ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

ASSESMENT OF BLACK CARROT, RED BEET, POMEGRANATE AND STRAWBERRY AS STARTER CULTURE SOURCE FOR SOURDOUGH BREAD

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KARA KAVUÇ, KIRMIZI PANCAR, NAR VE ÇİLEĞİN EKŞİ MAYA EKMEKLERİNDE STARTER KÜLTÜR KAYNAĞI OLARAK DEĞERLENDİRİLMESİ

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MAY 2015

Ayça Ayfer Paslı, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID **506121502**, successfully defended the **thesis** entitled "**ASSESMENT OF BLACK CARROT, RED BEET, POMEGRANATE AND STRAWBERRY AS STARTER CULTURE SOURCE FOR SOURDOUGH BREAD**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

FOREWORD

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May 2015

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ABBREVIATIONS

AACC	**:** American Association of Cereal Chemists
AIBI	**:** International Association of Plant Bakeries
ANOVA	**:** Analysis of variance
CFU	**:** Colony forming units
DAG	**:** Diacylglycerol
EPS	**:** Exopolysaccharides
IFU	**:** International Federation of Fruit Juice Producers
ISO	**:** International Organization for Standardization
LAB	**:** Lactic acid bacteria
MAG	**:** Monoacylglycerol
SPSS	**:** Statistical Package for the Social Science
TTA	**:** Total titratable acidity

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a*	**:** red/green (color parameter)
b*	**:** blue/yellow (color parameter)
CO_2	**:** Carbon dioxide
g	**:** gram
L*	**:** lightness/darkness (color parameter)
ml	**:** milliter
NaCl	**:** Sodium chloride
p	**:** probability (statistical analysis)

ASSESMENT OF BLACK CARROT, RED BEET, POMEGRANATE AND STRAWBERRY AS STARTER CULTURE SOURCE FOR SOURDOUGH BREAD

SUMMARY

In this study, investigations were carried out on the development of sourdough, derived from various fruits and vegetables, for the production of sourdough bread that meets the consumers demand for natural, tasty and healthy food. Strawberry, pomegranate, black carrot and red beet were preferred as starter culture source in preparation of sourdough. Sourdough preparation carried out in three-stage based on traditional procedure. For determining characteristics of sourdough; pH, total titratable acidity, lactic acid bacteria and yeast count analysis were performed. In evaluation of bread properties; bake loss, specific volume, color characteristic, moisture, pH, water activity and textural analysis were achieved. Furthermore, sensory analysis was conducted to determine consumer preferences in choice of bread.

pH and total titratable acidity value of preferment used in sourdough preparation, varied according to substrate source ($p<0.05$). pH value ranged between 3.58 which is average value for pomegranate and 5.08 which is the pH of red beet. Titratable acidity was lowest for red beet (4.53 ml) and highest for black carrot (7.93 ml). Lowest pH value (5.15) for Dough I was measured in pomegranate samples as in used preferment. The titratable acidity of Dough I was found between 2.37 and 4.97 ml for red beet and pomegranate, respectively. Amoung the Dough I samples , the highest yeast counts ($6.0*10^5$ cfu/g) were determined for strawberry and the highest count of lactic acid ($7.8*10^4$ cfu/g) were obtained in the black carrot while the lowest cell counts were obtained in pomegranate samples. Afterwards refreshments steps, the measured pH and titratable acidity did not vary according to substrate for Sourdough III ($p<0.05$). pH and titratable acidity values were found between 3.90 (red beet) and 4.08 (pomegranate); 14.07 (strawberry and pomegranate) and 14.83 (red beet), respectively. Among the Sourdough III samples, the highest yeast counts ($1.8*10^6$ cfu/g) were determined for strawberry and the highest count of lactic acid ($1.4*10^8$ cfu/g) were obtained in the red beet while the lowest cell counts were obtained in black carrot samples.

