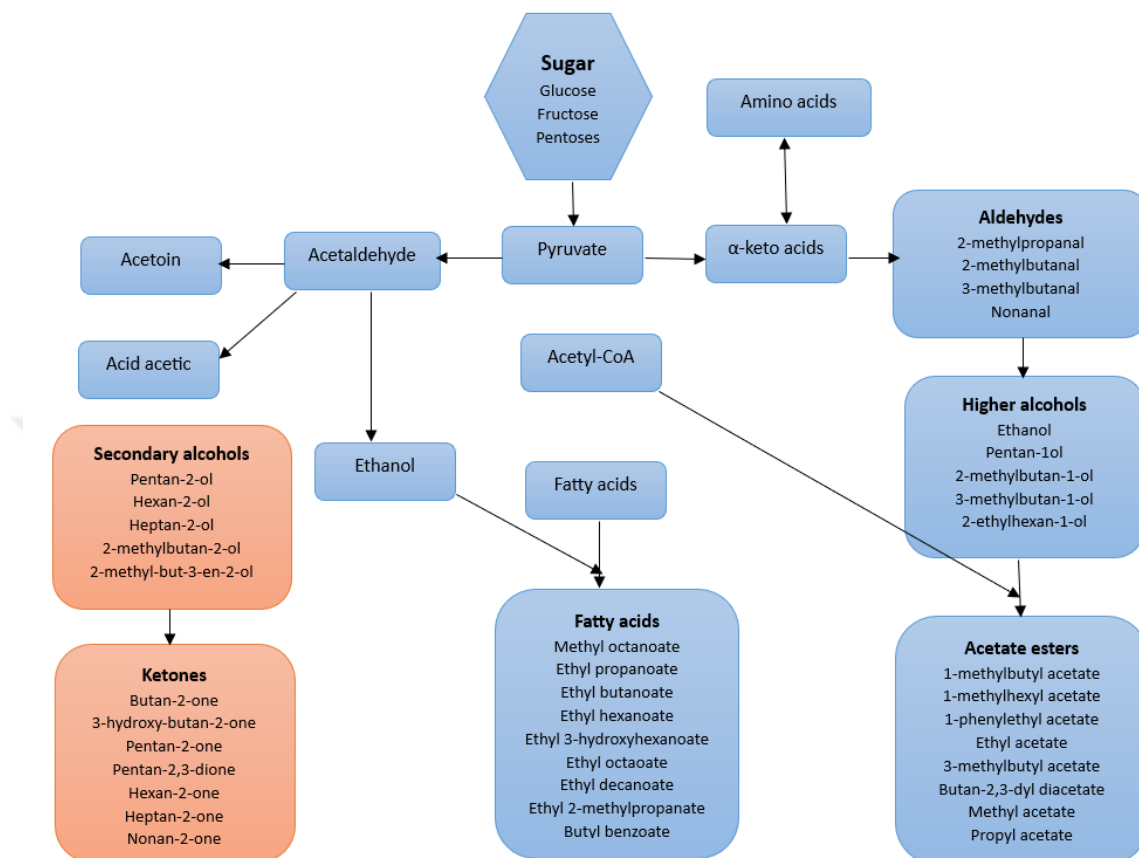


Figure 2.3. The major pathways (fatty acids, amino acids and sugars degradations) involved in the generation of the ultimate volatile compounds of cocoa beans (adapted from Dzialo et al., 2017).

A gas chromatography (GC) equipment is a chemical substances analysis instrument widely used for partitioning chemical compounds in a complex mixture sample. It employs a flow-through capillary tube known as column, through which diverse chemical substances of a mixture of sample pass in a gas stream (carrier gas, mobile phase) at different rates and interaction within chemical and physical column filling, known as the stationary phase. The objective of the stationary phase in the column is to separate various compounds, making each one exit the column at a different time (retention time) (Escuderos, 2011; Liu et al., 2021). Numerous studies using GC apparatus to identify and quantify volatile components in cocoa beans and products have been published in the literature (Afoakwa et al., 2012; Dzialo et al., 2017; Ramos et al., 2014; Rodriguez-Campos et al., 2012). Furthermore, to determine which volatiles significantly contribute to the odor of a product or which volatiles are responsible for the differences between the odors of two products, it is necessary to ensure that the method of extraction provides an extract with an odor that is representative of the original sample. This representativeness study should be a prerequisite to further aroma analyses

such as GC–Olfactometry (GC–O) (Amanpour et al., 2015; Amanpour et al., 2016). Several extraction techniques have been developed to isolate the volatile compounds of cocoa beans and products, including purge and trap extraction, solid-phase microextraction, steam distillation, simultaneous distillation extraction (Escuderos, 2011; Ducki, 2008; Saunshia, 2018; Mohamadi Alasti, 2019).

As aforementioned, the number of volatile substances detected in cocoa beans has reached over 600 (Crafack et al., 2013). GC coupled with FID (identification) and MS (quantification) is employed for characterization of volatile compounds. However, volatile compounds of cocoa beans research with many other foods shows that only a very small part of a large number of volatiles occurring in food, including cocoa beans and products actually contributes to the characteristic aroma (Amanpour et al., 2015). Thus, gas chromatography-mass spectrometry/olfactometry (GC–MS/O) has demonstrated to be a powerful technique in aroma research for the direct characterization of key aroma compounds in food matrices (Reiners, 1998; Issa-Issa et al., 2020; Mahmoud et al., 2024; Oussou et al., 2022). In other words, the technology of GC–O made it possible to distinguish identified volatiles into odor-active and non-odor-active compounds with regard to their existing concentration in the studied sample (Luna et al., 2006; Morales & Aparicio, 1999). Numerous techniques have been extended to gather and provide GC–O data and to assess the sensory contribution of single powerful aroma compounds, which can be classified in the following four techniques (van Ruth & O'Connor, 2001):

- ❖ **Dilution analysis methods**, used for the determination of potency concentrations according to stepwise dilution to the threshold, e.g. combined hedonic response measurement (Charm Analysis) and aroma extraction dilution analysis (AEDA).
- ❖ **Detection frequency methods**, employed for registering discovered aromas by a class of assessors. The number of assessors discovering an aroma (detection frequency) is applied as an evaluation of intensity of aroma.
- ❖ **Posterior intensity methods**, applied for generating evaluations of comprehended intensity, being registered after a peak has eluted.
- ❖ **Time-intensity methods**, used for generating assessments of comprehended intensity registered at the same time with the elution of the chromatographic peak, e.g.

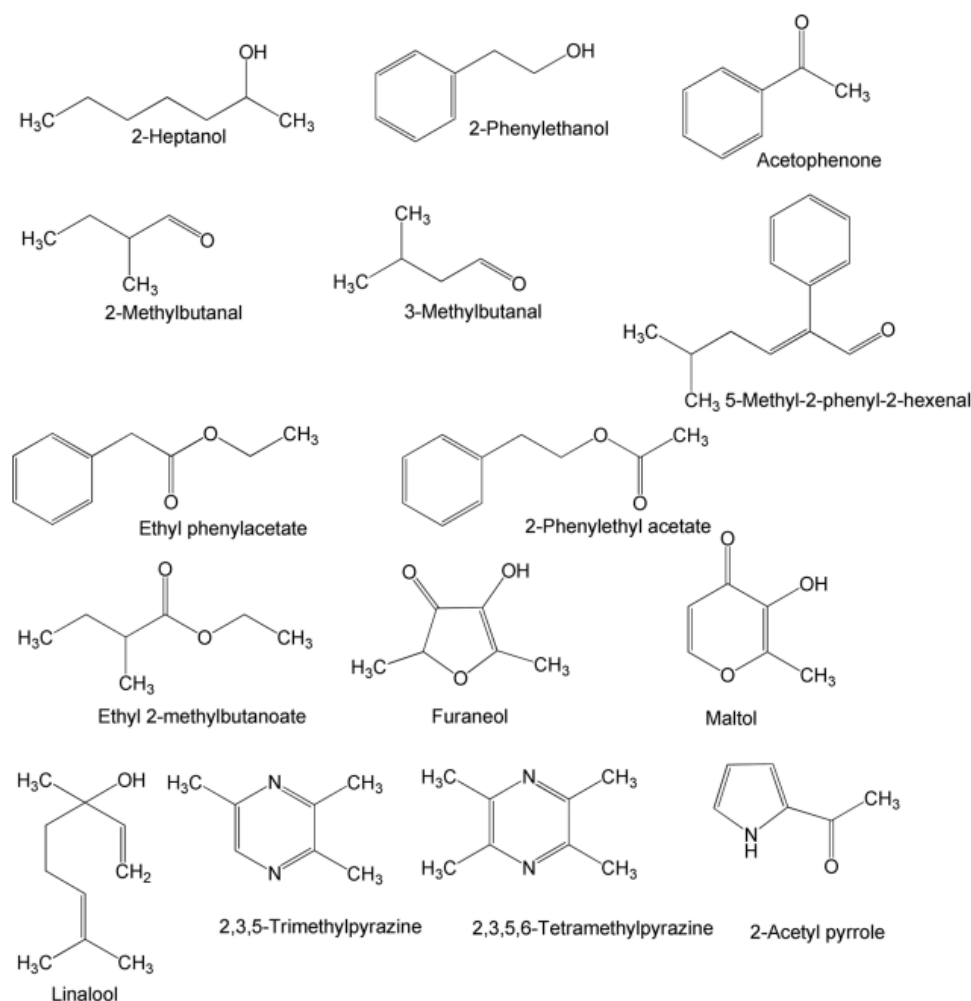


Figure 2.4. Chemical structures of some major aroma active compounds in dried fermented cocoa Beans (adapted from Aprotosoaie et al., 2016)

Volatile compounds are not produced in significant amounts during cocoa fruit growth but arise during the ripening stage, fermentation, drying and roasting process. During fermentation process, microorganisms induce biochemical, physical and chemical changes and an increase in some, fatty acids, protein and enzyme activities, thereafter these precursors are degraded during drying and roasting through Maillard reaction, oxidation and Strecker degradation. As mentioned earlier, the volatiles of cocoa beans are attributed to aldehydes, alcohols, esters, pyrazines, ketones, furans and, probably, others as yet unidentified volatile compounds. The major volatile compounds reported in fermented dried and roasted cocoa beans and products are the C6 and the C5 and C4-containing nitrogen volatile compounds (Afoakwa et al., 2008; Ramos et al., 2014; Rodriguez-Campos et al., 2012).

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Rodriguez-Campos et al. (2011) investigated the dynamic of volatile and non-volatile compounds in Forastero cocoa beans variety obtained from Cunduacan, Tabasco, México during fermentation and drying processes using principal components analysis. Thirty-nine different compounds were isolated and identified by SPME-HS/GC-MS and related to the desirable notes and off-flavor. 3-methyl-1-butanol acetate, pentanal and 2,3-pentanedione, and 1,3-butanediol and 2,3-butanediol were detected as main volatile compounds in fermented dried cocoa bean samples.

Chagas Junior et al. (2021) attempted to evaluate the profile of volatile compounds of on-farm fermented and dried cocoa beans inoculated with *Saccharomyces cerevisiae* KY794742 and *Pichia kudriavzevii* KY794725 isolated and analysis through SPME/GC-MS technique. The most important compounds with high content were detected as aldehydes, esters, ketones and alcohols and low concentration of off-flavours. The major volatile compounds present in cocoa bean samples were 2-nonanol, 2-phenylethanol, phenylacetaldehyde, benzaldehyde, isoamyl benzoate, 2-phenethyl acetate. Cocoa bean samples inoculated with *Saccharomyces cerevisiae* demonstrated the highest content of volatile compounds. The authors concluded that the incorporation of mixed culture starter can be an excellent alternative for cocoa producers, suggesting obtaining cocoa beans with pleasant characteristics for chocolate production, as well as creating a product identity for the growing region.

Barbosa-Pereira et al. (2019) studied the analytical dataset on volatile compounds of cocoa bean shells obtained from various cultivars and collected from different geographical origins. 101 volatile compounds, including aldehydes, esters, alcohols, furans, pyrazines were found as the main class compounds using HS-SPME/GC-MS. The predominant class of compound in cocoa bean shells was attributed to aldehydes from Brazil origin.

Qin et al. (2017) researched the volatile compounds in in Criollo, Forastero, and Trinitario cocoa seeds (*Theobroma cacao* L.) obtained from China in attempt to evaluate if they can be used as roadmap for future breeding or biotechnological applications. The aroma compounds emitted by these varieties of cocoa beans were detected and quantified by HS-SPME-GC-MS. Fifty-three compounds were identified, in which Trinitario cocoa had high contents of furfuryl alcohol, 3-carene, 2-pentanol, 1-pentanol, 2,3-butanediol, 2-heptanol, and benzyl acetate. Criollo cocoa contained high amounts of α -limonene, β -caryophyllene, β -myrcene, α -phellandrene, β -linalool, and acetic acid. Forastero cocoa also exhibited high contents of 3-methylbutanoic acid, 2-(2-butoxyethoxy) ethanol, anethole, and 2,4-pentanediol. Their results showed that the differences in the volatile compounds of the three morpho-genetic groups were essentially quantitative, and only a few compounds were found

exclusively specific to a certain group, mainly in Trinitario. The predominant volatile compound in the three cocoa beans varieties were aldehydes, terpenoids and esters.

Marseglia et al. (2020) carried out a study on volatile fingerprint of unroasted and roasted cocoa beans (*Theobroma cacao L.*). A total of 58 fermented and dried Forastero variety cocoa beans obtained from 22 various regions (America: Mexico, Cuba, Santo Domingo, Grenada, Trinidad, Venezuela, Ecuador, Perú, Brazil; Africa: Sierra Leone, Ivory Coast, Ghana, Nigeria, Sao Thome, Uganda, Tanzania, Madagascar; Southeast Asia: Sulawesi- Indonesia, Malaysia, Java- Indonesia, Papua New Guinea -Indonesia, Flores- Indonesia) were employed in the study. Volatile substances were separated and identified via HS-SPME/GC-MS system. It was observed that 57 volatiles were detected in unroasted (dried) cocoa beans, while 71 volatiles were determined in roasted cocoa beans. The detected compounds belong to various chemical classes comprising esters, alcohols, organic acids, aldehydes, ketones and pyrazines. In addition esters and pyrazines were the most qualitative and quantitative aroma groups found in all cocoa beans from the 22 regions. The authors stated that these classes of compounds may strongly contribute to the overall aroma of these cocoa beans.

Quelal et al. (2023) characterized the most key-aroma compounds from dried and roasted cocoa beans, by aroma extract dilution analysis and GC–MS-Olfactometry. Aroma extracts were isolated from the cocoa bean samples by using a HS-SPME. The results of AEDA revealed 28 aroma-active compounds, in which 2- and 3-methylbutanoic acid (FD 8192; sweaty), acetic acid (FD 2048; acidic), and 3-hydroxy-4,5-dimethyl-2 (5H)-furanone (FD 1024; spiced) were detected with the highest flavor dilutions (FD) factor, suggesting that these compounds are among those contributing to the aroma of dried cocoa beans.

Frauendorfer and Schieberle (2008) evaluated the aroma active compounds of cocoa powder through the application of extraction/distillation process followed by application of an aroma extract dilution analysis. The GC-MS-O analysis revealed 35 odor active constituents in the flavor dilution (FD) factor range of 8-4096. The highest FD factors were attributed to 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), 2- and 3-methylbutanoic acid (sweaty, rancid), dimethyltrisulfide (cooked cabbage), 2-ethyl-3,5-dimethylpyrazine (potato-chip-like), and phenylacetaldehyde (honey-like). Quantitation of 31 key odorants by means of stable isotope dilution assays, followed by a calculation of their odor activity values (OAVs) (ratio of concentration to odor threshold) revealed OAVs > 100 for the five odorants acetic acid (sour), 3-methylbutanal (malty), 3-methylbutanoic acid, phenylacetaldehyde, and 2-methylbutanal (malty). In addition, another 19 aroma compounds showed OAVs > 1. The authors concluded that these compounds may be the main contributors of overall aroma of cocoa bean powder.

Rottiers, Tzompa Sosa, Van de Vyver, et al. (2019) using advanced electronic nose (E-nose) technology coupled with gas chromatography (GC) determined key aroma compounds in cocoa liquor obtained from cocoa beans in order to discriminate between cocoa beans and cocoa liquors from different origins. Further tentative detection of major volatile compounds allowed the

identification of compounds within a large range of chemical groups occurring in cocoa products, such as acids, alcohols, aldehydes, ketones, esters, pyrazines, pyrones, and pyrroles. The most key odorant identified in cocoa product samples as the principal contributors to cocoa liquor flavor were acetic acid, 2-heptanol, 2/3-methylbutanal, acetophenone, isoamyl acetate, tetramethylpyrazine, maltol, and 2-acetyl-1-pyrroline.

2.6.5. Phenolic Compounds and Antioxydant Activity of Cocoa Beans

The cotyledon of cocoa bean consists of two type of parenchyma storage cells (Afoakwa, 2010). The first type of storage cell is known as polyphenolic cells, representing about 14-20% of the dry weight of the cocoa bean. These cells contain a single large vacuole filled with phenolic and alkaloids compounds (Osman et al., 2004).

Phenolic compounds, characterized by the presence of at least one phenyl group represent a large family of secondary metabolites naturally synthesized by the plant, also the largest class of natural antioxidant (Andres-Lacueva et al., 2008; Arlorio et al., 2005; Miller et al., 2006; Othman et al., 2007). Nowadays, more than 8000 of these compounds have been listed and the diversity of structures makes their classification a bit complex (Kelebek, 2016; Wang & Ho, 2009). Some authors use the carbon skeleton of their molecules to classified them into 9 groups (C6-C1, C6-C2, C6-C3, C6-C4, C6-C1-C6, C6-C2-C6, C6-C3-C6), while others group them into 4 families (hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and flavonoids). The polyphenols in cocoa beans are stored in the pigment cells located within the cotyledons. These pigment cells, also known as polyphenol-storage cells, can vary in color from white to deep purple, depending on the amount of anthocyanins present (Wollgast & Anklam, 2000b). These compounds play an essential role, especially in chocolate and cocoa beans related products, they are responsible the cocoa beans colors, astringency and bitterness taste sensation along with maillard reaction during drying and roasting process (Ziegleder, 2017).

Cocoa beans with a rich source of natural antioxidants containing phenolic compounds is of great importance in the prevention of numerous diseases. Therefore, these natural antioxidants, particularly phenolic compounds, through free radical scavenging activity inhibiting the lipid peroxidation are able not only to prolong the shelf-life of foods, but also protect the human body against damage caused by free radicals.

Flavonoid Compounds of Cocoa Beans

The flavonoids form a wide family of phenolic compounds with a common basic structure of the C15 skeleton organized through the C6-C3-C6 configuration. Two aromatic (A and B) and a heterocyclic ring (C) containing one oxygen atom are linked by a 3-carbon chain, giving a flavone unit (Jaganath & Crozier, 2010; Manach et al., 2004). The A ring is phloroglucinol, B ring refers to catechol if there is an hydrogen in the C5' and pyrogallol ring while substituted by hydroxyl. C-ring

is a pyrene. Nowadays, over 5000 flavonoid compounds with different and complex structure have been characterized by several researchers in various food and plant matrices (Havsteen, 2002; Middleton et al., 2000).

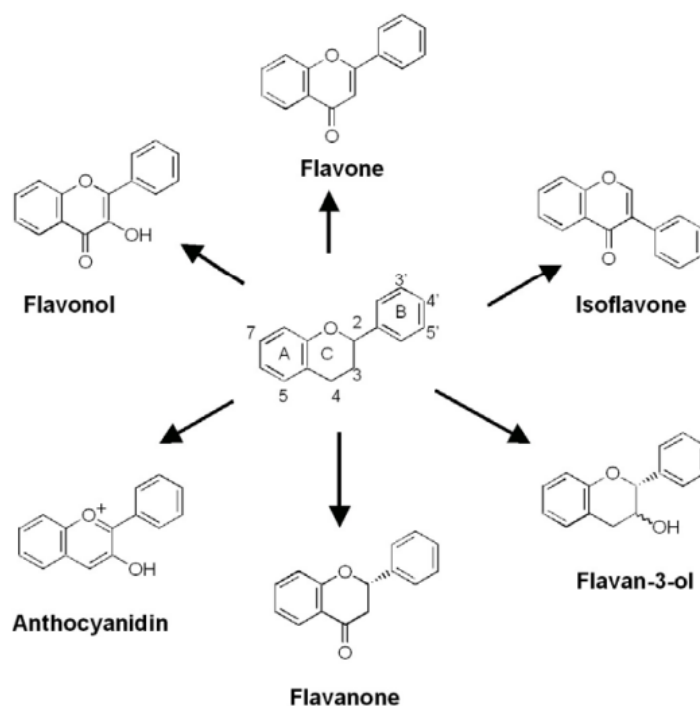


Figure 2.5. Basic structure of flavonoids and flavonoid subclasses. (adapted from Nishiumi et al., 2011)

Among the overall phenolic compounds found in cocoa beans, flavonoids represent the predominant and the most important compounds, they are plant secondary metabolites involved in different roles such as pigmentation, atmospheric nitrogen trapping, they protect plants against UV and fungal attacks (Chira et al., 2009; Quideau et al., 2003). Flavonoids are grouped in different subclasses, according to the degree of oxidation on the C pyrene ring except for chalcones. They include different type of compounds such as flavones, flavononols, flavonols, flavan-3-ols, and anthocyanins (figure 2.5). In particular, there are three group of flavonoids compounds present in cocoa beans that has been repoted of particular interrest: catechins or flavan-3-ols (ca. 37%) representing the monomeric form, proanthocyanidins also known as condensed tannins (ca. 58%) and anthocyanins (ca. 4%) (Bordiga et al., 2015; Wollgast & Anklaam, 2000a). These classes of compounds strongly contributes to the organoleptic aspect of cocoa beans products, providing color, astringency, and bitterness.

The main ubiquitous in cocoa beans is (-)-epicatechin covering up to 35 % of total content of phenolic substances in the cocoa beans (Gu et al., 2006; Andres Lacueva et al., 2008). Other catechins detected in smaller quantities are (+)-catechin, traces of (+)-gallocatechin, (-)-epicatechin-3-O-gallate and (-)-epigallocatechin (Afoakwa & Paterson, 2010; Belščak et al., 2009; Oliviero et al., 2009; Wollgast & Anklaam, 2000a). The amount of each fraction of these compounds depends on

the varietal characteristics of cocoa beans. Numerous studies have shown that the content of (-)-epicatechin and (+)-catechin depends on the geographical region of cultivation (Jalil & Ismail, 2008; Niemenak et al., 2006). (Kim & Keeney, 1984) demonstrated that the fermented cocoa beans may have up to 6-fold difference in contents of (-)-epicatechin, depending on growing region. The structures of monomeric flavan-3-ols present in cocoa are shown in Figure 2.6.

Anthocyanins constitute the largest and most important group of natural water-soluble pigments (Harborne, 1968; He & Giusti, 2010). To date, it is assumed that there are more than 635 anthocyanin compounds identified in nature, mainly in fruits vegetable flowers and grains (Byamukama et al., 2008; He & Giusti, 2010). They are characterized by a core glycosylated flavylum in position C3 that combines two benzene rings A and B. In raw cocoa beans, the anthocyanin fragment is mainly composed of cyanidin-3- α -L-arabinoside and cyanidin-3- β -D galactoside located in the vacuole of the beans, covering 4% of the total content of phenolic compounds (Belščak et al., 2009; Elwers, 2008; Niemenak et al., 2006; Oliviero et al., 2009; Wollgast & Anklam, 2000a).

Other flavonoids, including flavonols, flavones and flavanones in cocoa beans are present in aglycones or glycosylated forms with lower quantities (Jaganath & Crozier, 2010). These compounds are generally found in cocoa products in conjugated forms where the associated sugar moiety is often glucose or galactose, arabinose, and glucuronic acid may also be involved (L.-Z. Lin et al., 2008; L. Lin et al., 2008). The major representatives of flavonols glucosides are quercetin-3-O-D-arabinoside and quercetin-3-O-D glucoside (isoquercitrin), quercetin-3-O-galactoside (hyperoside) and quercetin-3-O-glucuronide (Andres-Lacueva et al., 2008; Arlorio et al., 2005; Wollgast & Anklam, 2000a). In addition, flavones such as apigenin, luteolin, and some glycosides of their respective aglycone like apigenin 8-C-glucoside (vitexin), apigenin-6-C-glucoside (isovitexin), luteolin-7-O-glucoside, luteolin-6- C-glucoside and luteolin-8-C-glucoside are also present cocoa powder (Sánchez-Rabaneda et al., 2003). In a recent study, flavanones, such as naringenin and its conjugates naringenin-7-O-glucoside (prunin) have ben repoted in cocoa beans (Oracz et al., 2014).

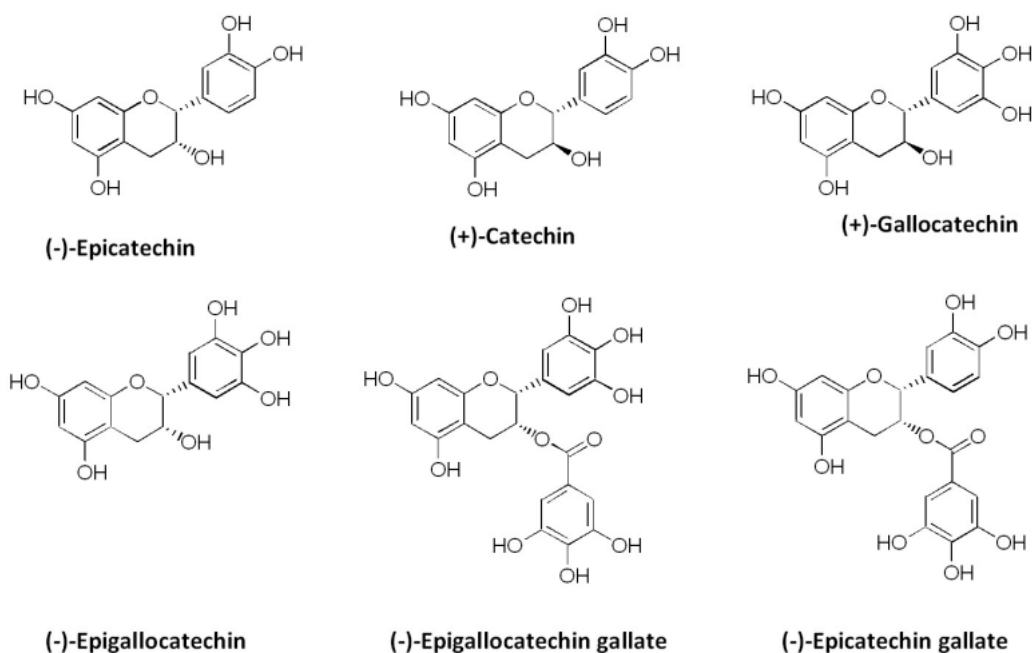


Figure 2.6. Some flavonoids present in cocoa beans (adapted from Patras et al., 2014)

Proanthocyanidins, also called condensed tannins are molecules able to combine and give stable complexes with polysaccharides (Riou et al., 2002), alkaloids and gelatin, and proteins as well (Haslam, 2007). Chemically proanthocyanidins are polymer of flavan-3-ols with C15 (C6-C3-C6) skeleton, divided into two subgroups: the procyanidins including (+)-catechin and (-)-epicatechin monomers and in another hand the prodelfhinidins which include (+)-gallocatechin and (-)-epigallocatechin (Da Silva et al., 1991; Koponen et al., 2007; Rigaud et al., 1991). Proanthocyanidins are predominately consisted of flavan-3,4-diols (leucoanthocyanidins), that are C4 → C8 or C4 → C6 bound (B-type) to condensed dimers, trimers or oligomers with epicatechin and/or catechin as the main extension subunit (Counet et al., 2004; Nazaruddin et al., 2006). In cocoa beans, procyanidins may also contain additional ether bond between C2→O7 (A-type) (Andres-Lacueva et al., 2008; Bonvehi & Coll, 1997; Jonfia-Essien et al., 2008). They account for approximately 58% of the total polyphenol content of fresh cocoa beans (Bauer et al., 2011; Dreosti, 2000; Jalil & Ismail, 2008). The insoluble and soluble tannins contribute to the typical astringency and bitterness of freshly harvested bean, which has been linked to the capacity of tannins to formulate complexes with proteins.

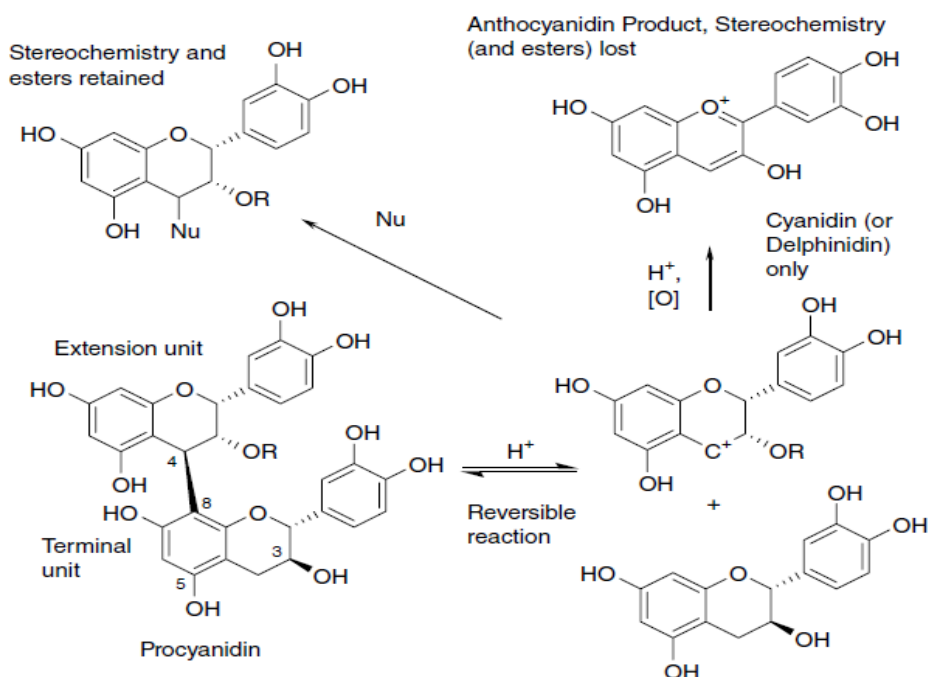


Figure 2.7. Reactions to analyse the proanthocyanidin structure. Strong acid product with oxidation shown on top right, nucleophile (thiolysis or phloroglucinolysis) product on top left (adapted from Waterhouse et al., 2024)

In an investigation, Karim et al. (2022) determined the phenolic components in Forastero and Nacional cocoa beans varieties obtained from Cote d'Ivoire. The authors found four main flavonoids, including rutin, quercetin, catechin, epicatechin and naringenin in all cocoa beans samples, in which naringenin (5.95 ± 0.35 mg/100g) demonstrated the highest amount in Nacional cocoa beans samples.

Ortega et al. (2008) characterized phenolic compounds in different part of cocoa bean samples (beans, nibs, liquor, and cocoa powder) by UPLC-MS/MS. Fourteen flavone components with sugars moiety were found in all the samples. The authors indicated that seven flavones including quercetin, quercetin-glucoside, dihydroquercetin, dihydroxykaempfero, kaempferol-rutinoside, myricetin-glucoside, luteolin-glucoside were predominantly detected in all the cocoa samples. In the same study, eleven procyanidins were also identified in the form of condensed dimer, trimer and oligomer with catechin and epicatechin subunit. Using DPPH and H-ORAC methods, the authors found that all cocoa sources are rich in antioxidant activity.

Fayeulle et al. (2018) attempted to identify two series of phenolic compounds indicating mass signals at m/z 605 and 893 (negative ionization mode) detected in fermented and dried cocoa beans using UHPLC-MS and UHPLC-ESI-IT-MS techniques. It was found that four isomers of ethyl-linked epicatechin and several isomers of epicatechin-ethyl-procyanidin B2 were successfully characterized in cocoa beans. The authors concluded that the study determined the presence of flavan-3-ol acetaldehyde condensation products in cocoa beans and provide the first report of epicatechin-ethyl-procyanidin B2.

2.6.5.1. Phenolic acids of cocoa beans

Phenolic acids are among the non flavonoid compounds consisting of two main groups including hydroxybenzoic and hydroxycinnamic acid derivatives found in cocoa beans and products (Lafay and Gil-Izquierdo, 2008). More than 17 phenolic acids and esters have been reported and the total amount of seven of them comprises not more than 23 ppm of the seed dry weight (gallic, phloroglucinol, protocatechuic acid, vanillic acid, o-hydroxyphenylacetic acid, p-coumaric acid, caffeic acid, ferulic acid (Ziegleder & Biehl, 2003).

Hydroxybenzoic acids are generally made up of C6-C1 carbon skeleton, which take origin from fresh cocoa beans in which five have been identified, mainly combined to glycoside moiety or as an ester (Gallic and ellagic tannins). In cocoa beans particularly, gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids are the main benzoic acid derivatives (Jalil & Ismail, 2008; Jonfia-Essien et al., 2008).

Hydroxycinnamic acids have a C6-C3 structure and are located in the cotyledon of cocoa beans as an ester of caffeic acid, but also form other esters with some organic acids, sugars, polyamides, and polysaccharides. In cocoa beans the major hydroxycinnamic acids are caffeic, ferulic, p-coumaric and chlorogenic acids (Arlorio et al., 2005; Jalil and Ismail, 2008; Jonfia-Essien et al., 2008). Recently, hydroxycinnamic acids polyamides with aromatic amino acids (tyrosine (deoxyclovamide) and (+)-N-(E)-caffeoyl-L-aspartate (caffeic acid aspartate) was found (Sanbongi et al., 1998; Arlorio et al., 2008; Elwers et al., 2009) such as (-)-N-[3'-4'-dihydroxy-(E)- cinnamoyl]-L-dihydroxyphenylalanine (clovamide), (-)-N-[4'-hydroxy-(E)-cinnamoyl]-L tyrosine (deoxyclovamide) and (+)-N-(E)-caffeoyl-L-aspartate (caffeic acid aspartate) was identified in cocoa liquor (Arlorio et al., 2008; Elwers et al., 2009; Sanbongi et al., 1998).

Table 2.2. Chemical structures of phenolic acids

Benzoic acids					Hydroxycinnamic acids
	R2	R3	R4	R5	
<i>p</i> -Hydroxybenzoic acid	H	H	O H	H	<i>p</i> -Coumaric acid
Protocatechuic acid	H	OH	O H	H	Caffeic acid
Vanillic acid	H	OCH 3	O H	H	Ferulic acid
Gallic acid	H	OH	O H	OH	
Syringic acid	H	OCH 3	O H	OCH ₃	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentistic acid	OH	H	H	OH	

Ortega et al. (2008) investigated the phenolic acids of various cocoa beans samples (beans, nibs, liquor, and cocoa powder) prepared from forastero variety using UPLC-MS/MS technique. Nine simple phenolic acids such as vanillic acid, caffeic acid, hydroxybenzoic acid, syringic acid, ferulic acid, coumaric acid, cinnamic acid, chlorogenic acid and protocatechuic acid were detected in all cocoa samples. The authors found that protocatechuic acid (64.14-226.0 mg/g) was the major phenolic acid identified in all the samples, in which cocoa powder found to contain the highest amount of phenolic acids.

Karim et al. (2022) attempted to evaluate the phenolic acids using HPLC technique in national and forastero cocoa beans obtained from Cote d'Ivoire. Eight phenolic acids, comprising cinnamic acid, protocatechuic acid, coumaric acid, ellagic acid, veratric acid, ferulic acid, vanillic acid and caffeic acids were detected in all cocoa beans varieties, in which ellagic acid is (18.48 ± 0.44 mg/100g) and cinnamic acid is (6.18 ± 0.63 mg/100g) demonstrated significant amount in national (Mercedes) variety.

2.6.6. Alkaloid Compounds of Cocoa Beans

Alkaloids characterized by heterocyclic nitrogen-containing compounds are mainly produced by higher plants and some microorganisms. Purine alkaloids are secondary plant metabolites derived from purine nucleotides, encompassing methylxanthine and methyluric acid (Zulak et al., 2006), that have been identified in about 100 plant species, including *Theobroma cocoa L* (Ashihara & Crozier, 1999). Tea, coffee and cocoa are the three most popular non-alcoholic beverage in the world that contain purine alkaloids such caffeine, theacrine, theophylline and theobromine. In cocoa beans, theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine) are the predominant purine alkaloids found (Figure 2.8), with theobromine being the most significant (Martínez-Pinilla et al., 2015; Urbńska & Kowalska, 2019). The psychoactive potential and the bitter taste are also attributed to theobromine and caffeine of cocoa beans and related products. The amount of theobromine (2.85-3.95%) determined in cocoa beans on the basis of dry matter is higher than caffeine (0.3-0.6%) (Aprotosoie et al., 2016). The amount of purine alkaloid in cocoa beans are strongly influence by post-harvest process, including pulp conditioning, fermentation, drying, roasting, alkalization (Aprotosoie et al., 2016; Febrianto & Zhu, 2020; Hernández-Hernández et al., 2018; Oduro-Mensah et al., 2018).

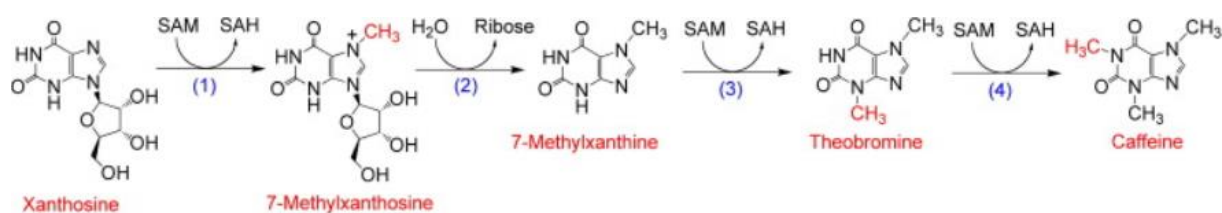


Figure 2.8. The major biosynthetic pathway of theobromine and caffeine from xanthosine: (1) 7-methylxanthosine synthase (xanthosine N-methyltransferase); (2) N-methylnucleosidase; (3) theobromine synthase (monomethylxanthine N-methyltransferase); (4) caffeine synthase (adapted from Apgar & Tarka, 2019)

Ortega et al. (2008) successfully characterized the purin alkaloids in cocoa beans from different sources (beans, nibs, liquor, and cocoa powder) through UPLC-MS/MS technique. Theobromine (64.87-89.66 mg/g) and caffeine (4.99-9.03 mg/g) were only the purin alkaloids determined in all studied cocoa samples. It was also observed that cocoa powder is rich in theobromine (89.66 mg/g) and caffeine (9.03 mg/g).

2.7 Factors Affecting Flavours Quality of Cocoa Beans

Numerous criteria are employed to evaluate the quality of cocoa beans, encompassing indicators like beans count and size, beans color, and beans acidity. However, the primary and most significant indicator of cocoa beans quality is the quantity and composition of volatiles flavor compounds present in the beans after processing (Krähmer et al., 2015; Magi et al., 2012). The flavor profile plays a central role in the acceptability of cocoa beans and cocoa-based products, including chocolate (Afoakwa et al., 2008), and thus plays a crucial role in determining overall quality (Owusu, 2010). Flavor compounds in cocoa beans are formed during drying and roasting, and they are liberated from flavor precursors generated during fermentation process. Flavor compounds in cocoa beans are generally influenced by factors such as type of cocoa (genotype), bean composition, soil type, age of cocoa pod and tree, postharvest treatments such as pulp pre-conditioning, fermentation and drying, industrial processes such as roasting as well as storage and transportation (Afoakwa & Paterson, 2010; Afoakwa et al., 2008; Craack et al., 2013; Owusu et al., 2012). Among all these factors, fermentation, drying and roasting processes are the main factors impacting the aroma and taste of cocoa beans and products.

Rodriguez-Campos et al. (2012) evaluated the influence of time and drying temperature on the profile of volatile compounds after 2, 4, 6, and 8 fermentation days followed by drying at 60, 70 and 80 °C. Samoa drier and by a sun-drying process were also applied as control for comparison to hot air convective drying. A total of 58 volatile compounds were detected by HS-SPME/GC-MS as esters (20), alcohols (12), acids (11), aldehydes and ketones (8), pyrazines (4) and other compounds (3). The authors found that six days of fermentation were enough to produce aroma compounds with desirable flavor in cocoa beans, as well as to avoid the production of compounds with off-flavour notes. In addition, drying at 70 and 80 °C after six fermentation days exhibited a volatile profile

similar to the one obtained by sun drying. They also observed that the optimal conditions for fermentation and drying of cocoa beans were 6 days of fermentation, followed by drying at 70°C.

Utrilla-Vázquez et al. (2020) characterized the volatile compounds of five varieties of Maya cocoa (three Criollo type and two Trinitario types) during fermentation and drying (sun drying) processes by Venn diagram and PCA. The volatiles fraction were isolated and determined via SPME-HS GC-MS system. The predominant volatiles related to desirable aromatic notes of dried fermented were produced by esters, aldehydes, ketones, and alcohols. Furthermore, dried unfermented cocoa beans presented a few numbers of aroma compounds, in the five cacao types, where alcohols, ketones and hydrocarbons dominated.

Mohamadi Alasti et al. (2019) examined industrial processes affecting the flavor of cacao beans obtained from Ivory Coast. The volatile components were extracted using Likens–Nickerson simultaneous distillation–extraction (SDE) apparatus and characterized through. Several compounds (alcohols, carboxylic acids, aldehydes, ketones, esters, and pyrazines) were identified. The authors stated that alkalization and roasting were shown to be two important steps in the cacao beans processing that can affect the final cocoa powder flavor. In addition, pyrazines and esters were found to be two predominant classes of flavor compounds generated during the roasting process by the Maillard reaction. It was observed that the cocoa powder contained 2.69% of tetramethylpyrazine, 3.22% isobutyl benzoate, and 1.38% linalool. The highest percentage of increase in the mean amounts of 2,3,5,6-tetramethylpyrazine, isobutyl benzoate, and linalool were perceived in the roasting stage, after which the percentages decreased.

Jinap et al. (1994) investigated the effect of different drying process on the acidity and volatile fatty acid compounds of 128 clones of cocoa beans obtained from Malaysia. Four drying treatments, including oven-drying at 60°C, air-blow drying, shade-drying and sun-drying were performed. The results showed no significant differences ($P > 0.05$) between samples that have undergone sun-drying and air-blow drying in the acidity and C3-C5 volatile fatty acids content; both were assessed favourably by taste assessors. Oven-dried beans had a high concentration of volatile fatty acids and generated chocolates with a high intensity of off-flavour.

In their study, Ascrizzi et al. (2017) evaluated the impact of different phases of the processing chain, including fermented and drying processes on volatile emission profiles of Blanco de Criollo cocoa beans and chocolate obtained from the beans. The volatile fraction were firstly isolated by HS-SPME and identification was carried out through GC-MS system. It was found that pyrazines, esters and aldehydes, alcohols, acids, monoterpene, ketones constituted the most important aroma compounds in cocoa beans and chocolate samples. 2-methylbutanal, isopentyl alcohol, phenylethyl alcohol, 2-phenyl-2-butenal, isopentyl acetate, ethyl phenylacetate, myrcene, tetramethylpyrazine, 2,3,5-trimethylpyrazine were identified as the major aroma compounds of cocoa beans. It was observed that the volatile content were more intense in dried fermented cocoa beans compared to only fermented beans. Moreover, monoterpene and oxygenated terpene

compounds were drastically reduced during drying process. Pyrazines, esters and aldehydes represented the most predominant aroma active groups of compounds in all cocoa beans samples.

In their study, Frauendorfer and Schieberle (2008) characterized the key aroma compounds of dried (unroasted) and roasted Criollo cocoa beans by the application of aroma extract dilution analysis (AEDA). The identification of key volatiles in the samples revealed 42 compounds in the flavor dilution (FD) factor range of 1-4096 for the unroasted and 4-8192 for the roasted cocoa beans. The authors detected similarity of compounds in both samples with difference in their intensity. 2- and 3-methylbutanoic acid (rancid) and acetic acid (sour) showed the highest FD factors in the unroasted beans, while 3-methylbutanal (malty), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), and 2- and 3-methylbutanoic acid (sweaty) were detected with the highest FD factors. Moreover, the determination of odor activity values (ratio of the concentration/odor threshold) revealed concentrations above the odor threshold for 22 compounds in the unroasted and 27 compounds in the roasted cocoa beans, respectively. In particular, a strong increase in the concentrations of the aldehydes (3-methylbutanal and phenylacetaldehyde as well as 4-hydroxy-2,5-dimethyl-3(2H)-furanone) was measured, suggesting that these odorants may strongly contribute most to the changes in the overall aroma of unroasted and roasted cocoa beans.

2.8 Factors Affecting Phenolic Compounds in Cocoa Beans

Drying and fermentation are crucial factors that significantly impact the phenolic and alkaloid compounds present in cocoa beans, which contribute to their flavor, aroma, and potential health benefits. Fermentation, a microbial-driven process, plays a vital role in the breakdown of proteins and polysaccharides, leading to the development of desirable flavors and the transformation of complex phenolic compounds into simpler forms. The duration, temperature, and aeration during fermentation are critical parameters influencing the phenolic composition and alkaloid content of cocoa beans (Dang & Nguyen, 2019; Menezes et al., 2022; Permatasari et al., 2016). Subsequently, the drying process reduces the moisture content, preventing microbial growth and preserving the quality of cocoa beans. Different drying methods, such as sun-drying or mechanical drying, affect the phenolic profile and alkaloid content. The drying conditions, including temperature, humidity, and airflow, play a significant role in the retention of phenolic compounds and alkaloids in cocoa beans (Cortez et al., 2024; Dang & Nguyen, 2019). Understanding and carefully managing the factors of drying and fermentation are essential for maintaining the phenolic and alkaloid compounds, ensuring their potential health benefits, and producing high-quality cocoa beans for the production of premium cocoa-based products.

During fermentation, oxidative reactions occur, involving both enzymatic and nonenzymatic processes (Redovniković et al., 2009). These reactions cause biochemical changes in polyphenolic compounds, leading to a reduction in the content of soluble polyphenols, resulting in the alleviation of the bitter and astringent taste and an unpleasant aroma of cocoa beans (Arlorio et al., 2005;

Misnawi et al., 2004; Serra Bonvehi & Ventura Coll, 2000). Enzymatic browning processes are among the reasons for the loss of monomeric flavan-3-ols during fermentation. Polyphenols are oxidized to quinonic compounds, which then condense with other flavanols and anthocyanins to form mostly insoluble high molecular weight tannins (Bonvehi & Coll, 1997; Bruinsma & Taren, 1999; Ramli et al., 2006). Polyphenol oxidase, a key enzyme, plays a significant role in these processes. Under the influence of polyphenol oxidase, (-)-epicatechin, (+)-catechin, and free anthocyanidins are oxidized to corresponding quinones, which can react with other phenolic compounds, amino acids, and peptides, resulting in the formation of yellow and brown pigments (Bittner, 2006; Camu et al., 2008; Niemenak et al., 2006). Additionally, polymerization of (-)-epicatechin and (+)-catechin leads to the formation of high molecular weight tannins, which have lower digestibility compared to monomers (Kyi et al., 2005; Schinella et al., 2010). Studies have shown a rapid decrease in (-)-epicatechin content between the second and third day of fermentation, with a notable reduction in both (-)-epicatechin and (+)-catechin content after five days of fermentation (Cooper et al., 2007; Kyi et al., 2005; Wollgast & Anklam, 2000a). Other researchers also observed a reduction of (-)-epicatechin and (+)-catechin contents by approximately 10-70% during fermentation (Nazaruddin et al., 2006; Wollgast & Anklam, 2000a).

During the drying process of cocoa beans, the degradation of simple flavanols and methylxanthins occurs (Bonvehi & Coll, 1997). The reduction in these compounds during drying is influenced not only by the residual activity of polyphenol oxidase (2%), but also by the potential migration of polyphenols with evaporated water (Camu et al., 2008; Misnawi et al., 2004; Redovniković et al., 2009). Exposure of fresh unfermented cocoa beans to sunlight during two-day drying under natural conditions can lead to a 50% decrease in (-)-epicatechin concentration (Camu et al., 2008; Jalil & Ismail, 2008). Kim and (Cienfuegos-Jovellanos et al., 2009) Keeney observed a 90% reduction in (-)-epicatechin concentration after fermentation and drying (Cienfuegos-Jovellanos et al., 2009). Furthermore, variations in the reduction of catechin content during fermentation and drying have been reported, with the Criollo variety showing stronger reductions compared to Upper Amazon Forastero, Lower Amazon Forastero, Nacional, and Trinitario varieties (Elwers et al., 2009).

Deus et al. (2018) conducted a study to evaluate the impact of different drying methods on the development of phenolic compounds, methylxanthines, antioxidant activity, and the presence of ochratoxin A (OTA) in cocoa beans. Antioxidant activity was assessed using DPPH, FRAP, ORAC, and β -carotene-linoleic acid assays, while phenolic compounds and methylxanthines were quantified using high-performance liquid chromatography with an ultraviolet detector (HPLC-UV). The findings revealed that cocoa drying led to a reduction in antioxidant activity, accompanied by decreased phenolic compound content (from 0.04 mg/g of catechin before drying to 0.02 mg/g after drying) and reduced levels of methylxanthines (from 19.44 mg/g of theobromine before drying to 11.71 mg/g after drying). Among the drying methods tested, the traditional drying method exhibited the highest preservation of antioxidant activity, as well as methylxanthine and phenolic compound

content. This research highlights the importance of drying techniques in maintaining the desirable properties of cocoa beans, such as their antioxidant capacity and phenolic compound composition, which contribute to their potential health benefits and quality in cocoa-based products.

Peláez et al. (2016) investigated the variations in methylxanthine (theobromine and caffeine) and catechin levels in different stages of cocoa bean processing. They examined fresh and fermented cocoa beans, dried cocoa grains, and cocoa liquor derived from Trinitario, Criollo, and Forastero cacao varieties. The results revealed that the theobromine and caffeine contents in fresh cocoa beans were influenced by both the cocoa type and production area, with higher caffeine levels found in Trinitario cacao compared to Criollo and Forastero varieties. Upon drying, the theobromine (ranging from 1.449 ± 0.004 to 1.140 ± 0.010 g/100g dry weight) and caffeine contents (ranging from 0.410 ± 0.03 to 0.165 ± 0.02 g/100g dry weight) decreased and were influenced by the fermentation and drying methods employed. Additionally, the catechin and epicatechin contents were affected by the fermentation and drying methods, with reductions observed during the fermentation and drying processes. Overall, the findings demonstrated that higher concentrations of theobromine, caffeine, catechin, and epicatechin were present in fresh cocoa beans compared to the subsequent stages of processing.

In another study, Dang and Nguyen (2019) examined the influence of maturity at harvest and fermentation conditions on the bioactive compounds of cocoa beans. They assessed proanthocyanidins, caffeine, and theobromine levels in cocoa beans harvested at different stages of maturity and subjected to varying fermentation durations. Additionally, they investigated the impact of using the commercial enzyme Pectinex® Ultra SP-L during fermentation. The results demonstrated that the amounts of proanthocyanidins, caffeine, theobromine, and antioxidant capacities increased as the cocoa fruits matured. The values ranged from 16.12 to 27.28 g catechin equivalents (CE)/100 g dry weight (DW), 99.66 to 173.61 mg/100 g DW, 556.39 to 948.84 mg/100 g DW, and 23.23 to 26.32 mol Trolox equivalents (TE)/100 g DW, respectively, in the unfermented cocoa beans. However, fermentation with the enzyme significantly decreased the proanthocyanidins content and antioxidant capacity of the resulting cocoa powder, with mean values dropping from 8.93 to 4.93 g CE/100 g DW and from 15.81 to 12.95 g mol TE/100 g DW, respectively. Two-way ANOVA analyses revealed the strong dependence of proanthocyanidins, caffeine, theobromine contents, and antioxidant capacity of cocoa beans on their maturity stages, fermentation methods, and fermentation duration.

Mazor Jolić et al. (2011) investigated the influence of cocoa beans processing on the phenolic compounds. Cocoa nibs were subjected to roasting and alkalisation process, and the authors observed that these processes had strong effect on phenolic compounds and antioxidant capacity of cocoa beans. As results, the roasting process showed 14% loss of total phenolics content, while alkalisation exhibited 64% loss of total phenolics content. It was also found that procyanidins B1 and B2 as well

as (-)-epigallocatechin were the unstable substances, while caffeic acid derivate presented the greatest stability in all technological process.

Alean et al. (2016) examined the impact of the drying process on phenolic compounds in cocoa beans. The researchers observed that the least degradation of polyphenols occurred at a temperature of 40 °C, resulting in a concentration of 3329.76 mg Ac. Gallic/100 g dried fruit, corresponding to a 45% reduction. Conversely, the highest degradation of polyphenols was observed at a temperature of 60 °C. The study concluded that the degradation of polyphenols is influenced by temperature, moisture, and drying times. These factors play a role in the irreversible oxidative processes of polyphenols and can be influenced by cellular destruction. Furthermore, the researchers developed a phenomenological model to simulate both water desorption behavior and the degradation of polyphenols during the drying process. This model provides a valuable tool for understanding and predicting the behavior of phenolic compounds during cocoa bean drying.

Abhay et al. (2016) conducted a study to investigate the impact of temperature and heating time on the polyphenol content of cocoa beans. The researchers dried cocoa beans at different temperatures (60°C, 70°C, and 80°C) with a relative humidity (RH) level of 50% for varying durations ranging from 0 to 40 hours. They found that the total phenolic content was highest when the beans were dried at 70°C, and it decreased as the drying time increased. The study confirmed that high temperatures and prolonged exposure time led to thermal degradation of phenolic compounds, with minimal influence from enzymatic activity, particularly at low moisture levels. Additionally, sensory evaluation indicated that the cocoa beans dried at 70°C were associated with a higher perceived astringency taste. This research provides substantial evidence highlighting the negative impact of temperature and heating time on polyphenols in cocoa beans, emphasizing the importance of careful drying conditions to preserve their phenolic content and sensory attributes.

In a study by Valadez-Carmona et al. (2017), the effects of different drying methods on phenolic compounds, antioxidant capacity, enzyme activity, and microstructure of cacao pod husks (CPH) were evaluated. The researchers found that fresh CPH contained 323.7 ± 26.5 mg gallic acid equivalents (GAE)/100 g dry mass (d.m.) of total phenolic compounds. Dehydration processes had a positive impact on the phenolic content and antioxidant capacity of CPH. Analysis revealed the presence of catechin, quercetin, (-)-epicatechin, gallic acid, coumaric acid, and protocatechuic acid in both fresh and dried CPH samples. Microwave and freeze-drying were found to preserve the microstructure of the husks effectively. The authors concluded that microwave drying demonstrated superior performance compared to hot air drying and was on par with freeze-drying in terms of preserving the quality of CPH. These findings highlight the potential of microwave drying as an efficient method for preserving the phenolic compounds and antioxidant capacity of cacao pod husks, thus offering potential applications in the valorization of this by-product.

In their study, Jader Alean et al. (2020) aimed to propose a method for evaluating the in-situ oxidation of polyphenolic compounds during cocoa drying. The researchers investigated the

oxidation of phenolic compounds in cocoa beans by applying microwaves in an On-Off mode during the drying process. The quantification of phenolic compounds was conducted before and after drying, employing various analytical techniques such as Fourier transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC). The study findings revealed that the degree of coloration observed in the inner zone and surface of the dried cocoa beans indicated that the degradation of phenolic compounds during drying was not solely driven by a transport mechanism (diffusion), but rather by in-situ oxidative processes. These results shed light on the oxidative changes occurring in phenolic compounds during cocoa drying, providing insights into the underlying mechanisms that affect the quality and composition of cocoa beans. The proposed method offers a valuable approach for assessing the oxidation of polyphenolic compounds during cocoa drying and contributes to a deeper understanding of the drying process's impact on cocoa quality.





3. MATERIAL AND METHODS

3.1 Materials

3.1.1. Cacao Fruits and beans

The healthy and mature cocoa pods of the *cv.* Forastero, Criollo and Nacional (Mercedes) were collected from one of the research stations of National Agricultural Research Center (CNRA) of Goh region (6° 25' 0" N, 5° 55' 0" O) / Cote d'Ivoire during the big cocoa harvesting season from October to December 2021. Forastero, Criollo and Nacional mature cocoa pods were differently harvested with hand and perch. The harvested cocoa pods were differently grouped for spontaneous fermentation.



Figure 3.1. Cocoa fruits and beans from the three cocoa cultivars

3.2 Methods

3.2.1. Fermentation of Cocoa Bean Samples

To obtain the fermented cocoa bean samples, approximately 500 ripe cocoa of each variety containing approximately 20-40 beans were cut with knife to remove altogether the beans and the placenta from the pods husk. The beans were removed carefully by hand from placenta to exclude any germinated, black or diseased beans or pieces of shell or placenta fragments. After the removal of the fresh beans containing the mucilaginous part from the placenta, fresh cocoa beans were subjected to natural fermentation on the research station using wooden box (45 x 40.5 x 40.5 cm³) fermentation process covered with banana leaves for 5 days (Lopez and Dimick, 1995; Mounjouenpou et al., 2008). Each assay of cocoa fermentation was carried out using approximately 10 kg of wet cocoa beans. For all experimentations of fermentation, the beans were mixed after 48 and 96 h of fermentation to limit the growth of lactic acid bacteria. After the completion of the

fermentation process, the fermented beans, approximately 500 g of each variety were differently packaged into labaled humid proof plastic bags and brought to Cukurova University/ Food Engineering Department and stored at – 20°C until drying and analysis.



Figure 3.2. Natural cocoa beans fermentation process (wooden box)

3.2.2. Drying of Cocoa Bean Samples

Fermented cocoa beans from each cultivar were subjected to drying using sun drying, hot air convective drying, microwave drying and freeze drying techniques untill the moisture content reached 7%–8% as commonly recommended in cocoa producing chain. All the drying conditions followed in these experiments were based on previous research (Febrianto & Zhu, 2022; Guehi et al., 2008; Ndukwu, 2009), where high phenolics and aromas content recovery in cocoa beans were achieved, with more focused on phenolics content.

3.2.3. Sun Drying

The most common traditional drying process of cocoa beans by producers in all cocoa cultivation regions of Ivory Coast was employed. The drying was conducted by exposing cocoa bean samples to dry to direct sunlight. For each sun drying experiment, approximately 300g of each fermented cocoa bean samples, with an initial moisture content of 52-55% were spread on the roof terrace of the Food Engineering Department and exposed directly to solar radiation in Adana/Türkiye's average weather conditions, which is similar to tropical climate. It was carried out under conditions as close as possible to field drying. The cocoa beans were mixed manually each day to promote and enhance uniform drying. The sun drying of cocoa beans were conducted from 9 am to 6 pm for 6 days on 10-16th July to reach the humidity of 7-8% under the following conditions: Temperature (35-40°C), relative humidity (45-60%), precipitation (0-2%) wind speed (8-10 km/h). The process was carried out in three repetitions. Dried cocoa beans samples were powdered and vacuum-packed with an automatic vacuum packaging machine (DZ300/2SA, China) to prevent moisture entrapment and kept in a dry environment untill used.



Figure 3.3. Sun drying process of fermented cocoa beans used in this study

3.2.4. Hot Air Convective Drying

Drying experiments were carried out using a humidity controlled oven (Drying oven (NUVE KD-400 Oven- Ankara-TURKIYE) with an overall dimension of 25.2 cm $\times$ 47.24 cm $\times$ 19.64 cm. 1 kg of fermented cocoa beans, with an initial moisture content of 52-55% were subjected to hot air convective drying on the meshed tray of the oven. The beans were spread thinly on a meshed tray with square openings measuring 0.4 cm $\times$ 0.4 cm. Heat was generated by heaters integrated into the walls of the oven. The oven is equipped with a humidity controller, which helps in maintaining a standard relative humidity level within the oven during the drying process. The relative humidity level was set at 50 %. The beans were drying at 65°C for 1500 min to reach 7-8% humidity. The process was carried out in three repetitions. Dried cocoa bean samples were powdered and vacuum-packed with an automatic vacuum packaging machine (DZ300/2SA, China) to prevent moisture entrapment and kept in a dry environment until used.



Figure 3.4. Hot air convective drying process of fermented cocoa beans used in this study

3.2.5. Intermittent Microwave Drying

A modified microwave oven (MW71E, Samsung, South Korea) with a rated power of 900 W at 2450 MHz with internal dimension of 23 cm × 36 cm × 34 cm, equipped with a turntable and a fan inside and operating at a voltage of 220 VAC was employed in the drying of cocoa bean samples. To obtain the dried fermented cocoa beans, 300g of each fermented cocoa beans with an initial water content of 52-58% were subjected to microwave drying on the oven's turntable with a thickness of about 10-15 mm. The drying process was performed at power levels of 200 W corresponding to a specific power value of 0.66 W/g, air speed: 1-2 m/s for 540 min as drying time to reach 6-8% of humidity. Microwaves powers dry products almost instantly when compared to oven temperatures. High power intensity, for example, beyond 400 W, tends to roast cocoa beans, whereas low power dries products normally. For this last reason and based on the previous works, a power level of 200 W, considered low power intensity, was chosen and applied for modified microwave drying of cocoa beans. The process was carried out in three repetitions. Dried cocoa beans samples were powdered and vacuum-packed with an automatic vacuum packaging machine (DZ300/2SA, China) to prevent moisture entrapment and kept in a dry environment until used.



Figure 3.5. Microwave drying process of fermented cocoa beans used in this study

3.2.6. Freeze Drying

The experiments were conducted using a freeze dryer (Ilshin, FD8512 South Korea) -66°C, -5 mTorr pressure. This compact table-top unit generally operates under very low pressure conditions (0.015 mbar), facilitating efficient sublimation of the sample. The equipment used comprises a condenser/refrigeration unit with water outlet valves and a control unit, with dimensions of the condenser unit measuring 32 cm x 35 cm x 46 cm. The lyophilization chamber is a transparent acrylic cylinder (radius = 10 cm, height = 25 cm) that maintains the required vacuum pressure for the freeze drying process. It also features a three-level holding tray designed for samples. The vacuum pressure is regulated by a vacuum pump, which is controlled by the control unit. The drying parameters employed were adapted from the optimal freeze drying conditions reported by Hii et al. (2009) for

the recovery of cocoa polyphenols. To obtain dried cocoa beans, 300 g of each variety with an initial water content of 52-55% were subjected to freeze drying for 48 hours to reach a final humidity of 7-6%. The process was carried out in three repetitions. Dried cocoa beans samples were powdered and vacuum-packed with an automatic vacuum packaging machine (DZ300/2SA, China) to prevent moisture entrapment and kept in a dry environment until used.



Figure 3.6. Freeze drying process of fermented cocoa beans applied in this study

3.2.7. Physicochemical Analysis

3.2.7.1. Cocoa Fruit Weight

Randomly selected fresh cocoa fruits of each cultivar were weighed with an electronic scale with 0.01 g precision and fruit weights were recorded.

3.2.7.2. Number of Dried Cocoa Beans per Kilogram

For this purpose, 100 g of randomly selected dried cocoa beans of each variety were weighed with an electronic scale with 0.01 g precision and counted, and the number of grains per kilogram was calculated.

3.2.7.3. Dried Cocoa Bean Width and Length

The width and length of 100 randomly selected dried cocoa beans of each variety were measured with a digital caliper with 0.01 mm precision.

3.2.7.4. Moisture Content of Cocoa Beans

Approximately 50-70 g of each fermented cocoa bean samples were initially crushed using a mortar to ensure homogeneity. Then, 10 g (M_1) of crushed fermented cocoa bean samples were placed into pre-weighed nickel cups. These cups were subsequently placed in a vacuum oven (Blue Pard, DZF-6030A) and subjected to drying at 70°C. Prior to drying, the containers intended for drying were properly prepared, allowed to reach a constant weight within the oven, and subsequently cooled in a desiccator. The dried sample removed from the oven was transferred to a desiccator and cool to room temperature. The final weight (M_2) of the cups, along with the dried samples, was then recorded after cooling (AOC, 2000). All the determination were conducted in three replications and the value of moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = [(M_1 - M_2) / M_1] * 100$$

Where,

M_1 = weight (g) of sample before drying.

M_2 = weight (g) of sample after drying (AOAC, 2000).