In this study, the addition level of sourdough and type of substrate, used as a starter culture source in sourdough preparation, did not led to significantly variation in bake loss ($p<0.05$). In wheat bread (control bread) and sourdough bread samples, loss of moisture during baking was found between 14.00 % (20 % strawberry sourdough usage) and 16.07 % (control and 10 % strawberry sourdough usage). For specific volume, sourdough bread samples prepared with 30 % black carrot sourdough had lowest value (6.19 ml/g) while control bread without sourdough application had highest value (7.58 ml/g). Sourdough application provides differentation in color properties of bread crumb and crust. In bread samples, the lighter color of crumb and crust structure was achieved with sourdough utilization. The parameter L* (lightness)

value of bread crumb ranged between 71.17 which is average value for % 30 pomegranate sourdough usage and 76.20 which is for control bread. Sourdough breads prepared with pomegranate sourdough had the lowest parameter L* value (73.08). In case for bread crust, the L* parameter value was found between 44.41 (% 10 strawberry sourdough usage) and 54.89 (% 20 strawberry sourdough usage). The source of substrate did not significantly effect the parameter a* (redness) value of bread crumb whereas the addition of sourdough led to significantly increase in the parameter a* value of bread crust ($p<0.05$). The highest a* value (17.97) was observed in the sample prepared with %30 addition of sourdough originated from black carrot. The parameter b* (yellowness) value ranged from 23.69 (S-10) to 30.91 (RB-10 and S-20) in crust while in crumb from 12.24 (P-20) to 13.39 (BC-30).

Moisture content of bread samples on first day was measured between 33.61 % (30 % strawberry sourdough usage) and 34.69 % (10 % strawberry sourdough usage) while afterwards the storage, a decrease in moisture content was observed.The moisture content of bread samples on the end of the storage ranged from 32.48 % (BC-30) to 34.19 % (RB-10). End of the storage black carrot originated sourdough samples exhibited lowest moisture content (33.07 %) in which maximum moisture loss occurred through storage. In terms of sourdough application, the highest moisture content change (2.64 %) observed in bread prepared with 30 % sourdough application. The water activity (a_w) of control bread and sourdough bread was found to 0.94 in all samples and did not change throught storage. Fruits and vegetables, used in sourdough preparation, leads to differentiation in pH value of bread and also there was a decline in pH values with incremental sourdough application ($p<0.05$). The lowest value (5.05) was determined in bread prepared with 30 % red beet sourdough while this value was found in control bread as 5.58. Besides with storage a decrease in pH values was observed. Neither substrate type nor addition level of sourdough leaded to significantly variation in pH change throughout storage ($p<0.05$) Nevertheless, maximum change (%) was observed within the sourdough breads prepared by %30 application.

In consequence of texturel analysis it was determined that firmness of bread was significantly varied depending on origin of sourdough and level of use ($p<0.05$). Firmer bread crumb was achieved with incremental sourdough application irrespective of sourdough variety. Firmness of bread on first day was determined between 217.90 (g) for control bread and 449.12 (g) for sourdough bread prepared with 30 % black carrot sourdough. Throughout to storage, change in firmness did not significantly vary in terms of utilization ratio or substrate type ($p<0.05$); yet black carrot-originated sourdough breads exhibited higher firming rate and significantly different from control bread

As a result of sensory analysis carried out for bread samples, sourdough bread prepared with 10 % strawberry sourdough application exhibited highest score (41.10). In respect of sourdough addition, %10 sourdough application resulted in higher score (40.10) than control bread. The addition of sourdough provided an increase of the score in several attributes such as taste, odour and color whereas control bread was preferred mainly in respect to texture and appearance.Considering fruits and vegetables, strawberry sourdough breads had the highest score (38.30); followed by pomegranate (37.50), red beet (37.30) and black carrot (36.37). The exquisite flavour of strawberry provided to exhibit prominent taste and odour features in sourdough breads.