3.2.7.5. Cut Test

The cut test is a widely used method for assessing the sanitary and fermentation quality of cocoa beans, as described by the international standards adapted from Guehi et al. (2007), Yro et al. (2018), Santos et al. (2019), Santos et al. (2019), Hii et al. (2006). In this test, 200 dried cocoa beans were cut lengthwise using a penknife, and both halves of each bean were examined under natural light. The assessment encompassed parameters such as insect damage, mould infestation, germination, and bean color. Cocoa beans were classified in Figure 3.7 as: fully fermented (ridged, brown- class 1; 120) (B), partially fermented (partially brown – class 2; 30 beans) (C), under fermented (purple – class 3; 40 beans) (D), unfermented (smoothed/slaty – class 4, 10 beans) (E). Defective beans were determined by summing germinated, infested, and flat beans. Results were expressed as a percentage, and the official standard considers batches with over 60% fully brown beans as good-quality cocoa beans.



A



B



C



D



E

Figure 3.7. Representative images of cocoa bean samples: (A) Board with cocoa beans for cut-test; (B) fully fermented bean; (C) partially fermented bean; (D) under-fermented bean; (E) unfermented/slaty bean

3.2.7.6. Determination of Color, and pH

A representative number of samples from each class were ground using a TE-631/4 multipurpose mill (Brazil) for 30 seconds at 10,000 rpm. The ground samples were immediately placed in Petri dishes, and the color was measured on the exposed surface using a Minolta colorimeter (CM-600d, Japan). Prior to measurement, the colorimeter was calibrated with a standard ceramic tile. Color values were expressed as lightness (L^*), redness (a^*), and yellowness (b^*) based on the CIELab* color system established by the Commission Internationale de l'Eclairage. To aid in the interpretation of variation among samples, chroma (C^*) was calculated using Equation 1, and the hue angle was determined using Equation 2.

$$C^* = (a^{*2} + b^{*2})^{1/2} \text{ Eq (1)}$$

$$\text{Hue angle} = \arctan (b^*/a^*) \text{ Eq (2)}$$

Subsequently, 5 g of ground cocoa beans were homogenized in 45 mL of boiling water using a centrifuge (Sorvall ST 8R Centrifuge, Germany) operating at 5000 rpm for 300 seconds. The resulting solution was filtered, and the pH was measured using a pH meter (MB-10, Brazil). All measurements were conducted in triplicate for accuracy and precision.

3.2.7.7. Determination of Cocoa Bean Oil Content

The dried cocoa bean samples of each variety were crushed into fine powder, and then subjected to extraction using a Soxhlet extraction apparatus, employing n-hexane as the solvent. The extraction process lasts for approximately 6-8 hours, after which the solvent is evaporated, and the oil content is determined. The use of hexane as the extraction solvent follows the standard method (Kesen et al., 2014a).

3.2.8. Volatile Compounds Analysis in cocoa beans

3.2.8.1. Extraction of the Volatile Compounds

Purge and Trap system (PTE). The extraction of aroma compounds from cocoa bean samples was conducted using the purge and trap system, as described by Rodríguez-Bencomo et al. (2015). The system consisted of a nitrogen (N₂) source controlled by a flow-meter (LZT 4-M, Union-Tek Instrument, China), connected to a splitter system for purging six samples simultaneously. The N₂ needle and cartridge were inserted through the septum to trap the volatiles. Lichrolut EN resins (Merck) containing 200 mg sorbent, described as one of the most appropriate sorbents for the extraction of the aroma compounds with respect to the previous studies were selected as the adsorbent material for retaining aroma compounds (Rodríguez-Bencomo et al., 2015; Sonmezdag et al., 2016). The temperature of the vial sample was maintained using a thermostated bath. 10g of cocoa bean powder sample was placed in a 20 mL vial, and the headspace was purged with nitrogen to remove oxygen. Preincubation of the samples at the optimized purging temperature of 60 °C was carried out for 10 min. The extraction process was conducted for 90 min with a nitrogen flow rate of 500 mL/min. Subsequently, the compounds retained in the cartridge were eluted using 12 mL of dichloromethane at a slow flow rate. After dehydration with anhydrous sodium sulfate, the aromatic extract was concentrated to 5 mL using a Kuderna Danish concentrator (Sigma Aldrich, St. Louis, MO, USA), followed by further concentration to 200 µL under a stream of N₂. The resulting extracts were stored at -20 °C in 2mL glass vials until analysis.



Figure 3.8. Purge and Trap System for aroma compounds extraction



Figure 3.9. Kuderna Danish concentrator (Sigma Aldrich, St. Louis, MO, USA)

3.2.8.2. Representativeness Test of the Aromatic Extracts

Sample Preparation and Presentation. The sensory panel was consisted of eight assessors, three females and five males, aged between 25 and 45 years old, from the Department of Food Engineering at the University of Cukurova. All panelists had prior training in odor recognition, sensory evaluation techniques, and experience in GC-Olfactometry. The representativeness of the aromatic extracts obtained from PTE was evaluated using cardboard smelling strips (reference 7140 BPSI, Granger-Veyron, Lya, France) as described by Selli and Kelebek (2011), Kesen et al. (2014), Selli et al. (2014). For the representativeness test, 5 g of cocoa bean powder was placed in a 25 mL brown-coded flask as a reference. The aromatic extracts obtained by PTE was adsorbed onto the smelling strips. After 1 minute of solvent evaporation, the ends of the strips were cut off and placed in dark-coded flasks (25 mL). After a 15 min resting period, the samples were presented to the panelists under neutral conditions at room temperature (20°C). No odor of the solvents was detected by the panelists, indicating complete evaporation of dichloromethane.

Similarity and Intensity Tests. To assess the similarity and intensity of the odor of the extracts compared to the reference sample of cocoa bean powder, both similarity and intensity tests were conducted. The methodologies for these tests were previously described in detail in our earlier research (Selli and Kelebek, 2011). The obtained results were subjected to statistical analysis using analysis of variance (ANOVA) with SPSS 22 software (Manugistic, Inc., Rockville, MD, USA).

3.2.8.3. GC-MS-Olfactometry Analyses of Volatile Compounds

The gas chromatography (GC) system employed in our study consisted of several components. It involved a Shimadzu's Nexis GC-2030 chromatograph equipped with a flame ionization detector (FID) and a Shimadzu QP2020-NX network-mass selective detector (MSD), and a Gerstel ODP-2 (Tokyo, Japan) sniffing port using deactivated capillary column (30 cm $\times$ 0.3 mm) heated at 240°C and supplied with humidified air at 40°C. Visual representations of the setup can be found in Figures 3. and 3. This system provided the capability to acquire multiple types of data simultaneously. Through the use of a flame ionization detector (FID), a quantification signal was obtained, while compound identification was facilitated by a mass selective detector (MSD). Additionally, the system incorporated a sniffing port to detect the odor characteristics of each compound. To ensure efficient distribution of the GC effluent, a Dean's switch was utilized to split the output in a 1:1:1 ratio among the FID, MSD, and sniffing port mode. This configuration enabled comprehensive analysis and characterization of the compounds under investigation. The volatile compounds were separated using a DB-WAX column (30 m length $\times$ 0.25 mm i.d. $\times$ 0.5 μ m thickness; J&W Scientific, Folsom, CA, USA). For each injection, a total of 3 μ L of extract was used in pulsed splitless mode (40 psi; 0.5 min). The injector and FID detectors were set at temperatures of 270 °C and 280 °C, respectively. The carrier gas (helium) was set at a flow rate of 1.5 mL/min. The oven temperature of the DB-WAX column was programmed to increase from 40 °C to 200 °C at a rate of 5 °C/min. Subsequently, the temperature was further increase to 260 °C at 8 °C/min and held for 5 min. The same temperature program was applied to the MSD. The mass detector operated in scan mode with an electron impact ionization energy of 70 eV. The GC-MS interface and ionization source temperatures were set at 250 and 180 °C, respectively. Identification and quantification were performed in full scan mode with a mass/charge range of 30–300 amu at 2.0 scan/s scan rate. The volatile compounds identification were achieved using various methods, such as comparing retention indices and mass spectral data from the Wiley 6 and NIST 98 libraries installed in the GC-MS system, as well as an internal library created from previous laboratory studies. Some identifications were confirmed by injecting chemical standards into the GC-MS system. Retention indices of the compounds were calculated using retention data from the linear alkane series. Finally, the concentrations of aroma compounds were determined by employing an internal standard method (Kesen et al., 2013; Oussou et al., 2022; Selli et al., 2006). The response factor of each compound was taken into account in the calculation (Selli and Kelebek, 2011) using the following formula:

$$C_i = (A_i / A_{st}) \times C_{st} \times RF \times HF$$

Where,

C_i : Concentration of compound

A_i : The peak area of the compound

A_{st} : Peak area of internal standard

C_{st} : Concentration of internal standard (41.5 µg/L)

RF: Response factor

HF: Calculation factor (factor for converting sample amount to kilogram: 10)



Figure 3.10. Shimadzu GC and GC-MS system for volatile analysis

3.2.8.4. Aroma Extract Dilution Analysis (AEDA)

The aroma extracts of the three varieties of cocoa beans obtained from Purge and Trap were subjected to analysis through Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS-O) with the involvement of three experienced sniffers. To perform aroma extract dilution analysis (AEDA), the concentrated aromatic extract of cocoa sample (200 µL) was gradually diluted using dichloromethane as a diluent. Dilutions were prepared at ratios of 1:1, 1:2, 1:4, 1:8, 1:16, and up to 1:1024 of the original extract, following the methodology described by Fickert and Schieberle (1998) and Schieberle and Grosch (1987). The dilutions were sniffed and evaluated by the sniffers until no odor could be detected in correlation with the GC-MS-O results. Each perceived odor was assigned a flavor dilution factor (FD factor), representing the highest dilution at which the odor remained detectable.



Figure 3.11. GC-MS-Olfactometry device

3.2.9. Sensory evaluation

A sensory analysis of each crushed cocoa bean (nibs) sample was conducted by a trained panel consisting of 10 panelists from the Department of Food Engineering. The panel included four females and six males, ranging in age from 22 to 51 years old. The panelists were trained to recognize specific attributes of cocoa bean for organoleptic assessment. The crushed cocoa bean samples, each amounting to 15 g, were presented to the panelists in tasting glasses, which were coded with unique four-digit numbers to maintain anonymity. The temperature of the samples was controlled at $28 \pm 2^{\circ}\text{C}$ during the evaluation. The panelists utilized profile sheets to record their assessments. They first smelled the crushed beans and then tasted it from the tasting glass, assigning intensity ratings to positive attributes such as fruity, bitter, and pungent, as well as negative attributes including fusty/muddy, musty/humid, winery/vinegar, metallic, fermented, and rancid. The intensity ratings were provided on a non-structured scale measuring 10 cm. The results of the sensory analysis were expressed as the median intensity of sensory perceptions based on the evaluations provided by the 10 panelists.

3.2.10. Determination of Antioxidant Activity of Cocoa Beans

3.2.10.1. Extraction of Antioxidant Compounds

For the analysis of total phenolic compounds, total flavonoids, total anthocyanins, and antioxidant activity, the natural antioxidant compounds were extracted using the method described by Guclu et al. (2021) with slight modification. Briefly, 1 g of cocoa powder was placed into a centrifuge tube and mixed with 20 ml of 80% methanol. The mixture was then subjected to ultrasonic water bath at room temperature (25°C) for 20 minutes and subsequently was centrifuged at 4°C and 4430g for 10 min. The extract was filtered through a Teflon filter for further analysis.

3.2.10.2. Determination of Total Phenolic Content

The total phenolic content was determined by spectrophotometric measurement of the color formed as a result of the reaction with Folin-Ciocalteu solution in alkaline medium (Singleton & Rossi, 1965) with slight modification. 1 mL of diluted extract, 60 mL of distilled water, 5 mL of Folin–Ciocalteu reagent, and 15 mL of Na₂CO₃ solution (20%) were thoroughly mixed in a 100 ml flask to ensure homogeneity. The resulting solution was kept in the dark at room temperature for 2 hours, and the absorbance was measured comparatively with a blank at 765 nm using UV-Visible spectrophotometer (Schimadzu-1201, Kyoto, Japan). The total phenolic content was calculated in mg/kg using a standard calibration curve prepared with chemically pure gallic acid (50-500 ppm).

3.2.10.3. Determination of Flavonoid Content

The total flavonoid content was determined using a modified protocol described by Hossain and Shah (2015) and Lakka et al. (2019) with some modifications. In this method, 0.5 mL of diluted extract was mixed in a 50 mL flask with 0.3 mL of 5% NaNO₂ solution and allowed to stand in darkness for 5 minutes. Afterward, 0.5 mL of 5% AlCl₃ solution was added to the mixture and kept in dark for 6 minutes. Subsequently, a saturated solution of 0.5 mL 1 M NaOH was added to the mixture and thoroughly mixed. The solution was left to incubate in dark at room temperature for 10 minutes. Following the incubation period, the absorbance was measured at 510 nm against a blank using a UV-Visible spectrophotometer (Schimadzu-1201, Kyoto, Japan). The total flavonoid content was quantified in mg/kg using a standard calibration curve constructed with chemically pure epicatechin.

3.2.10.4. Measurement of Antioxidant Activity

DPPH assay. The electron donation ability of the obtained cocoa bean extracts were assessed by measuring the bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, following a slightly modified protocol described by Kelebek et al. (2015) and Kesen et al. (2014b). Briefly, an aliquot of 0,1 ml of the diluted cocoa bean extract was added to 3.9 mL of DPPH solution in methanol (6×10^{-5} M). The mixture was vigorously shaken and allowed to stand at room

temperature for 30 minutes. The absorbance of the resulting solution was then measured at 517 nm using a UV-Visible spectrophotometer (Shimadzu UV-1201, Kyoto-Japan). The antioxidant activity of the cocoa bean extracts was calculated using a trolox calibration curve and expressed as mM Trolox equivalent per kg of cocoa bean. The mean and standard deviation were calculated based on three replications. The trolox standard curve was defined by the equation $y = 0.0004x + 0.0082$, with an R^2 value of 0.9995, covering a concentration range from 5 to 150 $\mu\text{mol/L}$.



Figure 3.12. The changes observed in the DPPH reaction over time

ABTS assay. The antioxidant capacities of the cocoa samples were assessed using the decolorization method based on the radical cation of 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The ABTS assay followed a slightly modified protocol as described by Kelebek et al. (2015). For ABTS radical solution preparation, ABTS and potassium persulfate were dissolved in pure distilled water to achieve final concentrations of 7 mM and 2.45 mM, respectively. The two prepared solution were mixed and the mixture was kept in the dark at room temperature for 16 hours. Subsequently, the ready-made solution was diluted with 80% methanol until the absorbance reached 0.7 ± 0.01 at 734 nm, indicating the formation of the ABTS radical. The prepared cocoa bean extracts were diluted in a mixture of water and methanol (1:10, v/v). Then, 0.1 mL of the diluted extracts was mixed vigorously with 3.9 mL of the ABTS solution. After allowing 10 minutes for the reaction to occur, the absorbance was measured at 734 nm using a spectrophotometer (Schimadzu-1201, Kyoto, Japan). The antioxidant activity of the samples was calculated using a trolox calibration curve. The calibration curve equation for the Trolox standard was determined as $y = 0.0004x + 0.0089$, with an R^2 value of 0.9996, covering a concentration range from 5 to 150 $\mu\text{mol/L}$.



Figure 3.13. The changes observed in the ABTS reaction over time

3.2.11. LC-DAD-ESI-MS/MS Analysis of Phenolic Compounds

3.2.11.1. Extraction of Phenolic Compounds

The phenolic compounds extraction was conducted using the method described by Kelebek et al. (2015) with slight modification, the cocoa sample (2 g) was mixed with 5 mL of n-hexane and 10 mL of a methanol/water (70:30; v/v) solution in a 50 mL centrifuge tube. The mixture was vigorously mixed and then subjected to centrifugation at 5500 rpm for 15 minutes. The hydro-alcoholic phase (extract) was collected, while the hexane phase was subjected to re-extraction twice with 5 mL of methanol/water (70:30; v/v) solution each time. Subsequently, the hydro-alcoholic fractions were combined and washed with 2 mL of n-hexane to remove any residual oil. The combined fractions were then concentrated and evaporated under vacuum at 40°C. The resulting dry extracts were dissolved in 0.5 mL of a methanol/water (50:50, v/v) solution and filtered through a 0.2 µm nylon filter (Whatman Inc., Clifton, New Jersey, USA) before being injected into LC-ESI-DAD-MS/MS system.

3.2.11.2. Determination of Phenolic Compounds

Subsequently, an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) controlled by Windows NT-based ChemStation software was employed for the characterization of phenolic compounds (Figure 3.18). The HPLC system was composed of a binary pump, degasser, and auto-sampler. A diode array detector (DAD) was employed for detection purposes. The column used in the system was a Phenomenex reversed-phase C-18 column (4.6 mm × 250 mm, 5 µm) (Torrance, California, USA). The mobile phase consisted of two solvents: Solvent A, a mixture of water and formic acid (99.5:0.5; v/v), and Solvent B, a mixture of acetonitrile and Solvent A (60:40; v/v). The analysis was conducted with a flow rate of 0.5 mL/min and a column temperature set at 25 °C. The elution conditions included an isocratic phase from 0 to 5 minutes with 0% B, followed by a

gradient phase from 0% to 5% B in 20 minutes, from 5% to 15% B in 18 minutes, from 15% to 25% B in 14 minutes, from 25% to 50% B in 31 minutes, from 50% to 100% B in 3 minutes, and finally, column washing and preconditioning. These conditions allowed for the elution of phenolic compounds. The UV-visible spectra ranging from 200 nm to 600 nm were measured for all peaks. All the analyses were carried out in three repetitions for each sample. Compound identification and assignment were conducted by comparing retention times and UV spectra to authentic standards. Confirmation of compound identification was carried out using an Agilent 6430 LC-MS/MS spectrometer (Santa Clara, CA, USA) equipped with an electrospray ionization source. The LC-MS/MS detection was performed in negative ion mode with optimized parameters including a capillary temperature of 400 °C, N₂ gas flow rate of 12 L/min, and a nebulizer pressure of 45 psi (Kelebek et al., 2015). All the data acquisition was performed using the Multiple Reactions Monitoring (MRM) method, which monitors specific mass transitions during predetermined retention times. Standard curves were generated using commercial standards with concentrations typically found in cocoa beans (approximately 1–100 mg/kg), resulting in regression coefficients (r^2) above 0.995 for all cases.

3.2.12. Statistical Analysis

The results of this study were subjected to analysis of variance using the SPSS 22 software package, and Duncan's multiple-comparison test was used to find significant differences in this study.

4. RESULTS AND DISCUSSIONS

4.1 Evaluation of the trade quality parameters of cocoa beans

Quality of cocoa is determined by a combination of factors that determine the acceptability of the cocoa to a buyer. These factors include proper fermentation, dried to the proper moisture level, free from abnormal odours and free from mould contamination. During cocoa beans fermentation and drying process, a number of physical and chemical changes occur in the cocoa beans: variations in colors, pH, fermentation index, humidity, aroma as well as transformation in chemical composition, which can have a negative impact in the quality and sensory characteristics of the dried cocoa beans. The characterization of a food crop entails the analysis of its most representative chemical, physical and organoleptic properties (Rial & Falqué, 2003). Moisture content is regarded as one of the primary factor influencing the quality of cocoa beans. The moisture content of all cocoa beans are presented in Table 4.1. The average values of the moisture content of fresh cocoa beans (before fermentation) were 62.18, 58.46 and 65.5% for Forastero, Criollo and Nacional, respectively in this study. After 5 days of fermentation, a clear decrease in moisture content was observed, which is due to the drainage of cocoa juice from the beans. The fermented cocoa beans moisture content were 55.75, 48.33 and 51.85% (w.b) for Forastero, Criollo and Nacional, respectively. The same observation was made by Ackah and Dompey (2021) and other previous researchers (Dzelagha et al., 2020; Hernandez & Granados, 2021) . The moisture content of cocoa beans is linked to climat, weather conditions, and significantly decrease during fermentation and drying processes.

Fermentation index (FI) determines the degree of fermentation based on the brownness formed in the cocoa beans. Over-fermented bean has fermentation index more than 1.6 while under-fermented beans has fermentation index of less than 1.0, which shows the completeness of the fermentation process (Nsor-Atindana et al., 2012). The fermentation index of all fermented cocoa beans samples are indicated in Table 4.1. It was observed that all cocoa beans were well fermented after 96 h fermentation in the present study. The well fermented cocoa beans was attributed to Nacional variety with FI of 1.56, following by Criollo with FI of 1.24. All cocoa beans were well fermented after 96 h fermentation which were in accordance with previous work reported by Pereira et al. (2017). The degree of fermentation in cocoa beans arise from the gradual diffusion of polyphenols during fermentation, followed by oxidation and reduction reactions with other cellular constituents, which then converting into brown colour in cocoa beans (Hernández-Hernández et al., 2016; Kresnowati & Febriami, 2016). Polyphenol oxidase implied in oxidation reaction by catalysing o-diphenol to o-quinone leading to the brown colour formation in cocoa beans (Hernández-Hernández et al., 2016). The polyphenol oxidase reacts best at 42–45 °C which usually achieves on day three of fermentation (Caligiani et al., 2007). This further corresponded to the formation of well-fermented beans which is obtained after 72 h fermentation. The fermentation index of the studied samples were in line with those reported by León-Roque et al. (2016) in cocoa fermented bean from

Peru. In another study conducted by Romero-Cortes et al. (2013) revealed that 48 to 72 hours are sufficient to reach to a suitable fermentation index for good cocoa quality. However, the fermentation index values observed in their study were in accordance with those reported in the present study, although the fermentation time was 96 hours.

The average values of pH of fermented Forastero, Criollo and Nacional cocoa beans ranged from 4.8-5.1, 5.0-5.5, and 4.9-5.4 after 5 days of fermentation, respectively. The average values of the total acidity of all dried cocoa beans are shown in Table 4.1. The pH reduction and consequently increase in total acidity (TA) during fermentation are due to the diffusion of acids (predominantly acetic acid) through the cotyledon (Afoakwa et al., 2008; Afoakwa et al., 2013). The total acidity value of fermented Forastero, Criollo and Nacional are shown in the table 4.1. It was observed after 5 days of fermentation that Nacional had the highest content of acidity, followed by Criollo. As reported by Afoakwa et al. (2011) and Castro-Alayo et al. (2019), the pH range must be between 4.7-5.5 during fermentation, to obtain fermented and dried cocoa beans with a high level of aromatic compounds, and our findings are in this range. Numerous investigations also confirm that the pH value of cocoa beans increase at the end of fermentation, and the good pH value in order to preserve the quality of cocoa beans is between 4.7-5.5 (Afoakwa et al., 2008; Guehi et al., 2010). Prolonged fermentation, regardless of the process applied, resulted in over-fermentation, characterized by a disagreeable putrid ammonia odor. The rise in pH in fermented cocoa beans stemmed from a concurrent drop of about 55% in citric acid concentration within the pulp, as noted by (Ardhana & Fleet, (2003). This shift was further influenced by the migration of various organic acids, including ethanol, lactic acid, and acetic acid, produced through microbial activity, from the external pulp to the internal cocoa seeds, a phenomenon highlighted by Wollgast and Anklam (2000a). The genesis of these organic compounds was linked to the consumption of pulp sugars (fructose, glucose, and sucrose) by the microflora. The presence of these organic elements contributed notably to the heightened levels of lactic and acetic acids, as previously elucidated by Gálvez et al. (2007). Despite the increased production of lactic and acetic acids within the beans as reported by Ardhana and Fleet (2003), cocoa beans that underwent a thorough fermentation process of 5 days exhibited a reduction of acidity compared to partially fermented ones (fermented for 3 and 4 days). This observation might be attributed to the controlled or minimized formation of lactic acid, achieved through specific turning procedures implemented at 48 and 96 hours into the fermentation process. In our study, the increase in pH in the fermenting cocoa pulp-bean mass and fermented bean could be due to frequent stirring every 48 h, similar to what was found by Portillo and Farinas, (2007) in their study.

Table 4.1. Some quality attributes in fresh and fermented cocoa bean cultivars

Beans Properties	Forastero	Criollo	Nacional
Fresh Bean Humidity (%)	62.18 ± 0.22 ^{ab}	58.46 ± 0.45 ^b	65.5 ± 0.18 ^a
Fermented Beans Humidity (%)	55.75 ± 1.06 ^a	48.33 ± 0.88 ^a	51.83 ± 0.34 ^a
pH	4.8 ± 0.00 ^a	5.0 ± 0.00 ^a	4.6 ± 0.00 ^a
Acidity (mg/kg)	2.4 ± 0.13 ^a	2.0 ± 0.68 ^b	1.8 ± 0.27 ^b
Fermentation Index	1.06 ± 0.00 ^b	1.24 ± 0.00 ^b	1.56 ± 0.00 ^b

Different lower-case letters (a,b,c) within the same line of each cocoa bean variety display significant differences ($p < 0.05$) between the different cocoa beans varieties

4.2 Physicochemical properties of cocoa beans

The average values of the physicochemical analysis, sanitary and the quality of fermented cocoa beans of different varieties are presented in Table 4.2 and Figure 4.1. Physicochemical analysis of fermented dried cocoa beans such as weight, length, width, cut test, one hundred cocoa beans mass, crude oil content were determined. The weight and length of cocoa fruits and beans significantly vary depending on the variety, the practices and region of cultivation, the degree of ripening and harvest periods. Fermentation is one of the major step to prepare fine cocoa through the formation of potent aroma precursors (Amoa-Awua, 2015). Color changes ranging from purple to brown were reported during the fermentation of cocoa beans. Cut-test (degree of fermentation) was applied to evaluate the color of the beans, which can be performed on the field; and often used as fermentation index (FI) (Amoa-Awua, 2015; Misnawi et al., 2003). Cocoa is graded on the basis of the count of defective beans in the 'cut test'. The cut test reveals the presence of certain defects which may cause off-flavors and indicates the degree of fermentation of the beans which has a bearing on the flavor and quality of the beans. The results of cut test analyses of the three varieties of fermented cocoa beans are exhibited in Figure 4.1. The cut score showed that 9.78, 7.46 and 4.18% of defective beans, and 11.74, 5.15 and 2.44% of purple beans was detected in Forastero, Criollo and Nacional fermented cocoa beans, respectively. An increase score 76.48, 82.16 and 89.36% of brown beans were marked in fermented Forastero, Criollo and Nacional beans, respectively. A small percentage of slaty beans (1.68%) was only observed in fermented Criollo beans. While no moldy beans were found in all fermented cocoa beans. A good-quality fermented products marked by high percentage of brown cocoa beans was obtained. The higher score of defective and purple Forastero fermented beans could be due to the oxidation of ethanol produced by yeasts into acetic acid, which then may diffuse into the beans. The combined effect of alcoholic fermentation and heat halts cocoa bean embryo viability, hampering their inherent germination potential, as reported by Cleenwerck et al. (2007). This leads to the reduction of germinated beans. Biochemical changes following this process prompt precursor molecule formation and internal color changes in cocoa nibs, shifting from purple

to brown. This alteration notably reduces defective beans, particularly purple and slaty ones. This biochemical transformation improves the quality of final product (Cleenwerck et al., 2007). As slaty beans are an indicator of improperly fermentation of cocoa, slight percentage of such beans demonstrate that the natural fermentation conducted in this study was processed properly (Hii et al., 2009). In addition, these observations suggest a potential reduction in the astringency of our cocoa beans samples. This proposition stems from findings by Hii et al. (2009), indicating that a significant presence of salty and purple beans might contribute to an overabundance of astringency in the final products, thereby masking the nuanced flavors of chocolate.

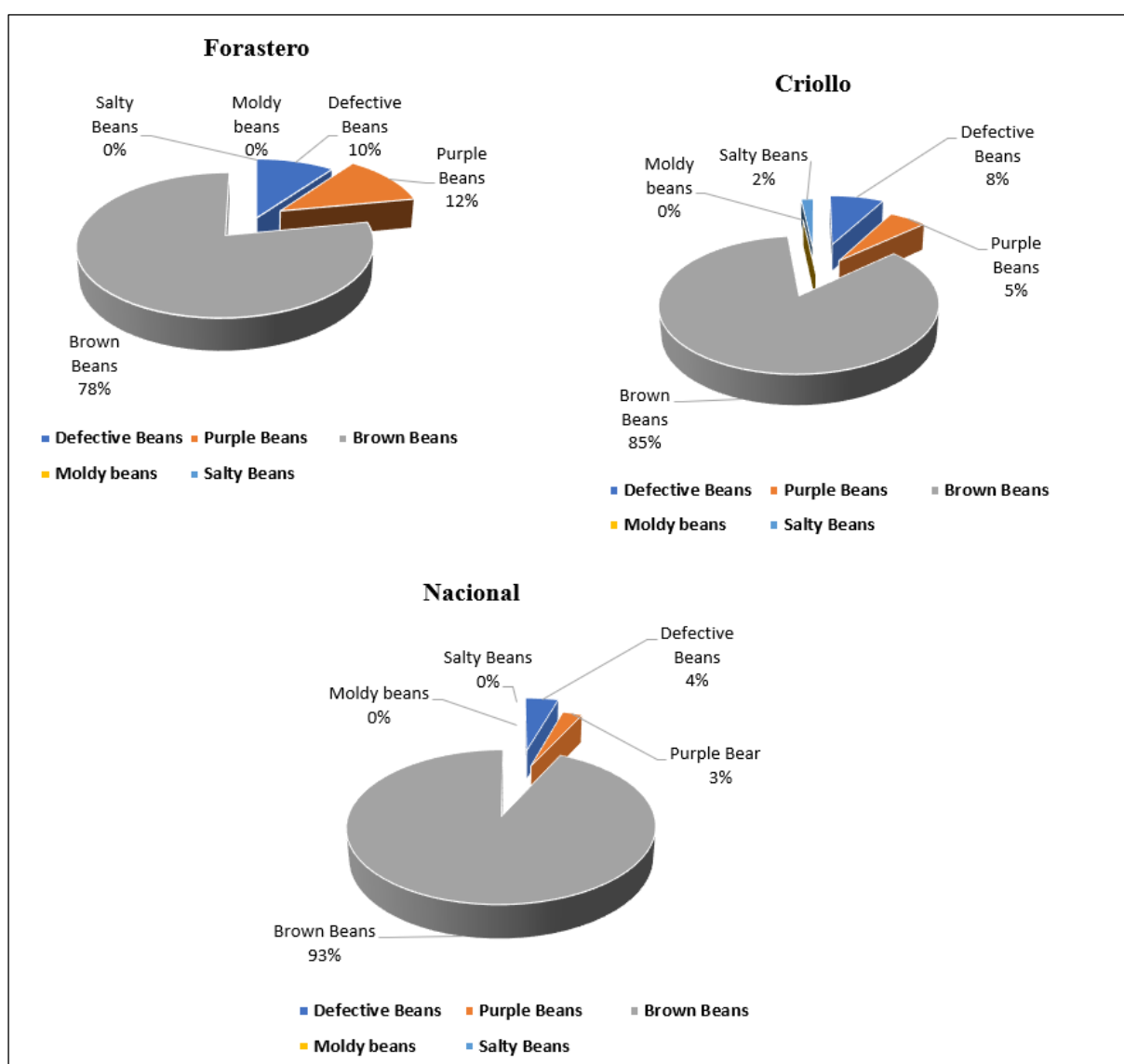


Figure 4.1. Some quality parameters of cocoa bean cultivars

The data of fermented dried Forastero beans are shown in Table 4.2. When using fresh-fermented beans as control, it was observed that the drying processes, especially microwave drying (MD) and convective drying (CD), caused substantial changes in the length-width of bean and beans

mass compared to freeze and sun-drying drying processes. However, no significant differences were seen in acidity and pH of all dried cocoa beans variety obtained from sun, microwave, convective and freeze drying methods. When the oil content of Forastero beans obtained from drying processes are compared, MD exhibited the highest oil content (59.2%), and the lowest oil content (28.6%) was extracted from freeze drying (FD) method. In their investigation, Apriyanto (2016b) and Apriyanto (2016a) reported pH values of 5-5.7 in sun dried Forastero beans. The average pH values of 5.16, 5.35 and 6.59 were reported by Guehi Guehi et al. (2010) in dried cocoa beans after 4, 5 and 6 days of fermentation, respectively. The total acidity (TA), considered a more reliable indicator of acidity than pH, exhibited a consistent downward trend over time across all treatment groups, according to findings by Nazaruddin et al. (2006). The acidity level observed in dried beans fermented for 4-5 days resembled that reported by Ardhana and Fleet (2003), although slightly lower than the figures documented by Bonaparte et al. (1998).

Regarding the findings of fermented dried Criollo beans, it was also seen that microwave drying (MD) and convective drying (CD) techniques significantly induced changes in the length-width of beans and bean weight in comparison with freeze drying (FD) (Table 4.2). However, no significant differences were seen in acidity and pH of all dried cocoa beans variety obtained from sun, microwave, convective and freeze drying methods. When the oil content of Forastero beans obtained from drying processes are compared, MD exhibited the highest oil content (53.1%), following by CD (44.63%). The lowest oil content (32.4%) was extracted from freeze drying (FD) method.

As of Nacional bean samples, significant differences was also observed in physical properties of beans, mostly the length-width, and bean weight. It was found that microwave drying (MD) and convective drying (CD) methods considerably effected the physical structure of cocoa beans (Table 4.2). However, no significant differences were seen in acidity and pH of all dried cocoa beans variety obtained from sun, microwave, convective and freeze drying methods. Furthermore the highest oil content (61.52%) was found in beans dry under microwave conditions, and the lowest oil content (38.9%) was attributed to freeze drying process. When the physicochemical properties of cocoa beans varieties are compared, Nacional presented an excellent characteristics based on the International Cocoa Beans trade acceptability conditions.

Table 4.2. General attributes of fermented and dried cocoa bean samples obtained from the three cocoa cultivars

Samples	Cocoa beans properties						
	pH	Acidity (mg/kg)	Width (mm)	Lenght (mm)	Bean weight (g)	100 beans mass (g)	Crude oil (%)
FFB (Control)	5.0 ± 0.10 ^b	1.6 ± 0.25 ^b	2.1 ± 0.4 ^a	3.7 ± 0.8 ^a	1.8 ± 0.3 ^a	156.4 ± 5.6 ^a	16.4 ± 2.4 ^d
SDFB	5.5 ± 0.20 ^a	2.1 ± 0.34 ^a	1.8 ± 0.3 ^{ab}	2.5 ± 0.6 ^b	1.2 ± 0.5 ^b	116.2 ± 3.4 ^b	50.9 ± 3.84 ^b
CDFB	5.6 ± 0.32 ^a	2.2 ± 0.63 ^a	1.4 ± 0.1 ^b	2.3 ± 0.1 ^b	1.1 ± 0.1 ^b	98.7 ± 2.7 ^d	48.0 ± 1.93 ^b
MDFB	5.6 ± 0.81 ^a	1.9 ± 0.20 ^b	1.6 ± 0.1 ^b	2.4 ± 0.6 ^b	1.0 ± 0.2 ^b	97.8 ± 3.80 ^d	59.2 ± 2.20 ^a
FDFB	5.8 ± 0.00 ^a	2.1 ± 1.20 ^a	1.9 ± 0.2 ^a	2.7 ± 0.1 ^b	1.3 ± 0.3 ^b	104.0 ± 5.4 ^c	28.6 ± 1.74 ^c
FCB (Control)	5.1 ± 0.50 ^b	2.3 ± 0.40 ^c	2.0 ± 0.3	3.8 ± 0.6 ^a	2.0 ± 0.4 ^a	184.6 ± 7.8 ^a	14.7 ± 1.8 ^d
SDCB	5.2 ± 0.15 ^b	3.5 ± 0.60 ^a	1.7 ± 0.2 ^a	2.9 ± 0.4 ^b	0.9 ± 0.1 ^b	96.5 ± 4.36 ^c	44.6 ± 3.4 ^a
CDCB	6.0 ± 1.02 ^a	2.5 ± 0.64 ^b	1.1 ± 0.1 ^b	2.2 ± 0.2 ^c	1.1 ± 0.1 ^b	94.5 ± 2.45 ^b	47.3 ± 1.3 ^a
MDCB	5.2 ± 1.04 ^b	3.3 ± 0.85 ^a	1.4 ± 0.2 ^b	2.1 ± 0.3 ^c	1.0 ± 0.1 ^b	91.5 ± 4.12 ^c	53.1 ± 3.7 ^a
FDCB	6.3 ± 1.25 ^a	2.8 ± 0.94 ^c	1.6 ± 0.1 ^a	2.7 ± 0.4 ^b	1.2 ± 0.2 ^b	105.0 ± 4.41 ^c	32.4 ± 1.7 ^c
FNB (Control)	5.0 ± 0.30 ^a	2.6 ± 0.3 ^b	2.3 ± 0.8 ^a	4.3 ± 0.8 ^a	2.4 ± 0.5 ^a	216.7 ± 8.9 ^a	19.8 ± 2.3 ^d
SDNB	5.3 ± 0.60 ^a	3.1 ± 0.2 ^a	1.9 ± 0.6 ^{ab}	3.2 ± 1.2 ^b	1.1 ± 0.1 ^b	114.7 ± 4.5 ^c	48.9 ± 4.6 ^b
CDNB	5.5 ± 0.80 ^a	3.2 ± 0.6 ^a	1.8 ± 0.4 ^c	2.9 ± 0.9 ^c	1.2 ± 0.3 ^b	115.4 ± 6.3 ^c	54.9 ± 3.4 ^a
MDNB	5.3 ± 0.30 ^a	3.1 ± 0.1 ^a	1.4 ± 0.4 ^c	2.8 ± 0.8 ^c	1.1 ± 0.1 ^b	110.2 ± 8.4 ^c	61.5 ± 2.3 ^a
FDNB	5.4 ± 0.50 ^a	3.0 ± 0.3 ^a	2.0 ± 0.5 ^a	3.5 ± 1.2 ^b	1.4 ± 0.4 ^b	126.4 ± 10.7 ^b	38.9 ± 3.4 ^c

SDFB: sun dried Forastero beans; CDFB: convective dried Forastero beans; MDFB: microwave dried Forastero beans; FDFB: freeze dried Forastero beans; SDCB: sun dried Criollo beans;

CDCB: convective dried Criollo beans; MDCB: microwave dried Criollo beans; FDCB: freeze dried Criollo beans; SDNB: sun dried Nacional beans; CDNB: convective dried Nacional beans; MDNB: microwave dried Nacional beans; FDNB: freeze dried Nacional beans. Each value represents mean ± standard deviation. Different lower-case letters (a,b,c) within the same column of each cocoa bean variety display significant differences ($p < 0.05$) between the different cocoa beans drying processes.

4.3 Moisture Content Loss

4.3.1. Sun Drying

Figure 4.2 shows the moisture loss profiles of the studied samples. Although the moisture content loss curve indicates a slight decrease with an initial moisture content of 55.75, 48.33 and 51.83% for Forastero, Criollo and Nacional, respectively, it was observed that, during natural drying, the moisture content variations of all cocoa varieties samples are closely related to environmental weather conditions. The required time which lasted 5 days (120 hours) to achieve the required moisture content of 7.29, 6.96 and 7.84% in Forastero, Criollo and Nacional, respectively, confirms this slow dehydration. During 5 days (120 hours), the humidity reduction was about 65.84 % for all cocoa varieties samples. Sun drying of cocoa beans at different layer thicknesses of 5 cm, 10 cm, and 15 cm reduced the average moisture content from an initial level of 54.39 % to a final level of approximately 13 % in 52 h, 56 h, and 66 h, respectively (Asante, 2015). These results show that the drying time of cocoa beans is not only related to the weather conditions that prevailed at the drying time moment but also the layers thickness of the cocoa beans destined to be dried. It was also observed that the variation in the moisture content over time is significant at the beginning of the drying operation and almost constant when the drying time towards the required moisture content.

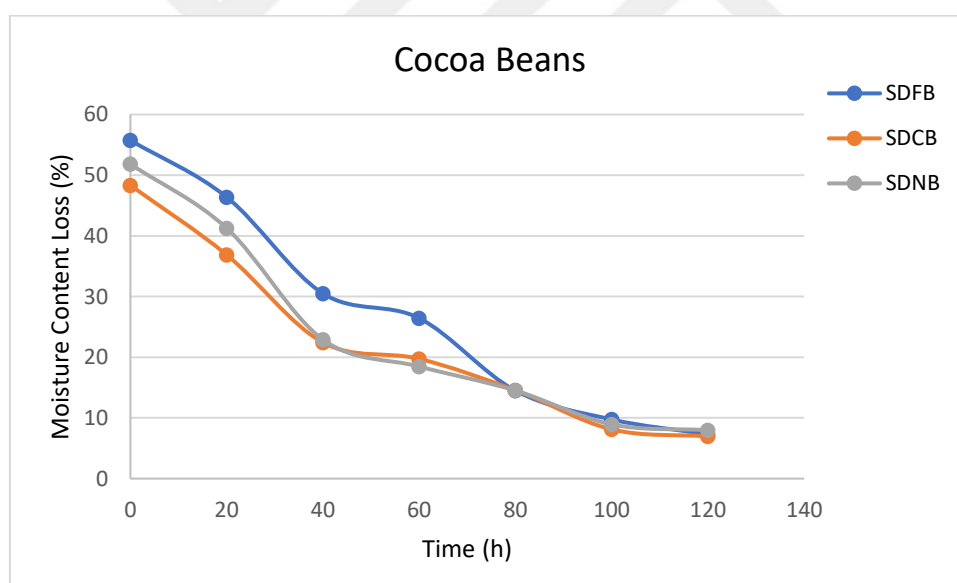


Figure 4.2. Effect of natural conditions on average moisture loss of sun-dried cocoa beans

4.3.2. Hot Air Convective Drying

The fermented cocoa beans obtained from the different varieties were hot air dried at 60°C for almost 3 days until the required moisture content was achieved. Figure 4.3 represent the drying curves for Forastero, Criollo and Nacional beans samples, showing the trends in moisture loss. The initial moisture content ranged from 55.75, 48.33 and 51.83% (w.b) to 7.61, 6.9 and 6.97% for Forastero, Criollo and Nacional, respectively. The drying process was characterised by a gradual decrease in

moisture content over time, followed by stabilization to achieve a constant value. Several previous studies on the drying of agro materials are unanimous on air temperature's positive effect, particularly for organic products with high internal resistance to water migration (Belghit et al., 1999; Mariem & Mabrouk, 2017). However, the required drying time to achieve the required moisture content varies widely depending on the temperature. Indeed, the drying times for cocoa beans to reach the required moisture content of 6 to 8% at 60°C were 50 h for Forastero and Criollo, and 55h for Nacional. In general, as drying temperatures rise, the moisture content decreases, resulting in a greater deficit in water vapour pressure (Abraham et al., 2016). This phenomenon is one of the driving forces behind the diffusing moisture process outwards. The increase in drying potential and decrease in drying time is explained by the fact that as temperature rises, so does the heat transfer intensity. The drying temperature increases the water molecules' energy, thereby accelerating its migration inside the product, allowing it to escape more easily and quickly.

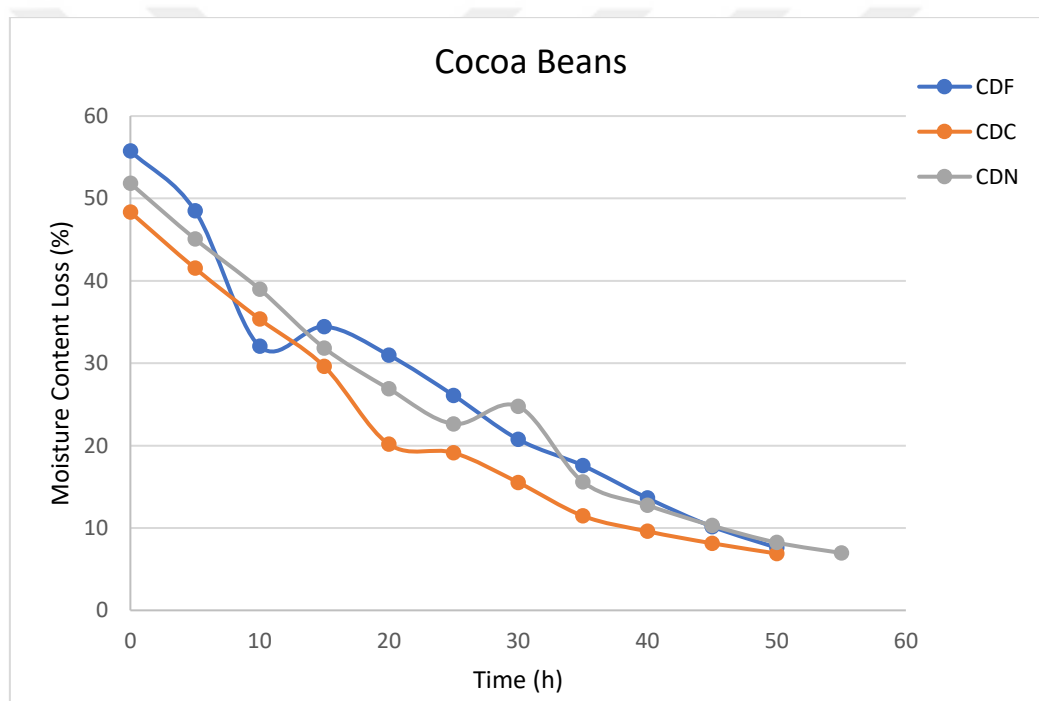


Figure 4.3. Effect of convective drying conditions (60°C) on the average moisture loss of cocoa beans

4.3.3. Intermittent Microwave Drying

The effect of exposure at 200 W for approximately 500 min on the moisture content loss of all cocoa beans samples was investigated. The moisture content loss profiles of Forastero, Criollo and Nacional beans samples is depicted in Figure 4.4. The initial moisture content was practically different for all samples of fermented cocoa beans, corresponding to 55.75 %, 51.83 and 48.33% (w.b) for Forastero, Nacional and Criollo beans respectively. With initial moisture of 55.75 % and 48.33% of Forastero and Criollo, respectively, reached the required moisture content of 6 to 8 % after 420 min,

While the Nacional fermented beans with an initial moisture content of 51.83 % (w.b) took 480 min to reach the required moisture content of 6 to 8 %.

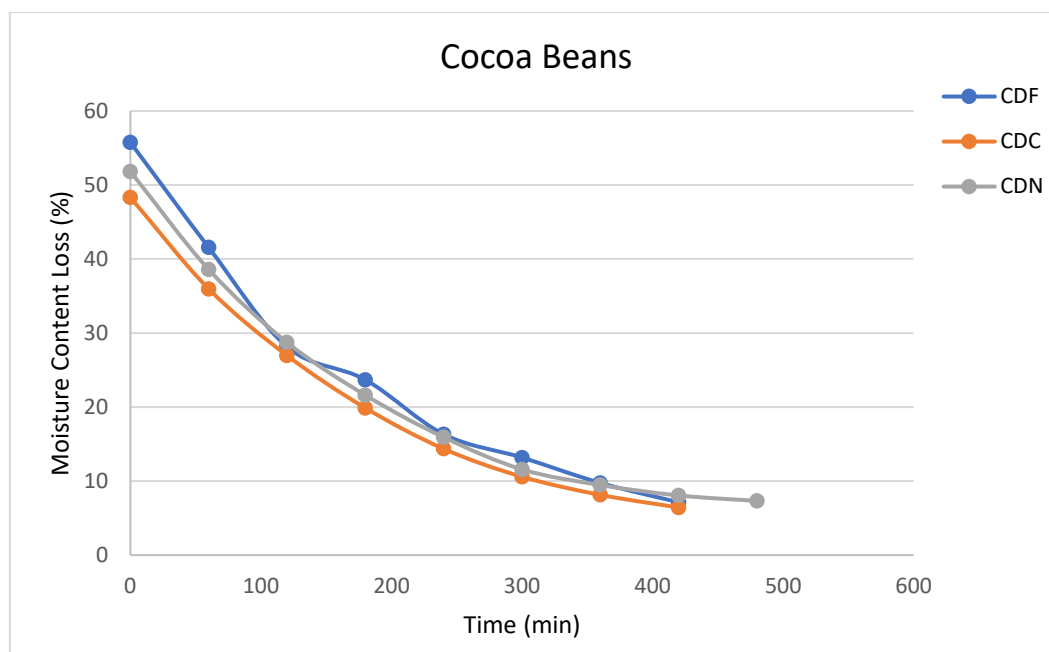


Figure 4.4. Effect of microwave drying conditions (200 W) on the average moisture loss of cocoa beans

It can be concluded in this section that all cocoa beans varieties were sun dried for about 72 h to achieve the appropriate moisture content. When they were hot air dried at 60 °C, the time was reduced to 50 and 55h, while under microwaves at 200 W and 6.2 mm/s (250 rpm), the time was reduced to 420 and 480 min. The results revealed that the moisture content loss and the drying times curves pattern are characteristics of agri food product drying for all drying methods and used operating parameters.

4.4 Effect of Drying on Colors Properties of Fermented Cocoa Beans

Colour is one of the most important aspects of food materials because it influences consumer acceptance. It is used for process control and consumer acceptability (Maskan, 2001). It is a considerable quality criterion influencing consumer choice (Ranalli et al., 2005). Thus, these factors might be concerned for coverage in testing the quality of cocoa beans. Food colour is governed by chemical, biochemical, microbial, and physical changes occurring during growth, maturation, handling, and post-harvest processing (Pathare et al., 2013). The effects of all drying methods used on colour parameters such as lightness L^* , redness a^* , and yellowness b^* for Forastero, Criollo and Nacional beans were investigated in this section. Many studies have focused on the colourimetric evaluation of food products such as carrot (Sulaeman et al., 2001), hazelnut (Shakerardekani et al., 2011), and pistachio (Salehi et al., 2023). Thus, in the same way, all cocoa beans varieties samples colour could also be described by the luminance parameters (L^*), redness (a^*), and yellowness (b^*). It is also important to remember that

the lightness L^* scale measures a product's relative lightness. Color data all dried cocoa beans samples obtained from sun drying (SD) and other drying methods used in this study are shown in Table 4.5. It was observed in all cocoa beans samples that the drying methods applied, especially microwave drying (MD) and convective drying, induced significant color changes in comparison with sun drying (SD) as control and freeze drying (FD).

When the lightness (L^*) parameters of all dried cocoa type samples were compared with those of the sun dried samples (control), it was observed that the MD and CD methods caused significant decreases in the L^* parameters of the dried cocoa samples, which means darker colors in dried samples while the FD method caused an increase in the L^* values meaning lighter colors especially in Nacional (52.80) and Forastero (52.24) beans (Table 4.5). These observations reveal that sun dried and freeze dried beans appear lighter than those of microwave and convective dried beans samples. The changes in L^* values of the dried and powdered cocoa samples can be attributed to the thermal degradation of the pigments and the interaction of the pigments with other substances, or Maillard reaction that causes browning during drying. It can be stated in our study that the increase of temperature or power decrease the lightness of cocoa beans and powder samples. Previous studies reported similar tendencies in dried cocoa beans using different drying techniques (García-Alamilla et al., 2017; Żyżelewicz et al., 2014). Increases were observed in the redness (a^*) color parameter of the dried cocoa bean samples as compared with the sun dried sample (Table 4.3). The results were significantly different ($p < 0.05$) for all drying methods within the same variety of cocoa beans samples. The a^* values of the Nacional (10.29) and Forastero (9.40) bean samples showed greater changes, while the highest value was found in the microwave drying (MD) process. These results revealed that microwave dried products tend to be reddish, whereas those of sun and freeze dried appear more greenish. There are no significant differences b^* values of the dried cocoa bean samples within the same variety. Overall, the results showed that the drying techniques affected the lightness and the redness but not the yellowness of all cocoa beans samples. Cocoa beans and powder color are affected by genetic of cocoa variety, ripening degree of beans, and especially drying and roasting methods. When the total color difference (ΔE^*) of the sun dried and other dried cocoa samples was assessed (Table 4.3), it was shown that FD method excepted for Nacional dried beans, caused significantly lower total color change ($P < 0.05$) while the MD method had the highest color difference in comparison with other techniques. Similarly, it has been reported in former studies that the total color difference differed based on the drying method in various products (Tekgül & Baysal, 2018; Yang et al., 2018).

Table 4.3. Effect of cocoa bean samples drying processes on color ($L^*a^*b^*$) parameters

Cocoa beans samples ¹	L^*	a^*	b^*	ΔE^*
Fermented Forastero beans (Control)	63.45 ± 2.4^a	1.68 ± 1.2^c	14.84 ± 3.6^a	00.00 ± 00.00^c
Sun dried Forastero beans (SDFB)	49.52 ± 3.7^c	5.38 ± 0.4^d	9.12 ± 1.3^b	15.50 ± 6.43^c
Convective dried Forastero beans (CDFB)	48.63 ± 4.5^c	8.42 ± 2.4^b	9.24 ± 2.2^b	17.21 ± 3.14^b
Microwave dried Forastero beans (MDFB)	48.75 ± 2.9^c	9.48 ± 1.3^a	8.77 ± 0.9^c	18.71 ± 6.24^a
Freeze dried Forastero beans (FDFB)	52.24 ± 3.8^b	7.00 ± 0.8^c	8.95 ± 1.3^c	13.73 ± 2.66^d
Fermented Criollo Beans (Control)	58.96 ± 1.2^a	-1.08 ± 0.9^e	20.19 ± 2.4^a	0.00 ± 00.00^e
Sun dried Criollo beans (SDCB)	50.58 ± 6.1^b	5.81 ± 0.7^d	9.27 ± 3.4^d	15.52 ± 2.44^c
Convective dried Criollo beans (CDCB)	46.57 ± 5.4^c	9.03 ± 1.2^b	9.82 ± 1.6^c	19.05 ± 9.24^b
Microwave dried Criollo beans (MDCB)	42.75 ± 2.2^d	9.79 ± 2.3^a	9.18 ± 1.1^d	22.41 ± 6.33^a
Freeze dried Criollo beans (FDCB)	51.62 ± 4.6^b	6.34 ± 0.5^c	10.73 ± 2.3^b	14.1 ± 2.78^d
Fermented National beans (Control)	66.23 ± 3.8^a	-3.74 ± 1.7^e	17.64 ± 2.8^a	0.00 ± 00.00^d
Sun dried National beans (SDNB)	46.73 ± 1.3^d	5.68 ± 1.2^d	9.69 ± 2.5^c	23.07 ± 4.18^b
Convective dried National beans (CDNB)	45.50 ± 2.3^e	8.36 ± 2.3^b	9.71 ± 3.8^c	25.28 ± 5.80^a
Microwave dried National beans (MDNB)	48.75 ± 5.6^c	10.29 ± 2.4^a	9.67 ± 1.3^c	23.79 ± 4.37^b
Freeze dried National beans (FDNB)	52.80 ± 3.3^b	6.25 ± 0.9^c	10.63 ± 1.6^b	18.15 ± 6.78^c

¹Numerical data are the means $\pm$ standard deviations of 21 measurements. Different superscript letters (a, b, c, etc.) on the numbers in same row demonstrate significant differences ($P < 0.05$) among drying methods within the same cocoa beans variety. ^{a,b,c,d} various superscript letters on the numbers of ΔE^* in same column indicate significant differences ($P < 0.05$) among drying processes within the same cocoa bean variety.

4.5 Effect of Drying on Natural Antioxydant Compounds of Fermented Cocoa Beans

4.5.1. Total Phenolic Content of Fermented and Dried Cocoa Beans

The beneficial effects obtained from phenolic compounds have been attributed to the antioxidant activities of these compounds. As already mentioned, phenolic compounds represent a class of more than 8000 compounds that occur naturally as secondary metabolites in plants (Kelebek, 2016; Khoddami, 2013; Tsimogiannis, 2019). Two groups of phenolic compounds particularly attract attention in cocoa beans, which includes catechins and flavonols. The amount of phenol compounds in cocoa beans and products varies depending on the processing methods, including fermentation, drying and roasting. The amount of the total phenolic content of each cocoa variety beans obtained from the four drying methods are shown in Table 4.4, and the mean differences were statistically significant ($p < 0.05$) for all measured parameters. As it can be also seen from the Table 4.4, when Forastero beans obtained from the control (fresh-fermented bean), sun-drying, convective drying, microwave drying and freeze-drying were compared, the highest amount of phenolic content (508.76 mg GAE/kg), followed by convective dried (CD) cocoa bean samples (387.44 mg GAE/kg), and no significant difference of

phenolic concentration was observed between Forastero convective-dried bean and freeze-dried Forastero bean samples ($p < 0.05$). The lowest (164.12 mg GAE/kg) was found in microwave dried (MD) cocoa bean samples. Regarding Crillo variety beans, the highest amount (386.42 mg GAE/kg) was also found in fresh-fermented bean samples, following by convective dried (CD) beans with an amount of 324.36 mg GAE/kg as it can be observed in the Table 4.4, while in Nacional variety beans, the highest amount of phenolic content (564.28 mg GAE/kg) was also attributed to fresh-fermented bean samples, and the lowest amount (188.36 mg GAE/kg) was found in Nacional microwave dried bean samples. When all the drying methods were compared, microwave drying process exhibited the lowest content of total phenolic content in all the varieties of cocoa beans samples. These observation may be due to the drying operated at high temperature and power, which significantly degraded the phenolic contents. In addition, when the phenolic content of all the cocoa varieties were compared based on the fresh-fermented bean, the highest phenolic content (564.28 mg GAE/kg) was observed in Nacional bean samples, followed by Forastero (508.76 mg GAE/kg) bean samples. The differences observed in phenolic content between the cocoa varieties may stem from the genotypes, chemical and soil compositions, cultural practices. Our results were in accordance with those reported by Othman et al. (2007) in dried cocoa bean from different regions. Mazar Jolić et al. (2011) investigated the changes of phenolic content and antioxidant capacity in different dried cocoa beans and their products. The authors observed that phenolic content values were varied between 3.49-15.21 GAE mg/g. Gu et al. (2006) have demonstrated a good correlation between total phenolic contents and antioxidant capacity in dried cocoa beans and their products. In another study performed by Abhay, et al. (2016) on the Effect of hot-air drying temperature on the polyphenol content and the sensory properties of cocoa beans, significant reduction of phenolic content in dried cocoa bean at high temperature was observed, which is in line with our findings. Similarly, Santhanam Menon et al. (2017) reported total phenolic contents in different dried cocoa beans, ranging from 94.4 mg/kg (microwave dried bean), 112.1 GAE mg/kg (oven dried bean), 126.3 GAE mg/kg (freeze dried bean). The authors concluded that drying process at high temperature degrades the phenolic content of cocoa beans and products. High values of total phenolic contents, ranging between 672.95-1380 GAE mg/g in three different cocoa hybrids. The authors also observed an increased amount in freeze dried beans, and concluded that thermal drying process significantly reduced the total phenolic content. Oracz and Nebesny, (2016) reported higher amount of total polyphenol values for the dried cocoa beans from Nacional, Trinitario, and Forastero cultivars, with concentration of 140.53, 167.23, and 173.58 mg GAE/g, respectively. In contrast, hybrid clones such as UAF-Upper Amazon Forastero and Trinitario-UAF had lower values, measuring 105.18 mg GAE/g from Ghana and 129.37 mg GAE/g from Indonesia, respectively.

4.5.2. Total Flavonoid Content of Fermented Dried Cocoa Beans

Flavonoids, including catechins (flavanols), anthocyanidins, flavonols are divided into subgroups as flavanones, flavones and isoflavonoids. Flavonoids can act as powerful chain-breaking antioxidants depending on the hydroxyl structures they contain (Shaïdi and Naczki, 2003, Kelebek, 2016). The flavonoid content of each cocoa variety is given in the Table 4.4, and expressed as mg epicatechin equivalent/kg (ECE/kg). In Forastero beans obtained from the four drying processes, the highest flavonoid content (218.48 mg ECE/kg) was found in fresh-fermented bean samples, and the lowest flavonoid content (89.47 mg ECE/kg) was attributed to Forastero microwave-dried (MD) cocoa bean samples. Criollo showed the highest flavonoid content (177.38 mg ECE/kg) in fresh-fermented cocoa bean sample, and the lowest amount (64.13 mg ECE/kg) was found in MD cocoa bean sample. Regarding, the cocoa bean samples obtained from Nacional variety, fresh-fermented cocoa bean sample exhibited the highest concentration (348.64 mg ECE/kg) of flavonoid content, followed by convective dried (CD) cocoa bean sample with an amount of 284.46 mg ECE/kg. The lowest flavonoid amount (92.64 mg ECE/kg) among the Nacional bean samples was determined in MD cocoa bean sample. Upon comparing the flavonoid content of various cocoa varieties using fresh-fermented cocoa beans, Nacional bean samples exhibited the highest flavonoid content, succeeded by Forastero bean samples (Table 4.4). Disparities in flavonoid content among cocoa varieties may probably arise from differences in genotypes, chemical and soil compositions, as well as cultural practices. Rining and Majid, (2018) Hafiz Majid, reported significant amount of total flavonoid content in different clones of dried cocoa bean, ranging from 2639 mgQE/g to 4536 mgQE/g. The authors highlighted that drying process at high temperature considerably reduce the content of flavonoid content. In an attempt to recover the flavonoid contents in different dried cocoa beans obtained from Colombia, Toro-Urbe et al. (2020) firstly reported total flavonoid content between 15.2-86.0 mg ECE/kg, which were lower than our findings. Zzaman et al. (2014) investigated total flavonoid content in dried and roasted cocoa beans ranked between 8.33-24.7 mg ECE/g. The study observed that the total flavonoid content gradually decreases with extending time and temperatures. Similar decreasing trends were observed in cocoa powder, with total flavonoid content loss reaching up to 67% during conventional drying and roasting methods (Donovan et al., 2006). Regarding flavonoid content, Cádiz-Gurrea et al. (2020) reported values ranging from 19 to 130 mg CE/g of dry extract for different dried cocoa bean cultivars. In contrast, Gu et al. (2013) found that the flavonoid content of cocoa beans from various origins ranged from 3.50 to 12.62 mg ECE/g. The observed differences in these contents can be attributed to several factors, including cocoa cultivars, geographical location, maturity stages, post-harvest processes, fermentation and drying methods and conditions, extraction procedures, and the analytical methodologies used in the assessments (Plaza et al., 2017; Rojas et al., 2020; Urbńska et al., 2019).

4.5.3. Total Anthocyanin Content of Fermented Dried Cocoa Beans

Anthocyanins are cocoa beans and related products components of great importance, contributing to organoleptic quality of cocoa beans derived products. Associated to the brown color of cocoa beans and related products like chocolate. Anthocyanins are mostly formed during fermentation of cocoa beans, therefore, their content in cocoa beans may differ from the cocoa cultivars, cultivation practices, and most importantly the fermentation method applied (Oracz et al., 2015;Febrianto et al., 2022). Table 4.4 listed the total concentration of the three cocoa varieties obtained from four drying processes. In Forastero beans, the highest amount of anthocyanin (108.33 CGE/kg), when the drying methods are compared, the highest content of anthocyanin is attributed to freeze drying process exhibiting an amount of 86.41 mg CGE/kg, following by convective-drying presenting an amount of 63.33 mg CGE/kg. This trend was also observed in Criollo and Nacional cocoa bean samples. As regards Criollo bean samples, the highest amount of anthocyanin (69.42 mg CGE/kg) was found in beans acquired from fresh-fermented Criollo beans, followed by convective dried bean samples, although no statistical difference was observed between the two samples ($p < 0.05$). The lowest amount of anthocyanin (23.78 mg CGE/kg) among the samples of this cultivar was found to be the microwave dried bean sample. Concerning Nacional cultivar samples, convective dried bean sample was determined with the significant amount of anthocyanin (115.24 mg CGE/kg), followed by fresh-fermented bean sample with an amount of 108.43 mg CGE/kg, while the lowest amount (46.89 mg CGE/kg) was attributed to microwave dried bean sample. When all the drying methods are compared, microwave drying process exhibited the lowest content of anthocyanin in all the varieties of cocoa beans samples. These observation may be due to the drying operated at high temperature and power, which significantly degraded the phenolic contents, including anthocyanins. The means difference between the three varieties, concerning the total anthocyanin contents using the fresh-fermented bean samples were significant at ($p < 0.05$) level, in which the Nacional cocoa beans variety presented the highest amount of anthocyanins. Afoakwa et al. (2012) reported a significant amount of anthocyanin in fermented and dried criollo and Forastero cocoa bean samples ranged from 15.68 to 180.87 mg CGE/kg. The authors observed a reduction of anthocyanin content at high drying temperature. In their study, Niemenak et al. (2006) also observed a variation in anthocyanin concentration of 101.3-143.6 mg CGE/kg in fermented and unfermented dried cocoa beans. These results were slightly similar to those reported in the present study.

4.5.4. Effect of Drying on Antioxidant Activity of Fermented Cocoa Beans

The values of antioxidant capacities of all fermented dried cocoa bean samples are given in Table 4.4. The antioxidant potency of cocoa beans was assessed by two different methods: DPPH and ABTS. Time-dependent changes of the reactions occurring in DPPH and ABTS methods were evaluated through UV-Vis spectrophotometric method. The principle of DPPH is based on the ability of

antioxidant compounds to reduce the DPPH radical, which is a purple stable compound. While the principle of ABTS is based on the ability of antioxidant compounds in a sample to decolorize the radical cation ABTS (Kelebek, 2016).