KARA HAVUÇ, KIRMIZI PANCAR, NAR VE ÇİLEĞİN EKŞİ MAYA EKMEKLERİNDE STARTER KÜLTÜR KAYNAĞI OLARAK DEĞERLENDİRİLMESİ

ÖZET

Bu çalışmadaki araştırmalar, tüketicilerin doğal, lezzetli ve sağlıklı gıda taleplerini karşılaşılayan ekşi maya ekmeği üretimi için çeşitli meyve ve sebzelerden ekşi maya geliştirilmesi üzerine gerçekleştirilmiştir. Ekşi maya hazırlanımında starter kültür kaynağı olarak kara havuç, kırmızı pancar, nar ve çilek tercih edilmiştir. Ekşi maya hazırlanımı geleneksel uygulama temel alınarak üç aşamalı olarak gerçekleştirilmiştir. Ekşi maya özelliklerinin belirlenmesinde pH, toplam titre edilebilir asitlik, laktik asit bakteri ve maya sayımı analizleri gerçekleştirilmiştir. Ekmek özelliklerinin değerlendirilmesinde ise pişme kaybı, spesifik hacim, renk özelliği, nem içeriği, su aktivitesi ve tekstürel analizleri gerçekleştirilmiştir. Ayrıca ekmek seçiminde tüketici tercihlerini belirlemek amacıyla duyusal analiz yapılmıştır.

Ekşi maya yapımında kullanılan önmaya örneklerinde pH ve titre edilebilir asitlik değerleri substrat kaynağına bağlı olarak değişkenlik göstermektedir. pH değeri nar için ortalama değer olan 3.58 ile kırmızı pancar için ortalama değer olan 5.08 arasında bulunmuştur. Titre edilebilir asitlik; kırmızı pancarda 4.53 ml ile en düşük, siyah havuçta 7.93 ml ile en yüksek bulunmuştur. Kullanılan önmayada olduğu gibi Hamur I için en düşük pH değeri nar örneğinde ölçümlenmiştir. Hamur I için titre edilebilir asitliğin 2.37 ml (kırmızı pancar) ve 4.97 ml (nar) arasında olduğu gözlemlenmiştir. Hamur I örnekleri arasında en yüksek maya sayımı ($6.0*10^5$ cfu/g) çilekde elde edilirken en yüksek laktik asit bakteri sayımı ($7.8*10^4$ cfu/g) ise kara havuçta elde edilmiştir. En düşük hücre sayımları ise nar örneklerinde elde edilmiştir. Tazeleme aşamaları sonrasında, Ekşi maya III örnekleri için ölçümlenen pH ve titre edilebilir asitlik değerleri besinsel bitki türlerine göre değişkenlik göstermemiştir ($p<0.05$). pH ve titre edilebilir asitlik değerleri sırasıyla 3.90 (kırmızı pancar) ile 4.08 (nar); 14.07 (çilek ve nar) ile 14.83 (kırmızı pancar) arasında bulunmuştur. Ekşi maya III örnekleri arasında en yüksek maya sayımı ($1.8*10^6$ cfu/g) çilekte elde edilirken en yüksek laktik asit sayımı ($1.4*10^8$ cfu/g) ise kara havuçta elde edilmiştir. En düşük hücre sayımları ise kara havuç örneklerinde elde edilmiştir.

Bu çalışmada, ekşi maya hazırlanımında starter kültür kaynağı olarak kullanılan substrat çeşidi ve ekşi maya kullanım düzeyi, pişme kaybında önemli değişikliklere yol açmamıştır ($p<0.05$). Buğday ekmeği (kontrol) ve ekşi maya ekmeklerinde, pişme sırasındaki nem kaybı % 14.00 (% 20 çilek ekşi mayası kullanımlı) ve % 16.07 (kontrol ve %10 çilek ekşi mayası kullanımlı) arasında bulunmuştur. %30 kara havuç ekşi mayası ile hazırlanan ekmek örneği en düşük spesifik hacim değerine (6.19 ml/g) sahipken ekşi maya kullanımı olmayan kontrol ekmeği en yüksek spesifik hacim değerine (7.58 ml/g) sahiptir. Ekşi maya kullanımı ekmek iç gözenek ve kabuk renk özelliklerinde farklılaşmayı sağlamaktadır. Ekmek örneklerinde ekşi maya kullanımı ile daha açık renkte iç gözenek ve kabuk yapısı elde edilmiştir. Ekmek iç gözeneğinde L* parametre (açıklık) değeri, % 30 nar ekşi mayası kullanımı