The antioxidant potential values, especially DPPH assay of studied fermented dried cocoa bean samples were between 0.51-0.91 mM Teq/kg for Forastero with the highest amount observed in convective dried bean samples, 0.42-0.87 mM Teq/kg for Criollo, in which the highest concentration was found in convective dried beans, and 0.57-1.046 mM Teq/kg for Nacional with a higher amount in convective dried bean samples compared to other drying processes. While ABTS assay ranked between 0.69-1.02 mM Teq/kg for Forastero, 0.53-0.90 mM Teq/kg for Criollo and 0.69-1.12 mM Teq/kg for Nacional cocoa bean samples based on the drying methods. As can be seen in Table 4.4, the antioxidant capacities of phenolic compounds were better reflected by the ABTS method than the DPPH method, and, in all cocoa bean samples obtained from different drying processes, the ABTS method provided higher values than DPPH method. Furthermore, another substantial finding is that the total phenolic compounds and antioxidant activity values demonstrate interdependent changes. Indeed, the amount of total phenolic compounds can be an important parameter of the antioxidant activity in cocoa beans; thus, dried cocoa beans with the greater content of total phenols may show the higher antioxidant activity. Moreover, when the cocoa variety were compared based on the sun dried beans, Nacional cocoa variety exhibited the highest antioxidant capacity. Jonfia-Essien et al. (2008), reported the total phenolic content ranging from 21.6 to 45.5 mM TE/g in Ghanaian traditional and hybrids dried cocoa beans. Gu et al. (2006) using an ethanolic extraction and an ORAC assay reported values of 103-826 mM TE/g in different dried natural cocoa powder. In the fermented and dried cocoa bean investigated in different locations of Peru by Balcázar-Zumaeta et al. (2023), the antioxidant capacity using DPPH assay ranged from 58.6 to 90.4 mM TE/g, and ABTS technique was between 16.7-26.9 mM TE/g. These reported results were higher than those reported in the present study. Gültekin-Özgüven et al. (2016) that reported that cocoa powder processing, including thermal drying and roasting processes significantly reduced the antioxidant capacity of cocoa bean and products. These observations were in agreement with our findings. In another study conducted by Ramos-Escudero et al. (2021), the total antioxidant capacity of the different Peruvian commercial dried cocoa bean from different cultivars ranged from 103.38 to 372.11 mmol TE/g (Table 3). Di Mattia et al. (2017) also reported antioxidant activity values between 240 and 490 mmol TE/g as measured by the DPPH assay. Additionally, Oracz and Nebesny, (2016) found that the antioxidant activity of the extractable fraction of cocoa cultivars from various geographical areas, measured using the DPPH test, ranged from 323.81 to 1370.12 μ mol TE/g of dry weight.

Table 4.4. Effect of drying methods on polyphenol content and antioxidant activities in cocoa bean samples

Samples	Polyphenol contents			Antioxidant Activities	
	Phenolic contents (mg GAE/kg)	Flavonoid content (mg ECE/kg)	Anthocyanin content (mg CGE/kg)	DPPH (mM Teq/kg)	ABTS (mM Teq/kg)
FFB (Control)	508.76 ± 9.4 ^a	218.48 ± 4.2 ^a	108.33 ± 7.8 ^a	1.68 ± 0.01 ^a	2.09 ± 0.80 ^a
SDFB	298.47 ± 3.4 ^c	108.96 ± 5.4 ^c	44.83 ± 2.3 ^c	0.81 ± 0.01 ^c	0.87 ± 0.01 ^c
CDFB	387.44 ± 5.6 ^b	124.63 ± 4.6 ^{cb}	63.33 ± 2.7 ^b	0.91 ± 0.03 ^b	1.02 ± 0.06 ^b
MDFB	164.12 ± 2.4 ^d	89.47 ± 1.2 ^d	24.56 ± 1.4 ^d	0.51 ± 0.00 ^d	0.69 ± 0.01 ^d
FDFB	325.74 ± 7.3 ^b	166.87 ± 1.3 ^b	86.41 ± 1.8 ^a	0.75 ± 0.01 ^c	0.88 ± 0.03 ^c
FCB (Control)	386.42 ± 6.2 ^a	177.38 ± 12.5 ^a	69.42 ± 6.4 ^a	1.35 ± 0.04 ^a	1.76 ± 0.07 ^a
SDCB	207.89 ± 4.1 ^b	89.18 ± 2.6 ^c	36.96 ± 1.3 ^b	0.61 ± 0.02 ^c	0.79 ± 0.02 ^c
CDCB	324.36 ± 6.4 ^a	145.26 ± 4.1 ^a	66.34 ± 2.4 ^a	0.87 ± 0.01 ^b	0.90 ± 0.01 ^b
MDCB	133.42 ± 2.6 ^d	64.13 ± 1.2 ^d	23.78 ± 1.2 ^c	0.42 ± 0.00 ^d	0.53 ± 0.00 ^d
FDCB	234.36 ± 1.5 ^b	110.68 ± 3.4 ^b	49.12 ± 2.3 ^a	0.68 ± 0.01 ^c	0.81 ± 0.02 ^{cb}
FNB (Control)	564.28 ± 11.3 ^a	348.64 ± 13.4 ^a	108.43 ± 7.2 ^a	0.97 ± 0.08 ^a	1.34 ± 0.06 ^a
SDNB	368.61 ± 5.7 ^c	177.68 ± 3.8 ^c	64.33 ± 1.8 ^c	0.73 ± 0.0 ^b	0.84 ± 0.01 ^c
CDNB	484.33 ± 7.2 ^b	284.46 ± 6.2 ^b	115.24 ± 3.9 ^a	1.04 ± 0.01 ^a	1.12 ± 0.06 ^{ab}
MDNB	188.36 ± 3.9 ^d	92.64 ± 3.1 ^d	46.89 ± 1.8 ^d	0.57 ± 0.00 ^c	0.69 ± 0.02 ^d
FDNB	403.44 ± 4.4 ^c	236.58 ± 4.7 ^b	96.78 ± 2.4 ^b	0.93 ± 0.02 ^a	1.09 ± 0.04 ^b

SDFB: sun dried Forastero beans; CDFB: convective dried Forastero beans; MDFB: microwave dried Forastero beans; FDFB: freeze dried Forastero beans; SDCB: sun dried Criollo beans; CDCB: convective dried Criollo beans; MDCB: microwave dried Criollo beans; FDCB: freeze dried Criollo beans, SDNB: sun dried Nacional beans; CDNB: convective dried Nacional beans; MDNB: microwave dried Nacional beans; FDNB: freeze dried Nacional beans. Each value represents mean ± standard deviation. Different lower-case letters (a,b,c) within the same column of each cocoa bean variety display significant differences (p<0.05) between the different cocoa beans drying processes.

4.6 Similarity and Intensity Assessment of Aromatic Extract of Fermented Dried Cocoa Beans

The selection of an appropriate extraction method in volatile substances analysis is based on the similarity and intensity of extracts to that of the studied sample before GC-MS-O analysis. The aim of the similarity and intensity evaluation tests was to compare the representativeness of the odor of the aromatic extract with that of reference (Selli et al., 2014; Selli et al., 2008; Selli et al., 2011). According to results obtained by the selected panelists, the mean similarity was detected to be 82.8 mm on a 100-mm unstructured scale and intensity scores was indicated as 72.6 mm on a 100 mm a 100-mm unstructured scale. These results are in accordance with previous studies conducted on different food matrixes such as orange wine (Selli et al., 2008), shea butter (Oussou et al., 2022), edible red algae (Le Pape et al., 2004) and Hibiscus sabdariffa (Zannou et al., 2020). Moreover, Prost et al. (2004) underscored the paramount importance of employing representative tests for the evaluation of aroma extracts. Such tests significantly enhance the sensitivity of the study and are crucial preliminary steps before initiating aroma analyses on food materials. Comparative assessments with other investigations have reported valuable insights. For instance, Selli et al. (2014)

reported a similarity score of 70.4 mm for a cherry tomato extract, while Amanpour et al. (2015) found that Iranian saffron extracts acquired through the SAFE method scored 71.8 mm. Additionally, (Kesen et al., 2014a). Selli et al. (2012) reported a similarity score of 66.7 mm for banana extract, Selli and Kelebek (2011) found a score of 60.6 mm for blood orange juice extract. The result of the similarity and intensity analysis showed that representative aroma extract was reached with the purge and trap extraction method to obtain the aroma and aroma-active compounds of the samples. Therefore, this method was found to be a suitable extraction method for the analysis of aroma compounds in fermented and dried cocoa beans.

4.7 Aroma Compounds of Fermented and Dried Cocoa Beans

The distribution of aroma compounds in all fermented Forastero, Criollo and Nacional cocoa bean varieties obtained from sun drying, convective drying, microwave drying and freeze drying processes according to the compound groups (%) are exhibited in Figure 4.5, Figure 4.6 and Figure 4.7, respectively. The aroma compounds detected in all cocoa bean samples attained from four drying processes and linear retention index values on the DB-Wax column for these compounds are displayed in Table 4.5, Table 4.6, and Table 4.7. Regarding to the results obtained from the analysis in all cocoa bean samples acquired from four drying processes;

- In the fresh-fermented Forastero beans, a total of 48 compounds (8 acids, 10 alcohols, 2 aldehydes, 9 esters, 5 ketones, 1 pyrroles, 1 pyrroline, 2 lactones, 10 terpenes, 1 phenol),
- In the fermented sun dried Forastero beans, a total of 58 compounds (8 acids, 9 alcohols, 6 aldehydes, 9 esters, 3 furans, 5 ketones, 4 pyrazines, 2 lactones, 10 terpenes, 1 phenol, 1 pyrroline),
- In the fermented convective dried Forastero beans, a total of 57 compounds (7 acids, 10 alcohols, 6 aldehydes, 8 esters, 5 furans, 3 ketones, 7 pyrazines, 1 pyrrole, 1 pyrroline, 2 lactones, 5 terpenes, 1 phenol, 1 hydrocarbon),
- In the fermented microwave dried Forastero beans, a total of 50 compounds (6 acids, 9 alcohols, 6 aldehydes, 6 esters, 5 furans, 2 ketones, 7 pyrazines, 1 pyrrole, 1 pyrroline, 2 lactones, 3 terpenes, 1 phenol, 1 hydrocarbon),
- In the fermented freeze dried Forastero beans, a total of 49 compounds (8 acids, 9 alcohols, 3 aldehydes, 10 esters, 5 ketones, 2 lactones, 10 terpenes, 1 pyrroline, 1 phenol) were detected and quantified.

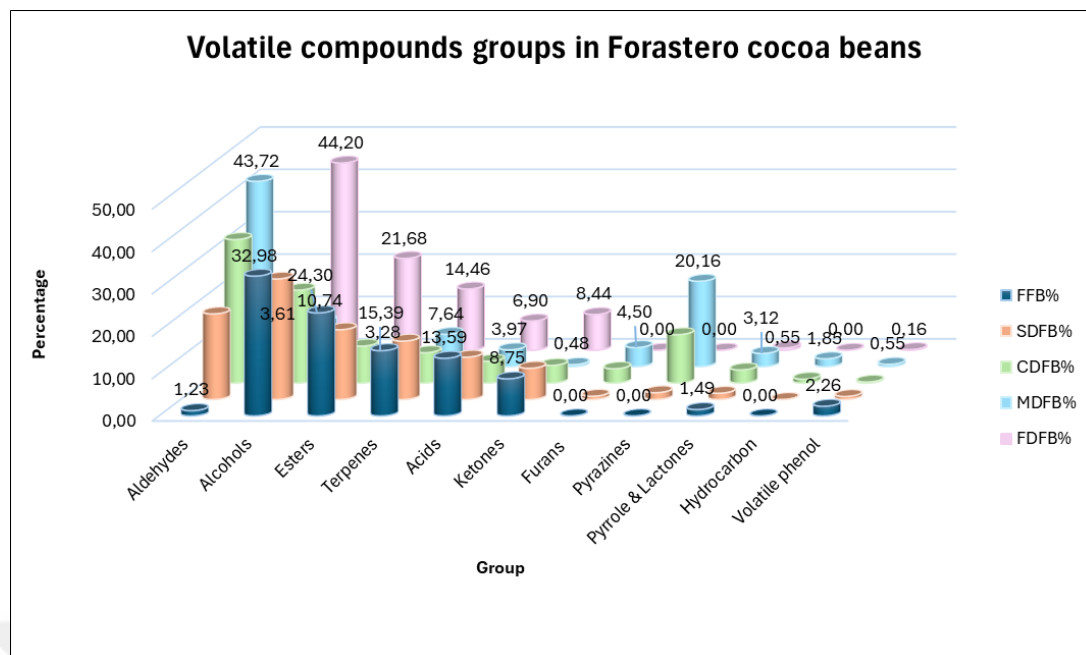


Figure 4.5. Distribution of aroma compounds according to compound groups (%) in Forastero cocoa beans obtained from fresh-fermented and drying processes

Table 4.5. Aroma compounds of Forastero fermented and dried cocoa bean samples

No.	Aroma compounds	LRI	Concentration (Mean $\pm$ SD)					Identification
			FFB	SDFB	CDFB	MDFB	FDFB	
	Acids							
1	Acetic acid	1418	318.4 $\pm$ 7.6 ^b	496.0 $\pm$ 5.3 ^a	209.5 $\pm$ 3.5 ^c	107.1 $\pm$ 2.3 ^d	209.5 $\pm$ 3.3 ^c	LRI, MS, Std
2	2-Methyl-propanoic acid	1587	479.2 $\pm$ 2.4 ^a	55.9 $\pm$ 2.8 ^e	68.2 $\pm$ 1.2 ^d	85.8 $\pm$ 1.5 ^c	106.7 $\pm$ 1.7 ^b	LRI, MS, tent
3	3-Methyl butanoic acid	1680	24.7 $\pm$ 0.5 ^e	78.8 $\pm$ 0.8 ^c	156.9 $\pm$ 1.6 ^b	188.3 $\pm$ 2.4 ^a	50.4 $\pm$ 0.3 ^d	LRI, MS, tent
4	Pentanoic acid	1762	136.7 $\pm$ 2.6 ^a	106.4 $\pm$ 1.9 ^b	20.3 $\pm$ 0.2 ^d	19.9 $\pm$ 0.8 ^d	65.5 $\pm$ 0.6 ^c	LRI, MS, tent
5	Hexanoic acid	1820	64.9 $\pm$ 1.8 ^d	170.3 $\pm$ 2.3 ^a	98.4 $\pm$ 0.6 ^c	105.3 $\pm$ 3.4 ^b	41.5 $\pm$ 0.5 ^e	LRI, MS, Std
6	Octanoic acid	2046	107.4 $\pm$ 1.2 ^a	41.3 $\pm$ 0.4 ^c	nd	nd	78.3 $\pm$ 0.8 ^d	LRI, MS, Std
7	Nonanoic acid	2180	42.8 $\pm$ 0.3 ^c	69.0 $\pm$ 1.2 ^b	29.1 $\pm$ 0.2 ^d	nd	87.8 $\pm$ 1.3 ^a	LRI, MS, Std
8	Phenylacetic acid	2554	68.2 $\pm$ 0.8 ^c	126.9 $\pm$ 2.8 ^b	49.7 $\pm$ 0.4 ^d	18.9 $\pm$ 0.2 ^e	288.2 $\pm$ 5.6 ^a	LRI, MS, Std
	Total		1242.1	1144.6	632.1	525.3	927.9	
	Alcohols							
9	2-Methyl-3-butanol	1089	495.7 $\pm$ 6.4 ^a	357.4 $\pm$ 2.8 ^c	194.8 $\pm$ 4.5 ^d	111.5 $\pm$ 1.8 ^e	406.2 $\pm$ 4.9 ^b	LRI, MS, tent
10	2-Methylpropanol	1094	84.7 $\pm$ 1.1 ^b	nd	nd	nd	239.5 $\pm$ 3.6 ^a	LRI, MS, Std
11	2-Pentanol	1138	674.3 $\pm$ 8.7 ^b	238.2 $\pm$ 2.7 ^c	99.9 $\pm$ 0.9 ^d	67.1 $\pm$ 0.4 ^e	757.8 $\pm$ 3.2 ^a	LRI, MS, tent
12	2-Methylbutanol	1206	469.3 $\pm$ 5.7 ^a	135.6 $\pm$ 4.8 ^c	96.2 $\pm$ 2.4 ^d	45.9 $\pm$ 1.2 ^e	269.0 $\pm$ 2.6 ^b	LRI, MS, Std
13	3-Methylbutanol	1240	324.9 $\pm$ 5.4 ^d	nd	856.0 $\pm$ 9.4 ^b	487.8 $\pm$ 3.7 ^c	1283.7 $\pm$ 11.4 ^a	LRI, MS, tent
14	Hexanol	1359	128.7 $\pm$ 2.4 ^e	1690.7 $\pm$ 9.4 ^b	942.9 $\pm$ 4.2 ^c	475.4 $\pm$ 6.7 ^d	2114.8 $\pm$ 16.1 ^a	LRI, MS, Std
15	2-Octanol	1412	nd	39.0 $\pm$ 0.8 ^b	96.9 $\pm$ 0.9 ^a	nd	nd	LRI, MS, Std
16	2,3-Butanediol	1545	68.9 $\pm$ 0.8 ^e	343.6 $\pm$ 2.4 ^b	179.9 $\pm$ 1.6 ^c	128.1 $\pm$ 2.7 ^d	486.9 $\pm$ 3.2 ^a	LRI, MS, Std
17	Benzylalcohol	1861	128.7 $\pm$ 4.3 ^a	63.6 $\pm$ 0.8 ^b	54.5 $\pm$ 0.4 ^c	21.7 $\pm$ 0.1 ^e	33.8 $\pm$ 0.7 ^d	LRI, MS, Std
18	Phenylethanol	1922	164.4 $\pm$ 2.4 ^a	80.5 $\pm$ 1.2 ^c	21.2 $\pm$ 0.7 ^d	11.6 $\pm$ 0.2 ^e	103.4 $\pm$ 2.4 ^b	LRI, MS, Std
19	2-Phenylethanol	1944	474.5 $\pm$ 5.7 ^a	310.3 $\pm$ 2.6 ^b	154.2 $\pm$ 2.1 ^d	70.6 $\pm$ 0.8 ^e	244.9 $\pm$ 6.5 ^c	LRI, MS, Std
	Total		3014.1	3258.9	2696.5	1419.7	5940.0	
	Aldehydes							
20	2-Methylbutanal	896	nd	388.4 $\pm$ 4.8 ^c	793.8 $\pm$ 9.7 ^b	1267.8 $\pm$ 12.6 ^a	nd	LRI, MS, Std
21	3-Methylbutanal	916	nd	574.6 $\pm$ 2.7 ^c	986.8 $\pm$ 6.2 ^b	2039.5 $\pm$ 9.8 ^a	nd	LRI, MS, Std
22	Hexanal	1097	79.3 $\pm$ 1.7 ^e	1024.6 $\pm$ 9.8 ^c	1869.0 $\pm$ 17.4 ^a	1529.8 $\pm$ 11.3 ^b	367.9 $\pm$ 3.3 ^d	LRI, MS, Std
23	2-Furaldehyde	1468	nd	103.9 $\pm$ 3.8 ^c	168.2 $\pm$ 1.5 ^b	429.2 $\pm$ 4.7 ^a	nd	LRI, MS, Std
24	Benzaldehyde	1544	33.5 $\pm$ 0.5 ^e	155.1 $\pm$ 1.2 ^c	208.3 $\pm$ 1.9 ^b	415.2 $\pm$ 2.6 ^a	71.6 $\pm$ 0.6 ^d	LRI, MS, Std
25	5-Methyl-2-furfural	1607	nd	68.5 $\pm$ 0.2 ^d	137.1 $\pm$ 2.4 ^a	100.8 $\pm$ 0.9 ^b	46.1 $\pm$ 0.9 ^c	LRI, MS, tent
	Total		112.8	2315.1	4163.2	5782.3	485.6	
	Esters							
26	Ethyl acetate	887	204.8 $\pm$ 3.5 ^b	185.7 $\pm$ 2.4 ^c	87.5 $\pm$ 0.9 ^d	30.8 $\pm$ 0.1 ^e	376.1 $\pm$ 4.7 ^a	LRI, MS, tent

Table 4.5^a in Devami

27	Ethyl 2-methylbutanoate	1064	78.3 ± 2.7 ^e	297.4 ± 4.9 ^b	166.9 ± 3.8 ^c	88.5 ± 0.7 ^d	408.7 ± 7.6 ^a	LRI, MS, tent
28	2-Phenyl acetate	1066	347.1 ± 4.3 ^a	180.9 ± 2.6 ^c	58.6 ± 0.5 ^d	19.5 ± 0.2 ^e	192.7 ± 3.8 ^b	LRI, MS, tent
29	Ethyl 3-methylbutanoate	1072	19.0 ± 0.2 ^e	102.1 ± 2.8 ^b	76.8 ± 0.8 ^c	38.5 ± 0.1 ^d	164.6 ± 3.1 ^a	LRI, MS, Std
30	2-Methylbutyl acetate	1115	885.5 ± 9.4 ^a	603.2 ± 7.2 ^c	353.5 ± 4.1 ^d	188.9 ± 2.3 ^e	794.6 ± 8.3 ^b	LRI, MS, Std
31	3-Methylbutyl acetate	1126	314.9 ± 5.7 ^a	201.5 ± 2.3 ^c	154.6 ± 4.2 ^d	67.2 ± 0.9 ^e	268.6 ± 5.8 ^b	LRI, MS, tent
32	Ethyl hexanoate	1241	94.3 ± 1.4 ^c	113.2 ± 1.8 ^b	67.2 ± 0.7 ^d	nd	234.7 ± 4.2 ^a	LRI, MS, tent
33	Benzyl acetate	1742	nd	77.4 ± 0.6 ^a	nd	nd	20.2 ± 0.1 ^b	LRI, MS, Std
34	Phenylethyl acetate	1810	170.3 ± 2.8 ^b	124.9 ± 1.6 ^c	107.6 ± 2.4 ^d	nd	376.1 ± 5.7 ^a	LRI, MS, tent
	Total		2220.7	1886.3	1072.7	433.4	2914.1	
	Furans							
35	2-Pentyl-furan	1249	nd	nd	97.5 ± 0.8	121.9 ± 2.6	nd	LRI, MS, tent
36	2-Acetyl furan	1512	nd	29.7 ± 0.4 ^c	74.9 ± 0.2 ^b	126.9 ± 2.4 ^a	nd	LRI, MS, tent
37	2-Acetyl-5-methylfuran	1608	nd	44.9 ± 0.8 ^c	66.8 ± 1.3 ^b	185.7 ± 2.7 ^a	nd	LRI, MS, tent
38	2(5H)-furanone	1716	nd	nd	108.9 ± 3.2	45.7 ± 0.4	nd	LRI, MS, tent
39	5-(1-Hydroxyethyl)-2(5H)-furanone	1864	nd	12.45 ± 0.6 ^c	67.6 ± 1.5 ^b	115.2 ± 2.3 ^a	nd	LRI, MS, tent
	Total		nd	87.1	415.7	595.4	nd	
	Ketones							
40	Diacetyl	971	106.5 ± 2.4	nd	nd	nd	77.8 ± 1.9	LRI, MS, tent
41	2-Heptanone	1184	124.3 ± 2.5 ^c	178.7 ± 3.1 ^b	238.5 ± 5.4 ^a	nd	86.6 ± 0.6 ^d	LRI, MS, Std
42	5-Methyl-2-hexanone	1190	79.0 ± 0.3 ^d	145.3 ± 1.9 ^c	178.5 ± 2.6 ^b	31.8 ± 0.6 ^e	390.8 ± 6.2 ^a	LRI, MS, tent
43	3-Hydroxy-2-butanone	1291	89.5 ± 1.4 ^c	295.3 ± 4.7 ^a	nd	nd	176.5 ± 3.2 ^b	LRI, MS, Std
44	2-Hydroxy-3-pentanone	1368	402.8 ± 7.4 ^b	155.6 ± 5.2 ^c	95.4 ± 1.3 ^d	31.4 ± 0.3 ^e	456.4 ± 8.3 ^a	LRI, MS, tent
45	Acetophenone	1652	103.7 ± 1.4 ^a	69.1 ± 0.3 ^b	nd	nd	24.7 ± 0.8 ^c	LRI, MS, Std
	Total		799.3	844.0	511.9	63.2	1135.0	
	Pyrazines							
46	2-Methylpyrazine	1274	nd	89.5 ± 1.6 ^c	179.0 ± 3.2 ^b	423.4 ± 7.8 ^a	nd	LRI, MS, Std
47	2,6-Dimethylpyrazine	1319	nd	18.5 ± 0.3 ^c	101.6 ± 2.4 ^b	291.6 ± 1.6 ^a	nd	LRI, MS, Std
48	2-Ethylpyrazine	1358	nd	44.8 ± 0.9 ^c	122.3 ± 1.5 ^b	419.0 ± 4.7 ^a	nd	LRI, MS, Std
49	2,3,5-Trimethylpyrazine	1413	nd	nd	345.1 ± 8 ^b	678.7 ± 10.1	nd	LRI, MS, Std
	2-Ethyl-6-methylpyrazine	1408	nd	nd	126.7 ± 2.0	305.6 ± 4.2	nd	LRI, MS, Std
	2,3,5,6-Tetramethylpyrazine	1466	nd	nd	406.5 ± 5.1	218.6 ± 2.3	nd	LRI, MS, Std
50	2-Acetyl-3-methylpyrazine	1627	nd	29.9 ± 0.2 ^c	122.4 ± 3.8 ^b	328.8 ± 5.5 ^a	nd	LRI, MS, Std
	Total		nd	182.7	1403.6	2665.7	nd	
	Pyroles & Lactones							
51	2-Acetyl-1-pyrroline	1310	5.1 ± 0.6 ^d	19.4 ± 0.3 ^a	15.3 ± 1.47 ^c	17.4 ± 0.50 ^b	15.8 ± 1.20 ^c	LRI, MS, Std

Table 4.5 in Devami

52	γ -Valerolactone	1589	46.8 $\pm$ 0.5 ^b	50.3 $\pm$ 0.8 ^b	102.7 $\pm$ 5.4 ^a	68.7 $\pm$ 1.3 ^b	19.9 $\pm$ 0.1 ^c	LRI,MS,Std
53	γ -Butyrolactone	1617	89.5 $\pm$ 0.8 ^d	117.1 $\pm$ 1.2 ^c	166.8 $\pm$ 3.6 ^b	204.2 $\pm$ 1.8 ^a	53.4 $\pm$ 0.4 ^e	LRI,MS,Std
54	2-Acetylpyrrole	1974	nd	nd	111.1 $\pm$ 0.9	139.2 $\pm$ 3.3	nd	LRI, MS,tent
	Total		141.4	186.8	395.3	429.5	89.1	
	Terpenes							
55	α -Pinene	1034	209.4 $\pm$ 4.7 ^a	158.1 $\pm$ 2.8 ^c	nd	nd	172.7 $\pm$ 1.5 ^b	LRI, MS,Std
56	Linalool oxide I	1080	24.7 $\pm$ 0.2 ^c	67.6 $\pm$ 1.2 ^a	nd	nd	42.2 $\pm$ 0.4 ^b	LRI, MS,Std
57	Sabinene	1119	104.9 $\pm$ 0.9 ^c	187.4 $\pm$ 3.6 ^b	nd	nd	345.4 $\pm$ 7.2 ^a	LRI, MS, Std
58	β -Myrcene	1156	132.4 $\pm$ 3.1 ^b	101.8 $\pm$ 6.4 ^c	nd	nd	240.9 $\pm$ 1.4 ^a	LRI, MS,tent
59	1,4-Cineol	1177	19.0 $\pm$ 0.1 ^e	46.1 $\pm$ 0.7 ^d	117.4 $\pm$ 2.8 ^a	84.6 $\pm$ 0.6 ^b	64.9 $\pm$ 0.8 ^c	LRI, MS,tent
60	D-Limonene	1206	18.2 $\pm$ 0.9 ^e	178.1 $\pm$ 3.6 ^c	246.4 $\pm$ 7.8 ^b	404.9 $\pm$ 9.4 ^a	99.2 $\pm$ 1.4 ^d	LRI, MS,Std
61	Ocimene	1233	164.8 $\pm$ 0.8 ^c	286.3 $\pm$ 1.4 ^b	86.5 $\pm$ 0.6 ^d	nd	424.6 $\pm$ 2.7 ^a	LRI, MS, Std
62	γ -Terpinene	1242	118.4 $\pm$ 2.6 ^c	270.8 $\pm$ 2.9 ^b	nd	nd	337.2 $\pm$ 7.2 ^a	LRI, MS,Std
63	Linalool oxide II	1442	196.4 $\pm$ 2.2 ^a	71.6 $\pm$ 0.8 ^c	38.7 $\pm$ 0.5 ^d	nd	89.5 $\pm$ 1.9 ^b	LRI, MS,Std
64	Linalool	1537	418.5 $\pm$ 7.8 ^b	210.7 $\pm$ 9.4 ^d	391.1 $\pm$ 5.6 ^c	520.4 $\pm$ 11.3 ^a	126.8 $\pm$ 0.9 ^e	LRI, MS, Std
	Total		1406.7	1578.5	880.1	1009.9	1943.4	
	Hydrocarbon and Phenol							
65	Styrene	1254	nd	nd	112.3 $\pm$ 0.8	244.3 $\pm$ 2.8	nd	LRI,MS,tent
66	Guaiacol	1960	206.8 $\pm$ 1.6 ^a	80.9 $\pm$ 0.4 ^b	30.8 $\pm$ 0.2 ^d	73.3 $\pm$ 0.6 ^c	20.9 $\pm$ 0.1 ^e	LRI,MS,tent
	Total		206.8	80.9	143.1	317.6	20.9	
	General Total		9143.9	11484.0	12314.8	13241.8	13456.0	

LRI: linear retention index calculated on DB-Wax capillary column. Concentration: results are the means of three repetitions as $\mu\text{g/kg}$. Identification: methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification. FFB: Fermented Forastero beans; SDFB: sun dried Forastero beans; CDFB: convective dried Forastero beans; MDFB: microwave dried Forastero beans; FDFB: freeze dried Forastero beans; Each value represents mean $\pm$ standard deviation. Different lower-case letters (a,b,c) within the same line display significant differences ($p < 0.05$) between the fresh fermented cocoa beans and the different cocoa beans drying processes; nd: not identified.

- In the fresh-fermented Criollo beans fraction, a total of 44 compounds (6 acids, 9 alcohols, 2 aldehydes, 11 esters, 3 ketones, 1 lactone, 1 pyrroline, 8 terpenes, 3 phenols),
- In the fermented sun dried Criollo beans extracts Criollo, a total of 52 compounds (5 acids, 8 alcohols, 7 aldehydes, 12 esters, 1 furan, 2 ketones, 4 pyrazines, 1 pyrrole, 1 pyrroline, 3 lactones, 8 terpenes, 2 phenols),
- In the fermented convective dried Criollo beans, a total of 59 compounds (5 acids, 8 alcohols, 8 aldehydes, 11 esters, 3 furans, 2 ketones, 7 pyrazines, 2 pyrroles, 1 pyrroline, 3 lactones, 7 terpenes, 3 phenols),
- In the fermented microwave dried Criollo beans, a total of 56 compounds (4 acids, 8 alcohols, 8 aldehydes, 10 esters, 3 furans, 2 ketones, 7 pyrazines, 2 pyrroles, 1 pyrroline, 3 lactones, 7 terpenes, 3 phenols),
- In the fermented freeze-dried Criollo beans, a total of 46 compounds (6 acids, 9 alcohols, 6 aldehydes, 11 esters, 2 ketones, 2 lactones, 9 terpenes, 3 phenols, 1 pyrroline) were determined.

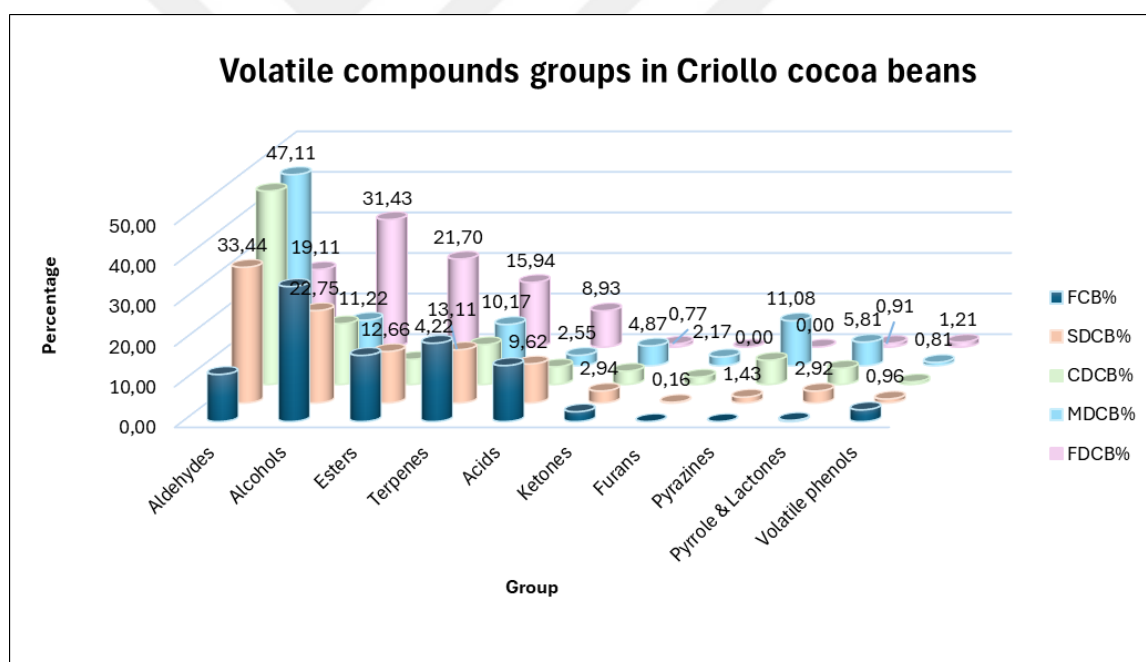


Figure 4.6. Distribution of aroma compounds according to compound groups (%) in Criollo cocoa beans obtained from fresh-fermented and drying processes

Table 4.6. Aroma compounds of Criollo fermented and dried cocoa bean samples

No.	Aroma compounds	LRI	Concentration (Mean ± SD)					Identification
			FCB	SDCB	CDCB	MDCB	FDCB	
	Acids							
1	Acetic acid	1418	714.9 ± 7.0 ^a	494.3 ± 1.4 ^c	238.0 ± 6.3 ^d	188.7 ± 4.5 ^e	573.9 ± 10.6 ^b	LRI, MS, Std
2	Pentanoic acid	1762	68.3 ± 0.5 ^a	nd	nd	nd	15.5 ± 0.1 ^b	LRI, MS, Std
3	Hexanoic acid	1820	87.4 ± 0.8 ^d	294.2 ± 5.6 ^a	211.6 ± 1.4 ^b	62.6 ± 0.5 ^e	110.4 ± 1.9 ^c	LRI, MS, Std
4	Octanoic acid	2046	124.0 ± 1.3 ^a	61.3 ± 0.4 ^c	41.0 ± 0.8 ^d	18.9 ± 0.2 ^e	78.3 ± 1.2 ^b	LRI, MS, Std
5	Nonanoic acid	2180	72.8 ± 2.1 ^d	79.0 ± 0.8 ^b	29.1 ± 0.1 ^c	17.6 ± 0.8 ^e	110.8 ± 3.4 ^a	LRI, MS, Std
6	Phenylacetic acid	2554	136.8 ± 4.3 ^b	85.0 ± 1.7 ^c	31.9 ± 0.2 ^d	nd	178.2 ± 5.4 ^a	LRI, MS, Std
	Total		1204.2	1013.8	551.6	287.8	1067.1	
	Alcohols							
7	2-Methyl-3-butanol	1089	486.7 ± 9.4 ^a	199.8 ± 1.8 ^c	87.4 ± 0.4 ^d	52.9 ± 0.2 ^e	219.8 ± 2.5 ^b	LRI, MS, Std
8	2-Methyl-1-butanol	1217	112.9 ± 5.8 ^b	nd	nd	nd	302.7 ± 4.3 ^a	LRI, MS, Std
9	(E)-2-hexanol	1245	274.9 ± 3.9 ^b	64.3 ± 0.8 ^e	203.4 ± 3.4 ^c	70.7 ± 0.2 ^d	468.0 ± 1.8 ^a	LRI, MS, tent
10	2,3-Butanediol	1545	104.9 ± 1.4 ^e	256.5 ± 5.8 ^b	183.7 ± 1.8 ^c	128.2 ± 0.8 ^d	633.8 ± 6.4 ^a	LRI, MS, Std
11	Hexanol	1359	1345.8 ± 24.7 ^a	1077.5 ± 12.4 ^c	827.0 ± 9.0 ^d	676.3 ± 7.8 ^e	1187.5 ± 9.4 ^b	LRI, MS, Std
12	Furfuryl alcohol	1650	84.6 ± 1.2 ^c	76.7 ± 0.5 ^d	220.5 ± 1.7 ^a	155.8 ± 2.5 ^b	nd	LRI, MS, tent
13	Benzyl alcohol	1861	33.9 ± 0.4 ^d	118.2 ± 1.3 ^b	56.6 ± 0.9 ^c	20.3 ± 0.1 ^e	136.4 ± 4.1 ^a	LRI, MS, Std
14	Phenylethanol	1922	144.9 ± 0.9 ^b	109.9 ± 2.4 ^c	45.1 ± 0.3 ^d	15.5 ± 0.1 ^e	164.2 ± 1.6 ^a	LRI, MS, tent
15	2-Phenylethanol	1944	312.9 ± 4.5 ^c	493.2 ± 7.8 ^b	288.7 ± 4.4 ^d	148.0 ± 0.8 ^e	644.2 ± 8.9 ^a	LRI, MS, Std
	Total		2901.5	2396.1	1912.4	1267.7	3756.6	
	Aldehydes							
16	2-Methylpropanal	801	nd	37.4 ± 0.8 ^b	92.0 ± 0.8 ^a	218.0 ± 6.3 ^b	nd	LRI, MS, Std
17	2-Methylbutanal	896	139.3 ± 0.9 ^e	959.3 ± 9.4 ^d	1287.2 ± 17.8 ^b	1468.5±12.4 ^a	423.3 ± 2.6 ^c	LRI, MS, Std
18	3-Methylbutanal	916	nd	182.4 ± 1.3 ^c	436.7 ± 4.3 ^b	862.7 ± 10.0 ^a	108.4 ± 1.7 ^d	LRI, MS, Std
19	Hexanal	1097	874.5 ± 7.5 ^e	2175.4 ± 19.8 ^b	3831.8 ± 32.4 ^a	2036.5±29.4 ^c	1660.4 ± 14.6 ^d	LRI, MS, Std
20	2-Furaldehyde	1468	nd	132.0 ± 1.4 ^c	206.7 ± 3.4 ^b	334.6 ± 4.8 ^a	48.9 ± 0.5 ^d	LRI, MS, Std
21	5-Methyl-2-furfural	1607	nd	17.0 ± 0.4 ^d	86.9 ± 0.8 ^a	69.0 ± 1.4 ^b	24.7 ± 0.2 ^c	LRI, MS, tent
22	Phenylacetaldehyde	1632	nd	nd	95.5 ± 1.3 ^b	228.2 ± 5.6 ^a	18.3 ± 0.1 ^c	LRI, MS, Std
23	5-Hydroxymethylfurfural	2528	nd	18.5 ± 0.1 ^c	68.5 ± 0.4 ^b	106.7 ± 0.8 ^a	nd	LRI, MS, tent

Table 4.6'in Devami

	Total		1013.8	3522.0	6105.3	5324.2	2284.0	
	Esters							
24	Ethyl acetate	887	92.5 ± 0.8 ^b	29.4 ± 0.7 ^d	79.6 ± 0.3 ^c	nd	166.8 ± 4.3 ^a	LRI, MS, tent
25	Ethyl 2-methylbutanoate	1064	164.2 ± 1.8 ^c	221.2 ± 4.3 ^b	137.6 ± 2.4 ^d	83.6 ± 0.9 ^e	426.7 ± 7.3 ^a	LRI, MS, tent
26	Ethyl 3-methylbutanoate	1072	208.6 ± 2.4 ^b	180.3 ± 1.3 ^c	87.8 ± 0.7 ^d	54.0 ± 0.6 ^e	342.6 ± 5.6 ^a	LRI, MS, tent
27	Isoamyl acetate	1126	264.9 ± 6.2 ^a	125.2 ± 1.9 ^c	68.2 ± 0.8 ^d	38.0 ± 0.2 ^e	169.3 ± 1.8 ^b	LRI, MS, tent
28	Ethyl valerate	1142	46.9 ± 0.4 ^d	155.4 ± 2.4 ^b	58.1 ± 0.2 ^c	21.9 ± 0.1 ^e	203.7 ± 1.4 ^a	LRI, MS, tent
29	Ethyl hexanoate	1241	77.1 ± 0.3 ^c	138.9 ± 3.8 ^b	43.7 ± 0.4 ^d	17.2 ± 0.4 ^e	218.4 ± 3.7 ^a	LRI, MS, tent
30	Ethyl octanoate	1446	100.7 ± 1.2 ^a	58.4 ± 0.7 ^c	29.6 ± 0.1 ^d	16.8 ± 0.2 ^e	72.6 ± 0.5 ^b	LRI, MS, tent
31	2-Furfuryl acetate	1534	nd	47.4 ± 0.5 ^c	89.4 ± 1.2 ^b	171.0 ± 3.1 ^a	nd	LRI, MS, tent
32	Methylphenyl acetate	1749	201.5 ± 4.4 ^b	109.4 ± 1.5 ^c	61.8 ± 0.2 ^d	24.6 ± 0.2 ^e	215.7 ± 3.6 ^a	LRI, MS, tent
33	Ethylphenyl acetate	1781	82.4 ± 0.6 ^c	107.0 ± 1.8 ^b	47.7 ± 0.4 ^d	23.4 ± 0.3 ^e	374.2 ± 1.8 ^a	LRI, MS, tent
34	Phenylethyl acetate	1810	94.6 ± 0.8 ^c	138.7 ± 0.9 ^b	86.9 ± 0.9 ^d	26.6 ± 0.1 ^e	367.3 ± 8.3 ^a	LRI, MS, tent
	Total		1421.6	1334.0	787.4	477.1	2594.4	
	Furans							
35	2-Pentyl-furan	1249	nd	nd	57.0 ± 0.8 ^b	106.5 ± 1.7 ^a	nd	LRI, MS, tent
36	2-Acetyl-furan	1512	nd	16.5 ± 0.4 ^c	76.6 ± 0.6 ^a	29.7 ± 0.3 ^b	nd	LRI, MS, tent
37	5-Methyl 2(5H)-furanone	1664	nd	nd	92.8 ± 0.9 ^b	108.8 ± 2.4 ^a	nd	LRI, MS, tent
	Total		nd	16.5	226.4	245.0	nd	
	Ketones							
	Diacetyl	971	88.2 ± 1.2 ^b	22.7 ± 0.4 ^c	nd	nd	37.1 ± 0.4 ^a	LRI, MS, tent
38	2-Pentanone	978	127.9 ± 2.6	nd	nd	nd	45.9 ± 0.7	LRI, MS, tent
39	2-Heptanone	1184	66.8 ± 0.2 ^d	171.6 ± 3.8 ^c	238.0 ± 4.3 ^b	301.2 ± 3.5 ^a	nd	LRI, MS, tent
40	3-Hydroxy-2-butanone	1291	16.5 ± 0.1 ^e	138.3 ± 2.4 ^c	176.3 ± 0.5 ^b	249.7 ± 5.6 ^a	46.7 ± 0.3 ^d	LRI, MS, tent
	Total		211.2	309.9	414.3	550.9	92.6	
	Pyrazines							
42	2-Methylpyrazine	1274	nd	47.3 ± 0.5 ^c	64.6 ± 0.8 ^b	127.0 ± 2.4 ^a	nd	LRI, MS, tent
43	2,6-Dimethylpyrazine	1319	nd	24.5 ± 0.4 ^c	68.3 ± 0.7 ^b	165.2 ± 6.9 ^a	nd	LRI, MS, tent
44	2,5-Dimethylpyrazine	1336	nd	43.6 ± 0.6 ^c	246.7 ± 4.5 ^a	98.5 ± 0.8 ^b	nd	LRI, MS, tent
45	2,3-Dimethylpyrazine	1357	nd	nd	68.9 ± 1.2 ^b	112.7 ± 1.2 ^a	nd	LRI, MS, tent
46	2-Ethylpyrazine	1358	nd	35.7 ± 0.3	148.1 ± 2.3 ^b	418.0 ± 7.3 ^a	nd	LRI, MS, tent
47	2-Ethyl-5-methylpyrazine	1397	nd	nd	72.4 ± 0.7 ^b	201.8 ± 1.5 ^a	nd	LRI, MS, tent

Table 4.6 in Devami							
48	2-Ethyl-6-methylpyrazine	1408	nd	nd	89.4 ± 0.4b	129.0 ± 0.9a	nd
	Total		0.0	151.1	758.4	1252.2	0.0
	Pyrroles, Pyrrolines & Lactones						
49	1-Methylpyrrole	1149	nd	nd	15.7 ± 0.1	106.9 ± 0.5	nd
50	δ-Octenolactone	1264	nd	18.7 ± 0.1c	70.7 ± 0.5b	144.8 ± 4.7a	nd
51	2-Acetyl-1-pyrroline	1310	2.6 ± 0.19	21.8 ± 0.95	14.6 ± 0.94	20.8 ± 0.93	19.4 ± 2.17
52	γ-Butyrolactone	1617	26.7 ± 0.4e	180.2 ± 2.4c	249.2 ± 4.6a	208.6 ± 2.9b	84.6 ± 0.8d
53	2-Acetylpyrrole	1974	nd	41.8 ± 0.3c	65.0 ± 0.8b	149.9 ± 3.4a	nd
	γ-Decalactone	2103	nd	67.0 ± 0.2b	103.3 ± 1.5a	47.0 ± 0.6c	23.9 ± 0.2d
	Total		29.3	329.5	518.5	678.0	127.9
	Terpenes						
54	α-Pinene	1034	42.6 ± 0.4b	nd	nd	nd	98.3 ± 0.5a
55	Camphene	1082	317.5 ± 4.6b	204.6 ± 1.8c	122.0 ± 1.9d	nd	388.0 ± 6.4a
56	D-Limonene	1206	26.7 ± 0.7e	162.8 ± 1.5c	213.1 ± 2.9b	475.2 ± 2.5a	78.9 ± 2.1d
57	Ocimene	1233	434.1 ± 7.6a	94.8 ± 1.2c	nd	nd	267.9 ± 3.9b
58	p-Cymene	1272	122.9 ± 4.3e	288.7 ± 4.3b	457.7 ± 8.3a	157.9 ± 2.4c	144.3 ± 1.3d
59	Linalool	1537	nd	179.5 ± 1.7c	286.5 ± 2.3b	496.5 ± 6.4a	81.4 ± 0.4d
60	(E)-Caryophyllene	1598	322.3 ± 2.6b	219.8 ± 3.2c	91.0 ± 0.8d	19.5 ± 0.4e	451.9 ± 9.4a
61	Geraniol	1851	103.5 ± 2.3c	179.3 ± 3.5b	60.1 ± 0.3d	nd	278.1 ± 3.7a
62	Thymol	2153	314.9 ± 1.4a	51.1 ± 0.6c	20.2 ± 0.1d	nd	116.7 ± 2.4b
	Total		1684.5	1380.6	1250.6	1149.1	1905.5
	Phenols						
63	Guaiacol	1960	163.9 ± 4.9 ^a	77.7 ± 1.2 ^b	48.7 ± 0.5 ^d	19.7 ± 0.2 ^e	57.4 ± 0.4 ^c
64	Eugenol	2141	37.2 ± 0.2 ^b	23.6 ± 0.4 ^c	16.2 ± 0.1 ^d	13.9 ± 0.2 ^d	63.5 ± 1.2 ^a
65	3,4-Dimethylphenol	2189	51.3 ± 0.6 ^a	nd	12.5 ± 0.1 ^c	57.4 ± 0.3 ^a	23.7 ± 0.1 ^b
	Total		252.4	101.3	77.4	91.0	144.6
	General Total		8718.5	10554.8	12828.7	11322.8	11972.7

LRI: linear retention index calculated on DB-Wax capillary column. Concentration: results are the means of three repetitions as µg/kg. Identification: methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compound, it must be considered as an attempt of identification. FFC: Fermented Criollo beans; SDCB: sun dried Criollo beans; CDFB: convective dried Criollo beans; MDFB: microwave dried Criollo beans; FDFB: freeze dried Criollo beans; Each value represents mean ± standard deviation. Different lower-case letters (a,b,c) within the same line display significant differences ($p < 0.05$) between the fresh fermented cocoa beans and the different cocoa beans drying processes; nd: not identified.

- In the fresh-fermented Nacional beans fraction, a total of 48 compounds (6 acids, 10 alcohols, 7 aldehydes, 9 esters, 3 ketones, 1 pyrazine, 1 lactone, 1 pyrroline, 8 terpenes, 2 phenols),
- In sun dried beans extracts Nacional, a total of 55 compounds (6 acids, 12 alcohols, 8 aldehydes, 10 esters, 3 ketones, 3 pyrazines, 1 pyrrole, 1 lactone, 1 pyrroline, 8 terpenes, 2 phenols),
- In convective dried Nacional beans, a total of 51 compounds (6 acids, 10 alcohols, 7 aldehydes, 10 esters, 2 furans, 2 ketones, 5 pyrazines, 1 pyrrole, 1 lactone, 1 pyrroline, 4 terpenes, 2 phenols),
- In microwave dried Nacional beans, a total of 52 compounds (6 acids, 9 alcohols, 7 aldehydes, 10 esters, 2 furans, 3 ketones, 7 pyrazines, 1 pyrrole, 1 lactone, 1 pyrroline, 3 terpenes, 2 phenols),
- In freeze dried Nacional beans, a total of 48 compounds (6 acids, 10 alcohols, 7 aldehydes, 9 esters, 3 ketones, 1 pyrazine, 1 lactone, 1 pyrroline, 8 terpenes, 2 phenols) were determined.

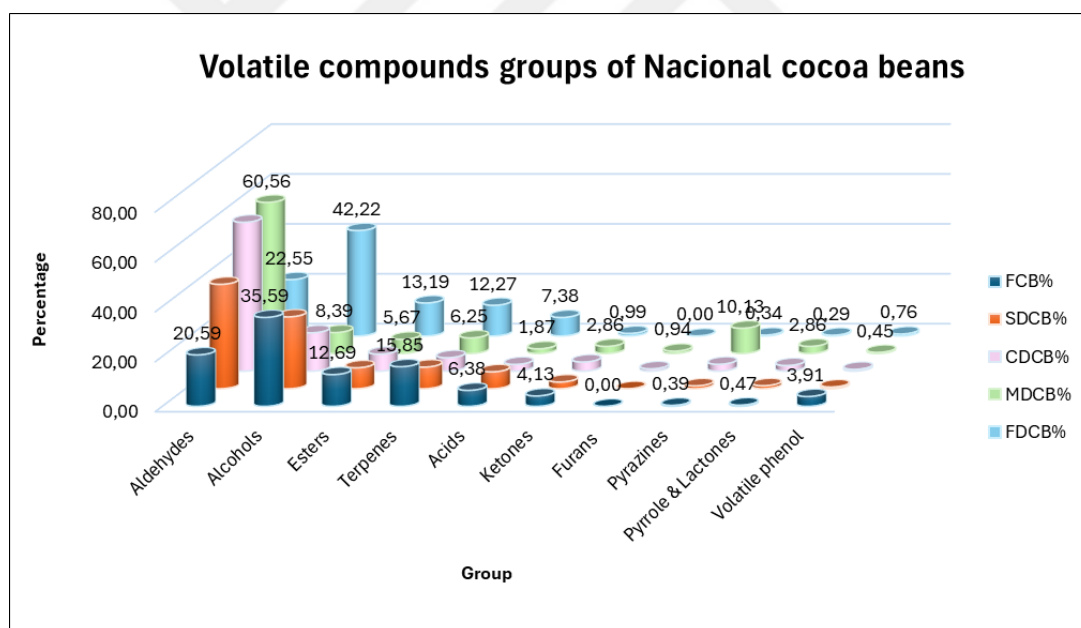


Figure 4.7. Distribution of aroma compounds according to compound groups (%) in Nacional cocoa beans obtained from fresh-fermented and drying processes

Table 4.7. Aroma compounds of Nacional fermented and dried cocoa bean samples

No.	Aroma compounds	LRI	Concentration (Mean $\pm$ SD)					Identification
			FNB	SDNB	CDNB	MDNB	FDNB	
	Acids							
1	Acetic acid	1418	219.4 $\pm$ 1.4 ^c	358.3 $\pm$ 8.4 ^b	152.1 $\pm$ 2.5 ^d	61.8 $\pm$ 1.3 ^e	573.9 $\pm$ 9.3 ^a	LRI, MS, Std
2	Pentanoic acid	1762	235.7 $\pm$ 7.3 ^a	80.3 $\pm$ 0.9 ^b	20.3 $\pm$ 0.1 ^c	19.9 $\pm$ 0.2 ^c	15.5 $\pm$ 0.2 ^d	LRI, MS, tent
3	Hexanoic acid	1820	47.6 $\pm$ 0.8 ^e	138.3 $\pm$ 1.2 ^a	78.5 $\pm$ 0.8 ^c	71.4 $\pm$ 0.6 ^d	110.4 $\pm$ 1.4 ^b	LRI, MS, Std
4	Octanoic acid	2046	69.9 $\pm$ 1.3 ^c	130.7 $\pm$ 3.4 ^a	30.7 $\pm$ 0.2 ^e	48.4 $\pm$ 0.4 ^d	78.3 $\pm$ 0.7 ^b	LRI, MS, Std
5	Nonanoic acid	2180	91.3 $\pm$ 0.7 ^b	65.0 $\pm$ 1.2 ^c	29.6 $\pm$ 0.2 ^e	47.8 $\pm$ 0.9 ^d	110.8 $\pm$ 1.6 ^a	LRI, MS, Std
6	Penylacetic acid	2554	77.5 $\pm$ 0.4 ^c	149.4 $\pm$ 2.3 ^b	48.4 $\pm$ 0.5 ^d	38.0 $\pm$ 0.6 ^e	178.2 $\pm$ 2.5 ^a	LRI, MS, Std
	Total		741.4	922.0	359.6	287.3	1067.1	
	Alcohols							
7	2-Methyl-2-butanol	975	514.9 $\pm$ 8.4 ^b	357.4 $\pm$ 5.2 ^c	nd	nd	792.0 $\pm$ 15.3 ^a	LRI, MS, Std
8	1-Penten-3-ol	1165	244.7 $\pm$ 4.6 ^d	479.1 $\pm$ 7.9 ^b	374.2 $\pm$ 5.8 ^c	196.8 $\pm$ 1.2 ^e	738.9 $\pm$ 11.3 ^a	LRI, MS, Std
9	(E)-2-Hexanol	1245	1068.4 $\pm$ 14.4 ^b	878.8 $\pm$ 10.6 ^c	321.7 $\pm$ 9.4 ^d	214.3 $\pm$ 0.8 ^e	1265.0 $\pm$ 24.1 ^a	LRI, MS, Std
10	3-Methylbutanol	1240	42.8 $\pm$ 0.2 ^d	69.0 $\pm$ 1.2 ^c	117.6 $\pm$ 4.3 ^a	nd	87.8 $\pm$ 0.9 ^b	LRI, MS, Std
11	2-Penten-1-ol	1324	458.7 $\pm$ 7.8 ^c	568.5 $\pm$ 7.3 ^b	328.8 $\pm$ 8.4 ^d	145.5 $\pm$ 1.4 ^e	863.8 $\pm$ 12.9 ^a	LRI, MS, tent
12	2-Heptanol	1327	nd	18.8 $\pm$ 0.4 ^c	60.5 $\pm$ 0.7 ^a	29.5 $\pm$ 0.2 ^b	nd	LRI, MS, tent
13	Hexanol	1359	622.9 $\pm$ 10.3 ^a	221.9 $\pm$ 1.5 ^c	193.7 $\pm$ 2.6 ^d	120.6 $\pm$ 0.4 ^e	477.8 $\pm$ 9.8 ^b	LRI, MS, Std
14	2,3-Butanediol	1545	344.2 $\pm$ 6.4 ^c	416.8 $\pm$ 6.3 ^b	202.5 $\pm$ 4.1 ^e	218.9 $\pm$ 2.8 ^d	621.9 $\pm$ 9.7 ^a	LRI, MS, Std
15	1,3-Butanediol	1578	47.3 $\pm$ 0.6 ^c	77.9 $\pm$ 1.2 ^b	nd	nd	108.4 $\pm$ 1.4 ^a	LRI, MS, tent
16	Furfuryl alcohol	1650	nd	61.2 $\pm$ 0.5 ^c	78.1 $\pm$ 0.4 ^b	107.9 $\pm$ 0.6 ^a	nd	LRI, MS, Std
17	Benzylalcohol	1861	324.6 $\pm$ 4.2 ^b	217.1 $\pm$ 3.8 ^c	144.7 $\pm$ 1.9 ^d	80.6 $\pm$ 1.4 ^e	346.8 $\pm$ 5.6 ^a	LRI, MS, tent
18	Phenylethanol	1922	468.7 $\pm$ 2.8 ^c	564.0 $\pm$ 6.5 ^b	236.7 $\pm$ 4.7 ^d	171.1 $\pm$ 3.7 ^e	799.6 $\pm$ 13.7 ^a	LRI, MS, tent
	Total		4137.2	3930.5	2058.5	1285.2	6102.0	
	Aldehydes							
19	2-Methylpropanal	801	24.9 $\pm$ 0.2 ^e	67.6 $\pm$ 1.5 ^c	76.2 $\pm$ 0.4 ^b	166.8 $\pm$ 3.9 ^a	46.9 $\pm$ 0.4 ^d	LRI, MS, Std
20	2-Methylbutanal	896	282.1 $\pm$ 2.7 ^e	636.6 $\pm$ 7.6 ^c	734.9 $\pm$ 2.7 ^b	1049.3 $\pm$ 7.2 ^a	493.2 $\pm$ 7.3 ^d	LRI, MS, Std

Table 4.7. Continued

21	3-Methylbutanal	916	894.2 ± 18.3 ^e	2750.9 ± 22.6 ^c	3125.9 ± 30.6 ^b	4695.1 ± 28.3 ^a	1287.8 ± 10.4 ^d	LRI, MS, Std
22	Hexanal	1097	788.7 ± 19.3 ^e	2019.0 ± 4.6 ^c	3682.5 ± 33.4 ^a	2978.4 ± 7.3 ^b	1093.1 ± 8.5 ^d	LRI, MS, tent
23	Nonanal	1395	317.3 ± 5.3 ^a	69.5 ± 1.2 ^e	109.6 ± 2.6 ^c	81.8 ± 0.5 ^d	198.2 ± 6.4 ^b	LRI, MS, tent
24	2-Furaldehyde	1452	nd	30.7 ± 0.2 ^c	75.1 ± 0.4 ^b	104.6 ± 1.7 ^a	nd	LRI, MS, Std
25	Benzaldehyde	1544	21.1 ± 0.6 ^d	116.9 ± 1.2 ^b	193.4 ± 4.5 ^a	203.6 ± 1.9 ^a	57.5 ± 0.9 ^c	LRI, MS, Std
26	(E,E)-2,4-Nonadienal	1681	64.8 ± 0.6 ^b	19.0 ± 0.1 ^c	nd	nd	81.8 ± 1.2 ^a	LRI, MS, tent
	Total		2393.1	5710.2	7997.5	9279.6	3258.5	
	Esters							
27	Ethyl acetate	887	246.0 ± 4.3 ^b	168.4 ± 4.3 ^c	96.8 ± 0.4 ^d	42.8 ± 0.3 ^e	317.3 ± 8.3 ^a	LRI, MS, tent
28	2-Methylpropyl acetate	1018	nd	nd	44.0 ± 0.7 ^b	155.5 ± 2.6 ^a	nd	LRI, MS, tent
29	Ethyl butanoate	1044	133.9 ± 2.6 ^b	135.0 ± 0.7 ^b	100.2 ± 0.9 ^c	58.9 ± 0.9 ^d	173.3 ± 2.7 ^a	LRI, MS, tent
30	Ethyl-2-methylbutanoate	1064	92.5 ± 0.8 ^e	191.4 ± 7.5 ^d	270.9 ± 3.7 ^a	218.9 ± 8.6 ^c	258.4 ± 6.4 ^b	LRI, MS, tent
31	2-Methylbutyl acetate	1115	126.4 ± 0.4 ^c	171.2 ± 3.4 ^b	69.6 ± 0.4 ^d	19.1 ± 0.2 ^e	352.3 ± 14.2 ^a	LRI, MS, tent
32	3-Methylbutyl acetate	1130	249.7 ± 7.1 ^a	61.3 ± 0.3 ^c	79.3 ± 0.4 ^b	19.5 ± 0.2 ^c	80.0 ± 0.3 ^b	LRI, MS, tent
33	Methyl octanoate	1437	86.6 ± 0.9 ^b	56.7 ± 0.6 ^d	78.0 ± 1.3 ^c	112.5 ± 0.4 ^a	38.7 ± 0.4 ^e	LRI, MS, tent
34	2-Furfuryl acetate	1534	nd	19.6 ± 0.1 ^c	52.8 ± 0.2 ^b	122.0 ± 0.7 ^a	nd	LRI, MS, tent
35	Methyl salicylate	1753	369.4 ± 2.8 ^a	120.9 ± 1.4 ^c	62.1 ± 0.5 ^d	31.0 ± 0.6 ^e	208.6 ± 5.4 ^b	LRI, MS, tent
36	Phenylethyl acetate	1810	108.7 ± 1.6 ^c	169.0 ± 1.5 ^b	48.7 ± 0.2 ^e	89.2 ± 1.6 ^d	373.3 ± 9.3 ^a	LRI, MS, tent
	Total		1475.4	1141.8	908.6	869.4	1906.6	
	Furans							
37	2-Acetylfuran	1512	nd	nd	62.8 ± 1.2 ^a	33.7 ± 0.8 ^b	nd	LRI, MS, tent
38	2-Acetyl-5-methylfuran	1608	nd	nd	59.0 ± 0.6 ^b	111.0 ± 1.4 ^a	nd	LRI, MS, tent
	Total		nd	nd	121.8	144.7	nd	
	Ketones							

Table 4.7. Continued

	Diacetyl	971	62.2 ± 1.5 ^b	48.3 ± 0.7 ^c	nd	nd	104.7 ± 1.5 ^a	LRI, MS, Std
39	2-Pentanone	978	76.4 ± 0.6 ^d	87.5 ± 1.5 ^c	117.2 ± 1.4 ^b	189.5 ± 2.4 ^a	58.8 ± 0.7 ^e	LRI, MS, Std
40	3-Hydroxy-2 butanone	1291	106.7 ± 1.4 ^d	228.8 ± 8.6 ^b	372.1 ± 7.3 ^a	140.3 ± 4.6 ^c	64.3 ± 0.3 ^e	LRI, MS, Std
41	Acetophenone	1652	297.4 ± 11.3 ^a	48.8 ± 0.9 ^c	nd	108.2 ± 0.8 ^b	20.7 ± 0.2 ^d	LRI, MS, Std
	Total		480.5	365.1	489.3	438.0	143.8	
	Pyrazines							
42	2-Methylpyrazine	1274	44.9 ± 0.6 ^d	97.8 ± 0.8 ^c	149.2 ± 4.2 ^b	221.2 ± 2.7 ^a	48.9 ± 0.2 ^d	LRI, MS, Std
43	2,6-Dimethylpyrazine	1319	nd	nd	nd	246.9 ± 9.4 ^a	nd	LRI, MS, Std
44	2,3-Dimethylpyrazine	1357	nd	37.9 ± 0.4 ^c	67.0 ± 1.4 ^b	444.4 ± 11.5 ^a	nd	LRI, MS, Std
45	2-Ethylpyrazine	1358	nd	19.4 ± 0.1 ^c	47.2 ± 0.5 ^b	122.0 ± 0.8 ^a	nd	LRI, MS, Std
46	2-Methyl-5-ethylpyrazine	1399	nd	nd	78.4 ± 0.6 ^b	128.3 ± 4.2 ^a	nd	LRI, MS, Std
47	2-Ethyl-6-methylpyrazine	1408	nd	nd	nd	318.3 ± 7.3 ^a	nd	LRI, MS, Std
48	2,3-Dimethyl-5-ethylpyrazine	1445	nd	nd	23.9 ± 0.4 ^b	70.8 ± 0.3 ^a	nd	LRI, MS, Std
	Total		44.9	155.1	365.7	1551.9	48.9	
	Pyrroles, Pyrrolines & Lactones							
49	2-Acetyl-1-pyrroline	1310	4.1 ± 0.03	19.4 ± 0.3	21.3 ± 0.41	21.3 ± 0.21	20.6 ± 1.20	LRI, MS, Std
50	γ-Butyrolactone	1617	54.7 ± 0.4 ^d	92.4 ± 0.9 ^c	216.2 ± 4.3 ^b	308.3 ± 2.4 ^a	41.4 ± 0.3 ^e	LRI, MS, tent
51	2-Acetylpyrrole	1974	nd	69.0 ± 0.5 ^c	82.6 ± 0.4 ^b	130.6 ± 1.3 ^a	nd	LRI, MS, tent
	Total		58.8	180.9	320.1	460.2	62.03	
	Terpenes							
52	α-Phellandrene	1038	197.8 ± 1.5 ^b	109.1 ± 0.7 ^c	nd	nd	396.1 ± 11.3 ^a	LRI, MS, Std
53	Sabinene	1119	102.5 ± 0.8 ^c	151.4 ± 0.9 ^b	72.0 ± 0.9 ^d	nd	279.5 ± 8.4 ^a	LRI, MS, Std
54	Ocimene	1233	234.1 ± 4.8 ^a	59.3 ± 0.2 ^c	28.9 ± 0.4 ^d	11.5 ± 0.2 ^e	81.9 ± 0.4 ^b	LRI, MS, tent
55	P-Cymene	1272	206.7 ± 2.5 ^b	178.7 ± 4.3 ^c	nd	nd	244.1 ± 4.6 ^a	LRI, MS, Std

Table 4.7. Continued

56	Linalool	1537	432.2 ± 15.6 ^a	107.4 ± 0.9 ^c	nd	nd	337.7 ± 7.3 ^b	LRI, MS, Std
57	(E)-Caryophyllene	1598	362.9 ± 4.3 ^a	88.5 ± 1.2 ^c	nd	nd	123.9 ± 1.7 ^b	LRI, MS, Std
58	β-aromadendrene	1635	217.2 ± 5.8 ^d	247.5 ± 5.6 ^c	413.2 ± 7.3 ^b	624.5 ± 7.1 ^a	119.2 ± 0.8 ^e	LRI, MS, tent
58	Valencene	1715	89.5 ± 0.7 ^e	238.4 ± 4.3 ^b	204.4 ± 5.1 ^c	322.4 ± 9.3 ^a	191.4 ± 3.8 ^d	LRI, MS, tent
	Total		1842.9	1180.3	718.5	958.4	1773.8	
	Phenols							
59	Guaiacol	1960	187.3 ± 2.4 ^a	47.4 ± 0.2 ^d	60.7 ± 1.2 ^c	56.1 ± 0.5 ^b	27.2 ± 0.6 ^e	LRI, MS, tent
60	Isoeugenol	2271	266.9 ± 7.3 ^a	45.6 ± 0.4 ^c	34.5 ± 0.5 ^d	13.4 ± 0.1 ^e	83.3 ± 1.8 ^b	LRI, MS, tent
	Total		454.2	93.0	95.2	69.5	110.5	
	General Total		11628.4	13678.9	13434.8	15345.1	14473.2	

LRI: linear retention index calculated on DB-Wax capillary column. Concentration: results are the means of three repetitions as µg/kg. Identification: methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification. FFN: Fermented Nacional beans; SDCB: sun dried Nacional beans; CDFB: convective dried Nacional beans; MDFB: microwave dried Nacional beans; FDFB: freeze dried Nacional beans; Each value represents mean ± standard deviation. Different lower-case letters (a,b,c) within the same line display significant differences ($p < 0.05$) between the fresh fermented cocoa beans and the different cocoa beans drying processes; nd: not identified

4.7.1. Effect of Drying Methods on Aroma Compounds in Cocoa Beans

The sensory characteristics of cocoa beans are significantly influenced by aroma compounds, making them a crucial factor in determining overall flavor. The distinctive and incomparable flavor profile of cocoa beans, encompassing chocolate, creamy, and fruity notes, is attributed to a myriad of volatile compounds. Consequently, the identification and quantification of compounds which contribute to the flavor or off-flavor are regarded as the key aspect for quality control. As demonstrated in Table 4.5, Table 4.6 and Table 4.7, the drying processes, especially convective drying and microwave drying have proven effective in influencing aroma compounds across all cocoa beans, with statistically significant differences observed among the volatile compounds in all samples ($p < 0.05$). Examining the findings related to each chemical group, it is important to highlight that the composition of aldehydes and alcohols in all cocoa bean varieties was influenced by the metabolic cascades governing the biosynthesis and degradation of major volatile compounds, occurring during the fermentation and drying processes of cocoa beans. The concentrations of overall aroma compounds in the different varieties of cocoa beans were significantly declined during drying processes. The fresh-fermented Forastero bean samples exhibited a total aroma compounds of 9143.9 $\mu\text{g/kg}$. When the drying effect was compared, the highest amount of total aroma compounds in Forastero beans (13456.0 $\mu\text{g/kg}$) was found in freeze-drying process, followed by the beans acquired from microwave drying (13241.8 $\mu\text{g/kg}$), convective drying (12314.8 $\mu\text{g/kg}$) and sun drying process (11484.0 $\mu\text{g/kg}$).