için ortalama olan 71.17 ile kontrol ekmeği için ortalama değer olan 76.20 arasında bulunmuştur. Nar ekşi mayası ile hazırlanan ekşi maya ekmekleri en düşük L* parametre değerine (73.08) sahiptir. Ekmek kabuğu için ise L* parametre değeri 44.41 (% 10 çilek ekşi mayası kullanımlı) ile 54.89 (% 20 çilek ekşi mayası kullanımlı) arasında bulunmuştur. Substrat kaynağı ekmek gözeneğinin a* (kırmızılık) parametre değerini önemli ölçüde etkilemez iken ekşi maya ilavesi ekmek kabuğunun a* parametre değerinde önemli ölçüde artışa yol açmıştır ($p<0.05$). En yüksek a* değeri (17.97) % 30 kara havuç orijinli ekşi maya kullanımıyla hazırlanan ekmek örneğinde gözlemlenmiştir. b* (sarılık) parametre değeri, kabukta 23.69 (% 10 çilek ekşi mayası kullanımlı) ile 30.91 (% 10 kırmızı pancar ekşi mayası kullanımlı ve %20 çilek ekşi mayası kullanımlı) arasında iken gözenekte 12.24 (% 20 nar ekşi mayası kullanımlı) ile 13.39 (% 30 kara havuç ekşi mayası kullanımlı) arasında değişmektedir.

Ekmek örneklerinin nem içeriği ilk günde % 33.61 (% 30 çilek ekşi mayası kullanımlı) ve % 34.69 (% 10 çilek ekşi mayası kullanımlı) arasında ölçümlenirken depolanma sonrasında nem düzeyinde azalma gözlenmiştir. Depolama süresi sonunda ekmek örneklerinde nem içeriği 32.48 % (% 30 kara havuç ekşi mayası kullanımlı) ile 34.19 % (% 10 kırmızı pancar ekşi mayası kullanımlı) arasında değişmektedir. Depolama süresince en çok nem kaybı meydana gelen havuç ekşi orijinli ekşi maya örnekleri, en düşük nem içeriğini sergilemiştir (% 33.07). Ekşi maya kullanımı açısından ise en çok nem içeriği değişimi (% 2.64) % 30 ekşi mayası ile hazırlanan ekmek örneklerinde gözlemlenmiştir. Kontrol ve ekşi hamur ekmeklerinin su aktivitesi tüm örnekler için 0.94 olarak bulunmuş ve depolama süresince değişiklik gözlenmemiştir. Ekşi maya hazırlanımında kullanılan meyve ve sebzeler ekmek pH değerinin farklılaşmasına yol açmaktadır. Ayrıca artan ekşi hamur kullanımı ile birlikte pH değerlerinde azalma söz konusudur ($p<0.05$). En düşük pH değeri % 30 kırmızı pancar ekşi mayası ile hazırlanan ekmekte saptanırken kontrol ekmeğinde bu değer 5.58 olarak bulunmuştur. Bunun yanı sıra depolama ile birlikte pH değerlerinde azalma gözlemlenmiştir. Depolama süresince substrat çeşidi ya da ekşi maya kullanım düzeyi pH değerinde önemli ölçüde değişime neden olmamıştır ($p<0.05$). Bununla beraber en yüksek değişim (%) 30 % kullanım ile hazırlanan ekşi maya ekmeklerinde gözlemlenmiştir.