In fresh-fermented Criollo bean samples, a total amount of 8718.5 $\mu\text{g/kg}$ of aroma compounds was quantified. When drying processes were compared, the highest concentration of aroma constituents found in Criollo beans was that obtained from convective drying (12828.7 $\mu\text{g/kg}$), followed by freeze-drying (11972.7 $\mu\text{g/kg}$), microwave drying (11322.8 $\mu\text{g/kg}$), and sun drying (10554.8 $\mu\text{g/kg}$).

Regarding Nacional beans, fresh-fermented Nacional bean sample had 11628.4 $\mu\text{g/kg}$ total amount of aroma compounds, and the highest concentration of aroma compounds in Nacional bean sample was observed in microwave drying (15345.1 $\mu\text{g/kg}$), followed by freeze-drying (14473.2 $\mu\text{g/kg}$), sun drying (11628.4 $\mu\text{g/kg}$), and convective drying (13413.5 $\mu\text{g/kg}$) drying process. Hence, there was a distinct increase in the total content of aroma compounds of all cocoa varieties under drying processes. Moreover, a clear increase in the amount of aldehyde compounds was observed in all cocoa varieties under the convective, roasted and microwave drying process. This trend confirms those reported by the former studies in the different cocoa cultivars by several scholars (Frauendorfer & Schieberle, 2008; Quelal et al., 2023).

As depicted in Table 4.5, Table 4.6 and Table 4.7, a broad range of aroma compounds was identified in the samples of all cocoa cultivars. Most of these compounds have been previously identified in various fermented and dried cocoa beans varieties through prior research efforts (Dulce et al., 2021; Frauendorfer & Schieberle, 2008, 2019). The primary source of the distinctive odor notes

in fermented and dried cocoa beans are attributed to the significant volatile compounds, specifically the C-6 and C-5 compounds. These compounds originate from the primary and secondary lipoxygenase cascades, respectively during the fermentation process and from Maillard reaction via the drying process. Furthermore, minor volatile compounds can serve as significant quality indicators, contributing to a deeper understanding of the formation or degradation of the major volatile compounds (Kalua et al., 2007). Indeed, the characteristic and exclusive aroma of fermented and dried cocoa bean, particularly its chocolate and fruity and creamy notes, can be attributed to numerous volatile compounds (Quelal et al., 2023). The volatile compounds in fermented and dried cocoa beans are widely recognized to be associated with the quantity and activity of enzymes involved in various pathways during fermentation by microorganisms population and Maillard reaction during the drying process. Additionally, some parameters such as fermentation degree, variety of cocoa bean, temperature, drying methods, geographic location significantly impact on the amount and quantity of the aroma constituents. Among these, the drying process is the most effective factors that influences the cocoa beans aroma (Erazo Solorzano et al., 2023; Rodriguez-Campos et al., 2012).

4.7.1.1. Effect of drying methods on Aroma compounds in Forastero beans

Ester Compounds

In the analyzed Forastero cocoa beans, nine esters such as ethyl acetate, ethyl 3 methylbutanoate, ethyl 2 methylbutanoate, 2-methylbutyl acetate, phenylethyl acetate, 2-phenyl acetate, 3-methylbutyl acetate, and ethyl hexanoate were detected and quantified in fermented beans, nine esters comprising the same composition in fermented beans, except benzyl acetate were observed in sun dried beans, eight esters including ethyl acetate, ethyl 3 methylbutanoate, ethyl 2 methylbutanoate, 2-methylbutyl acetate, phenylethyl acetate, 2-phenyl acetate, 3-methylbutyl acetate, and ethyl hexanoate were characterized in convective dried beans, six esters comprising ethyl acetate, ethyl 3 methylbutanoate, ethyl 2 methylbutanoate, 2-methylbutyl acetate, 2-phenyl acetate, 3-methylbutyl acetate, and ethyl hexanoate were present in microwave dried beans and 10 esters including the same composition in fermented beans, except benzyl acetate were identified and quantified in freeze dried beans. When the drying process and fresh fermented beans were compared, the highest amount of esters (2914.1 $\mu\text{g/kg}$) was observed in freeze-dried Forastero bean samples, followed by fresh-fermented (2220.7 $\mu\text{g/kg}$) bean and sun dried bean (1886.3 $\mu\text{g/kg}$) samples. Among the detected esters in Forastero beans, 2-methylbutyl acetate was identified in high quantity in fresh-fermented bean (885.5 $\mu\text{g/kg}$), and freeze-dried bean (794.6 $\mu\text{g/kg}$) samples. Some of esters identified in this study have been previously detected in other fermented and dried Forastero beans in prior assays (Rodriguez-Campos et al., 2012; Velásquez-Reyes et al., 2023). The concentration of total esters in Forastero beans exposed a decrement trend when sun , convective and microwave drying processes were applied (Table 4.5). Nonetheless, esters are generally considered minor

components compared to aldehydes and alcohols (Vekiari et al., 2010). Figure 4.8 illustrates the percentage distribution of esters in Forastero beans obtained across fermentation and drying processes, relative to the total compound content.

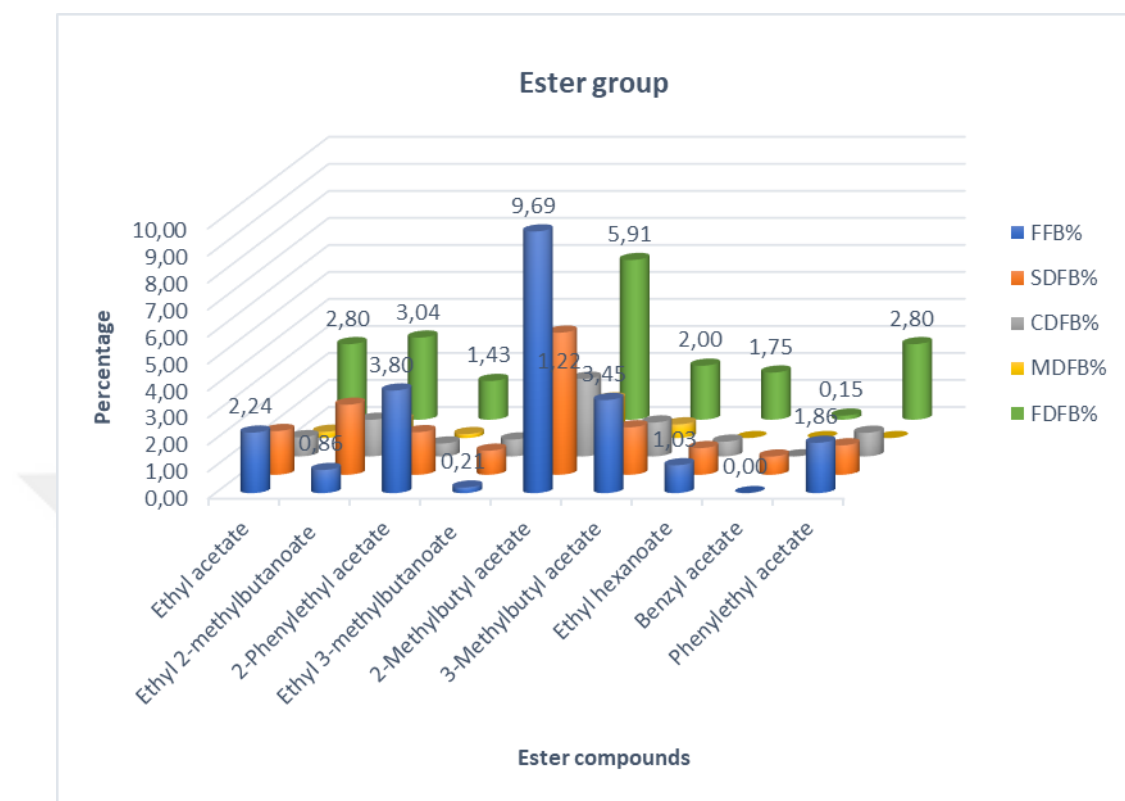


Figure 4.8. The ratio of ester compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Alcohol Compounds

The enzymes lipxygenase, alcohol dehydrogenase (ADH), prevalent in numerous plants, are responsible for the production of volatile alcohols that contribute to the overall aroma of cocoa beans through lipxygenase pathway. Alcohols are also derived from amino acid metabolism, which impart fruity and floral aroma odor to cocoa beans and related products. A total of 10, 9, 10, 9, 10 aroma compounds were identified in Forastero beans extract obtained from fresh fermented, sun dried, convective dried, microwave dried and freeze-dried bean samples, respectively. The percentage distribution of alcohol compounds in Forastero beans obtained across drying processes is illustrated in Figure 4.8. The concentration of total alcohols, as indicated in Table 4.5, exhibited statistically significant differences among the examined dried cocoa bean samples ($p < 0.05$). Total amount of alcohol of Forastero beans decrease during drying processes, and the freeze dried bean (5940.0 $\mu\text{g/kg}$) samples exhibited the highest content of alcohol, followed by sun dried bean (3258.9 $\mu\text{g/kg}$) samples. In this study, the C-6, C4 and C5 alcohols happened prevailingly in all fermented and dried Forastero bean sample. When compared to other investigations, the same obervation was made by Velásquez-Reyes et al. (2023), Cevallos-Cevallos et al. (2018), and Qin et al. (2017). A positive correlation between the drying processes and hexanol as a predominant alcohol was

determined, and its concentration displayed the lowest amount (128.7 µg/kg) in Forastero bean from only fermented bean samples, then increased up to 475.4 µg/kg, 942.9 µg/kg, 1690.7 µg/kg and 2114.8 µg/kg in Forastero microwave dried beans, convective dried bean, sun dried beans, and freeze-dried beans, respectively. This trend has already be observed in fermented and dried Forastero bean by Rodriguez-Campos et al. (2011), and Jinap et al. (1998). As mentioned, the volatile compound with the highest amount is hexanol, observed in freeze dried (2114.8 µg/kg) bean, and sun dried (1690.7 µg/kg) bean samples. This compound is followed by 3-methylbutanol in freeze dried (1283.7 µg/kg) bean and convective dried (856.0 µg/kg) bean samples. 2-pentanol was also found with an important quantity in freeze-dried (757.8 µg/kg), and only fermented (674.3 µg/kg) bean samples. The lowest amount of alcohols was observed in microwave dried (1419.7 µg/kg) bean, followed by convective dried (2696.5 µg/kg) and fresh-fermented (3014.1 µg/kg) bean samples, respectively. Alcohols fundamentally take into account as characterization markers, since their contents are different from each other when the drying process is applied. The significant decrease in the total content of alcohols in convective and microwave dried beans compared to sun dried and freeze-dried bean samples is probably link to the degradation and the conversion of volatile constituents due to the temperature and time applied.

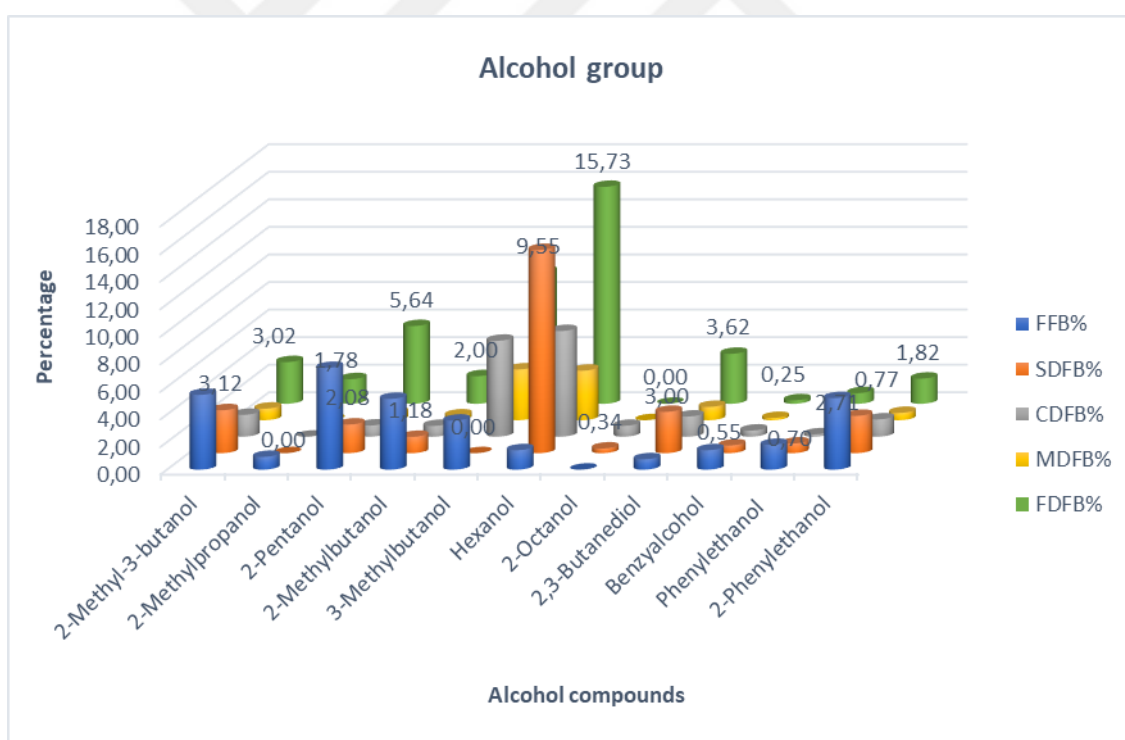


Figure 4.9. The ratio of alcohol compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Aldehyde Compounds

Aldehydes are one of the most abundant aroma substance groups in cocoa bean samples. These compounds are formed because of Stecker degradation of amino acids and have a significant effect on the aroma of many different foods (tea, wine, cocoa beans, roasted peanuts and coffee, etc.).

Aldehydes typically contribute to imparting the grassy, malty, sweet, floral, fruity and green plant aroma to food, especially cocoa beans and related products (Ullrich et al., 2023; Quelal et al., 2023; Rodriguez-Campos et al., 2012). The number of aldehydes detected and quantified in fresh-fermented Forastero beans was two, while the beans obtained from sun, convective, microwave and freeze-drying processes were six, and three respectively. These compounds constitute the second highest concentration within the aroma fraction of all Forastero bean samples when compared to other volatile compounds. Of all drying techniques applied to Forastero bean samples, microwave dried beans presented the highest amount of aldehyde (5782.3 µg/kg) with 43.72% proportion of total aroma substances, followed by convective dried beans (4163.2 µg/kg) accounting for 33.8% of total volatile constituents, sun dried beans (2315.1 µg/kg) with 20.19% of total volatile compounds, and freeze-dried beans (485.6 µg/kg) comprised 3.61% of total volatiles. The lowest amount of aldehyde (112.8 µg/kg) was attributed to fresh fermented Forastero bean samples accounting for 1.23% of the total volatile compounds. The amount of total aldehydes in Forastero beans significantly increased during drying stages. Rodriguez-Campos et al. (2012) indicated that the total concentration of aldehydes increased during drying process in Forastero beans, which is in compliance with the findings of our study. Among the characterized aldehyde in Forastero beans, hexanal was determined in high amount in microwave dried bean (1529.8 µg/kg), and convective dried bean (1869.0 µg/kg) samples. The second highest aldehyde detected in Forastero bean was 3-methylbutanal (2039.5 µg/kg) in microwave dried Forastero bean sample. These volatile compounds have already been mentioned in higher concentration in dried Forastero beans (Jinap et al., 1994; Portillo et al., 2009; Rodriguez-Campos et al., 2012). A high concentration of aldehydes and ketones is favourable for cocoa quality, producing fruity, green and flowery notes, specially hexanal, 2-methylbutanal and 3-methylbutanal (Bonvehí, 2005). The presence of 2 and 3-methylbutanal is appreciable in cocoa beans and products because of their malt notes. The formation of these two least aldehydes can be attributed to the degradation of isoleucine and leucine by lactic acid bacteria during fermentation (Counet et al., 2002; Frauendorfer & Schieberle, 2008). Figure 4.10 presents the ratio of aldehyde compounds in Forastero cocoa bean samples obtained across fermentation and drying processes, expressed as a percentage of the total compounds. In cocoa beans, aldehydes consistently occur in higher quantities compared to other volatile compounds.

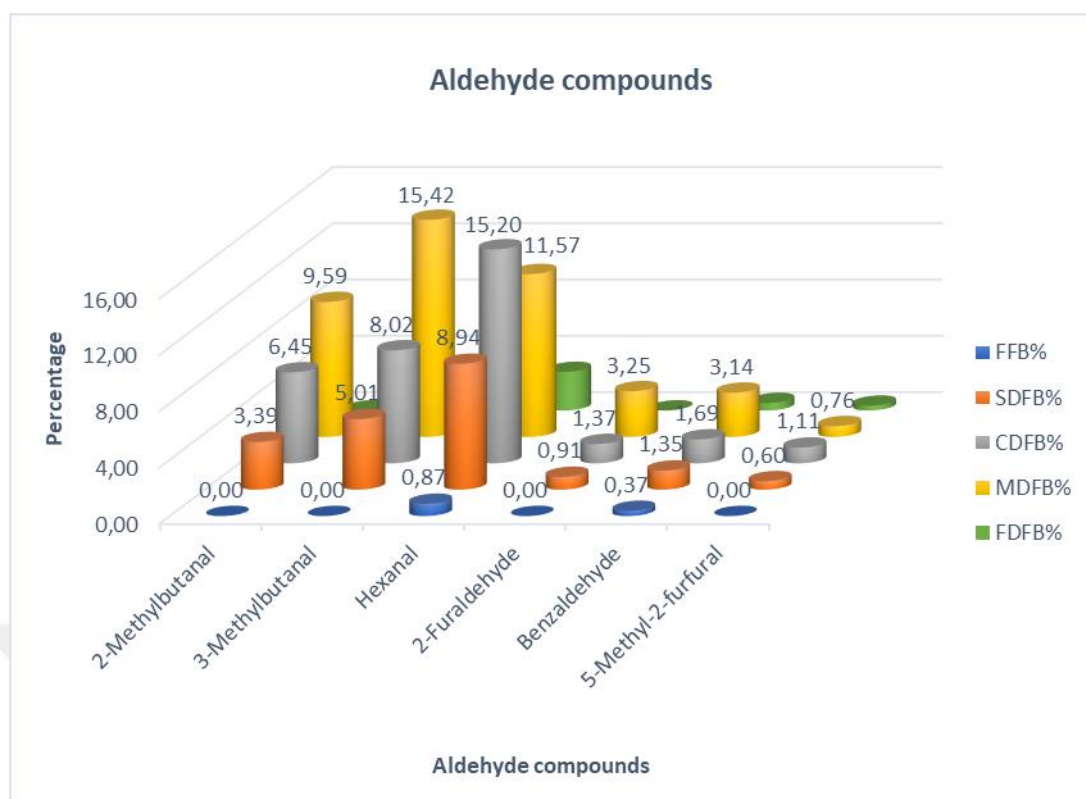


Figure 4.10. The ratio of aldehyde compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Terpene Compounds

Ten terpenes (ocimene, linalool, linalool oxide II, linalool oxide I, D-limonene, β -mycene, 1,4-cineol, γ terpinene, and sabinene) were identified and quantified in fresh-fermented Forastero beans, the same number of terpene were detected and quantified in sun dried and freeze-dried Forastero bean samples, while five terpene compounds (ocimene, linalool, linalool oxide II, D-limonene, and 1,4-cineol) and 3 terpene compounds (ocimene, D-limonene, and ,4-cineol) were found in convective dried and microwave dried Forastero beans, respectively. The total amount of these compounds was 1943.4, 1578.5, 1406.7, 1009.9, and 880.1 $\mu\text{g/kg}$ in freeze-dried, sun dried, fresh-fermented beans, microwave dried, and convective dried Forastero bean samples, respectively. When drying processes are compared within terpene compounds, freeze-dried Forastero beans presented the highest concentration (1943.4 $\mu\text{g/kg}$) of terpenes. Figure 4.11 illustrates the percentage distribution of terpene compounds in fresh fermented beans and dried beans, relative to the total compound content. These terpenes represented approximately 7.15%, 7.63%, 13.76%, 14.45%, and 15.39%, of the total volatiles analyzed in Forastero beans attained from convective dried, microwave dried, sun dried, freeze-dried, and fresh-fermented, bean samples, respectively. As can be demonstrated in Table 4.5, the amount of total terpenes showed an increasing trend along drying processes, specially in sun dried and freeze-dried bean samples compared to fresh fermented beans. The decrease in total terpenes obtained from convective dried and microwave dried bean extracts was probably due to its degradation resulted from dehydrogenations, epoxidations, double bond

cleavages, allylic oxidations, and rearrangements, being evidenced by previous reserchers using other food matrice (McGraw et al., 1999). The aromatic extract of fresh fermented and dried Forastero beans revealed the highest concentrations of linalool, ranging between 126.8 and 520.4 $\mu\text{g/kg}$, followed by ocimene among the terpenes. Some of the detected terpenes were also observed in Forastero beans by Qin et al. (2017) and from other regions by numerous reserchers (Acierno et al., 2016; Braga et al., 2018; Rodriguez-Campos et al., 2011). Terpenes are the lower molecular weight constituents of the terpenoid group, which are formed from two or three isoprene units. The biosynthesis of terpenes, along with enzymatic modifications like oxidation, reduction, and dehydration, has been extensively described by Skinkis et al. (2008).

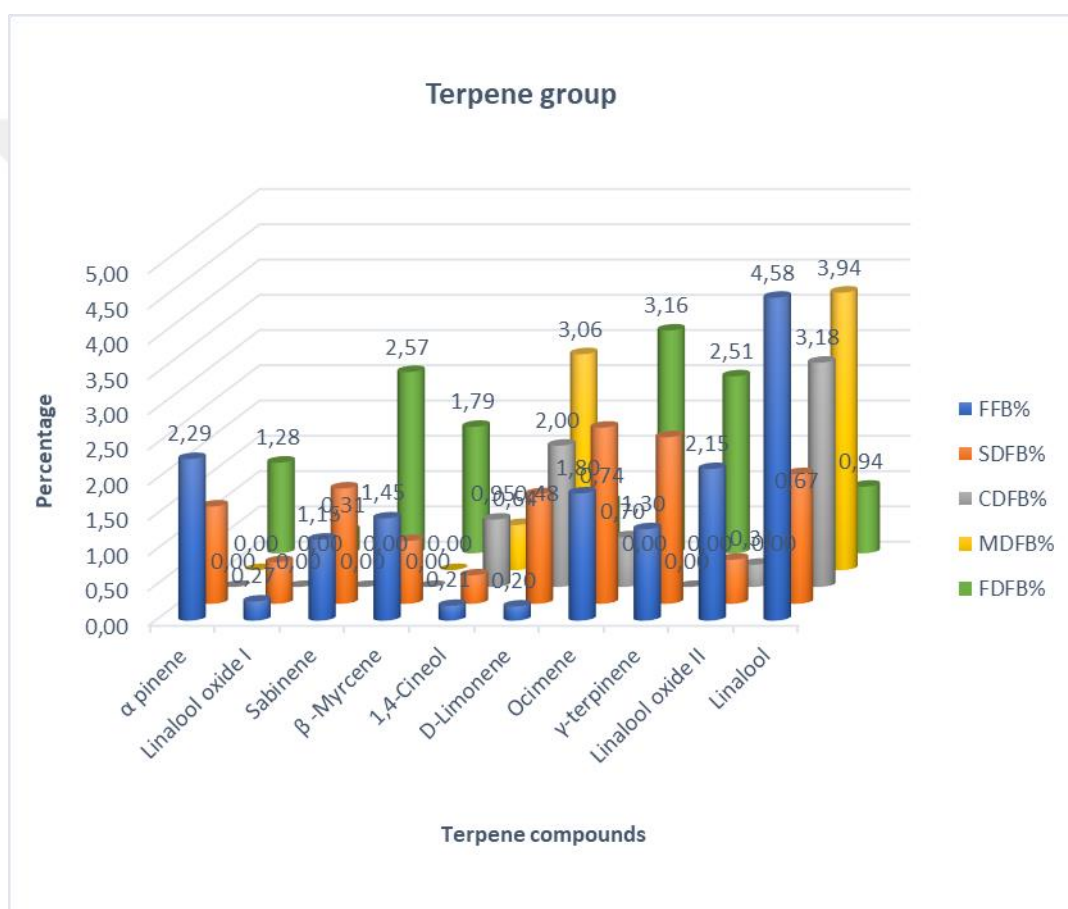


Figure 4.11. The ratio of terpene compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Ketone Compounds

Five, five, three, two, and five ketones were discovered in Forastero beans extracts attained from fresh-fermented, sun dried, convective dried, microwave dried and freeze dried bean samples, respectively. Figure 4. illustrates the percentage distribution of ketone compounds in Forastero beans obtained across drying processes, relative to the total compound content. The total content of ketones in Forastero bean samples obtained from fresh fermented bean extracts was 799.3 $\mu\text{g/kg}$, which increased up to 844.0 $\mu\text{g/kg}$ and 1135.0 in sun dried and freeze-dried bean samples, respectively. A

decrease was observed in convective dried (511.9 $\mu\text{g/kg}$), and microwave dried (63.2 $\mu\text{g/kg}$) bean samples. This part was also confirmed by previous experiments (Hinne, Abotsi, et al., 2019; Jinap et al., 1998; Ramli et al., 2006). From the ketone class, 2-hydroxy-3-pentanone represented the highest amount and its concentration content was 456.4 $\mu\text{g/kg}$, 402.8 $\mu\text{g/kg}$, 155.6 $\mu\text{g/kg}$, 95.4 $\mu\text{g/kg}$, and 31.4 $\mu\text{g/kg}$ in freeze-dried, fresh-fermented, sun dried, convective dried, and microwave dried Forastero bean samples, respectively, followed by 5-methyl-2-hexanone (390.8 $\mu\text{g/kg}$) with high concentration in freeze-dried bean samples, and 2-heptanone (238.5 $\mu\text{g/kg}$) in convective dried bean samples. 2-hydroxy-3-pentanone was also discovered in the high concentration among ketones in fermented and dried Forastero beans and genotypes from Amazon regions (Erazo Solorzano, Disca, et al., 2023; Utrilla-Vázquez et al., 2020).

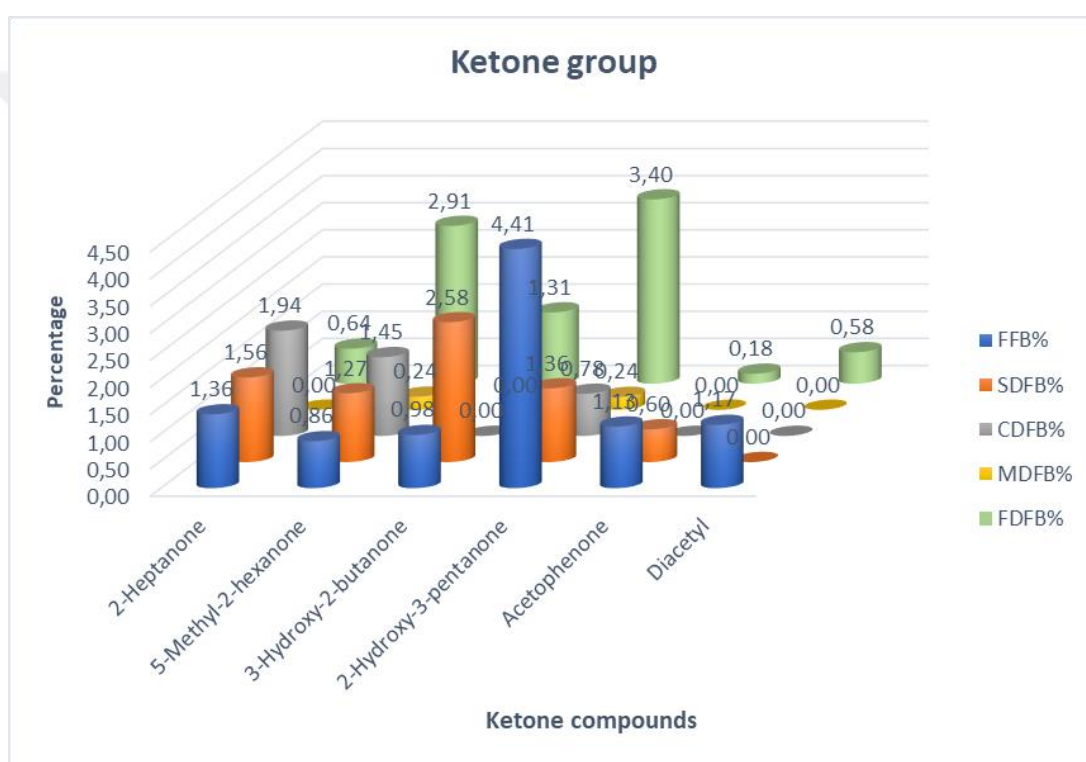


Figure 4.12. The ratio of ketone compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Furan and Furanone Compounds

Among furan and furanone compounds, a total of two furans and one furanone, including 2-acetylfuran, 2-acetyl-5-methylfuran, and 5-(1-hydroxyethyl)-2(5H)-furanone were detected and quantified in sun dried Forastero beans. Three furans and one furanone composing of 2-pentylfuran, 2-acetylfuran, 2-acetyl-5-methylfuran, and 5-(1-hydroxyethyl)-2(5H)-furanone were observed and quantified in convective dried Forastero beans. The same compounds were also determined in microwave dried Forastero beans. Microwave drying process exhibited the highest amount of volatile compounds (595.43 $\mu\text{g/kg}$), followed by convective drying process (415.73 $\mu\text{g/kg}$). Of all volatile

compounds characterized in all Forastero samples, 2-acetyl-5-methylfuran was found with the highest amount (185.73 $\mu\text{g/kg}$), followed by 2-acetylfuran (126.93 $\mu\text{g/kg}$) in microwave dried Forastero beans. It is well established that furans are formed from the interaction of amino and sugar through the Maillard reaction and Strecker degradation (Yıldız et al., 2010). It was observed in the study that furans and furanone were not detected in fresh fermented and freeze-dried beans, which confirmed Yıldız et al. (2010) statement. Some of the identified compounds have been previously reported in dried and roasted Forastero beans (Ramli et al., 2006, Serra Bonveh, 2005, Calva-Estrada et al., 2020).

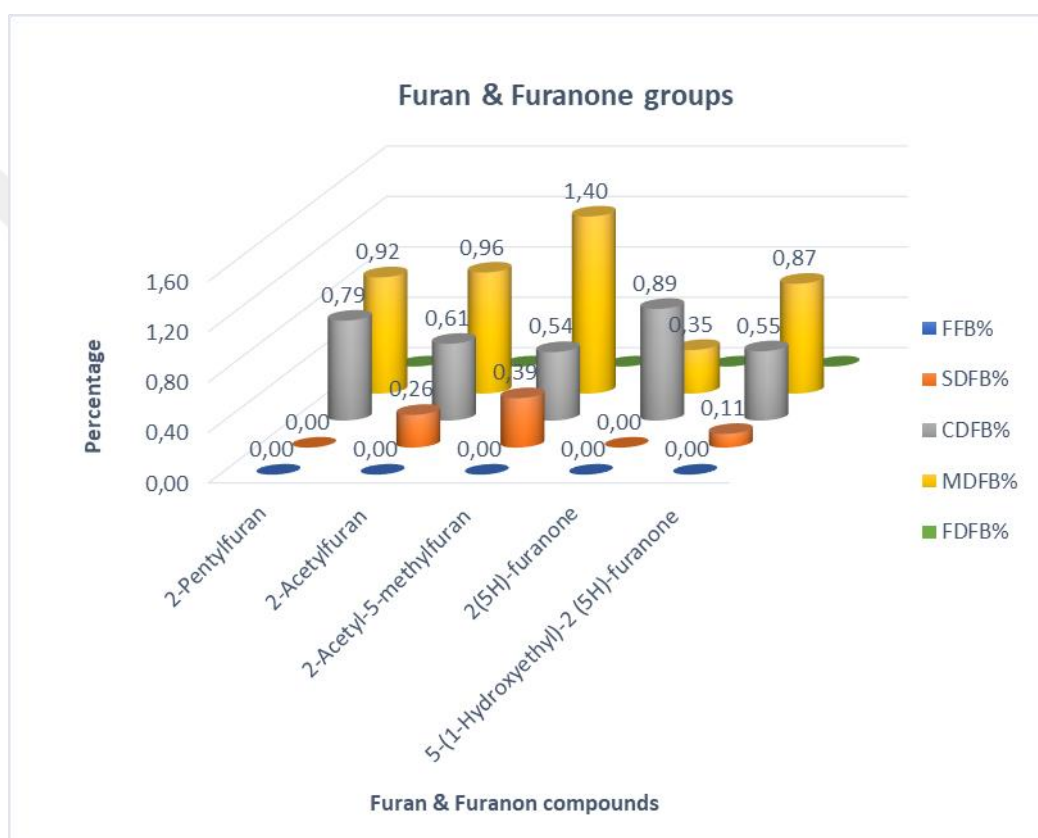


Figure 4.13. The ratio of furan and furanone compounds in the fermented and Forastero cocoa beans according to the amount of total compounds in percent

Pyrazine Compounds

Pyrazines are important aroma compounds in baked goods such as roasted peanuts, coffee beans, hazelnuts, and cocoa beans. It have been reported that pyrazine compounds are generated during the roasting of cocoa through the Maillard reactions that took place during the coffee and cocoa beans drying and roasting process (Liu et al., 2019). These compounds contributed desirable nutty and roast odor to the cocoa products (Liu et al., 2017; Schieberle & Pfner, 1999). Many pyrazines are known as the volatiles contributing to roasted aromas of cooked foods (Schieberle, 1995; Shimoda & Shibamoto, 1990), and in the case of cocoa bean coffee, such compounds have been associated to the roasty and earthy/musty flavors in dried and roasted cocoa bean and

coffee brews (Maeztu et al., 2001; Sanz et al., 2002). A total of seven different pyrazine compounds were quantitated in dried Forastero bean samples (Table 4.5). The total content of pyrazines in microwave dried bean (2665.7 $\mu\text{g/kg}$) sample was higher than in sun dried (182.7 $\mu\text{g/kg}$) and convective dried (1403.6 $\mu\text{g/kg}$) bean samples, while no pyrazine compounds were observed in fresh-fermented and freeze dried beans. This observation confirm the fact that pyrazines are mostly the products of Maillard reaction under heating conditions. The research conducted by Cros et al. (1994) and Álvarez et al. (2016) demonstrated that pyrazine concentrations experience a rapid increase throughout postharvest processes such as drying. These concentrations further evolve during the roasting phase. Of all the pyrazine compounds identified in dried Forastero bean samples, 2,3,5 trimethylpyrazine (678.7 $\mu\text{g/kg}$) had the highest concentration, followed by 2-methylpyrazine (423.4 $\mu\text{g/kg}$), and 2-ethylpyrazine (419.0 $\mu\text{g/kg}$) in microwave dried bean sample, and 2,3,5,6 tetramethylpyrazine (406.5 $\mu\text{g/kg}$) in convective dried bean samples. The pyrazines identified in the present study have been identified in different cocoa bean samples from different origins (Hinne, Van de Walle, et al., 2019; Jinap et al., 1998; Qin et al., 2017). In a similar investigation conducted by Velásquez-Reyes et al. (2023), the majority of the pyrazines detected in our study were discovered, and 2,3,5 trimethylpyrazine was also found as the dominant pyrazine in their study. The same observation was confirmed by Bonvehí (2005) in his study on volatile compounds of dried and roasted Forastero beans.

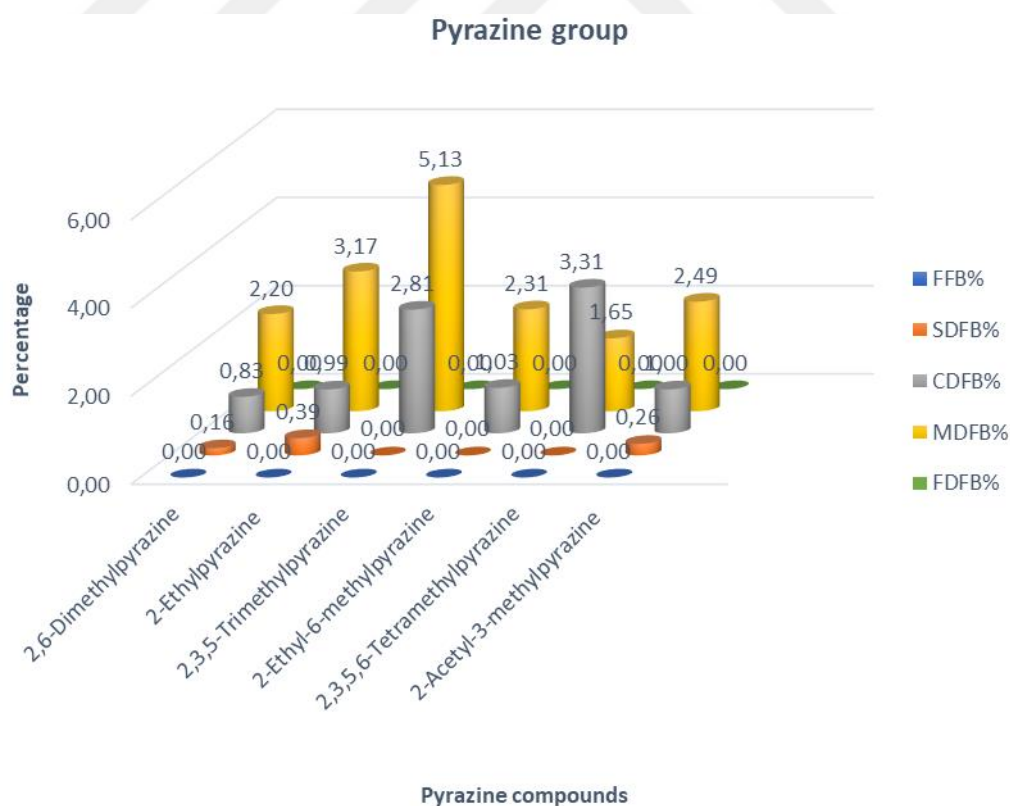


Figure 4.14. The ratio of pyrazine compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Acid Compounds

Eight acids such as 2-methylpropanoic acid, acetic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic acid, pentanoic acid, nonanoic acid, and phenylacetic acid were determined in fresh-fermented, sun dried, and freeze-dried Forastero bean samples, while seven acid compounds including the same cited above, except octanoic acid, and six acids similar to that observed in sun and freeze-dried bean, except octanoic and nonanoic acid were discovered in convective dried and microwave dried bean samples, respectively. Total amount of these compounds was between 525.3 and 1242.1 µg/kg, with the highest concentration observed in fresh fermented beans (1242.1 µg/kg), followed by sun dried beans (1144.6 µg/kg). The ratio of acid compounds in Forastero beans obtained through the drying processes according to the amount of total compounds in percent is illustrated in Figure 4.15. Among the acids (Table 4.5), acetic acid with 496.0 µg/kg exhibited the highest concentrations in Forastero beans obtained from sun drying process, while phenylacetic acid with 288.2 µg/kg, and 3-methylbutanoic acid with 188.3 µg/kg represented the highest concentrations in Forastero beans obtained from freeze-dried and microwave dried bean samples, respectively. It is well known that acetic acid is generated by the biochemical synthesis from the oxidation of ethanol during the first stages of fermentation (Schwan & Wheals, 2004). Frauendorfer and Schieberle (2008) reported that during the roasting process, 70% of the acetic acid concentration was eliminated, which confirmed the lowest concentration of acetic acid observed in microwave dried beans. Acids have been identified as providing sour and pungent sensations in fermented and dried cocoa beans. The majority of these compounds were discovered in previous investigations by numerous scientific (Ak & Aida, 2013; Bonvehí, 2005; Qin et al., 2017). In some similar study, acetic acid was determined as the most prominent short chain acid in Forastero fermented and dried beans (Frauendorfer & Schieberle, 2008; Koné et al., 2016). In another study, Jinap et al. (1994) also found acetic acid in the highest concentration in oven-dried and sun-dried beans.

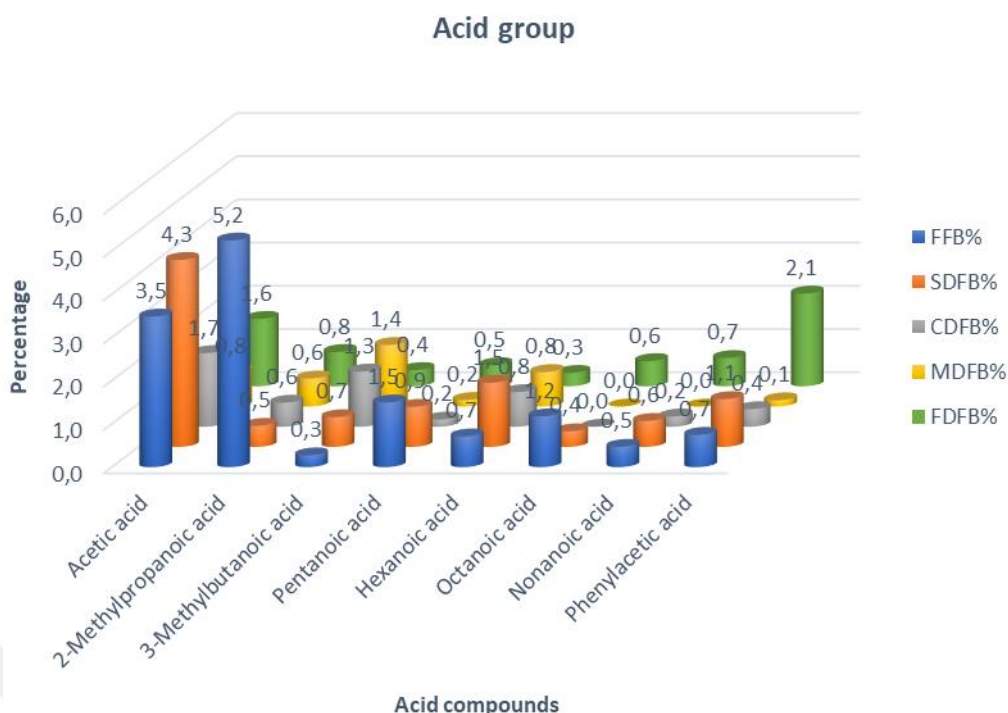


Figure 4.15. The ratio of acid compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

Other Compounds

Five different groups of aroma compounds, namely lactones, hydrocarbons, volatile phenols, pyrroles, and pyrroline were also determined in fresh-fermented and dried Forastero bean samples. Among the lactone compounds, a total of two were determined in all Forastero bean samples. Convective dried bean samples (269.6 $\mu\text{g/kg}$) had the highest amount of lactone compounds. The lowest total amount of lactone was quantified in freeze-dried bean sample (73.3 $\mu\text{g/kg}$). When lactone compounds were compared, γ -butyrolactone (204.2 $\mu\text{g/kg}$) was found in highest quantity in microwave dried bean samples. One hydrocarbon (styrene) compound was solely determined in convective dried bean (112.3 $\mu\text{g/kg}$), and microwave dried bean (244.3 $\mu\text{g/kg}$) samples. Among pyrrole, 2-acetylpyrrole compound was only detected in convective dried and microwave dried bean samples with different concentration. Microwave dried bean samples presented the elevated amount of pyrrole (139.2 $\mu\text{g/kg}$). This class of compound was not detected in fresh-fermented, sun dried and freeze-dried bean samples. Additionally, 2-acetyl-1-pyrroline was determined in all samples as a pyrroline compound. This compound, generally associated with roasting, is of utmost importance due to its low threshold value and contribution to overall aroma with a popcorn like note (Quelal et al., 2023). In our study, all drying techniques resulted an increase in this compound's concentration. Similarly, Frauendorfer and Schieberle (2019) reported that 2-acetyl-1-pyrroline was in the group of volatiles that their concentration increased during roasting. Other groups of compounds found in the samples are volatile phenol compounds, which are commonly present in significant amount in fermented and moderated dried cocoa beans. These compounds contribute not only to its fragrance

but also impart distinctive flavors, including bitter and pungent organoleptic notes. However, the presence of these compounds in cocoa beans is undesirable when they exist at high concentration. The total concentration of volatile phenol ranged from 20.9 to 206.8 $\mu\text{g/kg}$. As indicated in Table 4.5, the total amount of volatile phenol slightly decreased during drying process. Guaiacol was the only volatile phenol compound detected in all Forastero bean samples. This compound was predominant in fresh-fermented beans (206.8 $\mu\text{g/kg}$). Some of these compounds were also detected in Forastero fermented, dried and roasted beans (Bonvehí, 2005; Qin et al., 2017; Velásquez-Reyes et al., 2023).

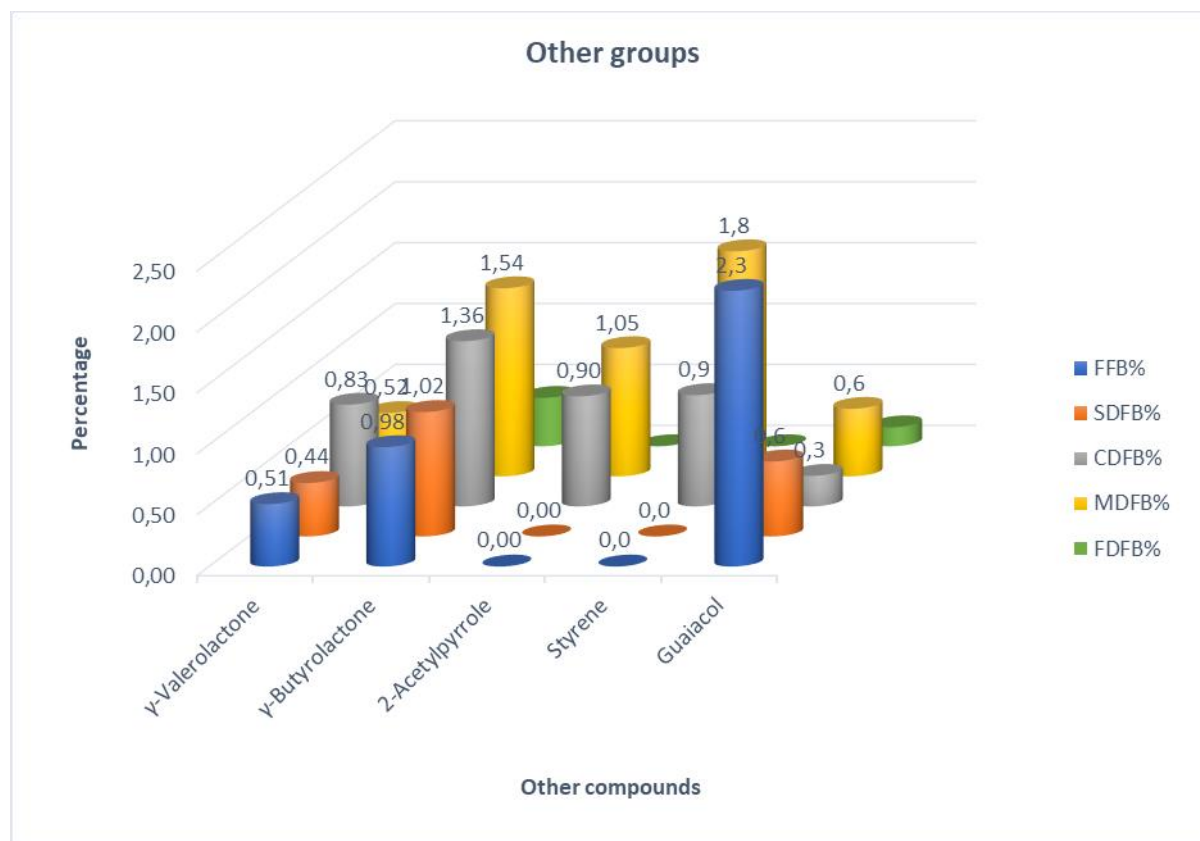


Figure 4.16. The ratio of other compounds in the fermented and dried Forastero cocoa beans according to the amount of total compounds in percent

4.7.1.2. Effect of Drying Methods on Aroma Compounds in Criollo Beans

Ester Compounds

Esters play a crucial role in cocoa's aroma profiles, imparting fruity flavors through the reaction between organic acids and ethanol (Afoakwa et al., 2008). In the analysis of Criollo beans, twelve esters were detected and quantified, which include ethyl acetate, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethylphenyl acetate, methylphenyl acetate, isoamyl acetate, 2-phenylethyl acetate, ethyl valerate, ethyl hexanoate, ethyl octanoate, and 2-furfuryl acetate. Sun dried Criollo beans exhibited the same composition with different concentration of ester as convective dried and microwave dried Criollo bean samples. As regard to fresh-fermented and freeze-dried bean samples,

only 2-furfuryl acetate was not present in both samples. The highest ester content was observed in freeze-dried Criollo bean samples (2594.4 $\mu\text{g/kg}$), followed by fresh-fermented (1421.6 $\mu\text{g/kg}$) and sun-dried (1334.0 $\mu\text{g/kg}$) beans. Based on the results, it can be confirmed that thermal drying has a significant impact on the ester compounds of Criollo beans. The same observation was made by Frauendorfer and Schieberle (2008) during drying and roasting processes of Criollo beans. The lowest amount of ester (477.1 $\mu\text{g/kg}$) was found in microwave dried bean samples. Notably, ethyl 2-methylbutanoate (426.7 $\mu\text{g/kg}$) were the most prominent ester, followed by 2-phenylethyl acetate (374.2 $\mu\text{g/kg}$), and phenylethyl acetate (367.3 $\mu\text{g/kg}$) in freeze-dried bean sample. Isoamyl acetate (264.9 $\mu\text{g/kg}$) and ethyl 2-methylbutanoate (221.2 $\mu\text{g/kg}$) were also abundant in fresh fermented and sun dried bean samples, respectively. Some of these esters identified in this study have been previously detected in other fermented and dried Criollo beans (Ducki et al., 2008; Portillo et al., 2009; Rodriguez-Campos et al., 2012). Figure 4.17 illustrates the percentage distribution of esters in Criollo beans under fermentation and drying processes relative to the total compound content. In the study performed by Pereira et al. (2017), a significant amount of ester compounds was determined in fermented and dried Criollo beans, in which phenylethyl acetate was identified as the second abundant ester compound.

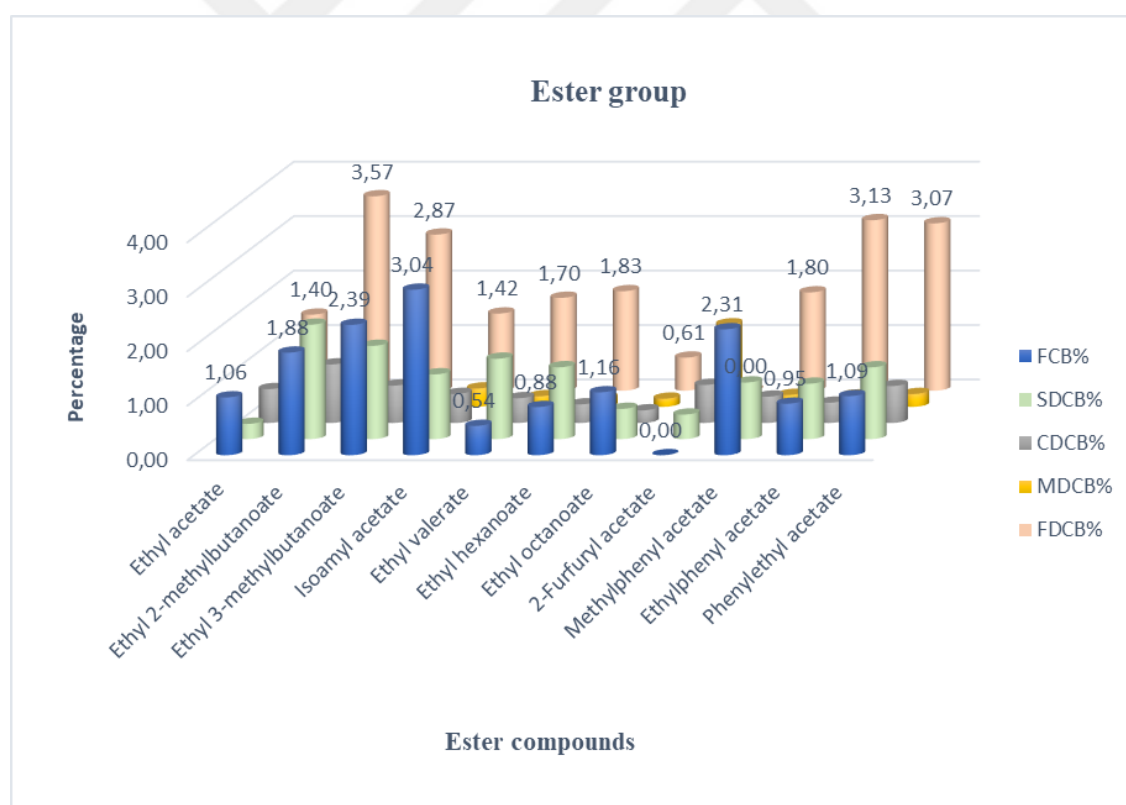


Figure 4.17. The ratio of ester compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Alcohol Compounds

Lipoxygenase and alcohol dehydrogenase (ADH) enzymes, common in various plants, play key roles in generating volatile alcohols that contribute to the aroma profile of cocoa beans via the

lipxygenase pathway. Alcohols, originating from amino acid metabolism, also impart fruity and floral aromas to cocoa beans and associated products (Saltini et al., 2013). In Criollo beans extract obtained from fresh-fermented, 9 alcohols were characterized, while, in sun-dried, convective dried, microwave dried, and freeze-dried bean samples, 8 alcohol compounds with distinct concentration were identified and quantified. Figure 4.18 illustrates the percentage distribution of alcohol compounds in Criollo beans across the different drying processes. Following aldehydes, alcohols constitute the second highest concentration within the aroma fraction of all Criollo bean samples when compared to other volatile compounds. The total alcohol concentration, detailed in Table 4.6, displayed statistically significant differences among the examined dried cocoa bean samples ($p < 0.05$). The total alcohol content of Criollo beans significantly increase in non-thermal drying process from 2901.5 to 3756.6 $\mu\text{g/kg}$, and decreased from 2901.5 up to 1267.7 $\mu\text{g/kg}$ during the thermal drying processes (Table 4.6). Freeze-dried bean samples exhibited the highest alcohol content (3756.6 $\mu\text{g/kg}$), followed by fresh fermented beans (2901.5 $\mu\text{g/kg}$), and sun-dried beans (2395.1 $\mu\text{g/kg}$). The lowest alcohol amounts were observed in microwave-dried (1267.7 $\mu\text{g/kg}$) bean samples, followed by convective dried (1912.4 $\mu\text{g/kg}$) bean samples. A significant decrease in the total alcohol content was noted in convective and microwave dried beans, likely linked to the degradation of volatile constituents due to the applied temperature and time. The majority of these compounds were previously reported in fermented and dried Criollo beans (Portillo et al., 2009; Qin et al., 2017). The volatile compound with the highest concentration was hexanol (1345.8 $\mu\text{g/kg}$) observed in fresh-fermented bean, and freeze-dried (1187.5 $\mu\text{g/kg}$) bean samples, respectively. Following hexanol, 2-phenylethanol was also prominent in freeze-dried (644.2 $\mu\text{g/kg}$) and sun dried (493.2 $\mu\text{g/kg}$) bean samples. Additionally, 2,3-butanediol (633.8 $\mu\text{g/kg}$), and 2-methyl-3-butanol (486.7 $\mu\text{g/kg}$) were notable in freeze-dried, and fermented bean samples, respectively. Kadow et al. (2013) in their study reported that the most predominant alcohol compound in Criollo beans was 2,3-butanediol. In another investigation carried out by Ascrizzi et al. (2019) and Ascrizzi et al. (2017), hexanol and 2-phenylethanol were also the most dominant alcohols in fermented and dried Criollo beans.

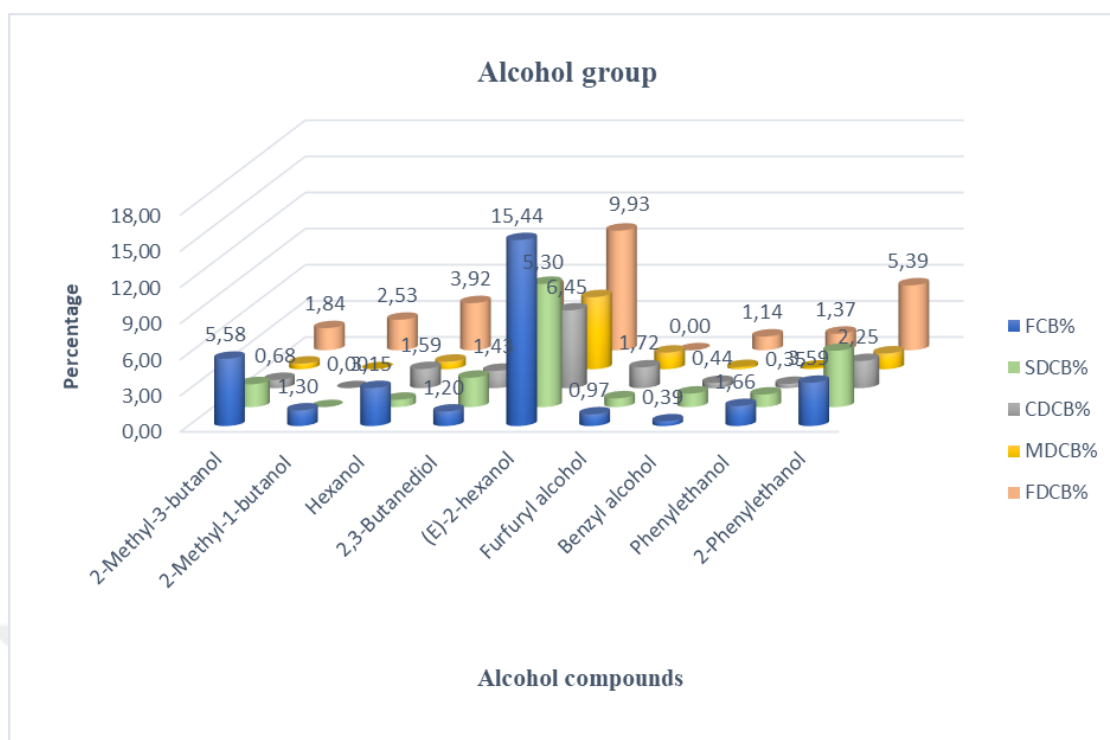


Figure 4.18. The ratio of alcohol compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Aldehyde Compounds

Aldehydes represent a significant group of aroma substances found abundantly in cocoa bean samples. These compounds arise from the Stecker degradation of amino acids and play a crucial role in shaping the aroma profiles of various foods such as tea, wine, cocoa beans, roasted peanuts, and coffee. Aldehydes typically contribute grassy, malty, sweet, floral, fruity, and green plant aromas to foods, especially cocoa beans and related products. In fresh-fermented Criollo beans, 2 aldehydes were detected and quantified, whereas beans obtained from sun, convective, microwave, and freeze-drying processes yielded 7, 8, 8, and 6 aldehydes, respectively. Interestingly, aldehydes constitute the highest concentration within the aroma fraction of all Criollo bean samples when compared to other volatile compounds. Among the drying techniques applied to Criollo bean samples, convective drying exhibited the highest amount of aldehydes (6105.3 $\mu\text{g/kg}$), followed by microwave drying (5324.2 $\mu\text{g/kg}$) and sun drying (3522.0 $\mu\text{g/kg}$) processes. Freeze-dried (2284.0 $\mu\text{g/kg}$) and fresh-fermented (1013.8 $\mu\text{g/kg}$) bean samples presented the lowest amount of aroma compounds. Ascrizzi et al. (2017) reported the majority of the detected aldehyde compounds in their investigation. Similarly, Valle-Epquín et al. (2020) discovered most of the identified aldehydes, and highlighted an important concentration of aldehyde components in sun dried beans. Notably, hexanal was found in high amounts in convective-dried (3831.8 $\mu\text{g/kg}$), sun-dried (2175.4 $\mu\text{g/kg}$), and microwave dried (2036.5 $\mu\text{g/kg}$) Criollo bean samples. The second highest aldehyde detected in Criollo beans was 2-methylbutanal in microwave-dried (1468.5 $\mu\text{g/kg}$) and convective-dried (1287.2 $\mu\text{g/kg}$) Criollo bean samples. Figure 4.19 illustrates the ratio of aldehyde compounds in Criollo bean samples obtained

across fermentation and drying processes, expressed as percentage of the total compounds. In cocoa beans, aldehydes consistently occur in higher quantities compared to other volatile compounds. 2-methylbutanal was previously identified in fermented and dried Criollo beans as the most represented aldehyde compounds (Ascrizzi et al., 2017; Bonvehí, 2005).

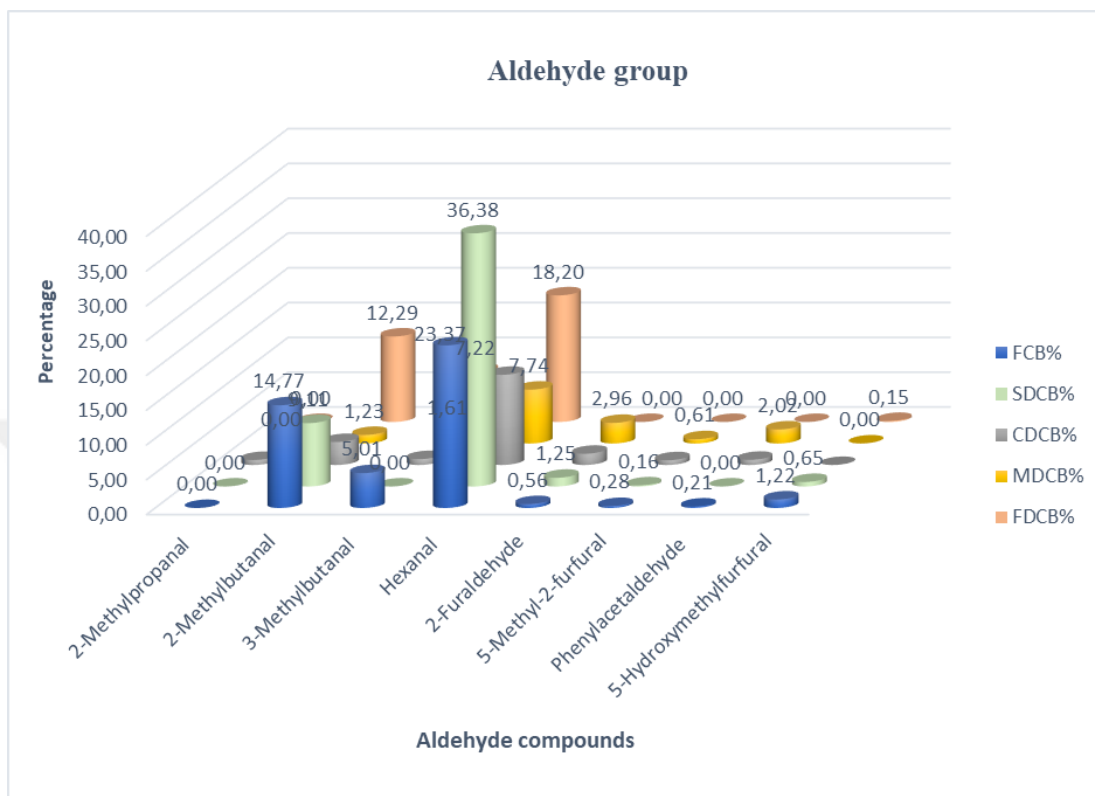


Figure 4.19. The ratio of aldehyde compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Terpene Compounds

Monoterpene compounds, especially the oxygenated ones, lend chocolate its sweet and floral aroma notes (Bonvehí, 2005). Eight terpenes, including p-cymen, ocimene, (*E*)-caryophyllene, D-limonene, thymol, geraniol, α -pinene, and camphene were identified and quantified solely in fermented Criollo beans. The same number of terpenes with different amount was detected and quantified in sun-dried Criollo bean sample, while convective dried and microwave dried Criollo beans contained seven (p-cymen, linalool, (*E*)-caryophyllene, D-limonene, camphene, thymol, geraniol) and four (p-cymen, linalool, (*E*)-caryophyllene, D-limonene) terpene compounds, respectively. The total amount of these compounds was 1905.5 $\mu\text{g/kg}$, 1684.5 $\mu\text{g/kg}$, 1380.6 $\mu\text{g/kg}$, 1250.6 $\mu\text{g/kg}$, and 1149.1 $\mu\text{g/kg}$ in freeze-dried, fresh fermented beans, sun dried, convective dried, and microwave Criollo bean samples, respectively. Freeze-dried Criollo beans exhibited the highest concentration (1905.5 $\mu\text{g/kg}$) of esters among the drying processes for ester compounds. These compounds have already been characterized in different fermented, dried and roasted Criollo beans (Ascrizzi et al., 2017; Frauendorfer & Schieberle, 2006). Figure 4.20 illustrates the percentage distribution of terpene compounds in only fermented and dried beans relative to the total compound

content. These terpenes represented approximately 9.75%, 10.16%, 13.10%, 15.94%, and 19.32% of the total volatiles analyzed in Criollo beans obtained from convective dried, microwave dried, sun dried, freeze-dried, and fresh fermented beans, respectively. As demonstrated in Table 4.6, the concentration of total terpenes exhibited a decreasing trend along drying processes. The aromatic extract of fresh fermented and dried Criollo beans revealed the highest concentrations of linalool, ranging between 0.0 and 496.5 $\mu\text{g/kg}$, followed by D-limonene (26.7-475.2 $\mu\text{g/kg}$), and (*E*)-Caryophyllene (19.5-451.9 $\mu\text{g/kg}$) among the terpenes. Interestingly, linalool was determined in elevated concentration in previous published papers (Ascrizzi et al., 2017; Frauendorfer & Schieberle, 2008).

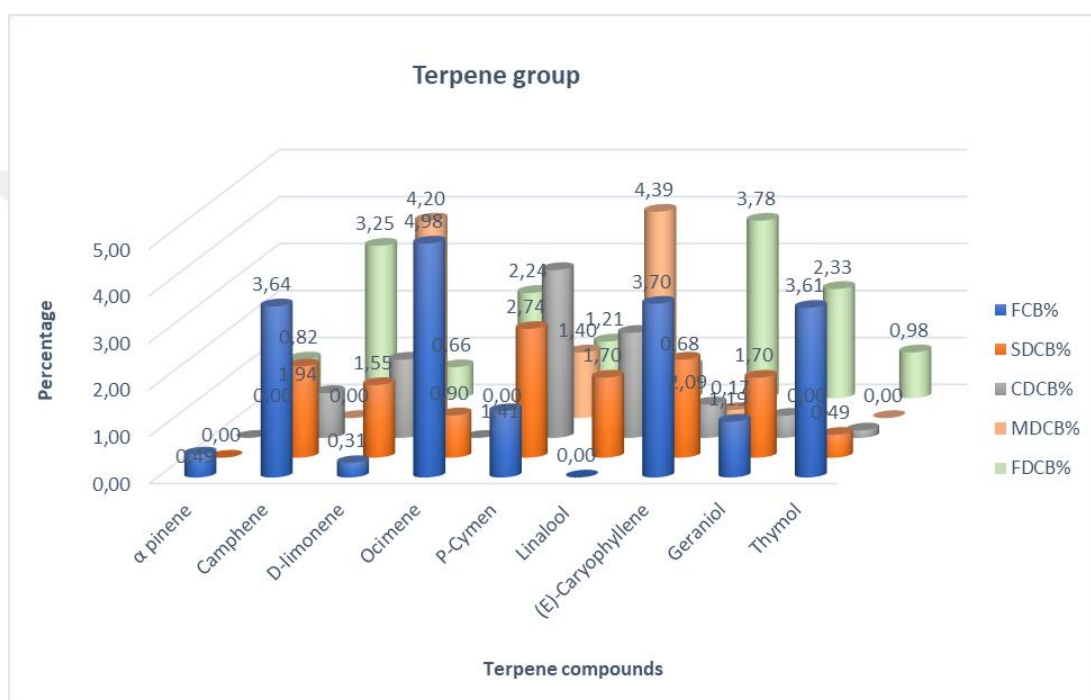


Figure 4.20. The ratio of terpene compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Ketone Compounds

In Criollo bean extracts obtained from fresh-fermented, sun-dried, convective dried, microwave dried, and freeze-dried samples, five, five, three, two, and five ketones were discovered, respectively. Figure 4.21 illustrates the percentage distribution of ketone compounds in Criollo beans through drying processes relative to the total compound content. The total content of ketones in freeze-dried and fresh-fermented bean samples were 92.6 and 221.2 $\mu\text{g/kg}$, respectively. An increase of aroma compounds was observed in microwave dried (550.9 $\mu\text{g/kg}$), convective dried (414.3 $\mu\text{g/kg}$), and sun dried (309.9 $\mu\text{g/kg}$) bean samples. Mohamadi Alasti et al. (2019) confirmed that the majority of ketones were formed during drying and roasted processes, which is in compliance with our results. Álvarez et al. (2019) and Álvarez et al. (2023) registered some of the detected ketone compounds in fermented and dried Venezuela Criollo beans. Moreover, Marseglia et al. (2020) also reported these detected ketones in their study, and highlighted that ketone was one of the most

important group, giving Criollo and its products a characteristic and fine flavor. Among the ketones class, 2-heptanone represented the highest amount, with concentrations of 301.2 and 238.0 $\mu\text{g/kg}$ in microwave dried and convective dried bean samples, respectively. Following, 3-hydroxy-2 butanone exhibited high concentration in microwave dried (249.7 $\mu\text{g/kg}$), and convective dried (176.3 $\mu\text{g/kg}$) bean samples. 2-heptanone was also found in high concentration in Criollo fermented and dried beans (Marseglia et al., 2020).

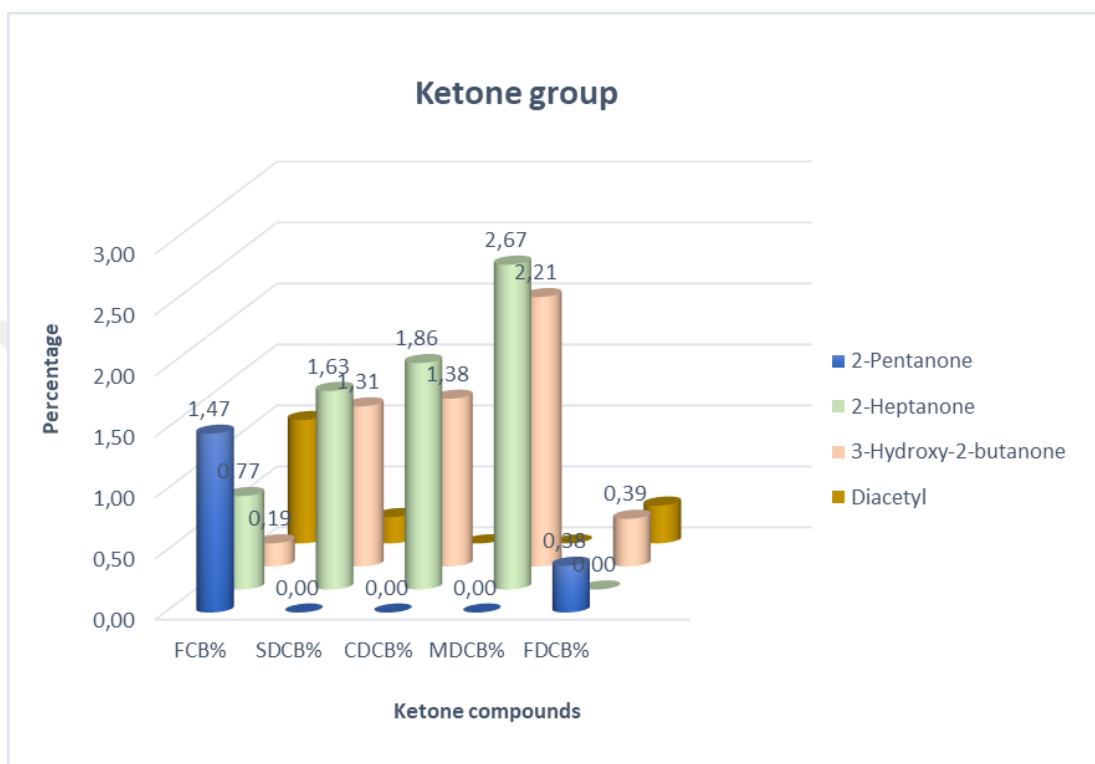


Figure 4.21. The ratio of ketone compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Furan Compounds

In sun-dried Criollo beans, one furan compound (2-acetylfuran) was detected and quantified, while in convective dried and microwave dried Criollo bean samples, two furans (2-pentylfuran and 2-acetylfuran,) and one furanone (5-methyl 2(5H)-furanone) with different concentration were determined. Microwave drying process exhibited the highest amount of volatile compounds (245.0 $\mu\text{g/kg}$), followed by convective drying process (226.4 $\mu\text{g/kg}$). These compounds have also been observed in roasted Criollo beans (Frauendorfer & Schieberle, 2008). Among all volatile compounds characterized in Criollo bean samples, 5-methyl 2(5H)-furanone was found with the highest amount (108.8 $\mu\text{g/kg}$), followed by 2-pentylfuran (106.5 $\mu\text{g/kg}$) in microwave dried Criollo beans. As already mentioned furans are typically formed from the interaction of amino acids and sugars through the Maillard reaction and Strecker degradation (Yıldız et al., 2010). Interestingly, in the study, furans and furanones were not detected in fermented and freeze-dried beans, which aligns with the findings reported by some researchers (Chetschik et al., 2019; Quelal et al., 2023).

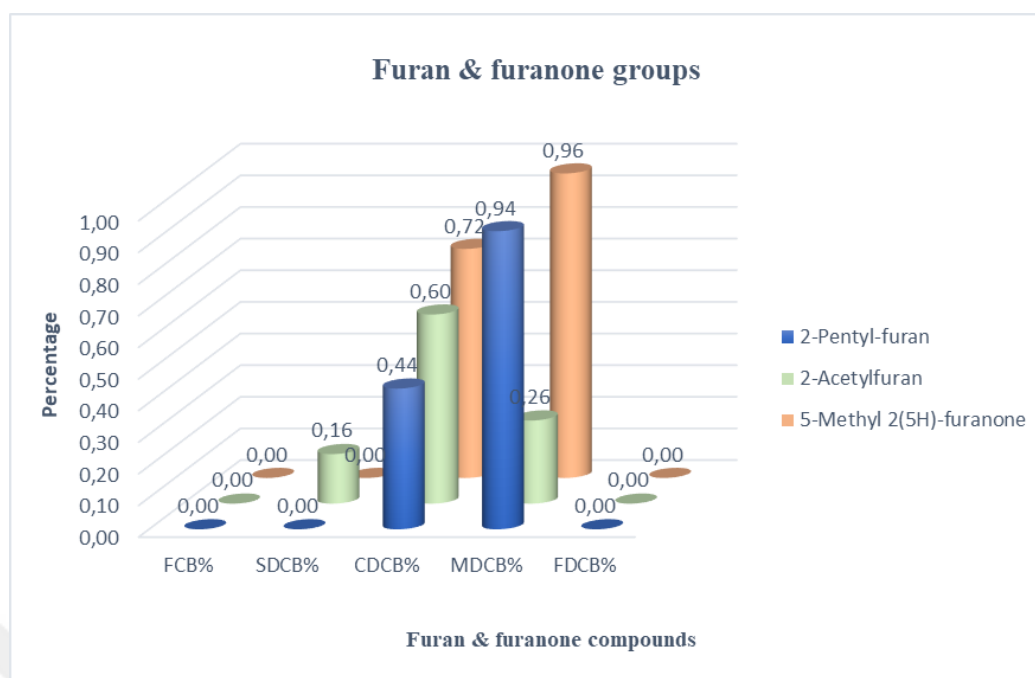


Figure 4.22. The ratio of furan and furanone compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Pyrazine Compounds

A total of seven different pyrazine compounds comprising, 2-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and 2,3-dimethylpyrazine were detected and quantified in dried Criollo bean samples (Table 4.6). The total content of pyrazines in microwave dried bean samples (1252.2 $\mu\text{g/kg}$) was higher than in convective dried (758.4 $\mu\text{g/kg}$) and sun-dried (151.1 $\mu\text{g/kg}$) bean samples, while no pyrazine compounds were observed in fresh fermented and freeze-dried beans. The majority of these pyrazines have been previously observed in dried and roasted Criollo beans from various origins (Álvarez et al., 2023; Marseglia et al., 2020; Rojas S et al., 2020; Velásquez-Reyes et al., 2023). As already mentioned, this observation confirms that pyrazines are primarily the products of the Maillard reaction under heating conditions. Among all the pyrazine compounds identified in Criollo bean samples, 2-ethylpyrazine (418.0 $\mu\text{g/kg}$) had the highest concentration, followed by 2,5-dimethylpyrazine (246.7 $\mu\text{g/kg}$) and 2-ethyl-5-methylpyrazine (201.8 $\mu\text{g/kg}$) in microwave dried, convective dried, and microwave dried bean samples, respectively. Velásquez-Reyes et al. (2023) reported 2,5-dimethylpyrazine as one of the most important pyrazine component in dried and roasted Criollo beans. Among the pyrazines detected, methylpyrazines may be the key markers in dried and roasting, as they significantly impart the cocoa aroma (Misnawi et al., 2004).

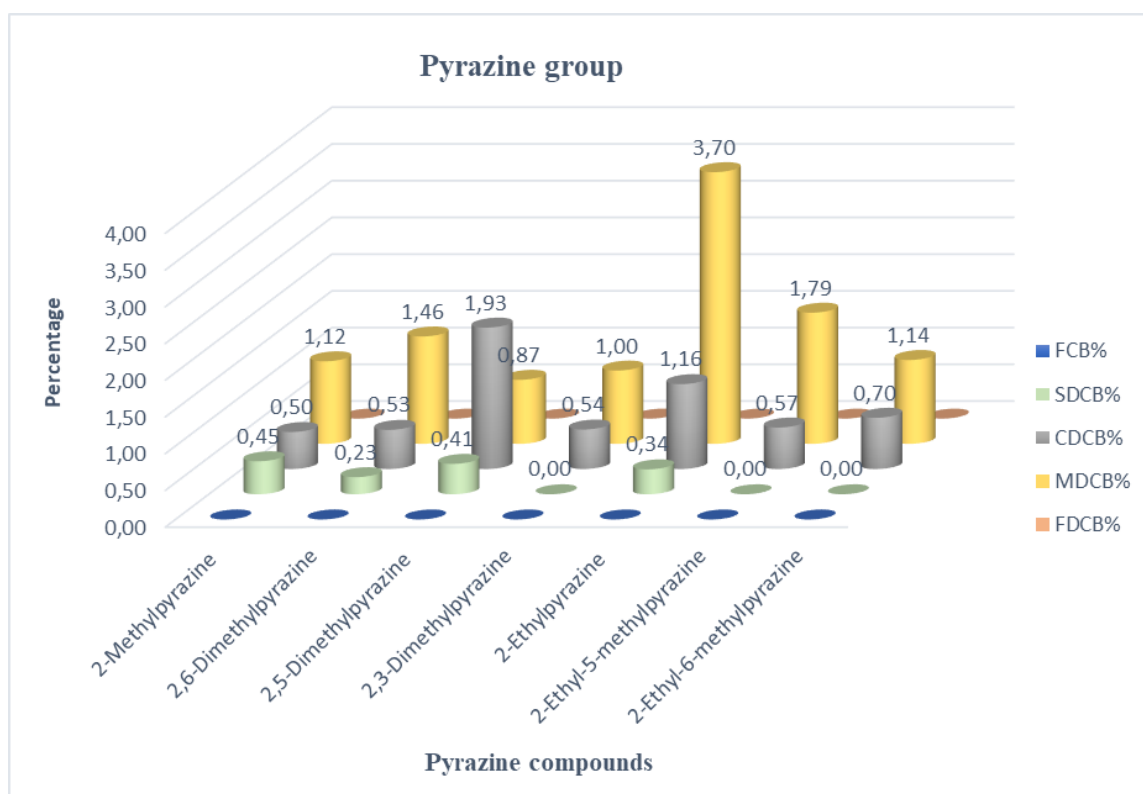


Figure 4.23. The ratio of pyrazine compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Acid Compounds

Six acids, including acetic acid, hexanoic acid, octanoic acid, nonanoic acid, phenylacetic acid, and pentanoic acid were determined in Criollo bean samples. In fresh-fermented and freeze-dried bean samples, a total of six acid compounds were discovered, whereas, five acids (acetic acid, hexanoic acid, octanoic acid, nonanoic acid, phenylacetic acid) were characterized in sun dried and convective dried bean samples. In microwave dried samples, only four acids (acetic acid, hexanoic acid, octanoic acid, nonanoic acid) were detected and quantified. The Total amounts of these compounds ranged between 18.9 and 714.9 $\mu\text{g/kg}$, with the highest concentration observed in fresh-fermented bean samples (1204.2 $\mu\text{g/kg}$), followed by freeze-dried bean samples (1067.1 $\mu\text{g/kg}$), and sun dried bean samples (1013.8 $\mu\text{g/kg}$). The ratio of acid compounds in Criollo beans obtained through the drying processes, relative to the total compound content in percent, is illustrated in Figure 4.23. Frauendorfer and Schieberle (2008) and Qin et al. (2017) also reported these volatile acids in fresh fermented and dried Criollo beans. Acids play a pivotal role in shaping cocoa flavor, particularly through the process of cocoa bean acidification by acetic acid during fermentation. This acidification triggers a cascade of biochemical transformations essential for the development of cocoa flavor profiles (Wang & Ho, 2009; Żyżelewicz et al., 2014). Acids have been identified as providing sour and pungent sensations in fermented and dried cocoa beans. Among the acid compounds listed in Table 4.6, acetic acid exhibited the highest concentration in Criollo beans obtained from fresh-fermented beans (714.9 $\mu\text{g/kg}$), freeze-dried beans (573.9 $\mu\text{g/kg}$), and sun dried beans (494.3 $\mu\text{g/kg}$).

Hexanoic acid (294.2 µg/kg) and phenylacetic acid (136.8 µg/kg) were also found in high concentration in Criollo beans obtained from sun dried and fresh fermented bean samples, respectively. Certain studies have demonstrated that chocolates made from Criollo beans tend to display more pronounced acidic tones, which are associated with acetic acid, compared to chocolates made from Forastero and other cocoa cultivars (Acierno et al., 2016; Frauendorfer & Schieberle, 2008). Acetic acid was reported in elevated concentration in fermented and dried Criollo beans (Qin et al., 2017). The same observation was made by (Velásquez-Reyes et al., 2023) in their recent study on fermented and Criollo beans.

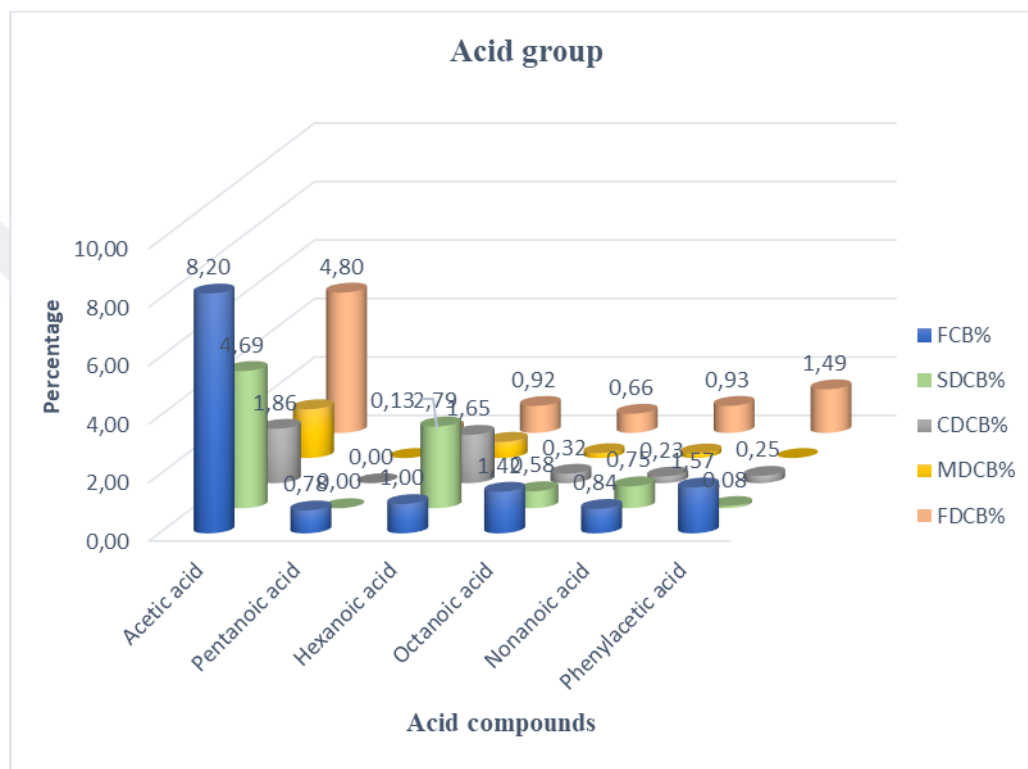


Figure 4.24. The ratio of acid compounds in the fermented and dried Criollo cocoa beans according to the amount of total compounds in percent

Other Aroma Compounds

Four distinct groups of aroma compounds lactones, pyrroles, pyrroline and volatile phenols, were determined in fresh-fermented and dried Criollo bean samples. Among these volatile compounds, three lactones were identified in dried Criollo bean samples, while only one (γ -butyrolactone) was determined in fresh-fermented bean samples. These identified lactones have been formerly observed in fermented and dried Criollo beans (Yang et al., 2024). Convective dried bean samples exhibited the highest amount (423.2 µg/kg) of lactone, followed by microwave dried (400.4 µg/kg) bean samples, while the lowest total amount (26.7 µg/kg) was quantified in fresh-fermented bean samples. Among lactone compounds, γ -butyrolactone (249.2 µg/kg) was determined in the highest quantity in microwave dried bean samples. This compound was previously detected as the most abundant lactone in fermented and dried Criollo beans (Qin et al., 2017; Yang et al., 2024),

which is in accordance with the findings of our study. Two pyrroles (2-acetylpyrrole and 1-methylpyrrole) were exclusively detected in microwave dried (256.8 $\mu\text{g/kg}$), convective dried (80.7 $\mu\text{g/kg}$), while one pyrrole (2-acetylpyrrole) was detected in sun dried bean samples. These compounds, like Pyrazines are the main compounds formed through the reactions that occur during drying and roasting, such as Strecker degradation and Maillard reaction (Bonvehí, 2005; Granvogl et al., 2006; Hoskin & Dimick, 1994; Serra Bonvehí & Ventura Coll, 2002), which confirmed the fact that no pyrroles were observed in fresh fermented and freeze-dried bean samples. Of all pyrroles detected, 2-acetylpyrrole represented the highest amount (149.9 $\mu\text{g/kg}$) in microwave dried bean sample. As already mentioned, volatile phenol compounds, commonly present in fermented and dried cocoa beans, contribute to fragrance and impart distinctive flavors, including bitter and pungent organoleptic notes. In Criollo bean samples, three volatile phenols, including guaiacol, 3,4-dimethylphenol, and eugenol were identified and quantified. The total amount of volatile phenols ranged from 12.5 to 163.9 $\mu\text{g/kg}$. The highest amount of volatile phenols was observed in fresh-fermented bean samples (252.4 $\mu\text{g/kg}$), followed by freeze-dried bean samples (144.6 $\mu\text{g/kg}$). Guaiacol (163.9 $\mu\text{g/kg}$) was the prominent volatile phenol detected in fresh-fermented Criollo bean samples, followed by eugenol (63.5 $\mu\text{g/kg}$), and 3,4-dimethylphenol (57.4 $\mu\text{g/kg}$) in freeze-dried and microwave dried bean samples, respectively. Some of these compounds were also detected in Criollo fermented, dried, and roasted beans (Velásquez-Reyes et al., 2023).

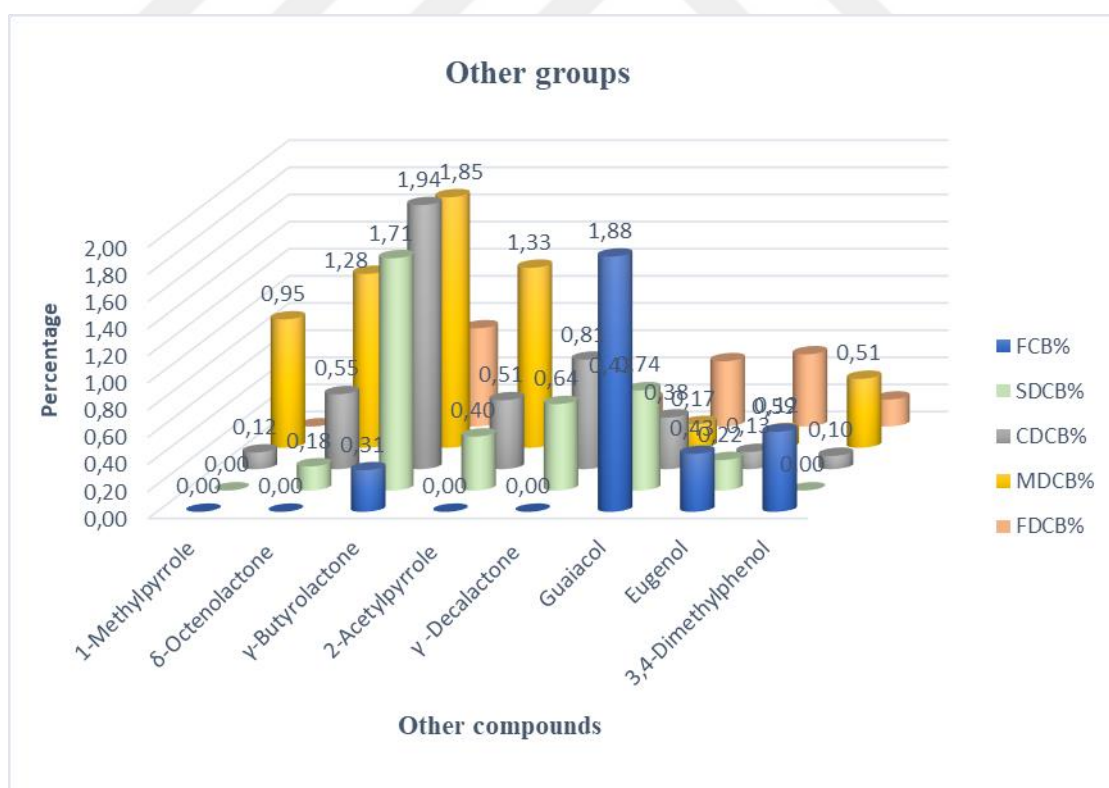


Figure 4.25. The ratio of other compounds in the fermented and Criollo cocoa beans according to the amount of total compounds in percent

4.7.1.3. Effect of Drying Methods on Aroma Compounds in Nacional Beans

Ester Compounds

In the analysis of Nacional beans, eleven esters, encompassing diacetyl, ethyl acetate, ethyl 2-methylbutanoate, ethylbutanoate, methylbutyl acetate, methyl salicylate, methyl octanoate, phenylethyl acetate, 2-methylpropyl acetate, 3-methylbutyl acetate, and 2-furfuryl acetate were identified and quantified. Interestingly, freeze-dried Nacional beans exhibited consistent ester compositions compared to fresh fermented bean, convective-dried and microwave dried samples, with variations observed in the concentrations of individual ester. For fresh-fermented and freeze-dried bean samples, only 2-furfuryl acetate and 2-methylpropyl acetate were absent in both cases. Freeze-dried Nacional bean sample displayed the highest ester content (1906.6 $\mu\text{g/kg}$), followed by fresh-fermented beans (1475.4 $\mu\text{g/kg}$) and sun-dried beans (1141.8 $\mu\text{g/kg}$). In contrast, microwave-dried bean samples exhibited the lowest ester content (869.4 $\mu\text{g/kg}$). These compounds have been previously characterized by numerous workers in fermented and dried Nacional beans (Cevallos-Cevallos et al., 2018; CRUZ-ROJAS et al., 2023; Ramos et al., 2014). Notably, phenylethyl acetate was the most prominent ester in freeze-dried bean samples (373.3 $\mu\text{g/kg}$), followed by methyl salicylate (369.4 $\mu\text{g/kg}$) in fresh fermented bean samples, and 2-methylbutyl acetate (367.3 $\mu\text{g/kg}$) in freeze-dried bean samples. Some of these predominant esters have been previously detected in other fermented and dried Nacional beans (Ramos et al., 2014). For instance, Huang and Barringer (2011) presented phenylethyl acetate as predominant ester in fermented, dried and roasted Nacional beans and cocoa hybrids. In a similar study conducted by Erazo Solorzano, Tuárez García, et al. (2023), phenylethyl acetate and methylbutyl acetate were the predominant esters in fermented and dried Nacional beans. These compounds have been already reported to be the products of fermentation produce by the yeasts (Koné et al., 2016; Rottiers, Tzompa Sosa, De Winne, et al., 2019; Rottiers, Tzompa Sosa, Lemarcq, et al., 2019), which confirmed the high concentration of these compounds in fermented and freeze-dried bean samples observed in our study. Figure 4.25 illustrates the percentage distribution of esters in Nacional beans during fermentation and drying processes relative to the total compounds content.

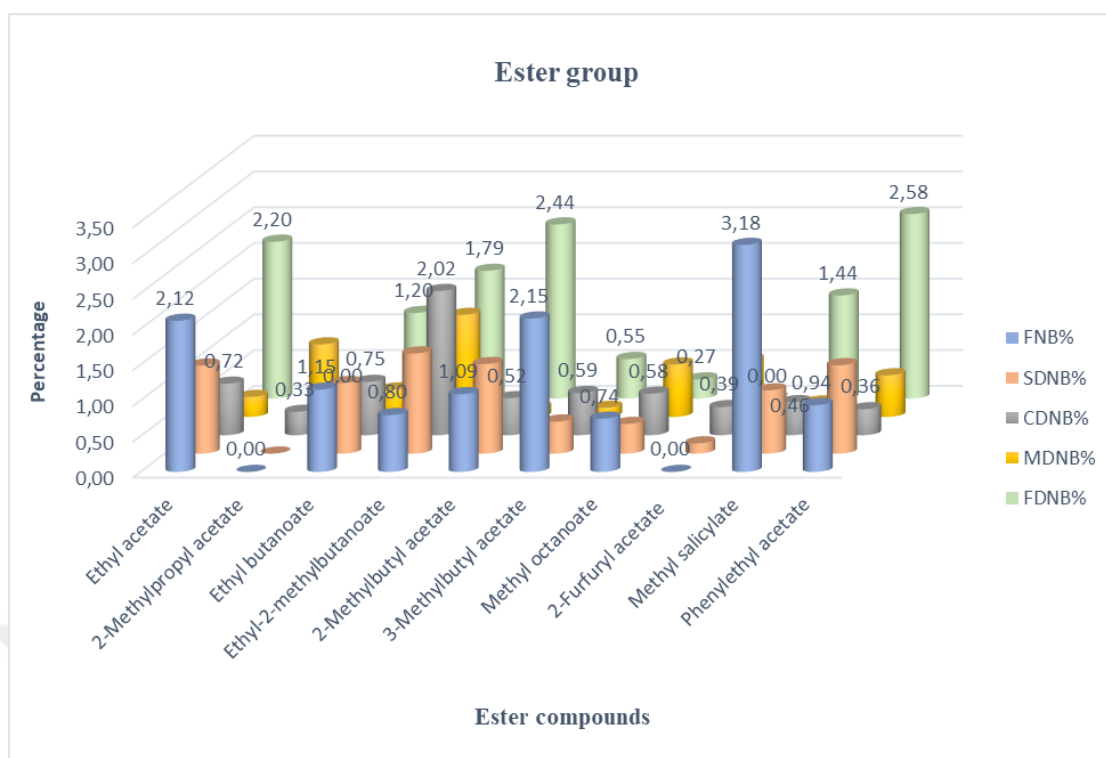


Figure 4.26. The ratio of ester compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Alcohol Compounds

In the analysis of Nacional beans, ten distinct alcohol compounds with different amount were characterized in the aroma extract obtained from fresh-fermented bean, convective-dried, microwave-dried, and freeze-dried bean samples. In comparison, sun-dried revealed twelve alcohol compounds with varying concentrations. Figure 4.26 illustrates the percentage distribution of alcohol compounds in Nacional beans across different drying processes. Table 4.7 details the total alcohol concentration, which showed statistically significant differences among the examined dried cocoa bean samples ($p < 0.05$). It was observed that the total alcohol content of Nacional beans significantly decreased during convective and microwave drying processes, while an increase in alcohols concentration was noted in freeze-dried bean sample (Table 4.7). Among the drying methods, freeze-dried bean sample exhibited the highest alcohol content (6102.0 $\mu\text{g/kg}$), followed by fresh-fermented bean sample (4137.2 $\mu\text{g/kg}$), and sun-dried beans (3930.5 $\mu\text{g/kg}$). The elevated amount of alcohol compound in freeze-dried beans compared to fresh-fermented beans could be explained by the fact that some compounds, such short chain acid may be converted to alcohol compounds, and increase its concentration by certain microorganisms, especially *Thermoanaerobacter strains* in moderated temperature (Scully & Orlygsson, 2020). Microwave-dried bean samples exhibited the lowest alcohol amounts (1285.2 $\mu\text{g/kg}$), followed by convective-dried bean sample (2058.5 $\mu\text{g/kg}$). The significant reduction of the total alcohol content in convective and microwave-dried beans could be attributed to the thermal degradation of volatile constituents due to the applied temperature and time.

The majority of the decripted alcohol compounds have been formerly determined in fermented, dried and roasted Nacional beans from different origins (Cevallos-Cevallos et al., 2018). The most abundant volatile compound was (*E*)-2-hexanol (1265.0 µg/kg), observed in freeze-dried bean sample (Table 4.7). In fact, numerous researchers (Calva-Estrada et al., 2020; Tuentner et al., 2020) also evidenced that (*E*)-2-hexanol was predominant in fermented and dried Nacional beans. Following (*E*)-2-hexanol, 2-penten-1-ol was also prominent in freeze-dried (863.8 µg/kg) and sun-dried (568.5 µg/kg) bean samples. Additionally, 2-methylbutan-2-ol (792.0 µg/kg), 1-penten-3-ol (738.9 µg/kg) and 2,3-butandiol (621.9 µg/kg) were notable in freeze-dried bean sample, while hexanol (622.9 µg/kg) was observed in high amount after (*E*)-2-hexanol (1068.4 µg/kg) in fresh-fermented bean sample. These compounds have demonstrated for contributing to the green, fruity, fresh and floral notes to Nacional beans and final products (Braga et al., 2018; Calva-Estrada et al., 2020). For instance, 2,3-butanediol, another desirable aroma component for high-quality cocoa products associated to sweet and floral odours, is present in substantial amounts to impact the aroma of chocolate due to its good stability during the manufacture of chocolate (Ascrizzi et al., 2017; Tran et al., 2016).

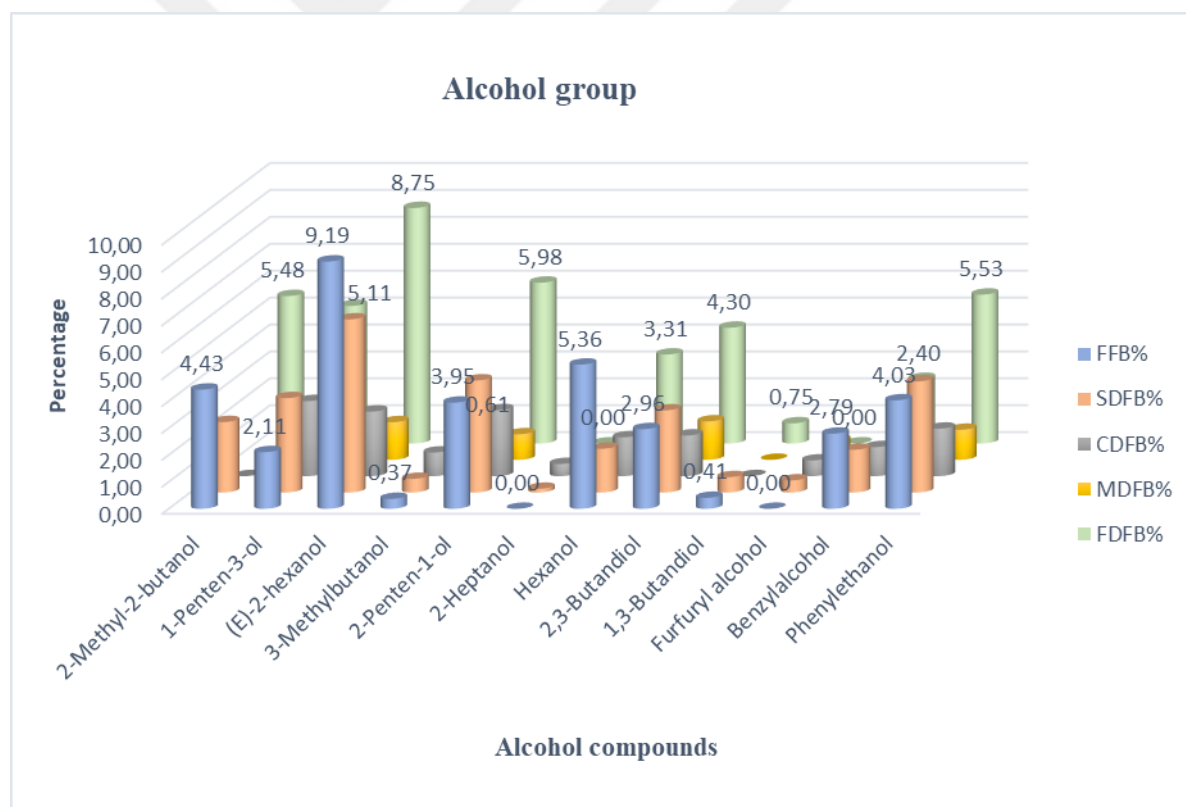


Figure 4.27. The ratio of alcohol compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Aldehyde Compounds

It is well known that aldehydes usually contribute to the grass and green plant aroma of cocoa beans and other fruits. Most of aldehyde compounds are the results of Strecker degradation of free amino acids during drying and roasting, which are crucial for the formation of cocoa aroma (Silwar,

1988). The number of aldehydes detected and quantified in Nacional bean extracts isolated from fermented and dried Nacional bean samples was a total of eight. As shown in table 4.7, 7 aldehydes were determined in Nacional beans obtained from freeze-drying, microwave drying, and convective drying process. The same number was also characterized in fresh-fermented bean samples, while 8 aldehydes were detected and quantified in sun-dried bean samples. Among the aroma compounds characterized, aldehydes was the most prominent in concentration through the Nacional bean samples, especially microwave dried and convective dried Nacional beans compared to other volatile compounds. Microwave drying exhibited the highest amount of aldehydes (9279.6 $\mu\text{g/kg}$) among the drying techniques applied to Nacional bean samples, followed by convective dried bean sample (7997.5 $\mu\text{g/kg}$), sun-dried bean sample (5710.2 $\mu\text{g/kg}$). Interestingly, freeze-dried and fresh-fermented beans presented the lowest amount of aroma compounds. The increasing amount of aldehyde in microwave-dried, convective-dried, and sun-dried bean samples is probably due to the significant formation of strecker aldehydes in Nacional dried bean samples. Certain of the identified and quantified compounds have been previously reported in fresh fermented, dried and roasted Nacional beans and cocoa hybrids from different regions by numerous researchers (Calva-Estrada et al., 2020; Cevallos-Cevallos et al., 2018; da Veiga Moreira et al., 2018). Of all aldehydes constituents detected, 3-methylbutanal was found in high amounts in microwave dried (4695.1 $\mu\text{g/kg}$), convective dried (3125.9 $\mu\text{g/kg}$), and sun dried (2019.0 $\mu\text{g/kg}$) Nacional bean samples. The second-highest aldehyde detected in Nacional beans was hexanal in convective-dried (3682.5 $\mu\text{g/kg}$), microwave-dried (2978.4 $\mu\text{g/kg}$), and sun dried (2019.0 $\mu\text{g/kg}$) Nacional bean samples. Figure 4.28 illustrates the ratio of aldehyde compounds in Nacional bean samples obtained through drying processes, expressed as a percentage of the total compounds. As already discussed, in cocoa beans, aldehydes and ketones consistently occur in higher quantities compared to other volatile compounds (Jinap et al., 1998; Tigrero-Vaca et al., 2022).

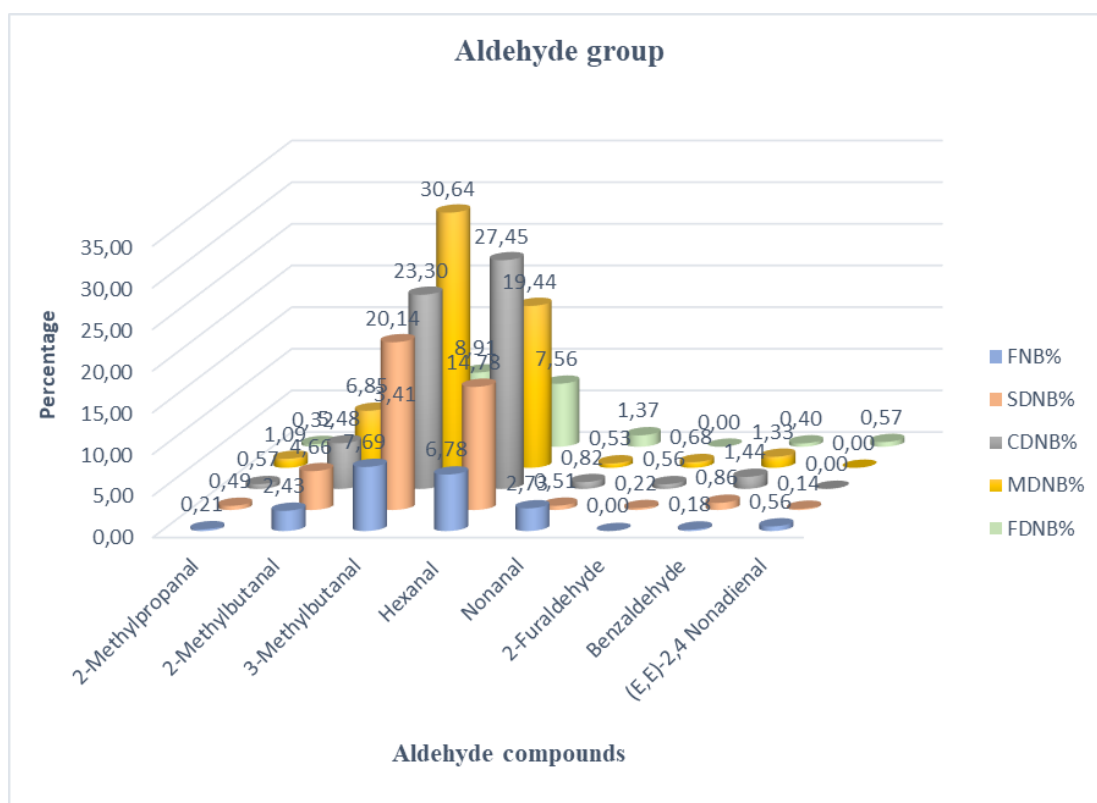


Figure 4.28. The ratio of aldehyde compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Terpene Compounds

In the analysis of Nacional bean samples, eight terpenes, including *p*-cymene, ocimene, (*E*)-caryophyllene, β -aromadendrene, valencene, α -phellandrene, linalool and sabinene were identified and quantified. Fresh-fermented bean sample, sun dried, and freeze-dried bean samples displayed the same number of terpene substances. In convective dried bean sample, 4 terpenes (sabinene, ocimene, β -aromadendrene, and valencene) was discovered, while in microwave dried bean sample, three terpenes (ocimene, β -aromadendrene, and valencene) with various amount were identified and quantified. The total amount of these compounds was 1842.9 $\mu\text{g/kg}$ in fresh fermented bean, 1773.8 $\mu\text{g/kg}$ in freeze-dried bean, 1180.3 $\mu\text{g/kg}$ in sun dried beans, 958.4 $\mu\text{g/kg}$ in microwave dried beans, and 718.5 $\mu\text{g/kg}$ in convective dried beans. Fresh-fermented Nacional bean exhibited the highest concentration (1842.9 $\mu\text{g/kg}$) of terpenes among the drying processes. The lowest concentration of terpenes was observed in microwave dried bean (958.4 $\mu\text{g/kg}$), followed by convective dried bean sample (718.5 $\mu\text{g/kg}$). From these observations, it is clear that thermal process had a significant effect on the composition of terpenes in dried Nacional beans. These detected terpenes have already been revealed in fermented and dried Nacional beans (Cevallos-Cevallos et al., 2018). Figure 4.29 illustrates the percentage distribution of terpene compounds in fresh fermented and dried beans relative to the total compound content. The terpenes identified, represented approximately 5.33%, 6.25%, 8.64%, 12.27%, and 15.85% of the total volatiles analyzed in Nacional beans obtained from convective dried, microwave-dried, sun dried, freeze-dried, and fresh-fermented bean samples,

respectively. As demonstrated in Table 4.7, the concentration of total terpenes exhibited a decreasing trend along the drying processes. Most of these constituents were reported from fermented and dried Nacional beans for the first time. The aromatic extracts obtained from microwave dried beans revealed the highest concentrations of β -aromadendrene (624.5 $\mu\text{g/kg}$), followed by linalool (432.2 $\mu\text{g/kg}$) in fresh fermented bean, and α -phellandrene (396.1 $\mu\text{g/kg}$) in freeze-dried bean sample among the terpene substances. β -aromadendrene, one of the main terpene, specially a sesquiterpene detected by Cevallos-Cevallos et al. (2018) in their study has been demonstrated to contribute to the fruity, green and woody notes in fermented, dried and roasted Nacional beans and products.

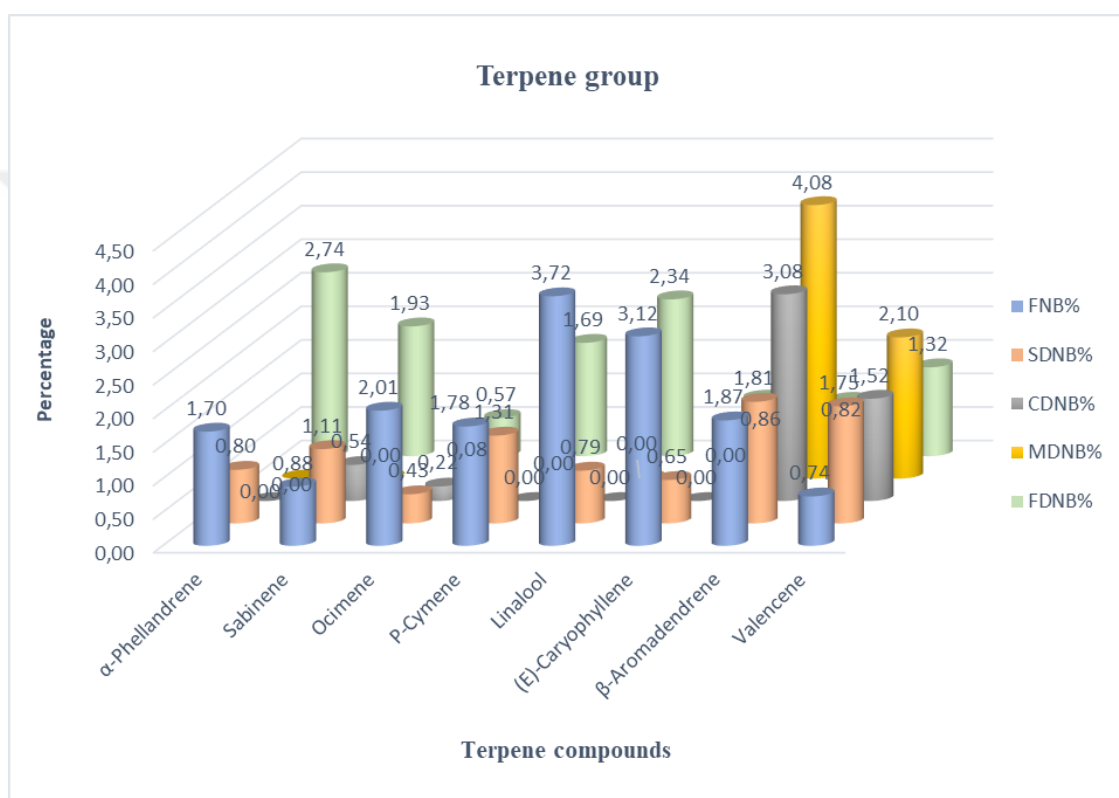


Figure 4.29. The ratio of terpene compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Ketone Compounds

In the analysis of aroma extracts from fresh-fermented bean, sun-dried, microwave dried, and freeze-dried Nacional bean samples, 3 ketones (3-hydroxy-2-butanone, 2-pentanone, and acetophenone) with different amount were identified and quantified, while 2 ketones (3-hydroxy-2-butanone, 2-pentanone) were determined in convective dried bean samples. The distribution of ketone compounds in Nacional beans during the drying processes relative to the total compound content is depicted in Figure 4.30. The total ketones content in fresh fermented bean and convective dried bean was 480.5 and 489.3 $\mu\text{g/kg}$, respectively. The lowest amount of ketones (143.8 $\mu\text{g/kg}$) was determined in freeze-dried bean sample. Among the ketone class analyzed in Nacional beans, acetophenone represented the highest amount (297.4 $\mu\text{g/kg}$) in fresh-fermented beans, followed by

3-hydroxy-2-butanone with a concentration 372.1 $\mu\text{g/kg}$ and 228.8 $\mu\text{g/kg}$ in convective dried and sun-dried samples, respectively. Following this, 2-pentanone (189.5 $\mu\text{g/kg}$) also exhibited high concentrations in microwave-dried bean sample. From our knowledge these ketone compounds identified and quantified in Nacional beans were reported for the first time. These compounds in high concentration have been associated to fruity and flowery notes, which is favorable for cocoa quality.

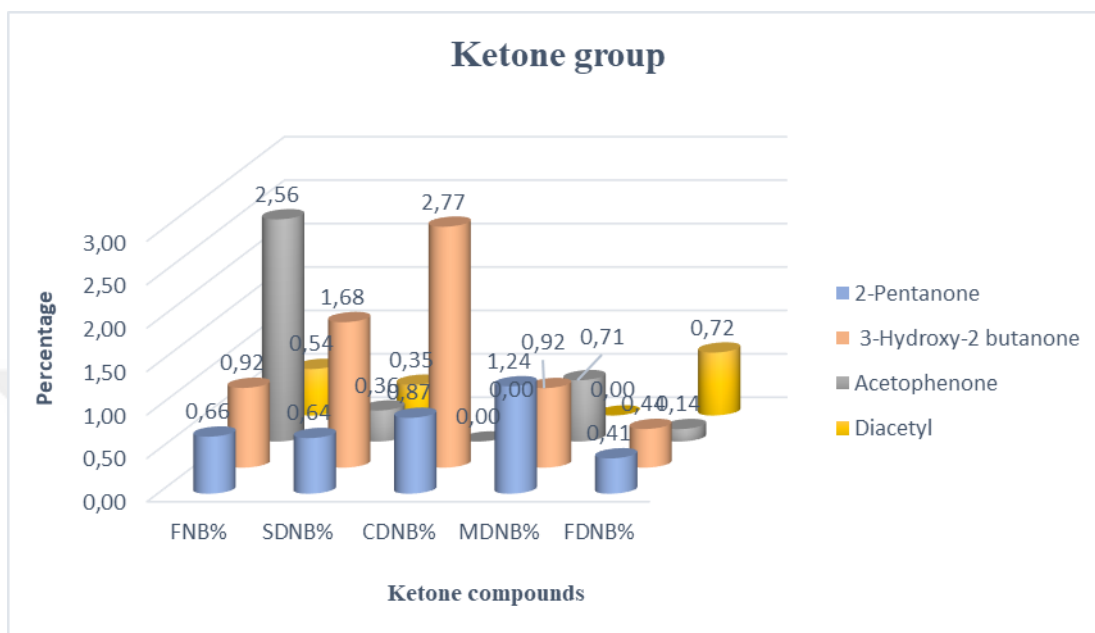


Figure 4.30. The ratio of ketone compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Furan Compounds

In sun-dried Nacional bean sample, 2-acetylfuran with low limit of detection ($< \text{LOD}$) was identified and quantified, while in convective-dried and microwave-dried Nacional bean samples, two furans (2-acetylfuran and 2-acetyl-5-methylfuran) with distinct concentration were determined. Among the drying processes, microwave drying exhibited the highest amount (144.7 $\mu\text{g/kg}$) of volatile compounds, followed by the convective drying process with a concentration of 121.8 $\mu\text{g/kg}$. Among all volatile compounds characterized in Nacional bean samples, 2-acetyl-5-methylfuran was found as the highest amount (111.0 $\mu\text{g/kg}$) of furans in microwave dried bean, followed by 2-acetylfuran (62.8 $\mu\text{g/kg}$) in sun-dried bean samples. Interestingly, in the study, furans were not detected in fresh-fermented and freeze-dried bean samples, which confirm the fact that furan compounds are typically formed via the interaction of amino acids and sugars through the Maillard reaction and Strecker degradation under heating conditions. As already mentioned, these observations is consistent with finding reported by some researchers (Yıldız et al., 2010). These two furan compounds were identified and quantified for the first time in dried Nacional bean samples. 2-acetyl-5-methylfuran has been reported to contribute to the roasted-nut and caramel-like notes to dried, roasted cocoa, coffee and some other seeds (Leite et al., 2013; Spada et al., 2024; Spada et al., 2022).

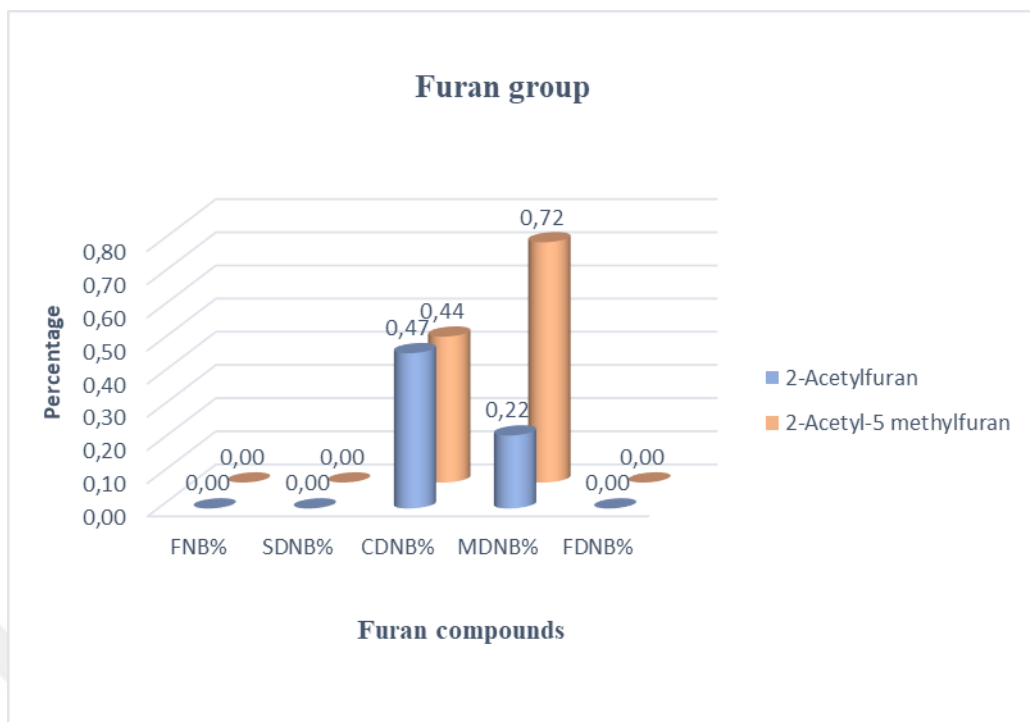


Figure 4.31. The ratio of furan compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Pyrazine Compounds

In dried Nacional bean samples, a total of seven different methyl and ethylpyrazine compounds were detected and quantified, these included 2-methylpyrazine, 2-ethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-methyl-5-ethylpyrazine, 2,3-dimethylpyrazine, and 2,3-dimethyl-5-ethylpyrazine (Table 4). Methylpyrazines stand out as significant flavor constituents in cocoa beans and related products. The total content of pyrazines was dominant in microwave-dried bean samples (1551.9 $\mu\text{g/kg}$), followed by convective-dried (365.7 $\mu\text{g/kg}$) and sun-dried (155.1 $\mu\text{g/kg}$) bean samples. Interestingly, one pyrazine (2-methylpyrazine) compound was observed in fresh-fermented and freeze-dried beans. It has been reported that some *Bacillus spp* are able to produce methylpyrazine compounds, which could explain the presence of 2-methylpyrazine in fresh-fermented and freeze-dried bean samples. Hruby et al. (1977) demonstrated the robust proteolytic activity of *B. subtilis*, *B. pumilis*, *B. megatarium*, and *B. cereus*. The low level of pyrazines in fresh-fermented and freeze-dried bean samples explain the fact that pyrazines primarily result from the Maillard reaction and Strecker degradation under heating conditions. During Maillard reactions, various alkylpyrazines were generated by the Strecker degradation of leucine, isoleucine, and glycine in numerous seeds, including cocoa beans as reported by Franklin et al. (2017) in their investigation. The majority of these pyrazines have demonstrated to strongly contributed roasted, baked, cocoa-like, and nutty aroma of numerous food matrices Franklin et al. (2018). Among all the pyrazine compounds identified, 2,3-dimethylpyrazine (444.4 $\mu\text{g/kg}$) exhibited the highest concentration, followed by 2-ethyl-6-methylpyrazine (318.3 $\mu\text{g/kg}$), and 2,6-dimethylpyrazine (246.9 $\mu\text{g/kg}$) in

microwave-dried bean samples. It's worth noting that the pyrazines identified in this study have been already found in different cocoa cultivars from various origins (Bonvehí, 2005; Farah et al., 2012; Ramli et al., 2006). However, based on our research from the literature, these characterized pyrazines were elucidated for the first time in Nacional dried bean samples.