Tekstürel analiz sonucunda ekmek sıkılığının ekşi maya kökeni ve kullanım düzeyine bağlı olarak önemli ölçüde değişkenlik gösterdiği belirlenmiştir ($p<0.05$). Ekşi maya çeşidinden bağımsız olarak, artan ekşi hamur kullanımı ile daha sıkı ekmek iç gözeneği elde edilmiştir. Ekmeklerin sıkılığı ilk günde kontrol ekmeği için 217.90 (g) ve % 30 kara havuç ekşi mayası ile hazırlanan ekşi maya ekmeği için 449.12 (g) arasında belirlenmiştir. Depolama süresince ekmek sıkılığı değişiminde substrat çeşidinin ya da kullanım düzeyinin önemli ölçüde etkisi bulunmamaktadır ($p<0.05$). Bununla birlikte kara havuç ekşi mayası kullanımı olan ekmeklerde daha yüksek düzeyde sertleşme oranı ve kontrol ekmeğine göre farklılaşma gözlemlenmiştir.

Ekmekler için yürütülen duyusal analiz sonucunda % 10 çilek ekşi maya kullanımı ile hazırlanan ekşi hamur ekmeği en yüksek skoru (41.10) sergilemiştir. Ekşi maya kullanım düzeyi açısından, % 10 ekşi maya kullanımı kontrol ekmeğine göre daha yüksek skor (40.10) elde edilmesini sağlamıştır. Ekşi maya kullanımı tat, koku ve

renk gibi çeşitli özellikler için puan artışını sağlarken kontrol ekmeği daha çok tekstür ve görüşünden dolayı tercih edilmiştir. Meyve ve sebzeler dikkate alınarak yapılan değerlendirmede çilek ekşi mayası kullanımlı ekmekler en yüksek skora sahip ike bu değeri sırasıyla nar (37.50), kırmızı pancar (37.30) ve kara havuç (36.37) ekşi mayası ekmekleri izlemektedir. Çileğin seçkin lezzet özelliği, ekşi maya ekmeğinde belirgin tat ve koku özellikleri sergilemesini sağlamıştır.

1. INTRODUCTION

Bread is one of the most widely consumed food products in the world and breadmaking technology is one of the oldest technologies dating back to the Neolithic era. The first bread was made around 10,000 years BC or over 12,000 years in the past, which may have been developed by deliberate experimentation with water and cereal flour (Scanlon and Zghal, 2001). Nutritionally, cereals are an important source of dietary proteins, carbohydrates, vitamins E and B - complex, iron, trace minerals, and fiber. Major cereal crops produced worldwide include wheat, rice, maize, rye and barley, their main application being in breadmanufacture (20 – 106 kg per capita annually). Only wheat and rye are suitable for the elaboration of leavened bread because of the presence of sufficientamounts (8 % – 14 %) of the complex protein gluten (De Valdez et al., 2010)

The ever growing consumer demand for high quality and healthy foods is a challenge for the baking industry to develop breads with improved sensory and nutritional properties (Mariotti et al., 2014) Technologies, imitating traditional processes, such as the use of sourdough, have been recently employed to satisfy the demand of consumers for natural or ‘‘clean” technologies (Plessas et al., 2007). Sourdough is an intermediate product for dough and bread preparation and contains metabolically active microorganisms. Due to their artisan and region-dependent handling, sourdoughs are an immense source of diverse lactic acid bacteria and yeast species and strains (De Vuyst and Neysens, 2005). Sourdough application is one of the key methods for enhancement of the sensory quality and shelf life of bread. Sourdough fermentation can also modulate the nutritional quality of bread in a number of ways, such as increasing the uptake of minerals, improving the content of bioactive compounds, and retarding starch digestibility (Flender et al., 2011).

However, bread production with the use of sourdough is a very sensitive method, which depends on various parameters that must be controlled. The most important are the pH value of fermentation, the fermentation temperature and the thorough selection of a starter culture with specific and desirable properties (Plessas et al.,

2011). Especially when sourdough is utilized, improvement of bread flavour requires a carefully controlled process to avoid e.g. excessive acidity (perceived as a sour or pungent flavour), and still enhance the positively charged flavour characteristics, such as roasted flavour of bread crust. Starter culture, ash content of flour, fermentation temperature and dough yield have been reported to influence bread flavor (Katina et al., 2006).

Fruits and vegetables are exclusive sources of water soluble vitamins C and B-complex, provitamin A, phytosterols, dietary fibres, minerals, and phytochemicals for the human diet. When favourable conditions of anaerobiosis, water activity, salt concentration and temperature occur, raw vegetables and fruits may be subjected to spontaneous lactic acid fermentation (Di Cagno et al., 2013). Vegetables have low sugar content but are rich in minerals and vitamins and have neutral pH and thus provide a natural medium for lactic acid fermentation. Lactic acid fermentation enhances the organoleptic and nutritional quality of the fermented fruits and vegetables and retains the nutrients and coloured pigments (Swain et al., 2014). Microorganism distribution found in wild pre-fermentations in sourdough may influence final properties of produced bread. Most sourdough cultures for bread production derive from sources associated with cereals, grain, or flour. However, other sources of yeast and lactic acid bacteria from other plant sources have not been well explored. The aim of this study was to develop the sourdough which derived from various fruit and vegetable sources and to evaluate these sourdoughs in bread making that meets the consumers demand for natural, palatable and nutritious food.

2. LITERATURE REVIEW

2.1 Bread

Bread products and their production techniques differ widely around the world depending on cultural habits. The objective of bread making is to convert cereal flours into attractive, palatable, and digestible food (Chavan and Chavan, 2011). Control of the production and distribution of bread has been used as a means of exercising political influence over the populace for at least the last two millennia (Mondel and Datta, 2008). The consumption of bread has declined last decades due to factors such as changing eating patterns and increasing number of choices of substitutes such as breakfast cereals and fast foods (Dewettincket et al., 2008). According to AIBI Market Report regarding to 2013, the bread production is relatively stable in a number of countries, but in most countries the bread production is slightly decreasing in Europe, while has been increased by about 30 % in Latin America and Africa.The bread consumption pattern differs widely in European countries. The highest consumption level is recorded in Turkey with an average of approximately 120 kg per head and the lowest consumption is in UK with approximately 32 kg. On average the European consumer eats 58 kg bread/head, which can be stated as stable in 2012 with a tendency of a slight decrease of bread consumption in Europe. Most countries with an average consumption of more than 50 kg bread/head can be considered as bread-eating countries, referring to bread as a key staple food (Selomulyo and Zhou, 2007; 'AIBI Bread Market Report', 2013).

Breadmaking technology has evolved continuously over the years as new materials, ingredients and equipment have been introduced to produce better quality bread while research has generated steady and impressive progress in breadmaking (Selomulyo and Zhou, 2007). The basic ingredients for breadmaking are flour, water, salt, fat and sugars. Water and flour are the most significant ingredients in a bread recipe, cause the texture and crumb structure mostly affected by this ingredients. Flour is always 100%, and the rest of the ingredients are a percent of that amount by

weight (Mondel and Datta, 2008). Leavening agents (microorganisms or chemical), improvers, a generic term for a wide range of additives used in bread formulations that include stabilizers, emulsifiers, oxidants, gums and supplementary enzymes (e.g. exogenous a-amylases, proteases, hydrolases for non-cellulosic polysaccharides, lipases, lipoxygenases) are also frequently added (Selomulyo and Zhou, 2007). Characteristics properties of fresh bread could be explained as an appealing golden brown crust, a pleasant roasted aroma, fine slicing, high volume, a soft and elastic crumb texture and a moist mouthfeel (Giannouet al., 2003; Selomulyo and Zhou, 2007).