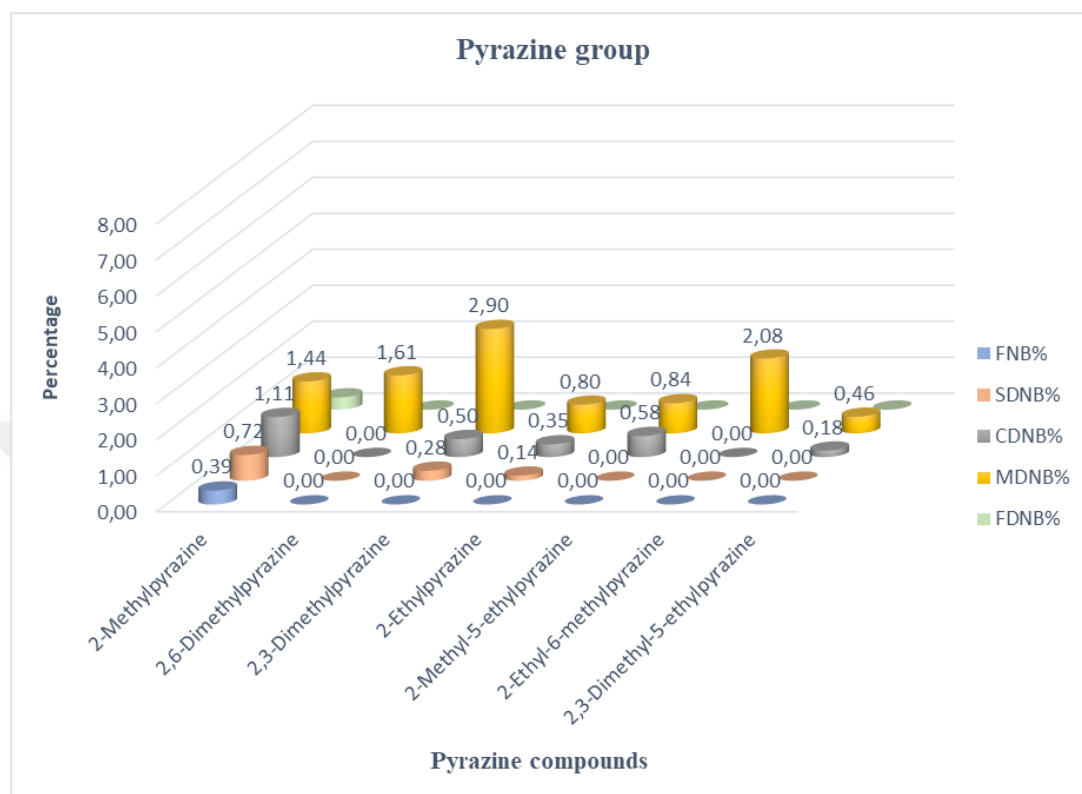


Figure 4.32. The ratio of pyrazine compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Acid Compounds

Six acids, including acetic acid, hexanoic acid, octanoic acid, nonanoic acid, pentanoic acid, and phenylacetic acid with distinct amount were determined in fresh-fermented beans and dried Nacional bean samples. The total amounts of these compounds ranged between 287.3 and 1067.1 $\mu\text{g/kg}$, with the highest concentration (1067.1 $\mu\text{g/kg}$) observed in freeze-dried bean samples, followed by sun-dried bean samples (922.0 $\mu\text{g/kg}$). The majority of these acids have been previously elucidated by numerous researchers (Cevallos-Cevallos et al., 2018; Luna et al., 2002). The lowest amount of acid (287.3 $\mu\text{g/kg}$) constituents was observed in microwave dried bean samples. The ratio of acid compounds in Nacional beans obtained through the drying processes, relative to the total compound content in percent, is illustrated in Figure 4.33. The formation of significant organic acids during cacao beans fermentation has been reported to start from day two and has been associated to the metabolism of sugars from the pulp, with slight increase in a moderated temperature during drying process (Bonvehí, 2005), which is in agreement with our findings. Among the acid compounds listed in Table 4.7, acetic acid with 573.9, 358.3, and 152.1 $\mu\text{g/kg}$ exhibited the highest concentration in Nacional beans obtained from freeze-dried, sun dried, and convective dried bean samples,

respectively. Pentanoic acid (235.7 $\mu\text{g/kg}$) and phenylacetic acid (178.2 $\mu\text{g/kg}$) were also found in high concentrations in fresh fermented and freeze-dried bean samples, respectively. Acids have been identified as providing sour and pungent sensations in fermented and dried cocoa bean samples.

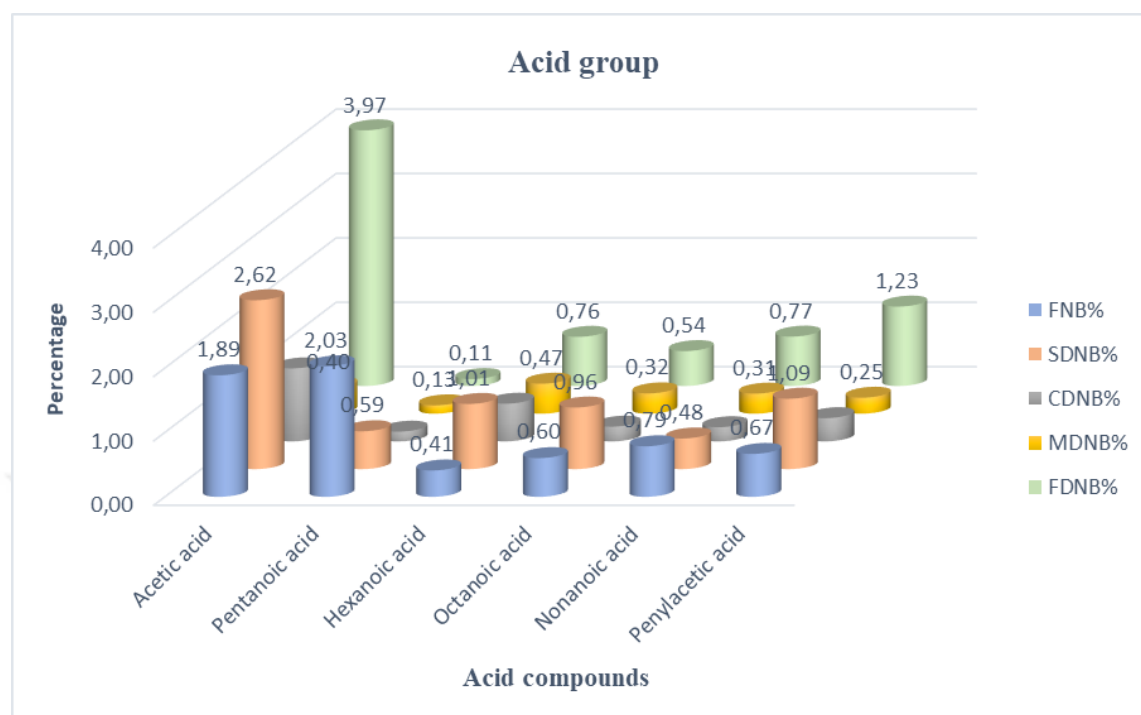


Figure 4.33. The ratio of acid compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

Other Aroma Compounds

In fresh-fermented and dried Nacional bean samples, three distinct groups of aroma compounds were identified, which included lactones, pyrroles, pyrroline, and volatile phenols. Among these volatile compounds, one lactone (γ -butyrolactone) was identified in all Nacional bean samples, while one pyrrole (2-acetylpyrrole) was only determined in sun dried, convective, and microwave dried bean samples. The microwave-dried bean samples exhibited the highest amount (438.9 $\mu\text{g/kg}$) of combined lactone and pyrrole, followed by the convective dried bean samples (298.8 $\mu\text{g/kg}$), while the lowest total amount (41.4 $\mu\text{g/kg}$) of combined lactone and pyrrole was quantified in the freeze-dried bean samples. Among lactone and pyrrole compounds, γ -butyrolactone (308.3 $\mu\text{g/kg}$) was determined in the highest quantity in microwave-dried bean samples. As can be shown in table 4.7, 2-acetylpyrrole (130.6 $\mu\text{g/kg}$) was also prominent in microwave dried bean samples. Pyrrole substances, associated with roasted, cocoa-like, and nutty note have been reported to be formed via Maillard reaction under heating conditions in foods (Franklin et al., 2017), which explained the elevated presence of 2-acetylpyrrole in microwave dried bean samples in our study. It is worth noted that volatile phenol compounds, commonly present in the aroma of fermented and dried cocoa beans, contribute to fragrance and impart distinctive flavors, including bitter and pungent organoleptic notes. In Nacional bean samples, two volatile phenols, including guaiacol and isoeugenol were

identified and quantified. The total amount of volatile phenols ranged from 13.4 to 163.9 $\mu\text{g/kg}$ in fresh-fermented and dried bean samples. The highest amount (454.2 $\mu\text{g/kg}$) of volatile phenols was observed in the fresh fermented bean samples, followed by the freeze-dried bean samples (110.5 $\mu\text{g/kg}$). Isoeugenol (266.9 $\mu\text{g/kg}$) was the prominent volatile phenol detected in fresh fermented Nacional bean samples, followed by guaicol (187.3 $\mu\text{g/kg}$). These two phenol compounds were detected for the first time in Nacional fermented and dried bean samples.

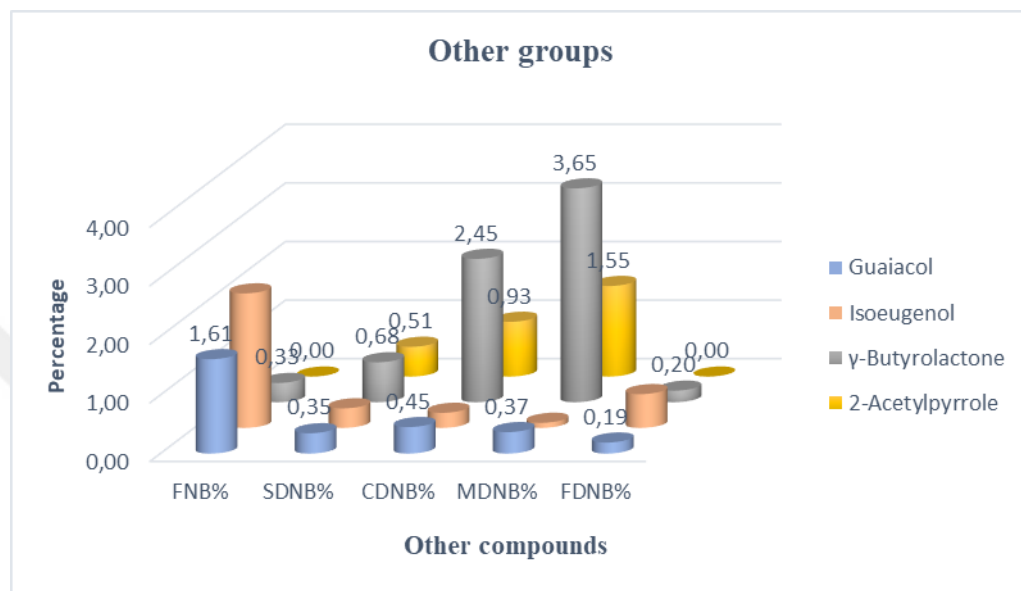


Figure 4.34. The ratio of other compounds in the fermented and dried Nacional cocoa beans according to the amount of total compounds in percent

4.8 Aroma-Active Compounds in Fermented and Dried Cocoa Beans

In an attempt to entirely understand the aroma profile of Forastero, Criollo, and Nacional fermented and dried bean samples, aroma extract dilution analysis (AEDA) was conducted to determine the key aroma-active compounds. The key odorants of all cocoa sample extracts were determined by sniffing the effluent of GC-MS-O, in which the odour description and the flavor dilution (FD) factors of these compounds were also determined and summarized in Table 4.8, Table 4.9 and Table 4.10.

4.8.1. Aroma-Active Compounds in Forastero Beans

The flavor dilution (FD) values of the aroma-active compounds of Forastero beans ranged from 4 to 128 in fresh fermented bean, from 4 to 512 in the sun-dried bean, from 4 to 128 in the convective dried bean, from 4 to 512 in the microwave dried bean, and from 4 to 2048 in the freeze-dried bean. A total of 24 different key odorant compounds were detected in only fermented bean, 28 in the sun dried bean, 27 in the convective dried bean, 24 in the microwave dried bean, and 24 in the freeze-dried bean.

As we can see from the Table 4.8;

- In aroma extract from fresh-fermented Forastero bean, 24 aroma-active compounds were found, which 24 of them were identified. The detected compounds consisted of 2 aldehydes, 7 alcohols, 3 terpenes, 2 lactones, 1 ketone, 1 pyrroline, 3 acids, and 3 esters. Two unknown aroma-active compounds (LRI: 1845 and 2006) were discovered in fresh-fermented bean sample. These compounds are often undetected in the GC-MS due to their very small peaks and/or their mass/charge (m/z) ratio. The flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 128. Among them, hexanal (green), D-limonene (citrusy), 2-hydroxy-3-pentanone (chemical), benzaldehyde (almond like), 2,3-butanediol (cheesy-creamy), hexanoic acid (cheesy-waxy), unknown compound with the 1845 LRI (roasted mushroom), exhibited the lowest value of FD factor (4), while 2-methylbutyl acetate (fruity), 2-pentanol (fermented), unknown compound with the 2006 LRI (green-herbal) exhibited the highest value of FD factor (128).
- In aroma extract isolated from sun dried Forastero bean, 28 aroma-active compounds were detected and 27 of them were clearly identified. The identified compounds were composed of 3 aldehydes, 6 alcohols, 4 terpenes, 2 ketones, 1 lactone, 1 pyrroline, 3 acids, and 3 esters. Three unknown aroma-active compounds (LRI: 1685, 1845 and 2006) were detected in the sun dried bean. In these cocoa bean samples, the flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 512. Of them, 2,6-dimethylpyrazine (roasted nut), 2(5H) furanone (caramel-sweet), phenylethanol (green-floral), and octanoic acid (fatty-rancid) were observed in the lowest FD value equal to 4, on the contrary, hexanol (flowery-spicy), and unknown compound with the 1845 LRI (roasted mushroom) were registered in the highest FD value equal to 512, followed by 3-methylbutanal (fresh-apple like) with FD value of 256.
- In aroma extract acquired from convective dried Forastero bean, 28 aroma-active compounds were determined and 25 of them were clearly identified. The identified compounds included 3 aldehydes, 6 alcohols, 3 pyrazines, 2 terpenes, 2 acids, 2 ketones, 2 lactones, 1 pyrrole, 1 pyrroline, and 3 esters. Three unknown aroma-active compounds (LRI: 1685, 1845 and 2006) were detected in convective dried cocoa bean. In these cocoa bean samples, the flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 128. Between them, ethyl acetate (fresh-fruity), 2-phenyl acetate (floral-fruity), 3-methylbutanol (solvent-like), and hexanoic acid (cheesy-waxy) displayed the lowest FD values (4), in the contrast, hexanal (green) demonstrated the highest FD values (2048).
- In aroma extract obtained from microwave dried Forastero bean, 25 key odorant compounds were discovered, in which 22 of them were completely characterized. The detected compounds consisted of 3 aldehydes, 6 alcohols, 3 pyrazines, 2 terpenes,

2 acids, 1 furanone, 1 lactones, 1 pyrrole, 1 pyrroline, and 2 esters. Among the identified aroma-active compounds, three unknown aroma-active compounds with LRI (1685, 1845 and 2006) were determined in microwave dried Forastero bean samples. The flavor dilution (FD) values of these aroma-active compounds ranged from 4 to 512. Among them, 2-phenyl acetate (floral-fruity), hexanal (green), 2-pentanol (fermented), acetic acid (vinegar), 2-phenylethanol (green cut grass), and unknown compound with the 2006 LRI (green-herbal) displayed the lowest FD values of 4, while 2-methylpyrazine (chocolate-like), hexanal (green), and 2,3,5-trimethylpyrazine (roasted nut) were determined in the highest value of FD (512).

- In aroma extract fractionated from freeze-dried Forastero bean samples, 25 aroma-active compounds were determined and 23 of them were fully analyzed. The identified compounds were composed of 2 aldehydes, 7 alcohols, 4 terpenes, 3 acids, 1 lactones, 1 pyrroline, 2 ketones, and 3 esters. Two unknown aroma-active substances (LRI: 1845 and 2006) were decrypted in freeze-dried Forastero bean samples. In the freeze-dried Forastero bean samples characterized, the flavor dilution (FD) factor of key odorant compounds ranged from 4 to 1024. Of all aroma-active compounds detected, 2-heptanone (green-fruity), γ -butyrolactone (burnt), hexanoic acid (cheesy-waxy), and unknown key odorant with the 2006 LRI (green-herbal), presented the lowest value of FD (4), whereas, hexanol (flowery-spicy) was identified in the highest FD value (1024), followed by 2-methylbutyl acetate (fruity), γ terpinene (fresh-citrusy) with FD value of 512.

Table 4.8. Aroma-active compounds of Forastero fermented and dried cocoa bean samples (FD $\geq$ 4)

No.	Aroma-active compounds	LRI ^a	Odor description ^b	FD factor ^c				
				FFB	SDFB	CDFB	MDFB	FDF B
1	Ethyl acetate	887	Fresh-fruity	32	8	4	-	128
2	3-Methylbutanal	916	Malty-apple like	-	128	256	512	-
3	2-Phenyl acetate	1066	Floral-fruity	64	16	4	4	32
4	2-Methyl-3-butanol	1089	Fruity	64	64	16	8	32
5	Hexanal	1097	Green	4	64	2048	512	32
6	2-Methylbutyl acetate	1115	Fruity	128	64	16	8	512
7	Sabinene	1119	Woody-herbal	8	32	-	-	64
8	2-Pentanol	1138	Fermented	128	64	8	4	128
9	2-Heptanone	1184	Green-fruity	32	8	128	-	4
10	D-Limonene	1206	Citrusy	4	16	32	64	8

Table 4.8. Continued

11	3-Methylbutanol	1240	Solvent-like	8	-	4	8	32
12	γ - Terpinene	1242	Fresh-citrusy	16	64	-	-	512
13	2-Methylpyrazine	1274	Chocolate like	-	8	64	512	-
14	2-Acetyl-1-pyrroline	1310	Popcorn-like	64	128	128	256	256
15	2,6-Dimethylpyrazine	1319	Roasted coffee	-	4	32	128	-
16	Hexanol	1359	Flowery-spicy	8	512	128	64	1024
17	2-Hydroxy-3-pentanone	1368	Chemical	4	64	-	-	16
18	2,3,5-Trimethylpyrazine	1413	Roasted nut, earthy	-	-	64	512	-
19	Acetic acid	1418	Vinegar	16	32	4	4	8
20	Linalool	1537	Fresh-citrusy	64	8	64	128	16
21	Benzaldehyde	1544	Almond like	4	8	16	64	4
22	2,3-Butanediol	1545	Cheesy-creamy	4	64	8	8	64
23	γ -Butyrolactone	1617	Burnt	8	16	16	32	4
24	Unknown	1685	Roasted bean	-	8	32	256	-
25	2-(5H)-furanone	1716	Caramel-sweet	-	4	8	64	-
26	Hexanoic acid	1820	Cheesy-waxy	4	32	4	16	4
27	Unknown	1845	Roasted mushroom	4	512	16	64	4
28	Phenylethanol	1922	Green-floral	16	4	4	-	8
29	2-phenylethanol	1944	Green cut grass	64	32	8	4	32
30	2-Acetylpyrrole	1974	Musty	-	-	8	32	-
31	Unknown	2006	Green-herbal	128	32	8	4	64
32	Octanoic acid	2046	Fatty-rancid	8	4	-	-	8

^a LRI: linear retention index calculated on DB-Wax capillary column.

^b Odor description: odor description as perceived by panalists through olfactometry analysis

^c FD factor is the highest dilution of the aroma extract at which an odorant is determined b.y aroma extract dilution analysis. FFB: fermented Forastero bean, SDCB: sun-dried Forastero bean, CDNB: convective dried Forastero bean, MDCB: microwave dried Forastero bean, FDNB: freeze-dried Forastero bean.

4.8.2. Aroma-Active compounds in Criollo Beans

The flavor dilution (FD) values of the aroma-active compounds in Criollo bean samples varied across different drying methods. Specifically, in sun-dried beans from 4 to 4096, in convective-dried beans, FD values ranged from 4 to 512, and in microwave-dried beans from 4 to 512, while in freeze-dried beans, FD values ranged from 4 to 2048. Furthermore, in fresh-fermented beans, FD values ranged from 4 to 2048. A panel of 26 different aroma-active compounds was

detected in fresh-fermented beans, 33 in sun-dried beans, 34 in convective-dried beans, 29 in microwave-dried beans, and 27 in freeze-dried bean samples.

As we can observed from Table 4.9;

- In the aroma extract from fresh-fermented Criollo beans, a total of 26 aroma-active compounds were identified, in which 26 of them were successfully characterized. These compounds comprised 2 aldehydes, 6 alcohols, 4 terpenes, 2 ketones, 1 lactone, 1 pyrroline, 4 acids, and 4 esters. Additionally, two unknown aroma-active compounds (LRI: 1297 and 1784) were discovered in the fresh-fermented bean samples. The flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 1024. Among them, D-limonene (citrusy), 2-hydroxy-2-butanone (buttery-creamy), γ -butyrolactone (burnt-like) benzaldehyde (almond-like), 2,3-butanediol (cheesy-creamy), and hexanoic acid (cheesy-waxy) exhibited the lowest FD factor of 4. Conversely, hexanol (flowery-spicy) showed the highest FD factor of 1024, followed by 2-methyl-3-butanol (fruity) with FD (512).
- In the aroma extract isolated from sun-dried Criollo beans, a total of 32 aroma-active compounds were detected, with 30 of them clearly identified. These compounds consisted of 3 aldehydes, 6 alcohols, 5 terpenes, 3 pyrazines, 2 ketones, 2 lactones, 1 pyrrole, 4 acids, and 4 esters. Additionally, two unknown aroma-active compounds (LRI: 1297 and 1784) were detected in the sun-dried bean samples. These compounds present challenges in identification due to their specific mass/charge (m/z) ratios and low peak intensities. The flavor dilution (FD) values of the aroma-active compounds in these cocoa bean samples ranged from 4 to 1024. Notably, 2-hexanol (green-herbal), δ -octenolactone (fresh-fruity), 2,6-dimethylpyrazine (roasted coffee), 2,5-dimethylpyrazine (dark chocolate), 2-Acetylpyrrole (musty), and phenylacetic acid (burnt honey) demonstrated the lowest value of FD (4). On the contrary, hexanal (green), was registered with the highest FD value of 1024.
- In the aroma extract obtained from convective-dried Criollo beans, a total of 34 aroma-active compounds were identified, with 32 of them successfully identified. These compounds comprised 4 aldehydes, 6 alcohols, 3 pyrazines, 4 terpenes, 4 acids, 2 ketones, 2 lactones, 1 furanone, 1 pyrrole, 1 pyrroline, and 4 esters. In addition, two unknown aroma-active compounds (LRI: 1297 and 1784) were determined in the convective-dried cocoa beans. The flavor dilution (FD) values of the aroma-active compounds in these cocoa bean samples ranged from 4 to 4096. Notably, ethyl 3-methylbutanoate (green-fruity), ethyl hexanoate (fruity), unknown compound with the 1297 LRI (mushroom-like), methylphenyl acetate (honey), phenylethyl acetate (sweet-fruity), phenyl ethanol (green-floral), octanoic acid (fatty-rancid), and phenylacetic acid (burnt honey) displayed the lowest values of FD (4), while, hexanal (green)

demonstrated the highest FD values of 4096, followed by 2-methylbutanal (green-fruity) with FD value of 2048 in convective dried Criollo bean samples.

- In the aroma extract obtained from microwave dried Criollo beans, a total of 29 key odorant compounds were discovered, with 27 of them completely characterized. These compounds included 4 aldehydes, 6 alcohols, 3 pyrazines, 3 terpenes, 2 acids, 2 ketones, 1 furanone, 2 lactone, 1 pyrrole, 1 pyrroline, and 3 esters. Notably, one unknown aroma-active compound with LRI of 1784 was also determined in the microwave-dried Criollo bean samples. The flavor dilution (FD) values of these aroma-active compounds ranged from 4 to 2048. Among them, ethyl 3-methylbutanoate (green-fruity), 2-methyl-3-butanol (fruity), ethyl hexanoate (fruity), 2-hexanol (green-herbal), (*E*)-Caryophyllene (musky-spicy), phenylethyl acetate (sweet-fruity), hexanoic acid (cheesy-waxy), phenyl ethanol (green-floral), were observed with the lowest FD values of 4. Conversely, 2-methylbutanal (green-fruity) was perceived as the highest FD factor of 2048, followed by hexanal (green) with FD value of 1024, 2-acetyl-1-pyrroline (FD: 512; popcorn-like) and D-limonene (FD: 512; citrusy).
- In the aroma extract fractionated from freeze-dried Criollo bean samples, a total of 28 aroma-active compounds were identified, with 25 of them fully analyzed. These compounds consisted of 4 aldehydes, 6 alcohols, 5 terpenes, 4 acids, 1 lactone, 1 ketone, 1 pyrroline, and 4 esters. Additionally, two unknown aroma-active substances (LRI: 1297 and 1784) were detected in the freeze-dried Criollo bean samples. In the freeze-dried Criollo bean samples characterized, the flavor dilution (FD) factors of the key odorant compounds ranged from 4 to 512. Among all the aroma-active compounds determined, phenylacetaldehyde (green cut grass) showed the lowest value of FD (4). However, hexanal (green), hexanol (flowery-spicy), (*E*)-caryophyllene (musky-spicy) were identified with the highest FD factor value of 512.

Table 4.9. Aroma-active compounds of Criollo fermented and dried cocoa bean samples (FD $\geq$ 4)

No.	Aroma-active compounds	LRI ^a	Odor description ^b	FD factor ^c				
				FC B	SDC B	CDC B	MDC B	FDC B
1	2-Methylbutanal	896	Green-fruity	16	512	2048	2048	64
2	3-Methylbutanal	916	Fresh apple like	-	16	32	128	8
3	Ethyl 3-methylbutanoate	1072	Green fruity	16	8	4	4	64
4	Camphene	1082	Woody	128	64	16	-	128
5	2-Methyl-3-butanol	1089	Fruity	512	16	8	4	64
6	Hexanal	1097	Green	64	1024	4096	1024	512
7	2-Heptanone	1184	Green-fruity	8	16	32	128	-
8	D-limonene	1206	Citrusy	4	32	64	512	16
9	Ocimene	1233	Fruity-citrusy	128	8	-	-	32
10	Ethyl hexanoate	1241	Fruity	8	32	4	4	64

Table 4.9. Continued

11	(E)-2-Hexanol	1245	Green-herbal	32	4	16	4	64
12	δ -Octenolactone	1264	Fresh-coconut-like, sweet	-	4	8	64	-
13	2-Methylpyrazine	1274	Chocolate like	-	8	8	64	-
14	3-hydroxy-2-butanone	1291	Buttery-creamy	4	16	32	64	8
15	Unknown	1297	Mushroom-like	32	8	4	-	128
16	2-Acetyl-1-pyrroline	1310	Popcorn-like	32	128	256	512	256
17	2,6-Dimethylpyrazine	1319	Roasted coffee	-	4	16	32	-
18	Hexanol	1359	Flowery-spicy	1024	128	64	64	512
19	2,5-Dimethylpyrazine	1336	Dark chocolate	-	4	32	16	-
20	Acetic acid	1418	Vinegar	128	64	16	8	64
21	Linalool	1537	Fresh-citrusy	-	16	32	128	8
22	2,3-Butanediol	1545	Cheesy-waxy	8	64	16	8	128
23	(E)-Caryophyllene	1598	Musky-spicy	64	32	16	4	512
24	γ -Butyrolactone	1617	Burnt-like	4	16	128	64	8
25	Phenylacetaldehyde	1642	Green cut grass	-	-	16	64	4
26	5-Methyl 2(5H)-furanone	1664	Caramel-sweet	-	-	16	16	-
27	Methylphenyl acetate	1749	Honey	32	16	4	-	32
28	Unknown	1784	Roasted nut	8	32	64	512	16
29	Phenylethyl acetate	1810	Sweet-fruity	8	32	8	4	128
30	Hexanoic acid	1820	Cheesy-waxy	4	64	64	4	8
31	Phenylethanol	1861	Green-floral	16	8	4	4	32
32	2-phenylethanol	1944	Floral-herbal	64	512	16	8	512
33	2-Acetylpyrrole	1974	Musty	-	4	8	32	-
34	Octanoic acid	2046	Fatty-rancid	32	8	4	-	8
35	Phenylacetic acid	2554	Burnt honey	8	4	4	-	16

^a LRI: linear retention index calculated on DB-Wax capillary column.

^b Odor description: odor description as perceived by panalists through olfactometry analysis

^c FD factor is the highest dilution of the aroma extract at which an odorant is determined by aroma extract dilution analysis. FCB: fermented Criollo bean, SDCB: sun-dried Criollo bean, CDNB: convective dried Criollo bean, MDCB: microwave dried Criollo bean, FDNB: freeze-dried Criollo bean.

4.8.3. Aroma-Active Compounds in Nacional Beans

The flavor dilution (FD) values of aroma-active compounds in Nacional beans varied among different drying processes. In fresh fermented beans, FD values ranged from 4 to 1024, while in sun-dried beans, they ranged from 4 to 512. Freeze dried beans exhibited FD values spanning from 4 to 1024, and in microwave-dried beans, it ranged from 4 to 512. Notably, the widest range of FD values was observed in convective dried beans, ranging from 4 to 4096. In terms of the key odorant compounds identified, a panel of 33 different aroma-active compounds was detected in fresh fermented beans, 35 in sun-dried beans, 31 in convective-dried beans, 31 in microwave-dried beans, and 33 in freeze-dried bean samples.

As can be indicated from Table 4.10;

- In the aroma extract acquired from fresh-fermented Nacional beans, a total of 33 aroma-active compounds were determined, with 31 of them fully characterized. These compounds included 6 aldehydes, 7 alcohols, 5 terpenes, 2 ketones, 1 volatile phenol, 1 lactone, 1 pyrroline, 3 acids, and 4 esters. Interestingly, 1 pyrrole was observed in fresh fermented bean samples, which could be generated by microorganisms' activities, especially by *Bacillus spp.* Additionally, two unknown aroma-active compounds (LRI: 1008 and 1325) were discovered in fresh-fermented bean samples. The flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 1024. Notably, D-limonene (citrusy), 2-methylpyrazine (chocolate-like), unknown compound with the 1325 LRI (caramel), and γ -butyrolactone (burnt-like) showed the lowest FD factor of 4. Conversely, hexanol (flowery-spicy) displayed the highest values of FD factor (512), followed by ocimene (fruity-citrusy) and 2-hexanol (green-herbal) with FD factor of 128.
- In the aroma extract isolated from sun-dried Nacional beans, a total of 35 aroma-active compounds were detected, with 33 of them clearly identified. These compounds comprised 6 aldehydes, 7 alcohols, 5 terpenes, 2 pyrazines, 2 ketones, 1 volatile phenol, 1 lactone, 1 pyrrole, 1 pyrroline, 3 acids, and 4 esters. Moreover, two unknown aroma-active compounds (LRI: 1008 and 1325) were characterized in the sun-dried bean samples, presenting challenges in identification due to their specific mass/charge (m/z) ratios and low peak intensities. The flavor dilution (FD) values of the aroma-active compounds in these cocoa bean samples ranged from 4 to 512. Among them, 2,3-dimethylpyrazine (roasted-like), nonanal (waxy-fatty), (E)-caryophyllene (musky-spicy), (E,E)-2,4 nonadienal (cooked-fatty) exposed the lowest FD values of 4. In contrast, 3-methylbutanal (apple-like), 2-acetyl-1-pyrroline (popcorn-like), and hexanal (green) were registered with the highest value of FD factor (512), which was followed

by 2-methylbutanal (green-leafy), hexanol (flowery-spicy), and 2,3-butanediol (cheesy-creamy) with FD value of 128.

- In the aroma extract obtained from convective dried Nacional beans, a total of 31 aroma-active compounds were characterized, among which 29 of them successfully determined. These compounds were composed of 5 aldehydes, 6 alcohols, 2 pyrazines, 2 terpenes, 3 acids, 2 ketones, 1 lactone, 1 furan, 1 pyrrole, 1 pyrroline, 1 volatile phenol, and 4 esters. Additionally, two unknown aroma-active compounds (LRI: 1008 and 1325) were identified in the convective-dried cocoa bean samples. The flavor dilution (FD) values of the aroma-active compounds in these cocoa bean samples ranged from 4 to 4096. Notably, 3-methylbutyl acetate (green), nonanal (waxy-fatty), 2-acetyl-5-methylfuran (nutty), phenylethyl acetate (fruity-sweet), phenylethyl acetate (sweet-fruity), and octanoic acid (fatty-rancid) displayed the lowest FD values of 4. Conversely, hexanal (green) showed the highest FD values of 4096 in convective-dried Nacional bean samples, followed by 3-methylbutanal (apple-like) with FD value of 1024.
- In the aroma extract isolated from microwave-dried Nacional beans, a total of 31 key odorant compounds were discovered, with 29 of them completely characterized. These compounds comprised 5 aldehydes, 6 alcohols, 3 pyrazines, 2 terpenes, 3 acids, 2 ketones, 1 furan, 1 lactone, 1 pyrrole, 1 pyrroline, and 4 esters. Impressively, two unknown aroma-active compounds with LRI of 1008 and 1325 were also determined in the microwave-dried Nacional bean samples. The flavor dilution (FD) values of these aroma-active compounds ranged from 4 to 1024. Among them, ethyl acetate (fresh-fruity), nonanal (waxy-fatty), acetic acid (vinegar), hexanoic acid (cheesy-waxy), and octanoic acid (fatty-rancid), were registered with the lowest FD values of 4. Conversely, hexanal (green) was observed with the highest FD factor of 1024, followed by linalool (fresh-citrusy) and two unknown compounds with the 1008 LRI (caramel-sweet) and the 1325 LRI (caramel) perceived with FD factor of 512.
- In the aroma extract fractionated from freeze-dried Nacional bean samples, a total of 33 aroma-active compounds were identified, with 30 of them fully characterized. These compounds included 6 aldehydes, 7 alcohols, 5 terpenes, 1 pyrazine, 3 acids, 2 ketone, 1 lactone, 1 pyrroline, 1 volatile phenol, and 4 esters. Remarkably, two unknown aroma-active compounds (LRI: 1008 and 1325) were determined in the freeze-dried Nacional bean samples. In the freeze-dried Nacional bean samples characterized, the flavor dilution (FD) factors of the key odorant compounds ranged from 4 to 1024. Among all the aroma-active compounds determined in the freeze-dried Nacional bean samples, 2-pentanone (plastic-chemical), unknown compound with the 1008 LRI (caramel-sweet), 2-methylpyrazine (chocolate-like), 3-hydroxy-2 butanone (buttery-creamy), unknown compound with the 1325 LRI (caramel), and γ -butyrolactone (burnt like)

showed the lowest FD value of 4. In contrast, hexanol (flowery-spicy) was registered with the highest FD factor of 1024, followed by 2,3-butandiol (cheesy-creamy) and 2-acetyl-1-pyrroline (popcorn-like) with FD value of 512.

Table 4.10. Aroma-active compounds of Nacional fermented and dried cocoa bean samples (FD $\geq$ 4)

No	Aroma-active compounds	LRI ^a	Odor description ^b	FD factor ^c				
				FNB	SDNB	CDNB	MDNB	FDNB
1	Ethyl acetate	887	Fresh-fruity	64	32	8	4	64
2	2-Methylbutanal	896	Green-fruity	16	128	512	1024	64
3	3-Methylbutanal	916	Apple like	32	512	1024	2048	128
4	2-Methyl-2-butanol	975	Sour-sweet	64	64	-	-	128
5	2-Pentanone	978	Plastic-chemical	8	8	16	32	4
6	Unknown	1008	Caramel-sweet	8	16	64	512	4
7	α pinene	1038	Green-floral	32	8	-	-	128
8	Ethyl-2-methylbutanoate	1064	Green-fruity	8	16	32	64	64
9	Hexanal	1097	Green	64	512	4096	1024	128
10	3-Methylbutyl acetate	1130	Green	64	16	4	8	16
11	1-Penten-3-ol	1165	Green-leafy	16	128	64	16	512
12	D-Limonene	1206	Citrusy	4	64	16	128	8
13	Ocimene	1233	Fuity-citrusy	128	32	-	-	128
14	(E)-2-Hexanol	1245	Green-herbal	128	32	16	8	64
15	2-Methylpyrazine	1274	Chocolate like	4	16	16	32	4
16	3-hydroxy-butanone	1291	Buttery-creamy	32	64	64	8	4
17	2-Acetyl-1-pyrroline	1310	Popcorn-like	64	512	512	256	512
18	2,6-Dimethylpyrazine	1319	Roasted coffee	-	-	-	64	-
19	2-Penten-1-ol	1324	Plastic-chemical	64	64	32	16	128
20	Unknown	1325	Caramel	4	16	64	512	4
21	2,3-Dimethylpyrazine	1357	Roasted like	-	4	8	128	-
22	Hexanol	1359	Flowery-spicy	512	128	64	32	1024
23	Nonanal	1395	Waxy-fatty	64	4	8	4	16
24	Acetic acid	1418	Vinegar	32	64	16	4	128
25	Linalool	1537	Fresh-citrusy	32	32	64	512	16
26	Benzaldehyde	1544	almond like	4	32	64	128	8
27	2,3-Butandiol	1545	Cheesy-creamy	64	128	16	16	512

Table 4.10. Continued

28	(E)-Caryophyllene	1598	Musky-spicy	64	4	-	-	16
29	2-Acetyl-5-methylfuran	1608	Nutty	-	-	4	16	-
30	γ -Butyrolactone	1617	Burnt like	4	8	16	32	4
31	(E,E)-2,4-Nonadienal	1681	Cooked-fatty	16	4	-	-	16
32	Phenylethyl acetate	1810	Fruity-sweet	16	32	4	8	128
33	Hexanoic acid	1820	Cheesy-waxy	4	16	8	4	64
34	Phenylethanol	1922	Green-floral	64	64	32	8	128
35	2-Acetylpyrrole	1974	Musty	-	8	16	64	-
36	Octanoic acid	2046	Fatty-rancid	8	32	4	4	8
37	Isoeugenol	2271	Sweet-floral	64	8	8	-	16

^a LRI: linear retention index calculated on DB-Wax capillary column.

^b Odor description: odor description as perceived by panalists through olfactometry analysis

^c FD factor is the highest dilution of the aroma extract at which an odorant is determined by aroma extract dilution analysis. FNB: fermented Nacional bean, SDNB: sun-dried Nacional bean, CDNB: convective dried Nacional bean, MDNB: microwave dried Nacional bean, FDNB: freeze-dried Nacional bean.

The majority of aroma-active compounds identified in the different fermented and dried cocoa beans obtained from the three cocoa cultivars have been previously reported by some reserachers (Liu et al, 2017; Frauendorfer & Schieberle, 2019; Utrilla-Vázquez et al., 2020). For instance, in the study conducted by Frauendorfer and Schieberle (2006) on different dried cocoa beans, 35 odor-active compounds were identified, among which 2-methylbutanal (malty), 3-methylbutanal (malty), 2-ethyl-3,5-dimethylpyrazine (potato-chip-like), 3-methylbutanoic acid (sweaty-rancid) and phenylacetaldehyde (honey-like) were the most potent in dried cocoa beans. In another investigation, Quelal et al. (2023) exhibited that hexanal, 2- and 3-methylbutanal were the most important aroma-active compounds in dried and roasted cocoa beans and products. Hexanal (green), 2-methylbutanal (green-fruity), 3 methylbutanal (fresh-apple like), hexanol (flowery-spicy) 2,3-dimethylpyrazine (roasted-like), α pinene (green-floral), 1-penten-3-ol (green-leafy), phenylacetaldehyde (green cut grass), 2-phenylethanol (floral-herbal), 2,3-butanediol (cheesy-waxy), γ terpinene (fresh-citrusy), acetic acid (vinegar), 2,3,5-trimethylpyrazine (roasted nut), γ -butyrolactone (burnt-like), octanoic acid (fatty-rancid) were detected as the key aromas in fermented and dried cocoa bean obtained from Coasta rica (Criollo) by Ullrich et al. (2021), in Forastero cocoa beans by Schlüter et al. (2022), from Colombia in Criollo and Trinitario cocoa clones by Tejeda et al. (2024), from Côte d'Ivoire in Forastero cocoa beans by Koné et al. (2016).

4.8.4. Organoleptic Assessment of Fermented and Dried Cocoa Beans

Sensory properties of all fermented and dried cocoa beans samples (nibs) were evaluated by a group of 10 panelists, aged between 24 to 45 years old. The sensory analysis results of the Forastero,

Criollo and Nacional fermented dried cocoa beans, as spider diagram profile, are given in Figure 4.35, Figure 4.36, and Figure 4.37. The assessment of the organoleptic quality of fermented dried cocoa beans entails conception of both desirable and undesirable sensory properties. As seen in Table 4.11, Table 4.12 and Table 4.13, the samples (nibs) were evaluated using ten parameters.

4.8.4.1. Organoleptic Assessment of Fermented and Dried Forastero Beans

The sensory profiles of the fresh fermented and four dried Forastero bean samples (nibs) were evaluated by the means of descriptive analysis in order to generate a more global plot of gustatory and orthonasal profiles of fermented and dried Forastero beans (nibs). Due to its high moisture content and the difficulties in obtaining sufficient nibs, the fresh fermented Forastero bean sample was not evaluated for its sensory profile. Each dried Forastero beans (nibs) was analyzed by a panel of 10 assessors, who were ask to describe the Forastero dried beans according to a set of 12 descriptors (bitterness, color, astringency, sourness, chocolate, caramel, floral, cocoa aroma, dried fruit, fresh fruit, mouldy, and overall quality) scored on a structural scale from 0 to 15 cm, and the results as mean score is indicated in the table 4.11. Forastero beans “color” and “overall quality” criteria were taken in consideration during the assessment sessions. The data of the results of descriptive analysis of fermented and dried Forastero beans (nibs) as mean score of the selected attributes is also reported in the figure 4.35 as spider diagram. All the attributes were evaluated by the panelist by smelling and tasting the fermented dried Forastero beans (nibs).

Table 4.11. Average scores of the sensory attributes and identification of statistically significant differences among the fermented and dried Forastero cocoa bean samples by one-way ANOVA and LSD test analyses.

Attributes	Dried Forastero cocoa beans				Sig.	Pr> F(Model)
	SDFB	CDFB	MDFB	FDFB		
Color	7.1 ^b	12.5 ^a	11.3 ^a	5.3 ^c	*	0.018
Bitterness	4.1 ^d	9.6 ^b	13.2 ^a	6.1 ^c	*	0.020
Astringency	9.8 ^b	5.3 ^c	2.03 ^d	11.2 ^a	*	0.022
Sourness	7.4 ^b	3.07 ^c	1.6 ^d	9.3 ^a	*	0.036
Chocolate	4.1 ^c	9.8 ^b	11.2 ^a	3.07 ^c	*	0.041
Caramel	3.7 ^c	6.4 ^b	8.1 ^a	3.1 ^c	**	0.008
Floral	8.4 ^a	8.1 ^a	7.8 ^a	9.2 ^a	ns	0.394
Cocoa aroma	4.1 ^d	8.9 ^b	11.3 ^a	5.6 ^c	*	0.032
Dried fruit	6.9 ^a	7.4 ^a	7.1 ^a	8.2 ^a	ns	0.186
Fresh Fruit	8.2 ^a	3.4 ^b	2.04 ^c	6.1 ^b	**	0.005
Mouldy	4.1 ^a	1.08 ^c	1.02 ^c	2.1 ^b	*	0.031
Overall quality	5.4 ^d	10.8 ^b	12.4 ^a	7.6 ^c	**	0.046

Sig.: significance at which means differ as shown by analysis of variance; ns.: notsignificant, significant at the * p<0.05 level, **p<0.01. Different letters in the same row indicate statistical differences at the p<0.05 level according to Duncan multiple test. P: sensory attribute evaluated orthonasally

The sensory profile scores for bitterness, chocolate, caramel, and cocoa aroma notes were higher in MDFB compared to other dried bean samples. Attributes such as sourness, bitterness, and astringency are considered undesirable in trade cocoa beans and cocoa products. The relatively high bitterness (13.20) in Forastero cocoa bean samples, as shown in Figure 4.35, is likely linked to the drying process applied. Indeed, dried cocoa beans processed using microwave and roasting methods tend to have a more bitter taste due to the high temperature and power application. Forastero cocoa beans subjected to freeze drying were characterized by high scores for undesirable sensory attributes such as sourness (9.30) and astringency (11.20). These elevated attribute scores can be explained by the significant contents of organic acids and polyphenols in the freeze-dried Forastero bean samples. Additionally, freeze-dried Forastero cocoa bean samples presented the highest score of floral note, which might be probably linked to the significant content of ester components. Regarding color attribute, CDFB demonstrated the highest sensory score of 12.5. Forastero cocoa beans obtained from the sun-drying process exhibited the lowest sensory scores for bitterness (4.10) and cocoa aroma (4.12). Additionally, sun-dried Forastero bean samples had the highest score (8.20) for the fresh fruit attribute, likely associated with the content of ester and terpenoid compounds. When comparing the overall quality of Forastero beans among the drying processes, significant difference was observed among the dried Forastero beans ($p < 0.01$), and the most appreciated samples were those from the microwave drying technique, with a sensory score of 12.4, followed by the convective drying process with a score of 10.8.

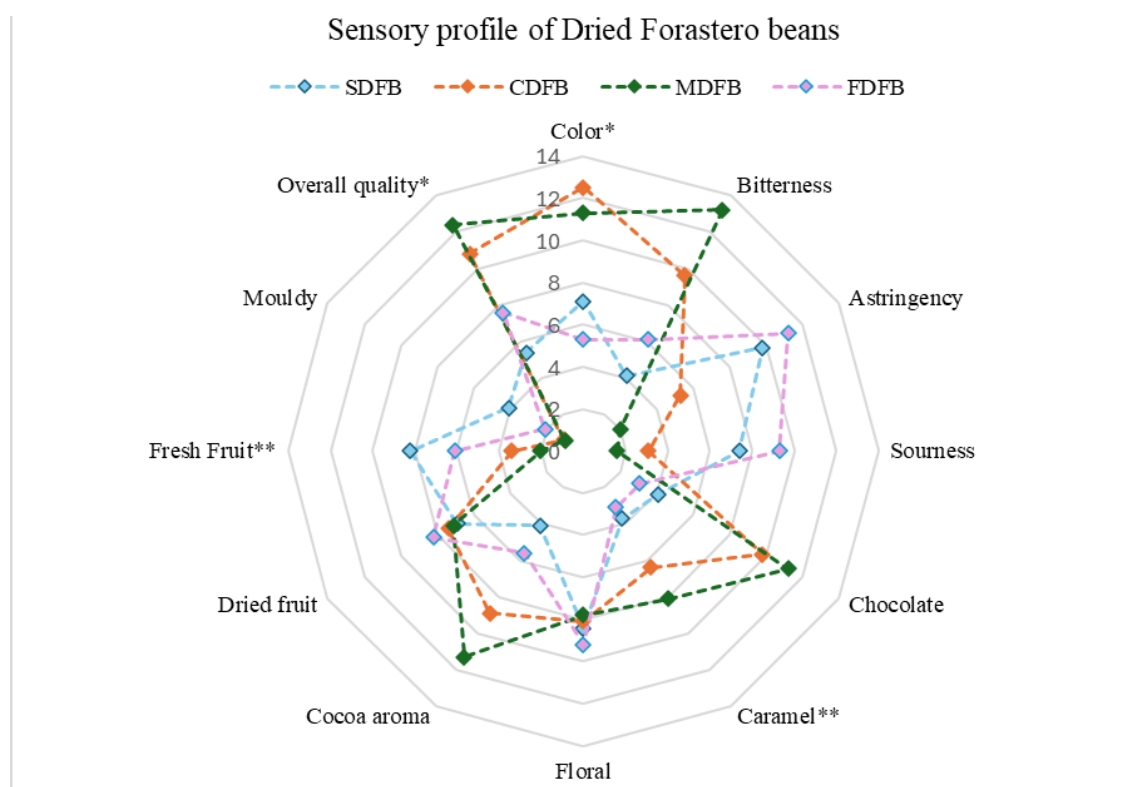


Figure 4.35. Spider web plot showing sensory profiles of the fermented and dried Forastero cocoa beans. Average scores of attribute intensity are shown as distance from the center.

4.8.4.2. Organoleptic Assessment of Fermented and Dried Criollo Beans

The sensory attributes of the fresh fermented and four dried Criollo bean samples (nibs) were evaluated using descriptive analysis. A panel of 10 assessors analyzed each sample, describing the dried beans based on a set of 12 descriptors, including bitterness, color, astringency, sourness, chocolate, caramel, floral, cocoa aroma, dried fruit, fresh fruit, mouldy, and overall quality. These descriptors were scored on a structured scale ranging from 0 to 15 cm, shown in Table 4.12. Criollo beans “color” and “overall quality” criteria were taken in consideration during the evaluation sessions. The results of the descriptive analysis of the dried Criollo beans are also presented in a spider diagram in Figure 4.36. All attributes were assessed by the panelists through the smelling and tasting of the fermented, dried Criollo beans (nibs).

Table 4.12. Average scores of the sensory attributes and identification of statistically significant differences among the fermented and dried Criollo cocoa beans, by one-way ANOVA and LSD test analyses.

Attributes	Dried Criollo Cocoa Beans				Sig.	Pr> F(Model)
	SDCB	CDCB	MDCB	FDCB		
Color	8.3 ^b	10.2 ^a	9.6 ^a	4.1 ^c	*	0.025
Bitterness	2.08 ^c	8.4 ^b	11.6 ^a	3.7 ^c	*	0.038
Astringency	10.2 ^a	5.3 ^c	3.2 ^d	8.7 ^b	*	0.014
Sourness	11.2 ^a	5.07 ^c	2.4 ^d	9.3 ^b	*	0.027
Chocolate	2.03 ^d	7.2 ^b	9.5 ^a	4.2 ^c	*	0.012
Caramel	2.3 ^c	9.4 ^b	11.2 ^a	1.08 ^d	*	0.016
Floral	6.3 ^a	7.05 ^a	7.6 ^a	6.8 ^a	ns	0.234
Cocoa aroma	5.2 ^c	6.3 ^b	9.4 ^a	4.3 ^c	*	0.021
Dried fruit	8.2 ^a	7.9 ^a	8.4 ^a	7.6 ^a	ns	0.194
Fresh Fruit	6.2 ^a	2.09 ^b	1.6 ^b	7.3 ^a	**	0.009
Mouldy	3.04 ^a	2.1 ^a	1.7 ^b	2.3 ^a	*	0.017
Overall quality	6.2	11.2	9.3	7.8	*	0.044

Sig.: significance at which means differ as shown by analysis of variance; ns.: not significant, significant at the * $p < 0.05$ level, ** $p < 0.01$. Different letters in the same row indicate statistical differences at the $p < 0.05$ level according to Duncan multiple test. P: sensory attribute evaluated orthonasally

The sensory profile scores, namely bitterness, chocolate, caramel, cocoa aroma, dried fruit, and floral were higher in MDCB than other dried bean samples. In fact, attributes such as sourness, bitterness, and astringency are considered undesirable in trade cocoa beans, and cocoa products. The relatively high bitterness (11.60) in Criollo cocoa bean samples as shown in Figure 4.36 is probably linked to the drying method. Indeed, dried cocoa beans using microwave and roasting process tend to have more bitter taste due essentially to the high temperature and power application. According to some scholars, the bitter taste of dried and roasted cocoa beans is the result of caffeine, theobromine, and some alkylpyrazines (Apriyanto, 2016a; Djaafar et al., 2019). Criollo cocoa beans issued from sun drying method was characterized by high scores for undesirable sensory attributes such sourness (11.20), and astringency (10.20). These elevated attribute scores could be explained by the significant contents of organic acids and polyphenols in sun-dried Criollo bean samples. Criollo cocoa beans

obtained from freeze-dried process exhibited the lowest sensory scores like 1.08, 4.30, 4.10 for caramel, cocoa aroma, and color attribute, respectively. Additionally, freeze-dried Criollo bean samples exhibited the highest score (7.30) of fresh fruit attribute, which could be associated to the highest concentration of esters. When the overall quality of Criollo beans among the drying processes were compared, the most appreciated cocoa bean samples were those from convective drying method, with sensory score of 11.2, followed by microwave drying process (9.3).

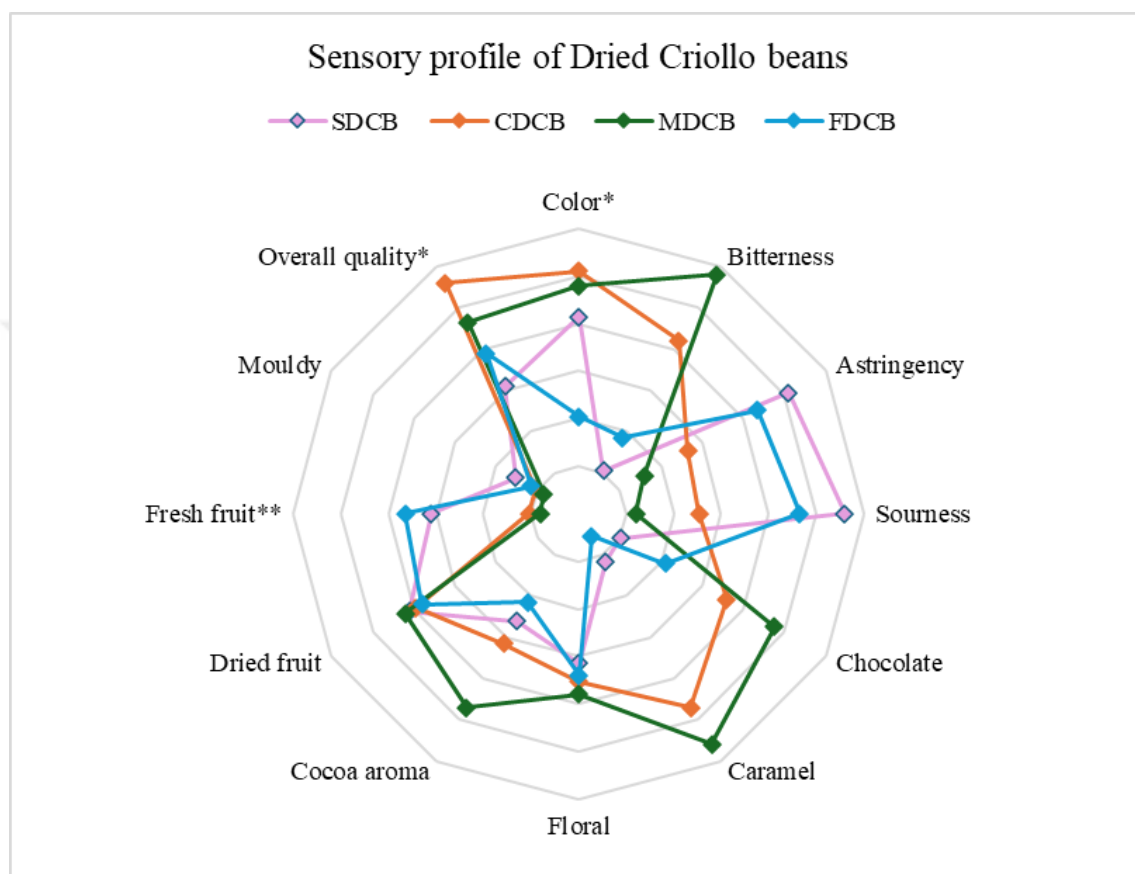


Figure 4.36. Spider web plot shows sensory profiles of the fermented and dried Criollo cocoa beans. Average scores of attribute intensity are shown as distance from the center.

4.8.4.3. Organoleptic Assessment of Fermented and Dried Nacional Beans

The sensory attributes of the four dried Nacional bean samples (nibs) were evaluated using descriptive analysis. Fresh-fermented Nacional bean sample was not evaluated for its sensory profile due to its high moisture content and some difficulties to have a considerable nibs. A panel of 10 assessors analyzed each sample, describing the beans based on a set of 11 descriptors (bitterness, color, astringency, sourness, chocolate, caramel, floral, cocoa aroma, dried fruit, fresh fruit, mouldy, and overall quality), which were scored on a structured scale from 0 to 15 cm, and the results are indicated in Table 4.13. Nacional beans “color” and “overall quality” criteria were taken in consideration during the assessment sessions. The results of this descriptive analysis are presented in a spider diagram in Figure 4.37. All attributes were assessed by the panelists through smelling and tasting the fermented and dried Criollo beans (nibs).

Table 4.13. Average scores of the sensory attributes and identification of statistically significant differences among the dried Nacional cocoa bean samples, by one-way ANOVA and LSD test analyses

Attributes	Dried Nacional Cocoa Beans				Sig.	Pr> F(Model)
	SDNB	CDNB	MDNB	FDNB		
Color	7.4	11.7	13.4	6.5	**	0.008
Bitterness	3.2	11.4	12.6	5.8	*	0.034
Astringency	8.6	6.02	3.1	10.3	*	0.019
Sourness	10.3	4.7	3.9	11.2	*	0.040
Chocolate	6.1	9.3	11.4	4.3	*	0.038
Caramel	3.7	7.08	9.02	2.8	*	0.017
Floral	4.9	4.6	4.02	5.01	ns	0.364
Cocoa aroma	3.07	7.6	10.3	4.2	*	0.036
Dried fruit	5.2	6.04	6.7	5.8	ns	0.028
Fresh Fruit	6.01	3.8	1.6	7.2	*	0.019
Mouldy	2.01	1.8	1.3	2.09	ns	0.254
Overall quality	7.1	11.3	12.4	8.2	*	0.015

Sig.: significance at which means differ as shown by analysis of variance; ns.: not significant, significant at the * $p < 0.05$ level, ** $p < 0.01$. Different letters in the same row indicate statistical differences at the $p < 0.05$ level according to Duncan multiple test. P: sensory attribute evaluated orthonasally

When the sensory analysis results of dried Nacional cocoa samples were statistically evaluated, the values obtained for all attributes and overall quality, excepted floral and mouldy notes were found to be statistically significant among all dried Nacional bean samples ($p < 0.05$, $p < 0.01$). The sensory profile scores, namely color, bitterness, chocolate, caramel, cocoa aroma, and dried fruit, were higher in MDNB than other dried bean samples. Regarding sourness, astringency, and fresh fruit, freeze-dried Nacional bean samples exhibited the highest scores such as 11.2, 10.3, 7.2, respectively compared to other dried Nacional bean samples. Additionally, no significant difference was observed when floral note score was compared between the samples ($p < 0.05$). The lowest undesirable attribute scores like sourness, astringency, and mouldy were determined in MDNB samples, followed by CDNB samples. In term of overall quality, the difference between samples was found to be statistically significant ($p < 0.01$), and the most like cocoa bean samples were that of microwave drying method, with sensory score of 12.4, followed by convective drying process (11.3).

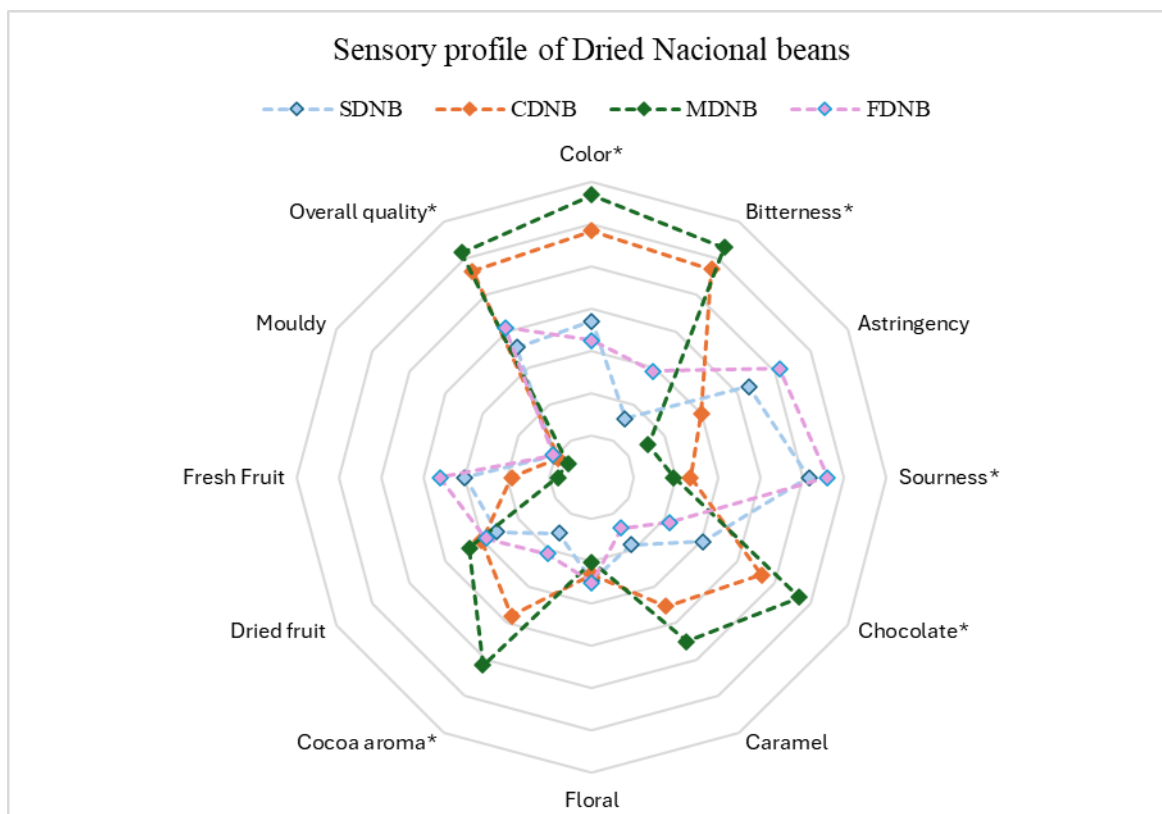


Figure 4.37. Spider web plot showing sensory profiles of the fermented and dried Nacional cocoa beans. Average scores of attribute intensity are shown as distance from the center.

4.9 Characterization of Volatile Compounds of the Three Fermented Cocoa Bean Varieties based on Sun Drying process

The distribution of aroma Compounds in sun-dried bean samples obtained from three different cocoa varieties (Forastero, Criollo, and Nacional) according to the compound groups (%) is shown in Figure 4.38. The aroma compounds identified in sun-dried bean samples attained from three different cocoa varieties and linear retention index values on the DB-Wax column for these compounds are illustrated in Table 4.14.

Regarding to the findings obtained from the analysis in sun-dried bean samples acquired from three different cocoa varieties;

- In the sun-dried beans obtained from Forastero variety, a total of 57 compounds (6 aldehydes, 9 alcohols, 5 ketones, 9 esters, 10 terpenes, 4 pyrazines, 2 lactone, 2 furans, 1 furanone, 8 acids and 1 volatile phenol),
- In the sun-dried beans extracted from Criollo variety, a total of 52 compounds (7 aldehydes, 8 alcohols, 5 acids, 2 ketones, 12 esters, 8 terpenes, 4 pyrazines, 2 lactones, 1 pyrrole, 1 furan, 2 volatile phenols) and,

- In the sun-dried beans belonging to the Nacional variety, a total of 48 compounds (8 aldehydes, 12 alcohols, 3 ketones, 10 esters, 8 terpenes, 3 pyrazines, 1 lactone, 1 pyrrole, 2 volatile phenols).

4.9.1. Aroma Compounds of Sun-Dried Cocoa Beans from the three Cocoa Cultivars

As already describe, aroma compounds are among the most critical factors influencing the sensory properties of dried , roasted cocoa beans and related products. The unique and distinctive flavor of cocoa beans and products, particularly its green, floral and fruity characteristics, is attributed to numerous volatile compounds (Qin et al., 2017; Rodriguez-Campos et al., 2011). Both concentration and odor threshold of these volatile compounds, whether they are major or minor components, play a crucial role in determining the quality of cooa beans and final products. Therefore, the identification and quantification of compounds that contribute to either the desired flavor or off-flavors are essential for quality control of cocoa beans and products (Qin et al., 2017; Spizzirri et al., 2019).

As can be exhibited in Table 4.14, there are significant differences among the compositions and content of volatiles in Forastero, Criollo and Nacional group ($p < 0.05$). Analyzing the findings for each chemical group reveals that the composition of aldehydes, esters and alcohols in the sun-dried beans from all cocoa cultivars was influenced by the metabolic pathways involved in the biosynthesis and degradation of the major volatile compounds in the beans. These processes occur throughout the beans fermentation, drying and roasting processes (Frauendorfer & Schieberle, 2008; Ramli et al., 2006). When cocoa cultivars were compared based on sun-drying process, the highest amount of total aroma compounds (13659.4 $\mu\text{g/kg}$) was found sun-dried beans obtained from Nacional cocoa type, followed by the dried bean sample acquired from Forastero cocoa cultivar (11464.6 $\mu\text{g/kg}$), whereas the lowest amount of the total aroma compounds (10533.0 $\mu\text{g/kg}$) was determined in sun-dried bean sample attained from Criollo cocoa group.

As shown in Table 4.14, a wide variety of aroma compounds were detected in the sun-dried bean samples of all cocoa varieties. Most of these compounds have been previously identified in different cocoa bean varieties and hybrids (Calva-Estrada et al., 2020; Schlüter et al., 2022). The primary contributors to the odor notes in cocoa beans are substantial volatile compounds, specifically the C-6 and C-5 compounds, which originate from the primary and secondary lipoxygenase pathways, respectively (D'Souza et al., 2017; Deuscher et al., 2020). Moreover, minor volatile substances can be utilized as a relevant quality indicators and conducted to a better conception of the formation or degradation of the major volatile compounds (Kalua et al., 2007). Indeed, the distinctive and unique scent of dried, roasted cocoa beans, particularly its green, floral, fruity, caramel, and chocolate features, is attributed to several volatile compounds (Quelal et al., 2023; Spizzirri et al., 2019). It is well-known that the volatile compounds in cocoa beans and products are related to the levels and activities of enzymes, microorganisms involved in various metabolic pathways, and most

importantly by various chemical reactions, including Maillard reactions (Marseglia et al., 2020). Nevertheless, further agents such as fruit ripening degree, variety of cocoa, drying and roasting process, geographic region and oil isolation techniques impacted the composition of aroma compounds. Among these drying and variety of cocoa beans are among of the most effective factors that impact the cocoa bean aroma.

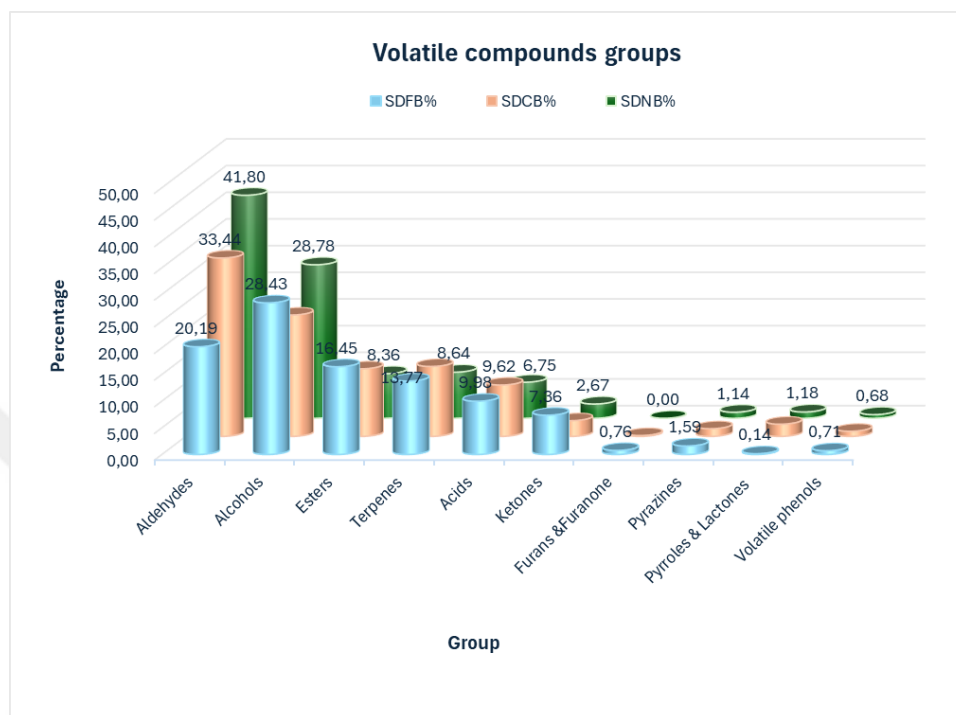


Figure 4.38. Distribution of aroma compounds according to compound groups (%) in sun-dried bean samples obtained from the three cultivars

Of all aroma compounds detected in sun-dried beans acquired from Criollo and Nacional group, aldehydes were identified and quantified the most dominant aroma compounds and main group of the aroma fractions in sun-dried Criollo and Nacional bean samples. However, alcohols were the most dominant compounds in sun-dried forastero bean samples, followed by aldehydes (Figure 4.38). When cocoa fresh beans (pulp) tissues are disrupted through fermentation, the lipoxygenase pathway is started by the release of enzymes via microorganisms activities. The reaction pathway consists an array of microorganisms and enzymes (lipoxygenase) that oxidize and cleave (hydroperoxide lyase) polyunsaturated fatty acids to yield aldehydes. These are subsequently reduced to alcohols (by alcohol dehydrogenase) and esterified to produce esters by alcohol acyltransferase (Żyżelewicz et al., 2014). Additionally, significant strecker aldehydes are also formed during the beans drying process. Aldehydes were also accounted for the largest proportion of the total aroma compounds content, which its concentration was 2315.1, 3522.0, and 5710.2 $\mu\text{g/kg}$ in sun-dried Forastero, Criollo, and Nacional bean samples, respectively. Aldehydes are generally found in greater quantities in dried and roasted cocoa beans compared to other flavor compounds. When aldehyde compounds are compared between the three cocoa cultivars, 3-methylbutanal (2750.9

µg/kg) was quantitatively the main aroma compound in sun-dried Nacional beans, followed by hexanal (2175.4 µg/kg) in sun-dried Criollo beans. 3-methylbutanal and hexanal were also discovered as a major aroma compound in most cocoa bean varieties from different origins (Ducki et al., 2008; Frauendorfer & Schieberle, 2008; Rodriguez-Campos et al., 2012).

4.9.1.1. Aldehyde Compounds

As already demonstrated, aldehydes are usually contributed to give the grass and green plant aroma to cocoa beans. The number of aldehydes detected and quantified in sun-dried Nacional bean extracts was eight, while from sun-dried Criollo and Forastero bean extracts was seven and six, respectively. These compounds form the main group of the aroma fraction cocoa beans in all cocoa bean varieties, except Forastero beans, making them more significant compared to other volatile compounds. Aldehydes were determined as the second most volatile compounds in Forastero cocoa group. The ratio of aldehyde compounds in sun-dried beans obtained from the three cocoa bean cultivars according to the amount of total aroma compounds in percent is presented in Figure 4.39. Aldehydes, like alcohols are always discovered in greater quantities in dried and roasted cocoa beans compared to further volatiles. In the present study, the aldehyde compounds of the three cocoa cultivars contributed 20.19-41.8% to the total volatile concentration. When the sun-dried cocoa bean varieties was compared, the highest concentration (5710.2 µg/kg) of aldehydes was observed in sun-dried Nacional bean samples accounting for of total aroma compounds, followed by sun-dried Criollo beans (3522.0 µg/kg) with proportion of the total aroma compounds and in sun-dried Forastero beans (2315.1 µg/kg) comprising in of total aroma compounds. The total concentration of aldehydes in the sun-dried Nacional samples was 1.62 times greater than in the sun-dried Criollo bean samples and 2.46 times greater than in sun-dried Forastero bean samples. These differences in aldehyde concentration in all cocoa bean varieties may be due to the chemical compositions, microorganisms and enzymes populations during fermentation, pH and the bioconversion rate (Cremer & Eichner, 2000).

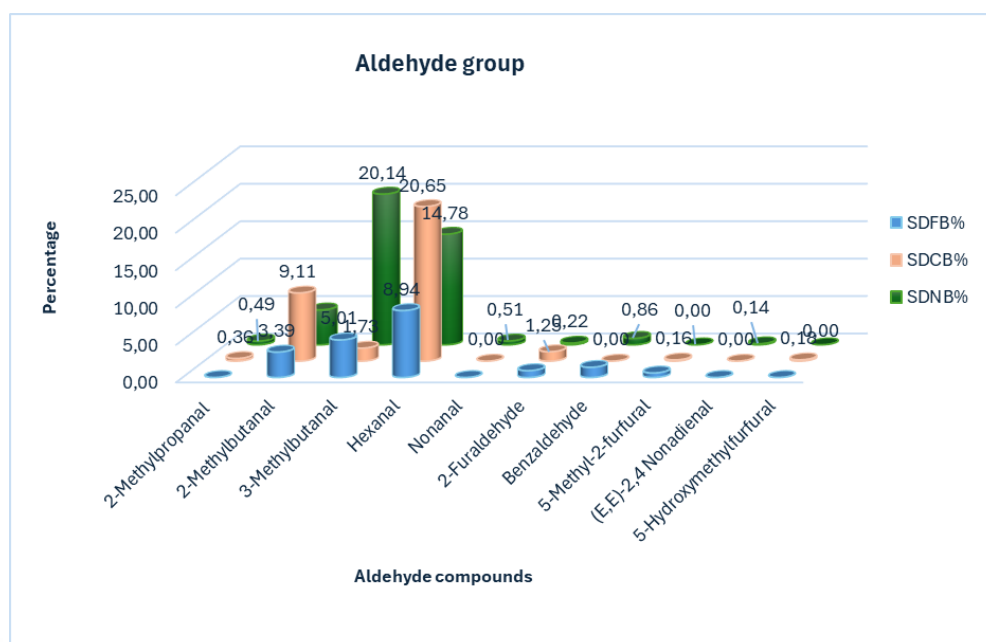


Figure 4.39. The ratio of aldehyde compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

3-Methylbutanal and hexanal were identified as major aldehyde compounds (Table 4.14 and figure 4.39), which accounted for 60% higher than of the total aldehydes. 3-Methylbutanal, a strecker aldehyde (green/apple-like) was present at the highest concentration among both the total aldehydes and the overall aroma compounds found in Nacional beans (2750.9 $\mu\text{g/kg}$), followed by Forastero beans (574.6 $\mu\text{g/kg}$); Criollo beans exhibited the lowest content at 182.4 $\mu\text{g/kg}$. These differences in 3-methylbutanal concentration between the three cocoa cultivars may be due to the different content of leucine and pH value of cocoa groups as revealed by Cremer and Eichner (2000). Hexanal (green) was found as the second dominant aldehyde compound in the three cocoa groups. The amounts of hexanal were significantly greater in the morphogenetic groups of Criollo beans (2175.4 $\mu\text{g/kg}$) and Nacional beans (2019.0 $\mu\text{g/kg}$) than those in Forastero beans (1024.6 $\mu\text{g/kg}$). In fact, numerous scholars Qin et al. (2017), Liu et al. (2017) and Liu et al. (2014) also evidenced that hexanal is the most prevalent volatile compound, responsible for green and cut grass-like sensory notes, which impart a positive characteristic to morphogenetic cocoa beans (Qin et al., 2017; Schnermann & Schieberle, 1997). The enzymatic breakdown of the 13-hydroperoxide of linolenic acid by aldehyde-lyase cause the production of hexanal compound (Kiritsakis et al., 1998). These aldehydes are prevalent, having been detected in many other cocoa cultivars such as Trinitario and hybrids (Portillo et al., 2009). Though the amount of 3-methylbutanal in the current study is more than that of hexanal in Nacional beans, hexanal could be contributed to green aroma notes more than 3-methylbutanal owing to its lower odor threshold (75–300 $\mu\text{g/kg}$). The odor threshold value of a compound is, in fact, the minimum concentration required to elicit an olfactory response (Baccouri et al., 2008).

Among aldehyde compounds discovered in the three cocoa bean groups, 2-methylbutanal has also been associated with quality status of dried, roasted cocoa beans and related products, which

has positive effect on cocoa beans and products. In fact, these compounds is positively associated with nutty and cocoa notes of cocoa beans and products (Misnawi, 2012; Schwan & Wheals, 2004). 2-methylbutanal was also found in significant amount, with greater amount in Criollo beans (959.3 µg/kg), followed by Nacional beans (636.6 µg/kg), and Forastero beans (388.4 µg/kg). Interestingly, nonanal (fresh/green) and 5-hydroxymethylfurfural (breadly/almond) were only present with small amount in Nacional beans (69.5 µg/kg) and Criollo beans (18.5 µg/kg), respectively. These volatile compounds have also been associated with positive flavor and good quality of cocoa beans and products as demonstrated by Liu et al. (2017) in their investigation.

4.9.1.2. Alcohol Compounds

Alcohols are generated by the action of the alcohol dehydrogenase (ADH) enzyme, which is found in many plants. This enzyme is responsible for producing the volatile alcohols that contribute to the overall aroma of dried, roasted cocoa beans and products. These compounds have also been reported to occur during fermentation as a results of microbial activity. Remli et al. (2006) demonstrated that alcohols may formed through heat degradation of aminop acids. They also reported that during drying and roasting, the concentration of alcohols decreases due to chemical degradation or volatilization, and at high temperatures (160°C to 170°C) and prolonged heat exposure promote the loss of alcohols. Alcohols are typically described as having "fruity and plant-like" notes (Saltini, R et al., 2013). A total of 9, 8 and 12 alcohols were detected across sun-dried beans acquired from Forastero, Criollo and Nacional groups, respectively. Alcohols quantitatively represented the second main class of volatile compounds in Nacional and Criollo cocoa groups, while representing the major group of volatile in Forastero cocoa group (Table 4.14), which has also been demonstrated in previous studies (Ducki et al., 2008; Saltini et al., 2013). The ratio of alcohol compounds in the three cocoa cultivars obtained from sun-dried beans, expressed as a percentage of the total compounds, is illustrated in Figure 4.40. The concentration of total alcohols, as shown in Table 4, revealed statistically significant differences between the examined cocoa bean groups ($p < 0.05$). The alcohol content was significantly higher in Nacional cocoa (3930.5 µg/kg) and Forastero cocoa (3258.9 µg/kg) compared to Criollo cocoa (2396.1 µg/kg). This obervation was also confirmed by Ducki, S et al. (2008) and Cambrai, A et al. (2010) in their investigation. Hexanol as the C-6 alcohol was predominantly present in Criollo and Forastero cocoa groups. Moreover, (E)-2-hexanol, phenylethanol and -penten-1-ol were also observed in a significant amount in Nacional cocoa group. Interestingly, 2-methyl-2-butanol, 1-penten-3-ol, 3-methylbutanol, 2-penten-1-ol, 2-heptanol, 1,3-butandiol, were only present in the Nacional cocoa bean samples. When compared to further investigations, the same observation of the mentioned C-6 alcohol (hexanol and (E)-2-hexanol) were found in cocoa groups from different origins (Colonges et al., 2021; Colonges, Seguire, et al., 2022).

As can be observed in the Figure 4.40, hexanol as the dominant volatile alcohol was determined, and its concentration exposed the highest amount in Forastero beans (1690.7 µg/kg),

followed by Criollo beans (1077.5 $\mu\text{g/kg}$), while Nacional cocoa group exhibited the lowest amount of hexanol (221.9 $\mu\text{g/kg}$). This trend has been previously confirmed by Xiao-Wei Qin et al. (2016) in Forastero, Criollo and Trinitario cocoa groups. (*E*)-2-Hexanol was determined as the second dominant alcohol in Nacional beans (878.8 $\mu\text{g/kg}$), followed by Criollo beans (64.3 $\mu\text{g/kg}$), while no (*E*)-2-hexanol was observed in Forastero beans. Kadow et al. (2013) and Oberparlaiter and Zieglede (1997) in their assays, reported that (*E*)-2-hexanol, 2-pentanol, 2,3-butanediol, 2-heptanol contributing to fresh and floral odors were the most abundant alcohols, accounting for 80% of total alcohols in Criollo, forastero and Trinitario cocoa groups. These compounds also contribute to the fruity and floral notes in fine cocoa beans and products (Ducki et al., 2008; Motamayor et al., 2008).

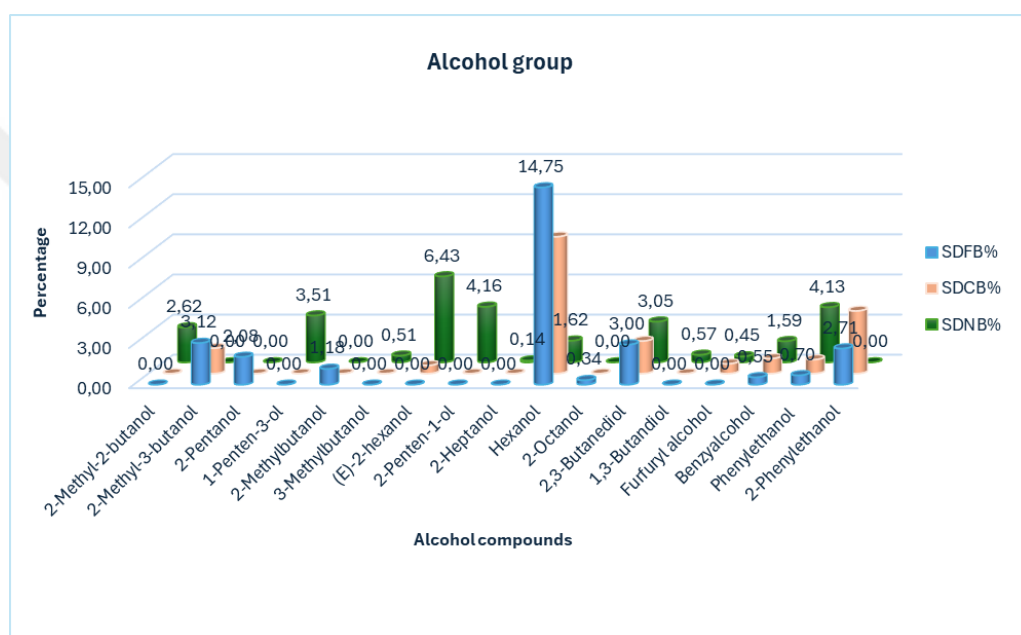


Figure 4.40. The ratio of alcohol compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.3. Ester Compounds

In the analyzed sun-dried cocoa beans acquired from the three cultivars, 9, 12 and 10 esters were identified and quantified in Forastero, Criollo and Nacional cocoa groups, respectively. Some of these esters were previously discovered in the investigated cocoa types and other cocoa cultivars (Ramli et al., 2006; Rodriguez-Campos et al., 2011). The ratio of ester compounds in sun-dried cocoa beans, obtained from the three cocoa types and expressed as a percentage of the total compounds, is demonstrated in Figure 4.41. Compared to alcohols and aldehydes, low level of total esters (between 1141.8 and 1886.3 $\mu\text{g/kg}$) were determined in cocoa beans acquired from Forastero, Criollo and Nacional groups. The low levels of esters in the three cocoa groups indicate a low activity of alcohol acyl transferase, which is likely the cause of the reduced ester levels observed in these cocoa groups (Ramli et al., 2006). These compounds confer a fruity note to unroasted cocoa based on the organic acid with ethanol (Afoakwa et al., 2008), and are the typical volatile substances, especially acetates

that arise from amino acids (Ziegler & Biehl, 2003). When the cocoa groups were compared, Forastero cocoa beans exhibited the highest total ester amount at 1886.3 $\mu\text{g/kg}$, followed by Criollo at 1334.0 $\mu\text{g/kg}$; Nacional cocoa beans showed the lowest concentration at 1141.8 $\mu\text{g/kg}$. The higher level of ester in Forastero cocoa beans may be probably due to the important concentration of organic acids and amino acids as demonstrated by Afoakwa et al. (2008) and Ziegler and Biehl (2003) in their researches, which confirmed the findings in our study. Furthermore, Forastero cocoa beans showed the highest 2-methylbutyl acetate at 603.2 $\mu\text{g/kg}$, followed by Nacional cocoa beans with 171.2 $\mu\text{g/kg}$, however, this compounds was not present in Criollo cocoa group. Ethyl 2-methylbutanoate was the second predominant ester observed in cocoa groups, with elevated amount in Forastero group (297.4 $\mu\text{g/kg}$), followed by Criollo type (221.2 $\mu\text{g/kg}$); the lowest amount was observed in Nacional cocoa type (191.4 $\mu\text{g/kg}$). Afoakwa et al. (2008) reported ethyl-2-methylbutanoate as an important flavor formed during cocoa bean fermentation. Interestingly, isoamyl acetate, a product of yeast metabolism, is an indicator of flavor defects in cocoa beans (Rodriguez-Campos et al., 2012). This compounds was only present with low amount (125.2 $\mu\text{g/kg}$) in Criollo cocoa group.

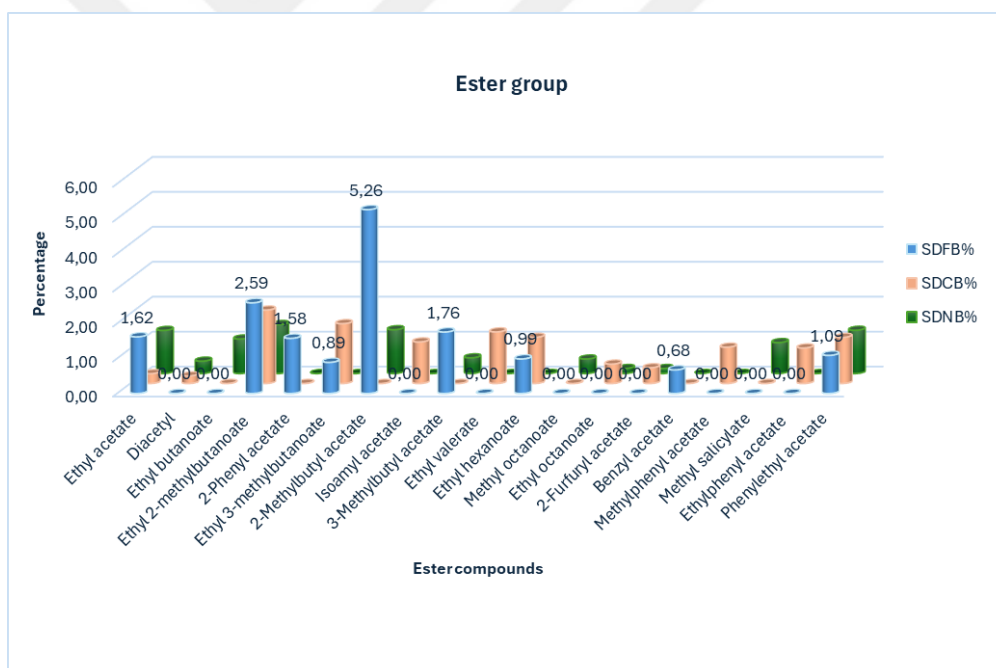


Figure 4.41. The ratio of ester compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.4. Acid compounds

As already described, acids are key contributors to cocoa flavor, particularly through the acidification of cocoa beans by acetic acid during fermentation. This process leads to various biochemical modifications essential for cocoa flavor development (Wang & Ho, 2009; Żyżelewicz et al., 2014). In the present study, eight acids such as acetic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, octanoic acid, nonanoic acid, and phenylacetic

acid in Forastero cocoa type, five acid containing acetic acid, hexanoic acid, octanoic acid, nonanoic acid, and phenylacetic acid in Criollo cocoa group, six acids including acetic acid, pentanoic acid, hexanoic acid, octanoic acid, nonanoic acid, and phenylacetic acid in Nacional cocoa cultivar were identified and quantified. Acids in the cocoa cultivars and hybrids are the result of sugar (cocoa pulp) metabolism through fermentation. The total amount of these compounds in the three cocoa group ranged between 922.0 and 1144.6 $\mu\text{g/kg}$. The proportion of acid compounds in sun-dried cocoa beans, expressed as a percentage of the total compounds and obtained from the three cocoa cultivars, is demonstrated in Figure 4. Most of volatile acids identified and quantified in all cocoa groups have been reported in previous study (Cevallos-Cevallos et al., 2018).

Acids content was significantly higher in Forastero cocoa (1144.6 $\mu\text{g/kg}$) and Criollo cocoa (1013.8 $\mu\text{g/kg}$) than those of Nacional cocoa (922.0 $\mu\text{g/kg}$), accounted for approximately 9.98%, 9.62%, and 6.74% of the total aroma compounds analyzed in sun-dried beans obtained from the three cocoa types, respectively. Among the acids (Table 4.14), acetic acid (vinegar-like) exhibited the most dominant acid in all cocoa cultivars, with the highest amount in Forastero cocoa (496.0 $\mu\text{g/kg}$), followed by Criollo (494.3 $\mu\text{g/kg}$); Nacional cocoa was observed with the lowest amount (358.3 $\mu\text{g/kg}$). Żyżelewicz et al. (2014) reported that acetic acid was the most dominant volatile acid in fermented and unfermented Trinitario cocoa and other types. Some studies demonstrate that chocolates produced from Criollo beans have higher acidic tones, attributed to acetic acid, compared to those made from Forastero and Trinitario beans (Frauendorfer, F.; Schieberle, P, 2008, Acierno, V et al., 2016). Hexanoic acid was determined as the second most dominant volatile acid in all cocoa cultivars, with significant amount in Criollo cocoa (294.2 $\mu\text{g/kg}$), followed by Forastero (170.3 $\mu\text{g/kg}$), while low amount was observed in Nacional cocoa (130.7 $\mu\text{g/kg}$). Organic acids have been detected as providing sour, buttery and pungent sensation in dried-fermented Forastero, Criollo and Nacional cocoa beans samples (Cevallos-Cevallos et al., 2018; Koné et al., 2016; Rodriguez-Campos et al., 2012).

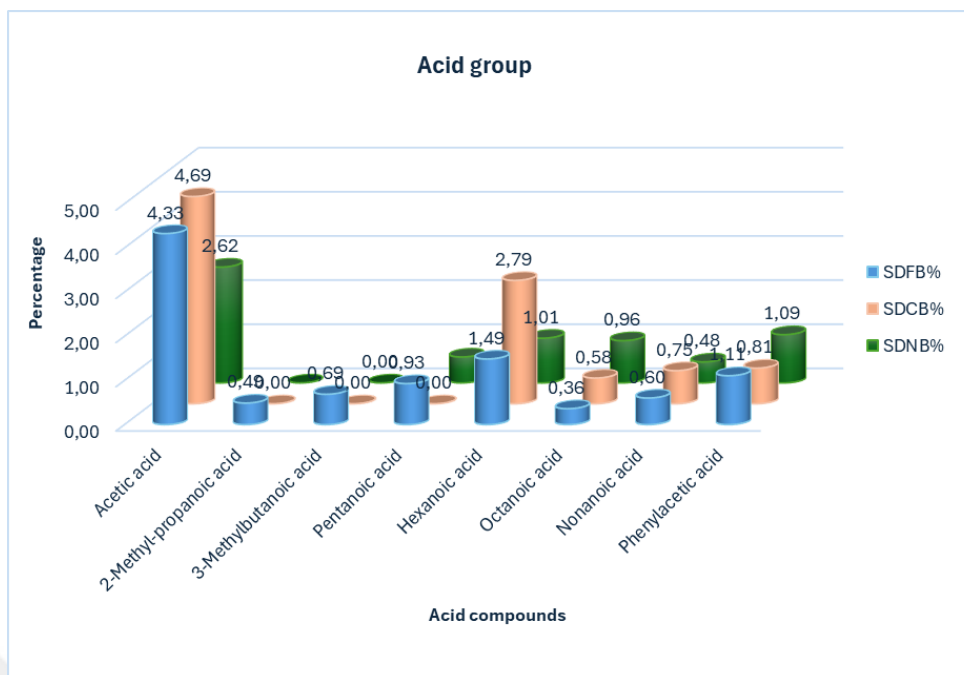


Figure 4.42. The ratio of acid compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.5. Ketone Compounds

Carbonyl compounds of the ketone type are crucial for developing good cocoa flavor. A high concentration of both aldehydes and ketones is favorable for cocoa quality (Rodriguez-Campos et al., 2012). Desirable ketones are obtained during a fermentation period of 6 to 8 days and drying at 70°C (Rodriguez-Campos et al., 2012). In the present study, five, two and three ketones were quantified and identified in sun-dried cocoa beans acquired from Forastero, Criollo and Nacional groups, respectively, quantitatively representing the fifth main group of volatile components in all the cocoa groups, which was also established in the former investigations (Qin et al., 2017; Spizzirri et al., 2019). The ratio of ketone compounds in cocoa groups, expressed as a percentage of the total compounds and obtained from sun-drying process, is displayed in Figure 4. The total content of ketones in cocoa beans was 844.0 µg/kg, the highest proportion in Forastero group, followed by Nacional group (365.1 µg/kg), while the lowest proportion was observed in Criollo group (309.9 µg/kg). From the ketone class, 3-hydroxy-2-butanone represented the most dominant in all cocoa cultivars, and its concentration was between 138.3-295.3 µg/kg. The highest amount of this compound was observed in Forastero cocoa group (295.3 µg/kg), while the lowest amount was found in Criollo cocoa group (138.3 µg/kg). 2-Heptanone was determined as the second most abundant ketone, and its concentration was almost similar in Forastero and Criollo cocoa groups, while no 2-heptanone was identified in Nacional cocoa type. The difference may be attributed to the postharvest techniques and the chemical composition of the cocoa beans. 3-Hydroxy-2-butanone was also identified with significant content of ketones in other cocoa cultivars and hybrids, including Maya cocoa (Utrilla-Vázquez et al., 2020).

4.9.1.6. Terpene compounds

Different terpenoid hydrocarbons, comprising monoterpenes, terpene alcohols and some of their oxides, were detected and quantified in cocoa bean groups. Indeed, ten terpenes (α pinene, linalool oxide I, sabinene, β -myrcene, 1,4-cineol, D-limonene, ocimene, γ -terpinene, linalool oxide II, and linalool), eight (camphene, D-limonene, ocimene, *p*-cymene, (*E*)-caryophyllene, geraniol, and thymol), and eight (α -phellandrene, sabinene, ocimene, *p*-cymen, β -aromadendrene, (*E*)-caryophyllene, valencene), were identified in sun-dried Forastero, Criollo, and Nacional cocoa cultivars, respectively. The total amount of these compounds ranged between 1180.3 and 1578.5 $\mu\text{g/kg}$, in which the highest content of terpene was observed in Forastero cocoa (1578.5 $\mu\text{g/kg}$), followed by Criollo (1380.6 $\mu\text{g/kg}$). Xiao-Wei Qin et al. (2017) reported lower terpenes content in Forastero cocoa group, which is in disagreement with our findings. This discord may probably due to the difference in chemical composition, geographical origin of cocoa cultivars, postharvest practices. The ratio of terpene compounds in sun-dried cocoa bean varieties, expressed as a percentage of the total compounds is demonstrated in Figure 4.43. Furthermore, terpenes accounted for approximately 8.64%, 13.10%, and 13.76% of the total volatile compounds analyzed in sun-dried cocoa beans obtained from Nacional, Criollo, and Forastero group, respectively. In the present study, ocimene, *p*-cymen, γ -terpinene, (*E*)-caryophyllene, β -aromadendrene, valencene, and camphene were the most dominant terpenes in Forastero, Criollo and Nacional cocoa group. Among the terpenes in all cocoa groups, *p*-cymen (288.7 $\mu\text{g/kg}$) was found with the highest concentration in Criollo cocoa group. In addition, ocimene (286.3 $\mu\text{g/kg}$) was the second most abundant terpene identified in Forastero cocoa group, followed by γ -terpinene (270.8 $\mu\text{g/kg}$) and β -aromadendrene (247.5 $\mu\text{g/kg}$) in Forastero and Nacional cocoa bean group, respectively. Some of these terpenes were also observed in other cocoa cultivars (Xiao-Wei Qin et al., 2017). These compounds have been associated to the characteristic pleasant odors (green, fruity and floral) in cocoa beans and related products (Kadow et al., 2013; Krings et al., 2006; Ziegleder, 1990).

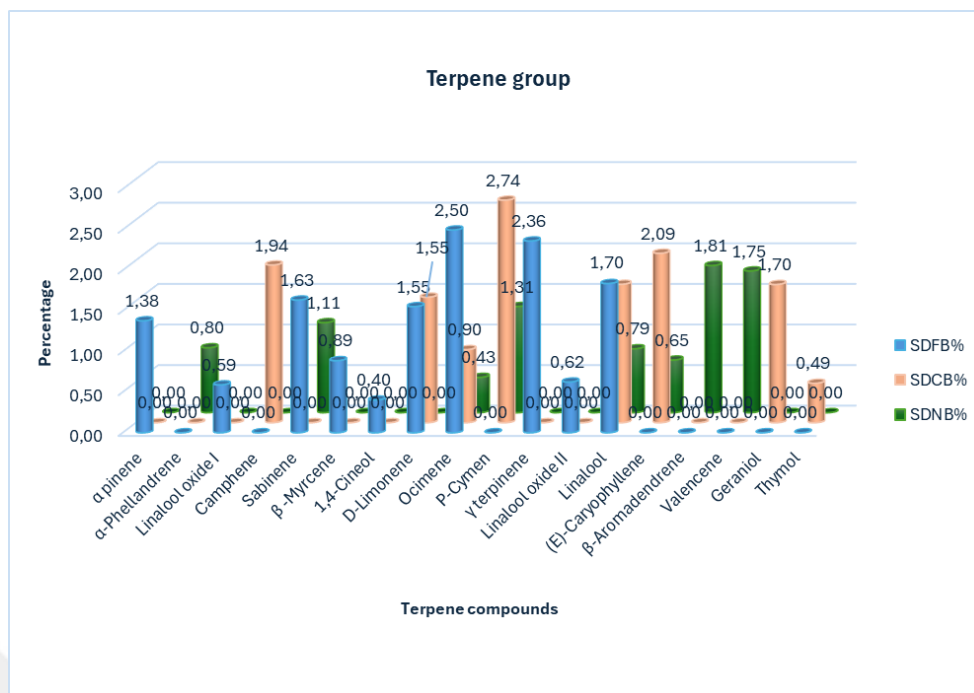


Figure 4.43. The ratio of terpene compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.7. Pyrazine Compounds

Pyrazines are key flavor compounds in baked goods, including roasted peanuts, coffee beans, hazelnuts, and cocoa beans. Most of the pyrazines are formed from α -aminoketones via Strecker degradation and Maillard reactions during drying and roasting of cocoa beans (Hashim et al., 1999; Rodriguez-Campos et al., 2012). The concentration of pyrazines is significantly affected by the temperature and duration of these thermal processes. These compounds contribute to desirable nutty and roasted aroma to cocoa beans and products (Liu et al., 2017; Schieberle & Pfner, 1999). A total of four different pyrazines (2-methylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-acetyl-3-methylpyrazine), four (2-methylpyrazine, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine), and three (2-methylpyrazine, 2,3-dimethylpyrazine, 2-ethylpyrazine) were determined in Forastero, Criollo, and Nacional cocoa types, respectively. da Veiga Moreira et al. (2018) also reported few number of pyrazines in cocoa beans from different hybrids cultivated in Brazil. The same observation was made by Visintin et al. (2017) in their studies. The authors highlighted that the presence of pyrazines can be influenced by factors such as weather conditions, pod ripeness, cocoa varieties, and postharvest processing. Figure 4.44 illustrates the ratio of pyrazine compounds in sun-dried cocoa bean varieties, expressed as a percentage of the total compounds. When pyrazine contents were compared among cocoa cultivars, Forastero cocoa type exhibited the highest amount of pyrazine at 182.7 $\mu\text{g/kg}$, while no significant difference was observed between Nacional cocoa (151.1 $\mu\text{g/kg}$), and Criollo cocoa (155.1 $\mu\text{g/kg}$). Of all pyrazines, 2-methylpyrazine was the most abundant in all cocoa bean cultivars, with the highest concentration observed in Nacional cocoa (97.8 $\mu\text{g/kg}$), accounted for over 60% of total pyrazines, followed by

Forastero cocoa (89.5 µg/kg); Criollo exhibited the lowest concentration of 2-methylpyrazine (47.3 µg/kg). Methylpyrazines compound are important substances of dried and roasted food flavor, including roasted cocoa flavor, and some of them can also be used as indicator for the degree of cocoa roasting as demonstrated by Heinzler and Eichner (1991) in their investigations.

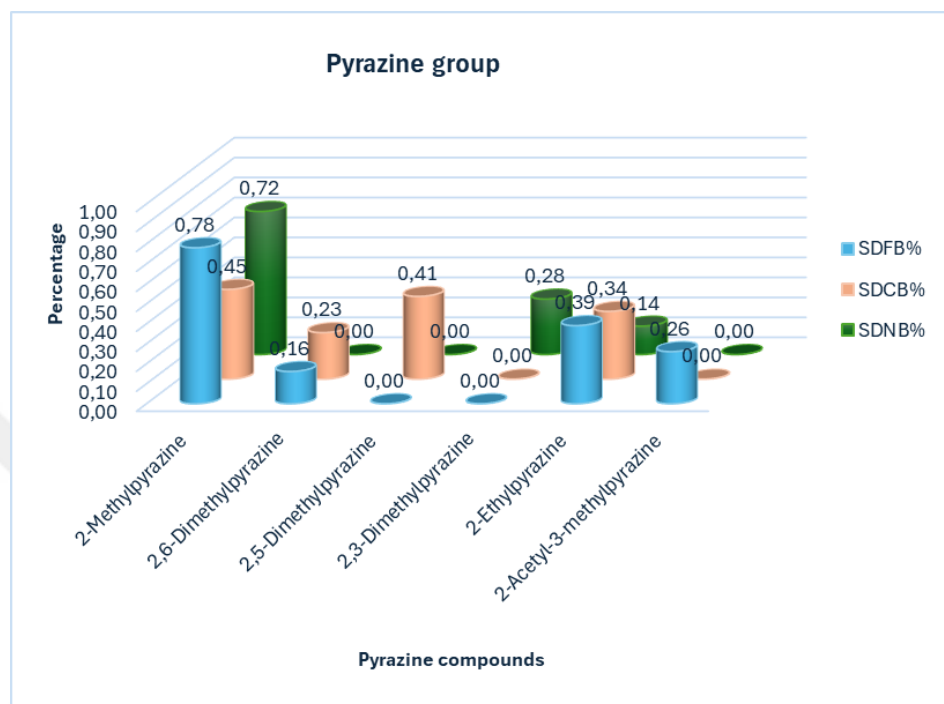


Figure 4.44. The ratio of pyrazine compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.8. Furans and Furanone Compounds

Two furans, namely 2-acetyl-5-methylfuran and 2-acetylfuran, and one furanone such as 5-(1-hydroxyethyl)-2-(5H)-furanone, were characterized in the present study, which accounted for 0.15-0.75% of the total volatile compounds. When furans and furanone were compared in number between the cocoa cultivars, two furans (2-acetyl-5-methylfuran and 2-acetylfuran) and one furanone (5-(1-hydroxyethyl)-2-(5H)-furanone) were observed in Forastero cocoa cultivars, only one furan (2-acetylfuran) was detected in Criollo cocoa type, while in Nacional cocoa cultivar low limit of detection (<LOD) of these compounds was observed. Forastero cocoa cultivar exhibited the highest total furan and furanone contents (87.1 µg/kg), followed by Criollo cocoa (16.5 µg/kg); Nacional presented the lowest amount of furan and furanone, which was not quantified due to the low limit of detection. Volatile furan and furanone are generally very low or absent in unfermented and sun dried cocoa beans. These compounds are responsible to the caramel-like, sweet and honey odors of cocoa beans and products (Żyżelewicz et al., 2014). The identified furans and furanone were potential derivative of strecker degradation and caramelization reaction developed in sun-dried cocoa beans. Indeed, the concentration of these compounds significantly increase during convective, microwave and roasting processes (Afoakwa et al., 2008). In this study, 2-acetyl-5-methylfuran and 2-

acetylfuran, representing 88% of the total furans and furanone. Additionally, Forastero cocoa cultivar exhibited the highest 2-acetyl-5-methylfuran and 2-acetylfuran contents at 44.9 and 29.7 $\mu\text{g/kg}$, respectively. Criollo cocoa type showed the lowest content of 2-acetylfuran at 16.5 $\mu\text{g/kg}$, whereas, none of them was quantitatively determined in Nacional cocoa cultivars. The ratio of furan and furanone compounds in dried cocoa beans, expressed as percentage of the total volatile components obtained from Forastero, Criollo and Nacional cocoa cultivars is illustrated in Figure 4.45.

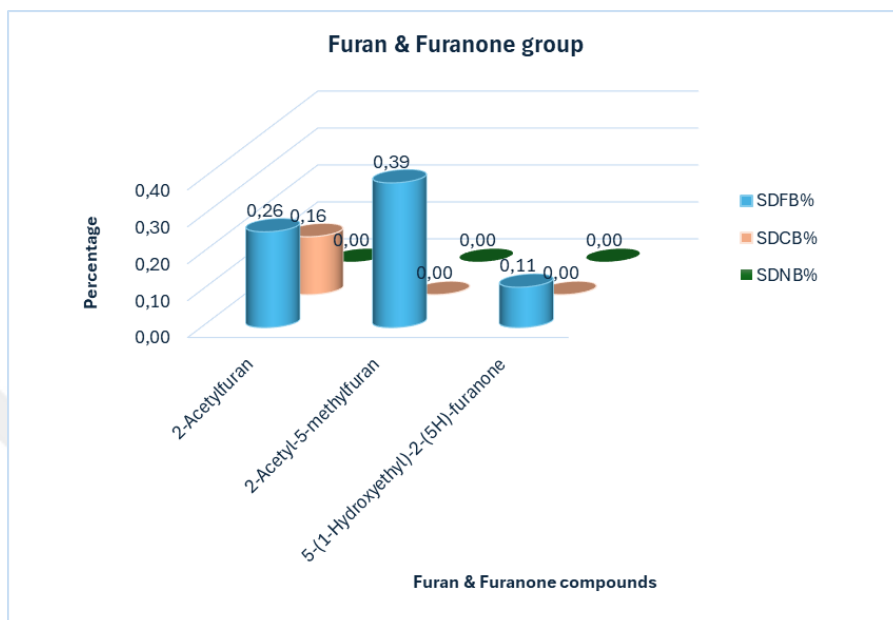


Figure 4.45. The ratio of furan and furanone compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

4.9.1.9. Other Compounds

In the analyzed cocoa groups, three distinct groups of aroma compounds were identified, including lactones, pyrroles, and volatile phenols. Among these, two lactones (γ -valerolactone, γ -butyrolactone), and one volatile phenol (guaiacol) were determined in Forastero cocoa groups, whereas, Criollo cocoa group exhibited two volatile phenols (guaiacol and eugenol), two lactones (δ -octenolactone and γ -butyrolactone), as well as one pyrrole (2-acetylpyrrole). As regards Nacional cocoa cultivars, one lactone (γ -butyrolactone), one pyrrole (2-acetylpyrrole), one pyrroline (2-acetyl-1-pyrroline), and two volatile phenols (guaiacol and isoeugenol) were observed in our study. When the total lactones and pyrrole concentration were compared, Criollo cocoa cultivar presented the highest amount (329.5 $\mu\text{g/kg}$), while no significant difference was shown between Forastero cocoa (86.8 $\mu\text{g/kg}$) and Nacional cocoa (180.9 $\mu\text{g/kg}$) groups. Among lactones, γ -butyrolactone was the most predominant in all cocoa cultivars, the highest content observed in Criollo cocoa (180.2 $\mu\text{g/kg}$), followed by Forastero cocoa (117.1 $\mu\text{g/kg}$). 2-acetylpyrrole was dominant in Nacional cocoa cultivar (69.0 $\mu\text{g/kg}$), followed by Criollo cocoa (41.8 $\mu\text{g/kg}$), while no pyrrole was observed in Forastero cocoa type. Regarding 2-acetyl-1-pyrroline, Criollo cocoa cultivar stood out with its highest concentration (21.0 $\mu\text{g/kg}$) while Nacional and Forastero cvs. had similar amounts. Overall, the sun

drying resulted an increase in this compound' concentration in all samples. This finding is in line with the literature as the production of this compound increases with heat application (Frauendorfer & Schieberle, 2008; Quelal et al., 2023).

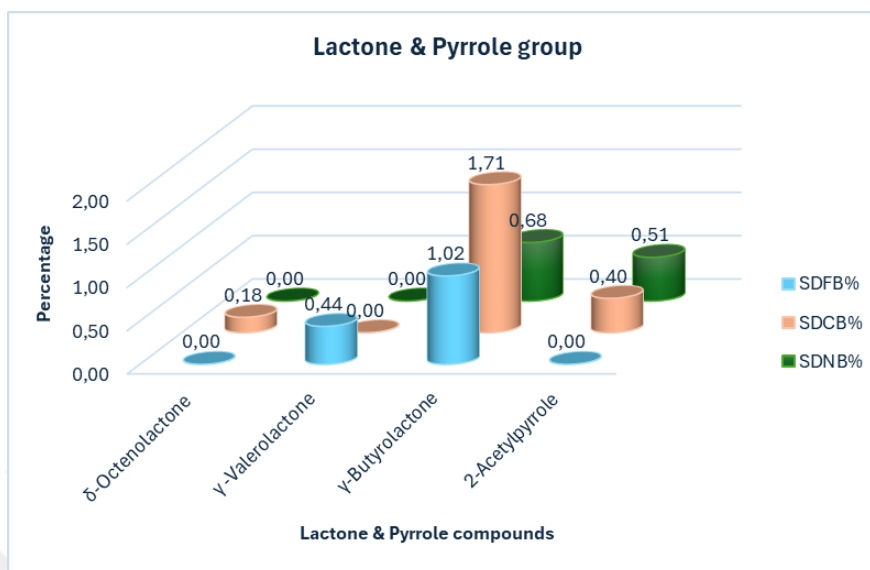


Figure 4.46. The ratio of lactone and pyrrole compounds of the sun-dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

Additionally, when volatile phenols were compared, no significant difference was reported between Criollo cocoa (101.3 $\mu\text{g/kg}$) and Nacional cocoa (93.0 $\mu\text{g/kg}$) cultivars. Although, the lowest amount of volatile phenols was reported in Forastero cocoa type (80.9 $\mu\text{g/kg}$), no significant difference was observed between Forastero cocoa and Nacional cocoa cultivars. Of all volatile phenols identified, guaiacol was the most abundant in all cocoa varieties, with the lowest amount in Nacional cocoa type (47.4 $\mu\text{g/kg}$); no significant difference was determined in Forastero cocoa (80.9 $\mu\text{g/kg}$) and Criollo cocoa (77.7 $\mu\text{g/kg}$) cultivars, which represented the highest concentration of guaiacol. Qin et al. (2017) also reported these compounds in Forastero and Criollo cocoas, and cocoa hybrids in their recent investigation.

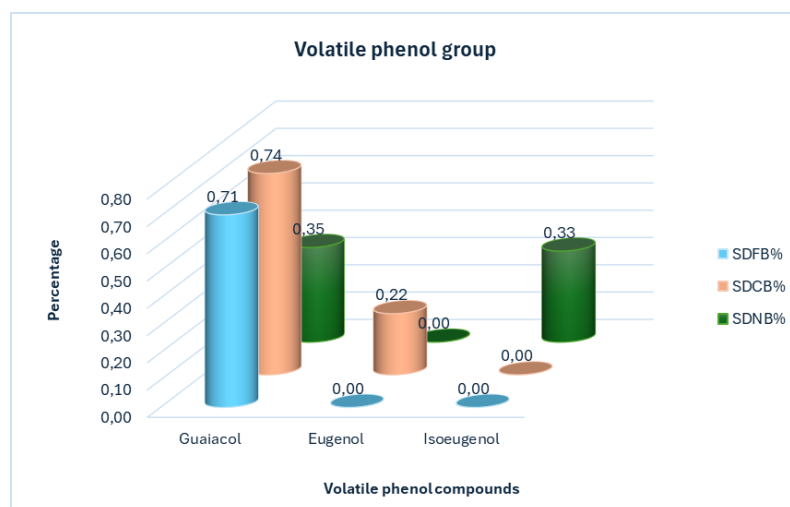


Figure 4.47. The ratio of volatile phenol compounds of the sun dried beans obtained from the three cocoa cultivars according to the amount of total compounds in percent

Table 4.14. Aroma compounds of sun-dried cocoa beans obtained from the three cocoa cultivars

No	Aroma compounds	LRI ¹	Concentration (Mean $\pm$ SD) ²			Identification ³
			SDFB	SDCB	SDNB	
	Acids					
1	Acetic acid	1418	496.0 $\pm$ 5.3 ^a	494.3 $\pm$ 1.4 ^a	358.3 $\pm$ 8.4 ^b	LRI, MS, Std
2	2-Methyl-propanoic acid	1587	55.9 $\pm$ 2.8 ^a	nd	nd	LRI, MS, tent
3	3-Methylbutanoic acid	1680	78.8 $\pm$ 0.8 ^a	nd	nd	LRI, MS, tent
4	Pentanoic acid	1762	106.4 $\pm$ 1.9 ^a	nd	80.3 $\pm$ 0.9 ^b	LRI, MS, tent
5	Hexanoic acid	1820	170.3 $\pm$ 2.3 ^b	294.2 $\pm$ 5.6 ^a	138.3 $\pm$ 1.2 ^c	LRI, MS, Std
6	Octanoic acid	2046	41.3 $\pm$ 0.4 ^c	61.3 $\pm$ 0.4 ^b	130.7 $\pm$ 3.4 ^a	LRI, MS, Std
7	Nonanoic acid	2180	69.0 $\pm$ 1.2 ^a	79.0 $\pm$ 0.8 ^a	65.0 $\pm$ 1.2 ^a	LRI, MS, Std
8	Phenylacetic acid	2554	126.9 $\pm$ 2.8 ^b	85.0 $\pm$ 1.7 ^c	149.4 $\pm$ 2.3 ^a	LRI, MS, Std
	Total		1144.6	1013.8	922.0	
	Alcohols					
9	2-Methyl-2-butanol	975	nd	nd	357.4 $\pm$ 5.2 ^a	LRI, MS, Std
10	2-Methyl-3-butanol	1089	357.4 $\pm$ 2.8 ^a	199.8 $\pm$ 1.8 ^b	nd	LRI, MS, Std
11	2-Pentanol	1138	238.2 $\pm$ 2.7 ^a	nd	nd	LRI, MS, tent
12	1-Penten-3-ol	1165	nd	nd	479.1 $\pm$ 7.9 ^c	LRI, MS, tent
13	2-Methylbutanol	1206	135.6 $\pm$ 4.8 ^a	nd	nd	LRI, MS, Std
14	3-Methylbutanol	1240	nd	nd	69.0 $\pm$ 1.2 ^a	LRI, MS, Std
15	(E)-2-hexanol	1245	nd	64.3 $\pm$ 0.8 ^b	878.8 $\pm$ 10.6 ^a	LRI, MS, Std
16	2-Penten-1-ol	1324	nd	nd	568.5 $\pm$ 7.3 ^a	LRI, MS, tent
17	2-Heptanol	1327	nd	nd	18.8 $\pm$ 0.4 ^a	LRI, MS, tent
18	Hexanol	1359	1690.7 $\pm$ 9.4 ^a	1077.5 $\pm$ 12.4 ^b	221.9 $\pm$ 1.5 ^c	LRI, MS, Std
19	2-Octanol	1412	39.0 $\pm$ 0.8 ^a	nd	nd	LRI, MS, Std
20	2,3-Butanediol	1545	343.6 $\pm$ 2.4 ^b	256.5 $\pm$ 5.8 ^c	416.8 $\pm$ 6.3 ^a	LRI, MS, Std
21	1,3-Butandiol	1578	nd	nd	77.9 $\pm$ 1.2 ^a	LRI, MS, tent
22	Furfuryl alcohol	1650	nd	76.7 $\pm$ 0.5 ^a	61.2 $\pm$ 0.5 ^a	LRI, MS, Std

Table 4.14. Continued

23	Benzylalcohol	1861	63.6 ± 0.8 ^c	118.2 ± 1.3 ^b	217.1 ± 3.8 ^a	LRI, MS, Std
24	Phenylethanol	1922	80.5 ± 1.2 ^c	109.9 ± 2.4 ^b	564.0 ± 6.5 ^a	LRI, MS, Std
25	2-Phenylethanol	1944	310.3 ± 2.6 ^b	493.2 ± 7.8 ^a	nd	LRI, MS, Std
	Total		3258.9	2396.1	3930.5	
	Aldehydes					
26	2-Methylpropanal	801	nd	37.4 ± 0.8 ^b	67.6 ± 1.5 ^a	LRI, MS, Std
27	2-Methylbutanal	896	388.4 ± 4.8 ^c	959.3 ± 9.4 ^a	636.6 ± 7.6 ^b	LRI, MS, Std
28	3-Methylbutanal	916	574.6 ± 2.7 ^b	182.4 ± 1.3 ^c	2750.9 ± 22.6 ^a	LRI, MS, Std
29	Hexanal	1097	1024.6 ± 9.8 ^b	2175.4 ± 19.8 ^a	2019.0 ± 4.6 ^a	LRI, MS, tent
30	Nonanal	1395	nd	nd	69.5 ± 1.2 ^a	LRI, MS, tent
31	2-Furaldehyde	1468	103.9 ± 3.8 ^b	132.0 ± 1.4 ^a	30.7 ± 0.2 ^c	LRI, MS, Std
32	Benzaldehyde	1544	155.1 ± 1.2 ^a	0.0 ^c	116.9 ± 1.2 ^b	LRI, MS, Std
33	5-Methyl-2-furfural	1607	68.5 ± 0.2 ^a	17.0 ± 0.4 ^b	nd	LRI, MS, tent
34	(E,E)-2,4-Nonadienal	1681	nd	nd	19.0 ± 0.1 ^a	LRI, MS, tent
35	5-Hydroxymethylfurfural	2528	0.0 ^b	18.5 ± 0.1 ^a	nd	LRI, MS, tent
	Total		2315.1	3522.0	5710.2	
	Esters					
36	Ethyl acetate	887	185.7 ± 2.4 ^a	29.4 ± 0.7 ^c	168.4 ± 4.3 ^b	LRI, MS, tent
38	Ethyl butanoate	1044	nd	nd	135.0 ± 0.7 ^a	LRI, MS, tent
39	Ethyl 2-methylbutanoate	1064	297.4 ± 4.9 ^a	221.2 ± 4 ^b	191.4 ± 7.5 ^c	LRI, MS, tent
40	2-Phenyl acetate	1066	180.9 ± 2.6 ^a	nd	nd	LRI, MS, Std
41	Ethyl 3-methylbutanoate	1072	102.1 ± 2.8 ^b	180.3 ± 1.3 ^a	nd	LRI, MS, tent
42	2-Methylbutyl acetate	1115	603.2 ± 7.2 ^a	nd	171.2 ± 3.4 ^b	LRI, MS, tent
43	Isoamyl acetate	1126	nd	125.2 ± 1.9 ^a	nd	LRI, MS, tent
44	3-Methylbutyl acetate	1130	201.5 ± 2.3 ^a	nd	61.3 ± 0.3 ^b	LRI, MS, tent
45	Ethyl valerate	1142	nd	155.4 ± 2.4 ^a	nd	LRI, MS, tent

Table 4.14. Continued

46	Ethyl hexanoate	1241	113.2 ± 1.8 ^b	138.9 ± 3.8 ^a	nd	LRI, MS, tent
47	Methyl octanoate	1437	nd	nd	56.7 ± 0.6 ^a	LRI, MS, tent
48	Ethyl octanoate	1446	nd	58.4 ± 0.7 ^a	nd	LRI, MS, tent
49	2-Furfuryl acetate	1534	nd	47.4 ± 0.5 ^a	19.6 ± 0.1 ^b	LRI, MS, tent
50	Benzyl acetate	1742	77.4 ± 0.6 ^a	nd	nd	LRI, MS, Std
51	Methylphenyl acetate	1749	nd	109.4 ± 1.5 ^a	nd	LRI, MS, tent
52	Methyl salicylate	1753	nd	nd	120.9 ± 1.4 ^a	LRI, MS, tent
53	Ethylphenyl acetate	1781	nd	107.0 ± 1.8 ^a	nd	LRI, MS, tent
54	Phenylethyl acetate	1810	124.9 ± 1.6 ^b	138.7 ± 0.9 ^b	169.0 ± 1.5 ^a	LRI, MS, tent
	Total		1886.3	1334.0	1141.8	
	Furans & Furanones					
55	2-Acetyl-furan	1512	29.7 ± 0.4 ^a	16.5 ± 0.4 ^b	nd	LRI, MS, tent
56	2-Acetyl-5-methylfuran	1608	44.9 ± 0.8 ^a	nd	nd	LRI, MS, tent
57	5-(1-Hydroxyethyl)-2-(5H)-furanone	1864	12.5 ± 0.6 ^a	nd	nd	LRI, MS, tent
	Total		87.1	16.5	nd	
	Ketones					
60	Diacetyl	971	nd	22.7 ± 0.4 ^b	48.3 ± 0.7 ^a	LRI, MS, tent
61	2-Pentanone	978	nd	nd	87.5 ± 1.5 ^a	LRI, MS, Std
62	2-Heptanone	1184	178.7 ± 3.1 ^a	171.6 ± 3.8 ^a	nd	LRI, MS, Std
63	5-Methyl-2-hexanone	1190	145.3 ± 1.9 ^a	nd	nd	LRI, MS, tent
64	3-Hydroxy-2-butanone	1291	295.3 ± 4.7 ^a	138.3 ± 2.4 ^c	228.8 ± 8.6 ^b	LRI, MS, Std
65	2-Hydroxy-3-pentanone	1368	155.6 ± 5.2 ^a	nd	nd	LRI, MS, tent
66	Acetophenone	1652	69.1 ± 0.3 ^a	nd	48.8 ± 0.9 ^b	LRI, MS, Std
	Total		844.0	309.9	365.1	
	Pyrazines					
67	2-Methylpyrazine	1274	89.5 ± 1.6 ^a	47.3 ± 0.5 ^b	97.8 ± 0.8 ^a	LRI, MS, Std
68	2,6-Dimethylpyrazine	1319	18.5 ± 0.3 ^a	24.5 ± 0.4 ^a	nd	LRI, MS, tent
69	2,5-Dimethylpyrazine	1336	nd	43.6 ± 0.6 ^a	nd	LRI, MS, tent

Table 4.14. Continued

70	2,3-Dimethylpyrazine	1357	nd	nd	37.9 ± 0.4 ^a	LRI, MS, Std
71	2-Ethylpyrazine	1358	44.8 ± 0.9 ^a	35.7 ± 0.3 ^a	19.4 ± 0.1 ^b	LRI, MS, Std
72	2-Acetyl-3-methylpyrazine	1627	29.9 ± 0.2 ^a	nd	nd	LRI, MS, Std
	Total		182.7	151.1	155.1	
	Pyrroles, Pyrroline & Lactones					
73	δ-Octenolactone	1264	nd	18.7 ± 0.1 ^a	nd	LRI, MS, tent
74	2-Acetyl-1-pyrroline	1310	19.4 ± 0.3	21.8 ± 0.95	19.4 ± 0.3	LRI, MS, Std
75	γ-Valerolactone	1589	50.3 ± 0.8 ^a	nd	nd	LRI, MS, Std
76	γ-Butyrolactone	1617	117.1 ± 1.2 ^b	180.2 ± 2.4 ^a	92.4 ± 0.9 ^b	LRI, MS, Std
77	2-Acetylpyrrole	1974	nd	41.8 ± 0.3 ^b	69.0 ± 0.5 ^a	LRI, MS, tent
	Total		186.8	262.5	180.8	
	Terpenes					
78	α-Pinene	1034	158.1 ± 2.8 ^a	nd	nd	LRI, MS, Std
79	α-Phellandrene	1038	nd	nd	109.1 ± 0.7 ^a	LRI, MS, Std
80	Linalool oxide I	1080	67.6 ± 1.2 ^a	nd	nd	LRI, MS, Std
81	Camphene	1082	nd	204.6 ± 1.8 ^a	nd	LRI, MS, tent
82	Sabinene	1119	187.4 ± 3.6 ^a	nd	151.4 ± 0.9 ^b	LRI, MS, Std
83	β-Myrcene	1156	101.8 ± 6.4 ^a	nd	nd	LRI, MS, tent
84	1,4-Cineol	1177	46.1 ± 0.7 ^a	nd	nd	LRI, MS, tent
85	D-Limonene	1206	178.1 ± 3.6 ^a	162.8 ± 1.5 ^a	nd	LRI, MS, Std
86	Ocimene	1233	286.3 ± 1.4 ^a	94.8 ± 1.2 ^b	59.3 ± 0.2 ^c	LRI, MS, tent
87	p-Cymen	1272	nd	288.7 ± 4.3 ^a	178.7 ± 4.3 ^b	LRI, MS, Std
88	γ-Terpinene	1242	270.8 ± 2.9 ^a	nd	nd	LRI, MS, Std
89	Linalool oxide II	1442	71.6 ± 0.8 ^a	nd	nd	LRI, MS, Std
90	Linalool	1537	210.7 ± 9.4 ^a	179.5 ± 1.7 ^b	107.4 ± 0.9 ^c	LRI, MS, Std
91	(E)-Caryophyllene	1598	nd	219.8 ± 3.2 ^a	88.5 ± 1.2 ^b	LRI, MS, Std
92	β-Aromadendrene	1635	nd	nd	247.5 ± 5.6 ^a	LRI, MS, tent
93	Valencene	1715	nd	nd	238.4 ± 4.3 ^a	LRI, MS, tent

Table 4.14. Continued

94	Geraniol	1851	nd	179.3 ± 3.5 ^a	nd	LRI, MS, Std
95	Thymol	2153	nd	51.1 ± 0.6 ^a	nd	LRI, MS, tent
	Total		1578.5	1380.6	1180.3	
	Volatile phenols					
96	Guaiacol	1960	80.9 ± 0.4 ^a	77.7 ± 1.2 ^a	47.4 ± 0.2 ^b	LRI, MS, tent
97	Eugenol	2141	nd	23.6 ± 0.4 ^a	nd	LRI, MS, tent
98	Isoeugenol	2271	nd	nd	45.6 ± 0.4 ^a	LRI, MS, tent
	Total		80.9	101.3	93.0	
			11484.0	10554.8	13678.8	

¹LRI: linear retention index calculated on DB-Wax capillary column. ²Concentration: results are the means of three repetitions as µg/kg. ³Identification: methods of identification; LRI (linear retention index), MS tent. (tentatively identified by MS), Std (chemical standard); When only MS or LRI is available for the identification of a compounds, it must be considered as an attempt of identification. Different lower-case letters (a,b,c) within the same line indicate significant differences (p<0.05) between the different cocoa bean cultivars. SDFB: Sun-dried Forastero beans, SDCB: Sun-dried Criollo beans, SDNB: Sun-dried Nacional beans.

4.9.2. Aroma-Active Compounds in Sun Dried Cocoa Bean from the Three Cocoa Cultivars

The aroma-active compounds of the cocoa cultivar samples obtained from the sun drying process, the characteristic odor provided by these compounds, and the flavor dilution factors (FD) are given in Table 4.15. The flavor dilution factors (FD) of the aroma-active compounds ranged from 4 to 1024. In the study, a total of 57 aroma-active compounds were identified in the three cocoa cultivar samples. When cocoa bean samples were analyzed, in Forastero cocoa cultivar, a total of 31 aroma-active compounds were identified, 32 key odorants in Criollo cocoa group, while 34 aroma-active substances were determined in Nacional cocoa cultivar. The number of aroma-active compounds and their flavor dilution factors (FD) values significantly varied among the cocoa cultivar samples.

As shown in the table 4.15, a total of 31 aroma-active compounds were characterized in Forastero cocoa cultivar samples, consisting of 4 aldehydes, 6 alcohols, 4 terpenes, 3 esters, 3 ketones, 1 lactone, 1 furanone, 1 pyrroline, 2 pyrazines, 3 acids. Three unknown aroma-active compounds (LRI: 1685, 1845 and 2006) were also detected in Forastero cocoa bean group samples. These compounds are often undetected in GC-MS analysis due to their minuscule peaks and low mass-to-charge (m/z) ratios. The flavor dilution values (FD) of the aroma-active compounds ranged from 4 to 512 for the Forastero cocoa group. Among these compounds, hexanol (flowery-spicy) and unknown aroma-active compounds (LRI: 1845) were determined the highest FD value (512), followed by 3-methylbutanal (apple-like, FD: 256). The compounds with the lowest value of FD factor (4) were phenylethanol (green, floral), octanoic acid (fatty, rancid), 2(5H) furanone (caramel, sweet), and 2,6-dimethylpyrazine (roasted coffee).

Regarding Criollo cocoa cultivar samples, a total of 32 aroma-active compounds were detected and 31 of them were fully identified. The identified compounds were composed of 3 aldehydes, 5 alcohols, 5 terpenes, 5 esters, 2 ketone, 2 lactone, 1 pyrrole, 3 pyrazines, and 4 acids. Interestingly, one unknown aroma-active compound (LRI: 1297) was also detected in Criollo cocoa bean groups, the flavor dilution (FD) factors of the aroma-active compounds ranged from 4 to 1024. Among them, ethyl acetate (fresh, fruity), (*E*)-2-hexanol (green, herbal), Octanoic acid (fatty, rancid), phenylacetic acid (burnt), δ -octenolactone (fresh, citrusy) exposed the lowest value of FD factor (4), while hexanal (green) exhibited the highest value of FD factor (1024), followed by 2-methylbutanal (green, fruity), 2-phenylethanol (green, cut grass) with FD factor of 512.

In cocoa beans obtained from Nacional cultivar, 34 aroma-active compounds were determined, among which 32 of them were identified. The detected compounds comprised 6 aldehydes, alcohols, 5 terpenes, 4 esters, 1 ketone, 1 lactone, 1 pyrrole, 1 pyrroline, 2 pyrazines, 3 acids, and 1 volatile phenol. Two unknown aroma-active compounds (LRI: 1008 and 1325) were detected in Nacional cocoa cultivar samples, the flavor dilution (FD) values of the aroma-active compounds ranged from 4 to 1024. Of them, nonanal (waxy, fatty), (*E,E*)-2,4 nonadienal (cooked, fatty), (*E*)-caryophyllene (musky, spicy), 2,3-dimethylpyrazine (roasted-like) presented the lowest

value of FD factor (4), whereas 3-methylbutanal (apple-like) was observed with the highest value of FD factor (1021), followed by hexanal (green) and 2-acetyl-1-pyrroline (popcorn-like) with FD value of 512.

As can be seen in the table 4.15, of all the 57 identified aroma-active compounds in the three cocoa cultivars, alcohols were found to be the most abundant group. A total of 10 different aroma-active alcohol compounds were identified, comprising 2-methyl-2-butanol (sour, sweet), 2-methyl-3-butanol (fruity), 2-pentanol (fermented), 1-penten-3-ol (green, leafy), (*E*)-2-hexanol (green, herbal), 2-penten-1-ol (plastic, chemical), hexanol (flowery, spicy), 2,3-butanediol (cheesy, creamy), phenylethanol (green, floral) and 2-phenylethanol (green, cut grass). The majority of these aroma-active alcohols compounds are prevalent, as they have already been discovered in various other cocoa cultivars (Jinap, S.W et al., 1998, Utrilla-Vázquez et al., 2020). In sun-dried cocoa bean obtained from Forastero cocoa group, alcohol compound with the highest FD factor was hexanol (flowery, spicy, FD:512). This compound was followed by 2-methyl-2-butanol (2-pentanol, FD:64), 2,3-butanediol (cheesy, creamy, FD:64), and 2-phenylethanol (green, cut grass, FD:64). Phenylethanol exposed the lowest FD value (4) among alcohol aroma-active compounds in Forastero cultivars. In sun-dried cocoa bean samples acquired from Criollo cultivar, alcohol compound with the highest FD factor was 2-phenylethanol (green, cut grass, FD:512). This was followed by hexanol (flowery, spicy, FD:128). Other alcohol aroma-active compounds identified in Criollo cocoa cultivar samples included 2-methyl-3-butanol (fruity), phenylethanol (green, floral), and the lowest FD value (4) was attributed to (*E*)-2-hexanol (green, herbal). In sun-dried cocoa bean samples obtained from Nacional coca type, alcohol compound with the highest FD factor were 1-penten-3ol (green, leafy, FD:128), hexanol (flowery, spicy, FD:128) and 2,3-butanediol (cheesy, creamy), followed by 2-methyl-2-butanol (sour, sweet, FD:64), 2-penten-1-ol (plastic, chemical, FD:64) and phenylethanol (green, floral, FD:64). Another alcohol aroma-active compound determined in Nacional cocoa type with low FD factors was (*E*)-2-hexanol (green, herbal, FD:32).

When the aroma-active components of cocoa cultivar samples were compared, the maximum number (7) of aroma-active alcohol compounds was determined in sun dried cocoa beans obtained from Nacional cocoa cultivar and the least number (5) was observed in Criollo cocoa cultivar. Hexanol and phenylethanol are aroma-active alcohols commonly identified in all cocoa cultivars, however, difference in FD factors of these compounds were determined, in which the highest FD value (512) of hexanol was characterized in Forastero cocoa cultivar. In general, alcohols generate less organoleptic properties than aldehydes owing to their higher odor threshold values. Among the aroma-active alcohol compounds, phenylethanol, which gives green and floral odors to dried and roasted cocoa bean samples, was perceived in Nacional cocoa group with the highest FD value (64). In addition, 2-methyl-3-butanol and 2-phenylethanol were detected in both Forastero and Criollo cocoa cultivars, while it was no present in Nacional cocoa type. The maximum FD value (64) of 2-methyl-3-butanol was determined in Forastero cocoa group, while the highest FD value (512) of 2-

phenylethanol was observed in Criollo cocoa cultivars. 2,3-butanediol with cheesy and waxy attributes was determined in Forastero and Nacional cocoa types, with the highest FD value (128) in Nacional cocoa group. Furthermore, (*E*)-2-hexanol was only detected in Criollo and Nacional cocoa cultivars, in which the maximum FD value (32) was found in Nacional cocoa group. These compounds were indicated as the most potent aroma-active compounds to contribute to the aroma profile of each sun-dried cocoa beans obtained from the three cocoa cultivars. The majority of the determined powerful alcohol compounds in our investigations have already been detected in different dried and roasted cocoa beans cultivars published by numerous scholars (Ramli et al., 2006; Rodriguez-Campos et al., 2012).

Other important aroma-active components detected in the cocoa cultivar samples were aldehydes. A total of 6 aroma-active aldehydes were determined, comprising 2-methylbutanal (green, fruity), 3-methylbutanal (apple-like), hexanal (green), nonanal (waxy, fatty), benzaldehyde (almond-like) and (*E,E*)-2,4 nonadienal (cooked, fatty). The majority of these aroma-active components have been previously determined in other cocoa cultivars and hybrids from different locations (Erazo Solorzano, Tuárez García, et al., 2023; Rottiers, Tzompa Sosa, De Winne, et al., 2019), which are also crucial for the development of good cocoa flavor and quality (Rodriguez-Campos et al., 2012). Regarding sun-dried cocoa samples obtained from Forastero cocoa group, 4 aroma-active aldehydes including 2-methylbutanal, 3-methylbutanal, hexanal and benzaldehyde. Among them, 3-methylbutanal was found with the highest FD value (256), followed by hexanal (green, FD:64). 2-methylbutanal and benzaldehyde were determined with the lowest FD value of 8. In sun-dried cocoa beans obtained from Criollo cocoa cultivar samples, 3 aroma-active aldehydes (2-methylbutanal, 3-methylbutanal and hexanal) were detected; hexanal was perceived with the maximum FD value (1024), followed by 2-methylbutanal (512), and the lowest FD value (16) was attributed to 3-methylbutanal. With respect to sun-dried cocoa beans from Nacional cocoa type samples, 6 aroma-active aldehydes were observed, among which 3-methylbutanal was identified with the highest FD value (1024), followed by 2-methylbutanal (green, fruity, FD:128). Moreover, nonanal and (*E,E*)-2,4 nonadienal were found to be the aroma-active components exposing the lowest FD value (4).

Among these identified aroma-active compounds, the maximum number (6) was found in Nacional cocoa cultivars, while the least number (3) was observed in Criollo cocoa type samples. 2-methylbutanal (green, fruity), 3-methylbutanal (apple-like) and hexanal (green) were commonly found in the three cocoa cultivar samples. However, difference in FD factors was observed among the cultivars. Hexanal was determined with 1024 FD factor in Criollo cocoa type samples, followed by Nacional cocoa group samples with a FD factor of 512. The lowest FD value of hexanal was found in Forastero cocoa type samples with FD value equal to 64. Beckett, S et al. (2017) postulated that hexanal is generally arise from lipid precursors. Regarding 3-methyl butanal, the maximum FD value (1024) was characterized in Nacional cocoa cultivar samples, followed by Forastero cocoa cultivar samples exhibited a FD factor of 256. 2-methylbutanal exposed the highest FD value (512) in Criollo

cocoa type samples, followed by Nacional cocoa group samples with the FD factor of 128. In addition, benzaldehyde was determined in Forastero cocoa type (FD:8) and Nacional cocoa cultivar (FD:32) samples. Interestingly, nonanal and (*E,E*)-2,4 nonadienal with FD value of 4 were only determined in Nacional cocoa type samples.

Another important class of aroma-active compounds detected in the three cocoa cultivar samples were esters. A total of 9 aroma-active ester compounds were identified, including ethyl acetate (fresh, fruity), ethyl-2-methylbutanoate (green, fruity), 2-phenylacetate (floral, fruity), ethyl 3-methylbutanoate (green, fruity), 2-methylbutyl acetate (fruity), 3-methylbutyl acetate (green), ethyl hexanoate (fruity), methylphenyl acetate (honey) and phenylethyl acetate (fruity, sweet). As can be seen in the Table 4.15, ester acetates were predominant esters in all cocoa cultivars. Adler et al. (2014) reported that the formation of these acetates is the key phase in the generation of desirable and fine cocoa and cocoa products flavors. Most of these aroma-active compounds have been previously characterized in dried and roasted cocoa beans and products from different cocoa cultivars (Jinap et al., 1998; Rodriguez-Campos et al., 2012). In sun-dried cocoa beans acquired from the Forastero cocoa group, ester compound with the highest FD factor was 2-methylbutyl acetate (fruity, FD:64). 2-phenyl acetate (floral, fruity) and ethyl acetate (fresh, fruity) exhibited the lowest FD value (16) among the ester aroma-active compounds in Forastero cultivars. In sun-dried cocoa bean samples from the Criollo cultivar, ethyl hexanoate (fruity) and phenylethyl acetate (fruity, sweet) were determined with the highest FD value (32), followed by methylphenyl acetate (honey, FD:16). Among the aroma-active ester compounds identified in Criollo cocoa type samples, the lowest aroma-active esters were attributed to ethyl 3-methylbutanoate (green, fruity, FD:8) and ethyl acetate (fresh, fruity, FD:4). In sun-dried cocoa bean samples from the Nacional cocoa type, the highest FD factors were for ethyl acetate (fresh, fruity, FD:32) and phenylethyl acetate (fruity, sweet, FD:32). These were followed by ethyl-2-methylbutanoate (green, fruity) and 3-methylbutyl acetate (green) exposing a FD value of 16.

When comparing the aroma-active compounds of cocoa type samples, the lowest aroma-active ester number (3) was found to be in sun-dried cocoa beans acquired from Forastero cocoa type samples and the highest number (5) was in Criollo cocoa cultivar samples (Table 4.15). It was seen that ethyl acetate (fresh, fruity) was commonly determined in all the cocoa cultivar samples, in which Nacional cocoa type samples exhibited the highest FD value equal to 32. 2-methylbutyl acetate (fruity) was only observed in Forastero cocoa type with the highest FD value (64) among the cocoa cultivars. Ethyl acetate, with pineapple odors, is a product of esterification from acetic acid and ethanol as demonstrated by Rodriguez-Campos et al. (2011). Phenylethyl acetate with fruity and sweet odors was identified in Criollo and Nacional cocoa type samples, with the FD value (32) in both samples. This aroma compound is postulated to be probably formed from the metabolism of yeasts, which was reported by Jinap et al. (1998) as the principal compound responsible for the unique aroma of Asian cocoa liquor.

Terpene compounds were also among the important aroma-active compounds determined in the three cocoa cultivar samples. A total of eight aroma-active terpenes compounds were found in all sun-dried cocoa cultivar samples, these included α pinene (green, floral), camphene (woody), sabinene (woody, herbal), D-limonene (citrusy), ocimene (fruity, citrusy), γ terpinene (fresh, citrusy), (*E*)-caryophyllene (musky, spicy). As indicated in the Table 4.15, terpenes give herbal, woody and fruity attributes to the cocoa beans. Aroma-active terpenes found in the three cocoa types samples exhibited different flavor dilution (FD) factors. In sun-dried Forastero cocoa type samples, terpene aroma-active compounds with the highest FD factors was γ terpinene (fresh, fruity, FD:64), followed by sabinene (woody, herbal, FD:32). Among the terpene aroma-active components detected in Forastero cocoa type samples, D-limonene and linalool were found with the lowest FD factors equal to 16 and 8, respectively. As regard to sun-dried Criollo cocoa cultivar samples, 5 terpene aroma-active compounds were identified, among which camphene exposed the highest FD value (64), followed by D-limonene and (*E*)-caryophyllene with FD factors of 32. In addition, linalool (fresh-citrusy, FD:16) and ocimene (fruity, citrusy, FD:8) presented the lowest FD factors. With respect to sun-dried beans from Nacional cocoa type, terpene compound with the highest FD factor was D-limonene, which has a citrusy odor and FD value of 64, followed by ocimene (fruity-citrusy, FD:32) and linalool (fresh, citrusy, FD:32). Of all the terpene aroma-active compounds in Nacional cocoa cultivar, α pinene (green, floral, FD:8) and (*E*)-caryophyllene (musky,spicy, FD:4) exhibited the lowest flavor dilution factors. Most of these aroma-active compounds are derived via the methylerythritol 4-phosphate (MEP) pathway in plastids, using carbohydrates as precursors (Erazo Solorzano et al., 2023).

When comparing the aroma-active components of cocoa cultivar samples, Nacional and Criollo cocoa cultivars presented the same number (5) of aroma-active alcohol compounds, which was found to be the highest number of terpene aroma-active compounds. In contrast, the Forastero cocoa cultivar had the fewest number, with only 4 terpene aroma-active compounds. Linalool and D-limonene were common aroma-active terpenes identified in all cocoa cultivars. However, there were significant differences in their flavor dilution (FD) factors. The highest FD value for D-limonene (64) was found in the Nacional cocoa cultivar, followed by Criollo cocoa group with FD value of 32. In addition, linalool exhibited a great FD value (32) in the Nacional cocoa type, followed by Criollo cocoa cultivar exhibited FD value of 16. Generally, terpene are known as potential key components contributing to the fruity, herbal and floral odors of fine-flavor cocoa (Rottiers, H et al., 2019). Among the aroma-active terpene compounds, camphene, which imparts woody odor to dried and roasted cocoa bean samples, was only perceived in the Criollo cocoa group with flavor dilution (FD) value of 64. Additionally, γ terpinene with fresh and citrusy attributes was only present in Forastero cocoa type with FD value of 64. Moreover, ocimene and (*E*)-Caryophyllene were only observed in the Criollo and Nacional cocoa cultivars. Among them, ocimene exhibited the highest FD value (32) in the Nacional cocoa type, whereas (*E*)-Caryophyllene was found in the Criollo cocoa group with

the elevated FD value (32). These compounds were identified as the most potent aroma-active compounds contributing to the aroma profile of each sun-dried cocoa bean sample from the three cocoa cultivars.

As can be shown in Table 4.15, the main methylpyrazine aroma active compounds, including 2-methylpyrazine (chocolate-like), 2,6-methylpyrazine (roasted coffee), 2,5-dimethylpyrazine (dark chocolate) and 2,3-dimethylpyrazine (roasted-like) were identified in the sun-dried cocoa beans obtained from the three cocoa cultivars. When pyrazine aroma-active compounds were compared, 2-methylpyrazine observed in all cocoa bean cultivars exhibited the highest FD value (16) in the Nacional cocoa cultivar sample. Similar FD value (8) of 2-methylpyrazine were determined in the Forastero and Criollo cocoa cultivar samples. In addition, 2,6-dimethylpyrazine with FD factor of 4 was determined in Forastero and Criollo cocoa types, while 2,5-dimethylpyrazine (dark chocolate, FD:4), and 2,3-dimethylpyrazine (roasted-like, FD:4) were detected in Criollo and Nacional cocoa cultivar samples, respectively.

Interestingly, 6 unknown aroma-active compounds were perceived in the three cocoa cultivar samples as can be seen in Table 4.15. With respect to Forastero cocoa group, 3 unknown aroma-active compounds, comprising unknown IV (LRI:1685, roasted bean, FD:8), unknown V (LRI:1845, roasted mushroom, FD:512) and unknown VI (LRI:2006, green, herbal, FD:32). Only one unknown aroma-active compound, unknown II (LRI:1297, mushroom-like, FD:8) was determined in sun-dried beans acquired from Criollo cocoa type. Regarding Nacional cocoa cultivar sample, 2 unknown aroma-active compounds, unknown I (LRI:1008, caramel, sweet, FD:16), unknown III (LRI:1325, caramel, FD:16) were observed. Forastero cocoa cultivar sample exhibited the maximum number (3) of unknown aroma-active compounds, in which unknown V was found to be the highest aroma-active compound with FD value of 512, followed by Nacional cultivar with a number of 2 unknown aroma-active compounds. These unknown aroma-active compounds might probably play a huge role in the characteristic odor of the sun-dried cocoa bean samples obtained from the three cocoa cultivars.

Table 4.15. Aroma-active compounds of sun dried cocoa bean samples obtained from the three cocoa cultivars (FD≥4)

No	Aroma-active compounds	LRI ^a	Odor description ^b			
	Esters			SDFB	SDCB	SDNB
1	Ethyl acetate	887	Fresh-fruity	16	4	32
2	Ethyl-2-methylbutanoate	1064	Green-fruity	-	-	16
3	2-Phenyl acetate	1066	Floral-fruity	16	-	-
4	Ethyl 3-methylbutanoate	1072	Green fruity	-	8	-
5	2-methylbutyl acetate	1115	Fruity	64	-	-
6	3-Methylbutyl acetate	1130	Green	-	-	16
7	Ethyl hexanoate	1241	Fruity	-	32	-
8	Methylphenyl acetate	1749	Honey	-	16	-
9	Phenylethyl acetate	1810	Fruity-sweet	-	32	32
	Aldehydes					
10	2-Methylbutanal	896	Green-fruity	8	512	128
11	3-Methylbutanal	916	Apple like	256	16	1024
12	Hexanal	1097	Green	64	1024	512
13	Nonanal	1395	Waxy-fatty	-	-	4
14	Benzaldehyde	1544	almond like	8	-	32
15	(<i>E,E</i>)-2,4 Nonadienal	1681	Cooked-fatty	-	-	4
	Alcohols					
16	2-Methyl-2-butanol	975	Sour-sweet	-	-	64
17	2-Methyl-3-butanol	1089	Fruity	64	16	-
18	2-pentanol	1138	Fermented	64	-	-
19	1-Penten-3-ol	1165	Green-leafy	-	-	128
20	(<i>E</i>)-2-Hexanol	1245	Green-herbal	-	4	32
21	2-Penten-1-ol	1324	Plastic-chemical	-	-	64
22	Hexanol	1359	Flowery-spicy	512	128	128
23	2,3-Butandiol	1545	Cheesy-creamy	64	-	128
24	Phenylethanol	1922	Green-floral	4	8	64
25	2-phenylethanol	1944	Green-cut grass	64	512	-
	Acids					
26	Acetic acid	1418	Vinegar	32	64	64
27	Hexanoic acid	1820	Cheesy-waxy	32	64	16
28	Octanoic acid	2046	Fatty-rancid	4	4	32
29	Phenylacetic acid	2254	Burnt honey	-	4	-
	Terpenes					
31	α pinene	1038	Green-floral	-	-	8
32	Camphene	1082	Woody	-	64	-
33	Sabinene	1119	Woody-herbal	32	-	-
34	D-Limonene	1206	Citrusy	16	32	64
35	Ocimene	1233	Fuity-citrusy	-	8	32

Table 4.15. Continued

36	γ terpinene	1242	Fresh-citrusy	64	-	-
37	Linalool	1537	Fresh-citrusy	8	16	32
38	(E)-Caryophyllene	1598	Musky-spicy	-	32	4
Ketones						
39	2-heptanone	1184	Green-fruity	8	16	-
40	3-hydroxy- 2 butanone	1291	Buttery-creamy	64	16	64
41	2-hydroxy-3-pentanone		Chemical	64	-	-
Lactone, Pyrroline & Pyrroles						
42	δ -Octenolactone	1264	Fresh-citrusy	-	4	-
43	2-Acetyl-1-pyrroline	1310	Popcorn-like	128	128	512
44	γ -Butyrolactone	1617	Burnt like	16	16	8
45	2-Acetylpyrrole	1974	Musty	-	4	8
Furanones						
46	2 (5H) furanone	1716	Caramel-sweet	4	-	-
Pyrazines						
47	2-Methylpyrazine	1274	Chocolate like	8	8	16
48	2,6-Dimethylpyrazine	1319	Roasted coffee	4	4	-
49	2,5-Dimethylpyrazine	1336	Dark chocolate	-	4	-
50	2,3-Dimethylpyrazine	1357	Roasted like	-	-	4
Unknown Compounds						
51	Unknown I	1008	Caramel-sweet	-	-	16
52	Unknown II	1297	Mushroom-like	-	8	-
53	Unknown III	1325	Caramel	-	-	16
54	Unknown IV	1685	Roasted bean	8	-	-
55	Unknown V	1845	Roasted mushroom	512	-	-
56	Unknown VI	2006	Green-herbal	32	-	-
Volatile Phenols						
57	Isoeugenol	2271	Sweet-floral	-	-	8

^a LRI: linear retention index calculated on DB-Wax capillary column.

^b Odor description: odor description as perceived by panalists through olfactometry analysis

^c FD factor is the highest dilution of the aroma extract at which an odorant is determined by aroma extract dilution analysis.

4.9.3. Organoleptic Assessment of the three Fermented and Dried Beans Varieties based on Sun drying Process

The sensory profiles of sun-dried beans (nibs) obtained from Forastero, Criollo and Nacional bean samples were evaluated using descriptive analysis to generate a comprehensive overview of the gustatory and orthonasal profiles fermented and sun-dried dried beans of the three cocoa varieties. A

panel of 10 assessors analyzed each dried bean samples (nibs), describing them based on 11 descriptors, including bitterness, astringency, sourness, chocolate, caramel, floral, cocoa aroma, dried fruit, fresh fruit, moldy, and overall quality. These descriptors were scored on a structured scale from 0 to 15 cm. During the assessment sessions, the criteria of “color” and “overall quality” were also considered. The results of the descriptive analysis, presented as mean scores of the selected attributes, are shown in Figure 4.48. All attributes were evaluated by the panelists through smelling and tasting the fermented and sun-dried beans (nibs).

Table 4.16. Average scores of the sensory attributes and identification of statistically significant differences among the three cocoa bean cultivars, by one-way ANOVA and LSD test analyses

Attributes	Cocoa cultivars			Sig.	Pr> F(Model)
	SDFB	SDCB	SDNB		
Color	6.1 ^c	7.8 ^b	9.3 ^a	**	0.003
Bitterness	3.07 ^a	4.8 ^a	3.6 ^a	ns	0.145
Astringency	9.2 ^a	8.9 ^a	8.4 ^a	ns	0.264
Sourness	8.1 ^b	10.3 ^a	7.2 ^c	**	0.002
Chocolate	3.6 ^a	3.2 ^a	4.08 ^a	ns	0.338
Caramel	1.3 ^c	2.08 ^b	4.7 ^a	*	0.015
Floral	7.2 ^a	5.8 ^b	5.2 ^b	*	0.026
Cocoa aroma	3.8 ^a	4.7 ^a	4.2 ^a	ns	0.248
Dried fruit	6.02 ^a	7.2 ^a	5.1 ^b	**	0.006
Fresh Fruit	8.9 ^a	6.1 ^c	7.4 ^b	*	0.018
Mouldy	4.3 ^c	5.7 ^a	1.08 ^c	*	0.005
Overall quality	10.4 ^b	8.4 ^c	12.8 ^a	*	0.024

Sig: significance at which means differ as shown by analysis of variance; ns.: not significant, significant at the * $p < 0.05$ level, ** $p < 0.01$. Different letters in the same row indicate statistical differences at the $p < 0.05$ level according to Duncan multiple test. P: sensory attribute evaluated orthonasally

When the sensory analysis results of dried Nacional cocoa samples were statistically evaluated, the values obtained for all attributes and overall quality, except for bitterness, astringency, chocolate, and cocoa aroma notes, were found to be statistically significant among all dried cocoa bean groups ($p < 0.05$). The sensory profile scores for attributes such as color and caramel notes were higher in SDNB compared to the other dried cocoa bean cultivars. Additionally, mouldy note, among the undesirable cocoa quality attribute was observed with the lowest value (1.08) in SDNB group. The scores of sourness (10.3) and dried fruit (7.2) attributes were statistically higher in SDCB cultivar than those of SDFB and SDNB cultivars. Regarding SDFB cultivar, floral (7.2) and fresh fruit (8.9) notes were significantly found in higher scores compared to other cultivars. The elevated floral and fresh fruit attribute scores might be linked to the significant amount of total esters in SDFB determined in the present study. When the three cocoa cultivars were compared in term of overall quality, SDNB group was the most appreciated with a sensory attribute result of 12.8, followed by

SDFB cultivar with a score of 10.4. Significant difference was observed in overall quality attribute among all cocoa bean cultivars ($p < 0.05$).

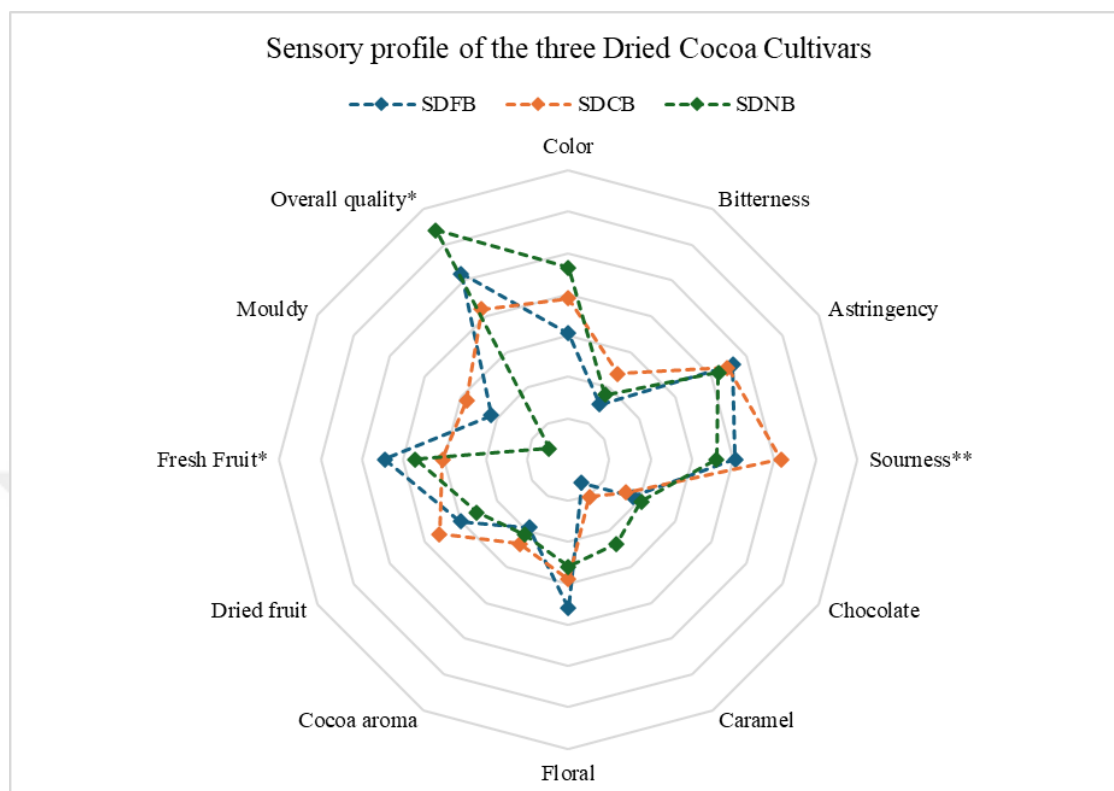


Figure 4.48. Spider web plot showing sensory profiles of the fermented and sun-dried cocoa beans from the three cocoa cultivars. Average scores of attribute intensity are shown as distance from the center.

4.10 Characterization of Phenolic Compounds of Sun-Dried Cocoa Beans

4.10.1. Characterization of Phenolic Compounds of Sun-Dried Cocoa Beans using LC-DAD-ESI-MS/MS

The quality and quantity of phenolic compounds are crucial factors in assessing cocoa bean, powder and cocoa butter's quality. These compounds are not only closely linked to the cocoa powder and butter resistance to oxidation but also play a significant role in its sensory properties, such as bitterness and pungency. The determination of phenolic compounds was carried out by comparing the retention times and MS and MS/MS spectral data from the three cocoa bean cultivars with those known standards. Table 4.18 presents a tentative characterization of various phenolic compounds, based on analyses conducted using LC-DAD and LC-ESI-MS/MS in multiple reaction monitoring (MRM) mode. This table includes key information such as retention time (RT), UV data (in nm), molecular ion $[M-H]^-$, molecular formula (MF), quantitative transition (m/z), primary MS/MS fragments, and proposed compounds. Figure 4.49 displays the base peak chromatogram of the tested cocoa bean phenolic extract samples. This chromatogram was obtained using LC-DAD and LC-ESI-MS/MS in both negative and positive ionization modes. A total of 26 phenolic compounds were identified in all the cocoa bean samples. These phenolic compounds, characterized from the phenolic

extracts, are classified into six categories: four phenolic acids, two flavan-3-ols, one catechin-3-O-glycoside, one flavonol-3-O-glycoside, one flavonols, two methylxanthines, five procyanidins B type (PCB) dimers, two procyanidins B type (PCB) trimers, two procyanidins A type glycosides and two other compounds. The results from LC-ESI-MS/MS showed no qualitative variations in the profile of phenolic compounds in the three cocoa cultivars samples studied. These findings were further supported by UV spectra obtained from DAD, which provided additional insights into the absorbance characteristics of the compound families. The chromatograms of some identified compounds from LC-DAD and LC-ESI-MS/MS are presented in Figures 4.49



Table 4.17. Main phenolic compounds identified in the three cocoa bean cultivar extracts by HPLC-DAD-ESI-MS/MS including: retention time (RT), Molecular Formula (MF), UV (nm), Molecular ion [M-H]⁻, Quantitative transition (m/z), Main fragments via MS/MS and Prop

Peak	Proposed Compounds	MF	RT (min)	UV (nm)	Ionization Mode	[M-H] ⁻ (m/z)	MS/MS (m/z)
1	N-Coumaroyl-L-aspartate_1	C ₁₃ H ₁₃ NO ₆	12.21	284; 306	[M-H] ⁻ 1	278	234, 163, 132
2	Caffeic acid	C ₉ H ₈ O ₄	14.32	180	[M-H] ⁻ 1	137	179,135
3	Theobromine	C ₇ H ₈ N ₄ O ₂	17.05	272	[M-H] ⁺ 1	181	167, 138, 137
4	Protocatechuic acid	C ₇ H ₆ O ₄	19.67	259;293	[M-H] ⁻ 1	153	109
5	Catechin-3-O-glucoside	C ₂₁ H ₂₄ O ₁₁	23.3	279	[M-H] ⁻ 1	451	289, 245
6	N-Caffeoyl-L-aspartate	C ₁₃ H ₁₃ NO ₇	24	282; 320	[M-H] ⁻ 1	294	276, 179, 132
7	Catechin	C ₁₅ H ₁₄ O ₆	28	278	[M-H] ⁻ 1	289	245, 205, 203
8	p-Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	29	-	[M-H] ⁻ 1	-	-
9	Procyanidin trimer	C ₄₅ H ₃₈ O ₁₈	32.2	280	[M-H] ⁻ 1	865	577, 425, 289
10	Procyanidin dimer	C ₃₀ H ₂₆ O ₁₂	32.8	280	[M-H] ⁻ 1	577	425, 407, 289
11	Caffeine	C ₈ H ₁₀ N ₄ O ₂	33.68	272	[M-H] ⁺ 1	195	181, 151, 138
12	Procyanidin dimer	C ₃₀ H ₂₆ O ₁₂	34	280	[M-H] ⁻ 1	577	425, 407, 289
13	C11H21O9S_1	C ₁₁ H ₂₁ O ₉ S	35.24	275	[M-H] ⁻ 1	329	241
14	Epicatechin	C ₁₅ H ₁₄ O ₆	36	278	[M-H] ⁻ 1	289	245, 205, 137
15	C11H21O9S_2	C ₁₁ H ₂₁ O ₉ S	37.86	278	[M-H] ⁻ 1	329	241
16	N-Coumaroyl-L-glutamate	C ₁₄ H ₁₅ NO ₆	38.4	286	[M-H] ⁻ 1	292	274, 248, 145, 119
17	Procyanidin trimer	C ₄₅ H ₃₈ O ₁₈	39	278	[M-H] ⁻ 1	865	577, 425, 407, 289
18	Procyanidin dimer	C ₃₀ H ₂₆ O ₁₂	41	280	[M-H] ⁻ 1	577	425, 289
19	Quercetin-3-O-glucoside	C ₂₁ H ₂₀ O ₁₂	41.67	255; 354	[M-H] ⁻ 1	463	301
20	Ferulic acid	C ₁₀ H ₁₀ O ₄	42.1	194	[M-H] ⁻ 1	248	193,149,178,134
21	Quercetin	C ₁₅ H ₁₀ O ₇	42.6	275; 365	[M-H] ⁻ 1	301	-
22	N-Feruloyl-L-aspartate	C ₁₄ H ₁₅ NO ₇	43	286; 318	[M-H] ⁻ 1	308	276, 264, 246, 193
23	Procyanidin dimer	C ₃₀ H ₂₆ O ₁₂	46	280	[M-H] ⁻ 1	577	425, 289
24	Procyanidin A-type hexoside	C ₃₆ H ₃₄ O ₁₇	47.9	280	[M-H] ⁻ 1	737	611, 449
25	Procyanidin A-type pentoside	C ₃₅ H ₃₂ O ₁₆	48.9	280	[M-H] ⁻ 1	707	449

4.10.1.1. Methylxanthines

The alkaloids, comprising theobromine and caffeine (compound 3 and 11, respectively), detected at a UV λ_{max} of 272 nm, were the most prominent chemical compounds identified in the three cocoa bean cultivar samples. Their identification was achieved by comparing retention times and MS/MS fragmentation data obtained in positive mode with commercial standards. These methylxanthines were isolated in cocoa bean fractions obtained from sun drying process to compare phenolic compounds among the cocoa cultivars. Theobromine, with a $[\text{M}-\text{H}]^+$ ion at m/z 181 and MS/MS fragments at 167, 138, and 137 m/z , and caffeine, with a $[\text{M}-\text{H}]^+$ ion at m/z 195 and MS/MS fragments at 181 and 138 m/z , are the primary alkaloids found in cocoa and cocoa-related products (Damm et al., 2016; Pedan et al., 2018; Pereira-Caro et al., 2013; Tomas-Barberán et al., 2007).

4.10.1.2. Phenolic Acids

The phenolic acids (compound 2, 4, 8 and 20, respectively), including caffeic acid (m/z 137), protocatechuic acid (m/z 153), ferulic acid (m/z 248), *p*-coumaroylquinic acid (m/z 475) were found in the beans. The retention time and MS/MS fragmentation pattern obtained with commercial standard confirmed the identity of these phenolic acids. In general, these three phenolic acids ionized better in the negative ionization mode and exhibited a loss of 44 u (CO_2) in MS/MS, as proved by (Ammar et al., 2015) in their studies. Caffeic acid, ferulic acid and *p*-coumaroylquinic acid are hydroxycinnamic acids derivatives, while protocatechuic acid is a hydroxybenzoic acid derivative, these acids compounds are widely distributed in nature, reported for cocoa beans and related products in several studies (Barnaba et al., 2017; Rodríguez-Carrasco et al., 2018). Recently, this compound was also quantified in cocoa bean and husks from Peru (Cádiz-Gurrea et al., 2020), Colombia (Cantele et al., 2020), Honduras (Rossin et al., 2019), and Ecuador (Rojo-Poveda et al., 2019; Rossin et al., 2021).

4.10.1.3. Flavan-3-ols and Their Glycosides

As shown in the Table 4.17, compounds 7 and 14, which exhibit characteristic UV–visible spectra with a λ_{max} at 278 nm, were identified in the three cocoa bean samples as catechin and epicatechin, respectively. They possess a $[\text{M}-\text{H}]^-$ ion at m/z 289, with MS/MS fragments at 245, 205, and 203 m/z , and their identities were confirmed by comparison with pure standards. These compounds are well-known as the primary constituents of cocoa beans, cocoa products, and cocoa byproducts (Barnaba et al., 2017; D'Souza et al., 2017; Pereira-Caro et al., 2013). Additionally, compound 5 showing a $[\text{M}-\text{H}]^-$ ion at m/z 451 and MS/MS fragments at 289 from catechin, were tentatively identified as an isomers of flavan-3-ols glycosides, specifically catechin-3-O-glucoside. This catechin glycoside has been previously documented in cocoa beans in some studies by Patras et al. (2014) and Cádiz-Gurrea et al. (2020).

4.10.1.4. Procyanidins

In addition to the monomers of flavan-3-ols and their glycosides, the cocoa bean samples from the three cultivars also revealed the presence of several oligomers, known as procyanidins, along with their derivatives. These compounds are composed of multiple units of catechin and epicatechin. A total of 8 compounds were tentatively identified among the various fractions obtained from all cocoa bean extracts, including B-type procyanidin dimers and trimers, as well as A-type procyanidin glycosides.

Four chemical compounds (compounds 10, 12, 18, and 23), as indicated in the Table 4.17 were identified as B-type procyanidin dimers, exhibiting the same UV–visible spectrum with a λ_{max} at 280 nm, a $[\text{M}-\text{H}]^-$ ion at m/z 577, and a fragmentation pattern yielding a fragment at 289 m/z , which corresponds to the monomers catechin and epicatechin. Some of these compounds have been documented in the literature in cocoa beans and other cocoa products, such as cocoa husks and beans from Peru (Cádiz-Gurrea et al., 2020; Patras et al., 2014). Additionally, two chemical compounds (9 and 17) exhibited in Table 4.17 were identified as B-type procyanidin trimers. These compounds displayed a λ_{max} at 278–280 nm, a $[\text{M}-\text{H}]^-$ ion at m/z 865, and a similar fragmentation pattern in negative ionization mode, resulting in fragments at 577, 425, 407, and 289 m/z (Calderon et al., 2009). Regarding B-type procyanidin dimers, the aforementioned studies have documented these polyphenols in cocoa beans and cocoa products. Moreover, two compounds (25 and 26) with typical UV–visible spectra at λ_{max} 280 nm were identified as A-type procyanidin glycosides. Among these, compounds 25 exhibiting a $[\text{M}-\text{H}]^-$ ion at m/z 737 and MS/MS fragments at 611 and 449 m/z , was identified as isomer of procyanidin A-type hexoside (D'Souza et al., 2017; De Taeye et al., 2017). In contrast, compounds 26, which possesses a $[\text{M}-\text{H}]^-$ ion at m/z 707 and exhibit a similar fragmentation pattern yielding fragments at 449 m/z , was identified as isomers of procyanidin A-type hexosides (Patras et al., 2014). These compounds were reported in this study for the first time in fermented and dried Nacional cocoa bean.

4.10.1.5. Flavonols and Their Glycosides

The UV–visible spectra, with typical λ_{max} values at 255 nm and 354 nm, along with a similar fragmentation pattern in negative ionization mode that yields a fragment at 301 m/z , indicated that compounds 19 as shown in the Table 4.18 is flavonol glycoside. Specifically, it was identified as quercetin-3-*O*-glucoside, which has a $[\text{M}-\text{H}]^-$ ion at m/z 463. The commercial standard of quercetin-3-*O*-glucoside (compound 19) allowed confirm the identity of these flavonol to be confirmed. Interestingly, this compounds was determined for the first time in Nacional bean sample.

4.10.1.6. N-Phenylpropenoyl-L-Amino Acid

Compound 1 characterized by a $[\text{M}-\text{H}]^-$ ion at m/z 278 and MS/MS fragments at 234, 163, and 132 m/z , along with UV–visible spectra typical of λ_{max} 284/306 nm, was identified as isomers

of N-Coumaroyl-L-aspartate. Alongside compounds 16, this molecule exhibited a similar fragmentation pattern in negative ionization mode, which is characteristic of coumaric acid, producing fragments at 274, 248, 145 and 119 m/z (Tomas-Barberán et al., 2007). The tentative identification of N-coumaroyl-L-glutamate (compound 16) was based on its [M-H]⁻ ion at m/z 292 and the MS/MS fragmentation pattern, which produced fragments at 274, 248, 230, 202, 163, 143, and 119 m/z, as previously described for cocoa beans. Additionally, the UV–visible spectra exhibited a typical λ_{max} of 286 nm (Stark & Hofmann, 2005).

In addition to the coumaric acid derivatives, N-caffeoyl-L-aspartate (compound 6) was tentatively identified based on its [M-H]⁻ ion at m/z 294, MS/MS fragments at 276, 179, and 132 m/z, and UV–visible spectra showing typical λ_{max} values at 282 and 320 nm. Part of its identity was confirmed by comparison with the fragmentation patterns of pure caffeic acid, which also yielded fragments at 179 and 132 m/z. Additionally, compound 22, characterized by a [M-H]⁻ ion at m/z 308, MS/MS fragments at 276, 264, 246, 193, 149, and 134 m/z, and UV–visible spectra with typical λ_{max} values at 286 and 318 nm, was tentatively identified as N-Feruloyl-L-aspartate. This identification was supported by the fragmentation pattern characteristic of ferulic acid, producing fragments at 193 and 134 m/z. The MS/MS fragmentation patterns for the hydroxycinnamic acids (p-coumaroylquinic acid, caffeic and ferulic acids) were confirmed by comparison with data from pure reference standards. While N-coumaroyl-L-glutamate and N-feruloyl-L-aspartate have been previously described in cocoa beans, especially Forastero and Criollo cultivars from diverse regions (Stark & Hofmann, 2005), this study marks the first identification of these compounds in Nacional cultivar.

4.10.1.7. Others Phenolic Compounds

Among the compounds described and classified above, two isomers of sulfated compounds containing sulfonic acid compounds 13 (C₁₁H₂₁O₉S_1) and 15 (C₁₁H₂₁O₉S_2), as indicated in the Table 4.18 were tentatively identified. These compounds exhibit UV–visible spectra with a typical λ_{max} at 278 nm, and their identification was based on the [M-H]⁻ ion at m/z 329 and MS/MS fragments at 241. These sulfated compounds have also been documented in the literature by several authors for cocoa beans and related products (Cádiz-Gurrea et al., 2014), however, no information regarding these compounds have been reported in Nacional cocoa bean and products in previous investigations.

4.10.2. Quantitative Distribution of Phenolic Compounds in the Three cocoa Bean cultivars Obtained from Sun Drying Process

The concentration of the total and individual phenolic compounds of the three cocoa bean samples obtained from sun drying process, declared in mg/kg, are represented in Table 4.19. The quantification of these compounds were tentatively performed based on the similarity of their

molecular structures as follows: procyanidins were expressed as equivalents of procyanidin B1; catechin-3-*O*-glycosides as equivalents of catechin; flavonol-3-*O*-glycosides as equivalents of quercetin-3-*O*-glucoside; and N-phenylpropanoid-L-amino acids as equivalents of the respective phenolic acids present in their structures, namely p-coumaric, caffeic, and ferulic acids. The amounts of each molecule and the total quantities of each group of molecules quantified for the three cocoa cultivar samples are detailed in Table 4.18. The concentration of total phenolic and alkaloid compounds, as can be indicated in in Table 4.18 revealed a significant differences between examined cocoa bean samples statistically ($p < 0.01$). The total amount of phenolic compounds ranged between 1842.27 and 2735.99 mg/kg cocoa bean in this study. In addition, when cocoa cultivars were compared, SDNB exhibited the highest amount of phenolic compounds, following by SDFB. Cocoa cultivars from sun drying process exposed a quite similar individual phenolic profile (Table 4.18). A total of 25 phenolic compounds including caffeic acid, protocatechuic acid, ferulic acid, p-Coumaroylquinic acid, catechin, epicatechin, catechin-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin, theobromine, caffeine, procyanidins B type dimer (4), procyanidins B type trimer (2), procyanidin A-type hexoside, procyanidin A-type pentoside, N-coumaroyl-L-aspartate, N-caffeoyl-L-aspartate, N-coumaroyl-L-glutamate, N-feruloyl-L-aspartate and isomers of sulfated compounds containing sulfonic acid (2) were identified and quantified in the three sun dried and fermented cocoa cultivar samples. As shown in Table 4.18, a comparison of the concentrations of individual phenolic compounds in the three cocoa bean cultivars obtained from sun drying process, significant differences among the group were observed statistically ($p < 0.01$).

Table 4.18. Phenolic content (mg/kg) of sun dried cocoa bean samples acquired from Nacional, Forastero and Criollo cultivars

Peak	Proposed compounds	SDNB	SDFB	SDCB
	Phenolic acids			
1	Caffeic acid	6.42 ± 0.2 ^a	1.6±0.3 ^c	2.36±0.8 ^b
2	Protocatechuic acid	7.81±0.6 ^c	9.16±1.2 ^b	11.20±1.8 ^a
3	Ferulic acid	1.76±0.1 ^c	8.45±0.9 ^a	4.92±0.7 ^b
4	p-Coumaroylquinic acid	266.51± 4.6 ^a	162.95±2.4 ^c	204.58±3.7 ^b
	Total	282.50±3.8^a	182.16±2.3^c	223.05±4.2^b
	Flavonoids			
5	Catechin	21.09±1.3 ^a	9.23±0.9 ^c	13.44±1.6 ^b
6	Epicatechin	156.18±2.4 ^c	163.24±1.8 ^b	192.19±3.5 ^a
7	Catechin-3- <i>O</i> -glucoside	16.23±0.4 ^a	11.77±1.3 ^c	13.50±1.8 ^b
8	Quercetin-3- <i>O</i> -glucoside	10.85±0.8 ^a	4.4±0.2 ^c	6.63±0.6 ^b
9	Quercetin	4.8±0.9 ^a	0.89±0.1 ^c	2.43±0.4 ^b
	Total	209.14±0.9^a	189.52±0.1^c	228.19±0.4^b
	Procyanidins B type (PCB) dimers			
10	PCB_1	59.01±1.4 ^b	67.16±1.8 ^a	58.12±1.6 ^b
11	PCB_2	15.20±0.8 ^b	18.50±0.2 ^a	12.57±0.3 ^c

Table 4.18. Continued

12	PCB_3	29.48±0.6 ^a	18.59±0.4 ^b	14.53±0.2 ^c
13	PCB_4	12.89±0.3 ^a	12.19±0.5 ^a	9.32±0.8 ^b
	Total	116.57±3.4^a	116.43±4.8^a	94.54±2.7^b
	Procyanidins B type (PCB) trimers			
14	PCB_1	45.41±1.8 ^a	37.70±1.4 ^b	27.87±0.9 ^c
15	PCB_2	36.12±2.1 ^a	16.29±0.9 ^c	25.52±1.6 ^b
	Total	81.53±3.6^a	53.99±1.7^b	53.39±1.3^b
	Procyanidins-glycosides			
16	Procyanidin A-type hexoside	9.03±0.6 ^a	4.07±0.2 ^c	6.38±0.5 ^b
17	Procyanidin A-type pentoside	16.10±0.8 ^c	21.83±1.2 ^b	26.90±0.9 ^a
	Total	25.13±1.4^b	25.90±1.3^b	33.28±1.8^a
	Methylxanthines			
18	Theobromine	1897.38±21.4 ^a	1329.49±17.8 ^b	1140.17±10.6 ^c
19	Caffeine	896.4±8.7 ^a	478.8±5.9 ^c	623.7±7.4 ^b
	Total	2793.78±19.2^a	1808.29±14.6^b	1763.87±11.8^c
	N-Phenylpropenoyl-L-amino acids			
20	N-Coumaroyl-L-aspartate	24.09±1.2 ^a	9.71±0.8 ^c	14.32±0.2 ^b
21	N-Caffeoyl-L-aspartate	49.35±1.8 ^a	25.24±0.9 ^c	30.83±0.7 ^b
22	N-Coumaroyl-L-glutamate	16.80±0.3 ^a	8.76±0.1 ^b	6.43±0.1 ^c
23	N-Feruloyl-L-aspartate	18.12±0.8 ^a	14.95±0.6 ^b	12.40±0.3 ^c
	Total	108.36±2.1^a	58.67±1.3^b	63.98±1.8^c
	Others			
24	C11H21O9S_1	5.12±0.2 ^a	1.04±0.05 ^c	2.45±0.4 ^b
25	C11H21O9S_2	11.67±0.8 ^a	1.79±0.3 ^c	3.41±0.6 ^b
	Total	16.79±1.3^a	2.83±0.5^c	5.86±0.4^b
	General Total	3626.99±26.4^a	2437.80±18.7^b	2464.80±16.8^b

SDNB: sun dried Nacional beans; SDFB: sun dried Forastero beans; SDCB: sun dried Criollo beans. Different lower-case letters (a,b,c) within the same line indicate significant differences ($p<0.05$) between the sun dried cocoa bean acquired from the three cocoa cultivars. Each value represents the mean of three determinations ($n = 3$) and three independent experiments.

Among the 25 chemical compounds identified in the the three cocoa cultivar samples, methylxanthines were the most prevalent chemical group, with concentrations ranging from 1763.87 to 2793,78 mg/kg, which represented up to 77.02%, 74.17% and 71.56% of total phenolics and alkaloids in Nacional, Forastero and Criollo bean samples by comparing with other groups, respectively. Cocoa beans and products rich in methylxanthines derivatives from the phenolic class are recognized for their enhanced stability (Barbosa-Pereira et al., 2021). The typical organoleptic characteristics of cocoa beans and associated products, such as bitterness, astringency and pungency, are partly attributed to methylxanthine compounds. Consistent with findings in the cocoa beans from the three cultivars , theobromine which amount varied from 1140.17-1897.38 mg/kg was present in higher concentrations compared to caffeine (478.8-896.4 g/kg). These observations was also made by Letricia Barbosa-Pereira et al., 2021 in their investigation in Criollo and Trinitario bean samples.

These findings are in agreement with those described by several authors in the literature for cocoa beans and related products, including the three cultivars studied (Barbosa-Pereira et al., 2021; del Rosario Brunetto et al., 2007). When cocoa cultivars were compared, Nacional variety exhibited the highest amount of methylxanthines (2796.78 mg/kg) and theobromine (1897.38 mg/kg), following by Forastero cultivar sample.

The other class of chemical constituents ($n=23$), characterized in this study, was mainly consisted of phenolic compounds in the range between 1.04 and 266.51 mg/kg of CBS. Fermented and sun dried cocoa bean obtained from Nacional cultivar was discovered with the highest amount (1.76 and 266.51 mg/kg) of these bioactive compounds, followed by Criollo cultivar sample. On the other hand, cocoa bean from Forastero cultivar was with the lowest contents of phenolic compounds, ranging between 0.89 and 163.24 mg/kg.

Phenolic acids represent a significant group within the phenolic composition of cocoa beans and related products. These compounds are secondary aromatic metabolites widely distributed across the plant kingdom (Andreasen et al., 2000; Exarchou et al., 2003; Prim et al., 2003). Phenolic acids can be categorized into two main carbon skeletons: hydroxycinnamic and hydroxybenzoic structures (Bendini et al., 2007). They contribute not only to the color and organoleptic qualities of foods but also to their health benefits and antioxidant properties (Maga & Katz, 1978). Several researchers affirmed the occurrence of phenolic acids as significant compounds in cocoa bean and products (Araujo et al., 2018; Granato et al., 2018). Four phenolic acids, including, protocatechuic acid (as the hydroxybenzoic acid), caffeic acid, ferulic acid and p-coumaroylquinic acid (as the hydroxycinnamic acids), which made the most abundant group of individual phenolic compounds in all tested cocoa bean cultivars were detected and quantified in this study. These compounds were previously reported in Criollo, Forastero and Trinitario varieties (Barbosa-Pereira et al., 2021; Bordiga et al., 2015; Carrillo et al., 2014). Phenolic acids contributed with 33.90%, 31.82% and 28.93% of total phenolic compounds in Nacional, Criollo and Forastero cultivar samples, respectively. Among the phenolic acids studied, p-coumaroylquinic acid was found to be the most abundant, followed by protocatechuic acid, which was the second most predominant phenolic acid in all cocoa bean cultivar samples analyzed. The contents of these individual phenolic acid compounds found in our study were higher than those discovered by Rodríguez-Carrasco et al. (2018) and Barbosa-Pereira et al. (2021) in cocoa bean samples. Difference in concentration of phenolic acids among the fermented and sun dried cocoa bean from the three cocoa cultivars may probably due to the activity of hydrolytic enzymes and microorganisms during fermentations. These results were previously observed by further researchers (Krähmer et al., 2015; Lee et al., 2003).

One of the most significant group of polyphenols identified in cocoa products, including fermented and sun dried cocoa bean samples derived from the three cocoa cultivars examined in this study, was flavonoids, which accounted for approximately 71.06%, 68.13% and 66.09% of total phenolic compounds. Flavonoids are widely recognized as prevalent secondary plant metabolites

(Kanadaswami et al., 2005; Le Marchand, 2002; Nestel, 2003). This group encompassing flavan-3-ols (2) and their derivatives (1), procyanidins (6) and their glycosides (2), and flavanols (1) along with their respective glycosides (1), phenylpropanoids (4) and other compounds (2) were identified and quantified in all cocoa bean samples investigated in this assay. These findings align closely with values reported in the literature for cocoa beans, comprising Criollo and Forastero and their products (Barnaba et al., 2017; Rodríguez-Carrasco et al., 2018). All of these compounds were also proved in former studies by Amandine Cambrai et al. (2016) in Criollo, Forastero, Nacional and Trinitario and Barbosa-Pereira et al. (2021) in Criollo and Forastero cocoa bean shell. The total amount of this group was between 447.34 and 550.71 mg/kg. Comparing the total concentration of this class of compound among the cocoa bean cultivar, Nacional type exhibited the highest total amount (550.71 mg/kg), followed by Criollo with a total concentration of 477.87 mg/kg.

The flavan-3-ols (catechin and epicatechin) were determined and quantified as the predominant compounds in all cocoa bean varieties, accounting for approximately 29.33% of the total phenolic content in Criollo type, about 27.39% in Forastero cultivar and 21.27% in Nacional variety. In addition, epicatechin was observed in higher concentrations in all samples. Moreover, the highest amount of epicatechin (205.53 mg/kg) was found in Criollo cocoa bean sample, followed by Nacional cocoa bean sample with a concentration of 177.26 mg/kg. Significant difference was not observed between sun dried Nacional bean and Forastero bean samples when epicatechin concentrations of both cultivars were compared. Catechin was found with highest amount (21.09 mg/kg) in Nacional cocoa bean sample. These values align with the lowest reported levels in the literature for Criollo and Forastero from various geographical origins (Barbosa-Pereira et al., 2021; Bordiga et al., 2015) and are lower than those observed in other studies on cocoa beans (Rodríguez-Carrasco et al., 2018).

Derivatives of flavan-3-ols, specifically the glycosides of catechin, catechin-3-*O*-glucoside was also detected and quantified in all fermented and dried cocoa bean from the three cocoa cultivars. This compound represented approximately 2%, 1.86% and 1.92% of the total phenolic compounds present in cocoa bean samples from Nacional, Criollo and Forastero cultivar, respectively. Fermented and dried Nacional bean sample exhibited the highest amount of catechin-3-*O*-glucoside (16.23 mg/kg) among others, however, statistical difference was not found between all cocoa bean cultivars, in which close similarity was observed between the concentration of Criollo and Forastero cultivar samples. This compounds has been reported in cocoa bean and products, mostly in Criollo, Forastero and Trinitario cultivars by numerous scholars (Calderon et al., 2009; Niemenak et al., 2006; Rodríguez-Carrasco et al., 2018).

The total content of flavan-3-ol oligomers, classified as B-type procyanidins (PCB), PCB trimers, and A-type procyanidins (PCA) glycosides, accounts for 31.18%, 26.79% and 25.85% of the total phenolic compounds present in the fermented and sun dried cocoa bean from Forastero, Nacional and Criollo cultivars, respectively. Among the three groups of procyanidins determined in

the three cocoa cultivars, PCB dimers were found in higher concentration, ranging between 94.54-116.57 mg/kg and represented about 13.48%, 13.99% and 18.49% of the total content of phenolic compounds in Forastero, Nacional and Criollo cultivars, respectively. It was also observed that no significant difference in concentration was observed among the cocoa cultivars. Regarding individual B type procyanidins (dimers and trimers), PCB_1 was the isomer determined in higher amounts, within the range of 58.12-67.16 mg/kg, and the cocoa bean sample from Forastero showed the highest concentration, while no significant difference was revealed between Criollo and Nacional cultivars. Considering procyanidin type A glycosides (PCA), fermented and sun dried cocoa bean sample obtained from Criollo was found with higher amount compared to others, particularly PCA pentoside (16.10-26.90 mg/kg). Similarity in concentration of procyanidin type A glycosides was found between Nacional and Forastero cultivars. These compounds were previously asserted in Criollo, Forastero and Trinitario of different geographical regions (Rodríguez-Carrasco et al., 2018), in cocoa bean shell from Criollo and Trinitario cultivars (Barbosa-Pereira et al., 2021) in dried Forastero bean from Ghana (Ortega et al., 2010).

Another group of chemical compounds identified in the cocoa bean samples from the three cultivars consists of N-phenylpropenoyl-L-amino acids, which have been suggested as chemical markers for cocoa products. These compounds account for 2.98%, 2.59% and 2.40% of the total bioactive compounds in Nacional, Criollo and Forastero cultivar samples, respectively. The total concentration ranges from 58.66 mg/kg in the Forastero bean sample to 108.35 mg/kg in the Nacional bean sample. Four of this class of compounds, including N-Coumaroyl-L-aspartate, N-Caffeoyl-L-aspartate, N-coumaroyl-L-glutamate, N-feruloyl-L-aspartate were detected and quantified in all the three cocoa cultivar samples. Among them, N-Caffeoyl-L-aspartate was the most dominant, with a concentration of 49.35 mg/kg in the Nacional bean, 30.83 mg/kg in the Criollo bean, and 25.24 mg/kg in the Forastero bean sample. Similar pattern was also observed by other researchers in different cultivar of cocoa beans and associated products from different regions (Damm et al., 2016; Pereira-Caro et al., 2013).

Finally, a small class of bioactive compounds, which contributes 7.5% to the total bioactive compounds, was quantified for the first time in Nacional bean, with concentrations ranging from 1.79 mg/kg in the Forastero bean sample to 11.67 mg/kg in the Nacional bean sample. The compounds C₁₁H₂₁O₉S_1 and C₁₁H₂₁O₉S_2 were found as this small group of chemicals, in which Nacional bean sample exhibited the highest total amount (16.72 mg/kg), followed by Criollo bean sample with a concentration of 5.86 mg/kg. In their study conducted in Venezuela, Barbosa-Pereira et al. (2021) also determined C₁₁H₂₁O₉S_2 in cocoa bean shell for the first time.



5. CONCLUSION AND RECOMMENDATIONS

Within the scope of this project, the effects of various drying techniques of three cocoa bean cultivars on the general and color characteristics, phenolic compounds, antioxidant capacity, aroma profile, aroma-active compounds, and sensory properties were primarily investigated, and secondarily, a comparative study on the phenolic compounds antioxidant capacity, aroma profile and aroma-active compounds between the three cocoa cultivars based on conventional drying technique (sun-drying) was also conducted in detailed.

The results of the study can be summarized as follows:

As a result of the quality trade and physical properties of fermented and dried cocoa beans

- The values of physical properties such as fermentation index, cut test, width, length, bean weight and 100 beans weight of all three cultivars were found in line with the standards which make them good quality trade cocoa beans.
- The values of color measurement results (L^* , a^* , b^*) of fermented and dried cocoa beans of all cultivars showed significant differences between the cocoa bean samples at four different drying processes ($p < 0.05$) with microwave and convective drying standing out. In addition, the color measurement results (L^*) were the highest in all the fresh-fermented bean obtained from the three cultivars.

As a result of phenolic content, total flavonoid content, total anthocyanin content and antioxidant activity of fermented cocoa beans according to the drying techniques

- The total phenols content, total flavonoid content and total anthocyanin content of cocoa bean were statistically different ($p < 0.05$) in the fermented and dried cocoa beans from the three cultivars, and the highest values were in fresh-fermented beans, followed by freeze-dried and convective dried beans. All the fermented and dried cocoa bean from the three cocoa cultivars showed the same pattern.
- When the total phenolic, total flavonoid and total anthocyanin contents of the three cocoa cultivars based on sun drying technique were compared, the highest values were in sun dried cocoa bean was from Nacional cultivar, followed by Forastero cultivar.
- As a result of antioxidant activity analysis with ABTS and DPPH methods, the highest antioxidant activity value was observed in the fresh fermented bean obtained from Forastero and Criollo cultivars, while in the Nacional cultivar, convective dried bean showed the highest value of antioxidant activity. Indeed, the antioxidant activity values

were decreased in cocoa bean when microwave drying technique was applied in all cocoa cultivars.

- When all the results were evaluated, Nacional cv. stood out in terms of total phenolics and antioxidant activity. Additionally, sun drying and convective drying is recommended to obtain higher phenolic content in cocoa beans.
- The total phenolic content and antioxidant activity values exhibited interdependence. In fact, cocoa bean with higher total phenolic content demonstrated greater antioxidant activity.

As a result of phenolic compounds of fermented cocoa bean from the three cultivars according to the sun drying techniques

- As sun drying displayed better results in total phenolics and antioxidant activity, a comprehensive determination of phenolic compounds was carried out in sun dried cocoa beans from three different cvs.
- When the bioactive compounds, including phenolic and alkaloid compounds were compared, statistically significant differences were found between the cocoa bean obtained from the three cocoa cultivars. Higher amounts of total bioactive compounds were detected in the sun dried cocoa bean obtained from Nacional cultivar similar to the results of total phenolic and antioxidant activity analysis.
- Methylxanthine class of alkaloid compounds was found as the most dominant group in all sun dried bean from the three cocoa cultivars, with the highest amount in Nacional cultivar.
- The amount of theobromine, followed caffeine were detected as the predominant bioactive compounds in all the sun dried cocoa bean samples obtained from the three cocoa cultivars
- Phenolic acids and flavan-3-ols, class of phenolic compounds were also observed as the second and third prevailing groups in all the sun dried cocoa bean, respectively, with an increase value in Nacional bean sample.
- The amount of caffeic acid, followed by epicatechin were determined as the most dominant phenolic compounds in all cocoa bean samples. Overallly, Nacional cv. Had the highest amount of phenolics and its use is recommended to obtain higher bioactivity cocoa beans.

As a result of analysis of aroma profile of fermented cocoa beans according to drying techniques

- The aroma content and profile was significantly and clearly changed with the different drying techniques of all cocoa cultivars ($p < 0.05$). The concentrations of aroma compounds displayed an increase with the drying application.
- The prevailing class of aroma compounds in all fermented and dried cocoa bean was aldehydes (especially hexanal and 3-methylbutanal), followed by alcohols, esters and terpenes in Forastero, Criollo and Nacional cocoa cultivars. Moreover, total amount of aldehyde compounds were significantly increased in microwave and convective dried bean samples.
- Each volatile group reacted differently to the drying process. For example, the amounts of pyrazines, furans, pyrrole and pyrrolines increased with the applied heating resulting higher concentrations in convective and microwave dried samples. In contrast, the amounts of alcohols, esters and terpene compounds were protected better with sun drying and freeze drying techniques giving the beans more desirable and distinct aroma.
- When the total amount of aroma compounds were compared between the cocoa cultivars on sun dried bean samples, Nacional cultivar exhibited the highest amount, followed by Forastero cultivar. Interestingly, aldehyde compounds were found as the most dominant class of compounds, and sun dried bean samples obtained from Nacional cultivar exhibited the highest total amount of aldehyde compounds, hexanal was also determined as the most dominant aldehyde in all sun-dried cocoa bean samples.

As a result of olfactometric analysis of fermented cocoa beans according to the drying techniques;

- The type and number of aroma-active compounds were differed in fermented cocoa bean samples of the three cocoa cultivars at the different drying techniques.
- In the fermented and different dried cocoa bean samples obtained from the Forastero cultivar, the flavor dilution (FD) values of aroma-active compounds ranged from 4 to 2048. When the drying process were compared among the fermented and different dried Forastero bean samples, sun drying and convective drying techniques exhibited the highest number of aroma-active compounds. Among them, hexanal (green) with 2048 FD, followed by hexanol (flowery-spicy) with 1024 FD in CDFB and FDFB samples, respectively, exhibited the highest value of FD factor.
- In Criollo cultivar, the flavor dilution (FD) values of aroma-active compounds ranged from 4 to 4096. When comparing the drying processes among these samples, sun drying

and convective drying techniques yielded the highest number of aroma-active compounds. Notably, hexanal (green) exhibited an FD value of 4096, while 2-methylbutanal (green-fruity) followed with an FD of 2048 in the CDFB and MDCB samples, respectively, representing the highest FD factors. In addition, hexanol with 1024 FD, (*E*)-Caryophyllene and 2-methyl-3-butanol with 512 FD were also observed with the significant value of FD factor in FCB and FDCB samples.

- In Nacional cultivar, the flavor dilution (FD) values of aroma-active compounds ranged from 4 to 4096. Among the drying methods analyzed, convective drying and microwave drying techniques produced the highest number of aroma-active compounds. Notably, hexanal (green) demonstrated an FD value of 4096, while 3-methylbutanal (green-fruity) followed with an FD of 2048 in the CDFB and MDCB samples, respectively, indicating the highest FD factors. Additionally, hexanol and 2-methylbutanal showed an FD of 1024 in FDNB and MDNB samples, respectively. 1-penten-3-ol, linalool and 2,3-butanediol each exhibited an FD of 512, highlighting their significant contributions in the FDNB, MDNB and FDNB samples, respectively.
- When the type and power of aroma-active compounds were compared between the cocoa cultivars on sun dried bean samples, the sun dried bean obtained from Nacional cultivar was more numerous and stronger than sun dried bean samples from the two other cultivars.
- In sum, Nacional cv. outshined the other two cvs. with its high bioactivity, high amount of aroma compounds and higher results of sensory analysis. The panelists preferred Nacional cv. cocoa bean more for its overall quality.
- Regarding the drying processes, sun drying displayed higher protection in both phenolics and aroma compounds resulting more preferable product. In conclusion, sun dried Nacional cv. cocoa beans are recommended for their overall quality.
- For upcoming studies, the optimization of fermentation and drying processes is highly recommended with the inclusion of novel non-thermal plasma treatments.

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