2.1.1 Bread composition

Flour is the most important ingredient in bread making because it modulates the specific characteristics of bakery products. It consists of protein, starch and other carbohydrates, ash,fibers, lipids, water, and small amounts of vitamins, minerals, and enzymes (Chavan and Chavan, 2011). Many different flours are produced by the milling operation, one from each grinding step. Flour extraction rates are used to compare yields of flour and are expressed as a percentage of the production of milled products.Flour yields typically vary from 72% for white flour to 100% for whole-meal flour (Sievert, 2012). Wheat flour is the most common flour used to make many types of breads that differ in shape, structure, and sensory characteristics. Milling removes the fibrous layers of the grain; therefore, refined cereals do not have the same nutritional and health benefits as the grain or wholemeal (Rosell, 2011). Nutritional improvement of wheat flours could be achieved by increasing the extraction rate, since high amounts of nutritional products are recovered from the kernel (Rosell, 2012). Without the bran and germ, approximately 45% of the grain proteins are lost, along with 80% of fiber, 50-85% of vitamins, 20-80% of minerals, and up to 99.8% of phytochemicals. In addition, important losses of amino acids (35-55%) occur during refining (Rosell, 2011). Unlike from the other nutrients % composition of starch increases with the milling due to abundance in the endosperm (Rosell, 2012). Starch is the main component in wheat flour with the level of approximately 70-75 %. The starch granules are composed of two classes of starch polymers: amylose and amylopectin.Amylose makes up about 25-28% of the starch, the remaining 72-75% is amylopectin. The amylopectin molecules are responsible for the crystallinity in the granule. Within the granule both the amylose and

amylopectin appear to be ordered at right angles to the surface of the granule. This ordering gives the granule the property of birefringence. When starch is heated in excess water at about 55 °C the crystallites start to melt, the granule swell, and the birefringence is lost over about a 7 °C temperature range, leading to the irreversible destruction of the molecular order of the starch granule. This process is termed gelatinization. The amylose content and the amylopectin branch chain-length distributions predominantly affect the gelatinization and pasting/gelation properties of the starch (Sievert, 2012). Upon cooling and storage, the starch polysaccharides reassociate to a more ordered or crystalline state. This process is defined as retrogradation which refers to the starch ‘‘going back’’ to its initial crystallinity (Goesaert et al., 2005). When the starch granules are subjected to some shear during roller milling operation starch damage occurs. Such shear can disrupt the crystallites within the starch granule.As a result of crystallite disruption the starch granules will swell in water at room temperature and will be readily hydrolyzed by α-amylase (Sievert, 2012). A certain amount of damaged starch is desirable to increase water absorption and fermentable sugars. Conversely with increase in starch–gluten interactions, makes it difficult for the strengthened dough to be expanded by the gases produced during fermentation. The destruction of the damaged starch is accompanied with a decrease in dough strength and allows the dough to expand much easier during proofing and baking (Schiraldi and Fessas, 2012). In wheat flour, the non-starch polysaccharide distinguished into three classes of compounds: arabinoxylans, β-glucans with different linkage point, and a glycoprotein with the carbohydrate moiety being an arabinogalactan. All these compounds are dietary fiber constituents. The water-extractable arabinoxylans impart viscosity to dough, and their water-unextractable counterpart have a high water holding capacity. The protein content of wheat varies over a rather wide range (6 to 18 %) depending on both environmental and genetic factors. In wheat flour about 15% of the protein is soluble in water or aqueous salt solutions (albumins or globulins) and the remaining 85% is storage protein (prolamins and glutelins). The storage proteins in wheat are forming the gluten (Sievert, 2012). The quantity and quality of gluten proteins largely determine rheological and viscoelastic properties of the dough, mixing requirements and sensitivity to overmixing and contribution to the gas retention properties,which in turn determine loaf volume and crumb structure of the resulting bread (Goesaert et al., 2005).

Water is essential for the formation of dough and is responsible for its fluidity. Once wheat flour is mixed with water to prepare a dough, the mixture undergoes substantial macroscopic changes. Along with proper kneading an apparently homogeneous sticky wet paste results in a rubber-like consistency (Schiraldi and Fessas, 2012; Chavan and Chavan, 2011). In particular, the consistency depends clearly on the amount of water used in making it. The water added to the flour fulfills four functions:it dissolves soluble molecules, activates enzymes, brings about the formation of new bonds between the macromolecules in the flour, and alters the rheological properties of the dough. Deficiency of water prevents to hydrating gluten and developing elasticity of the dough. Conversely, an excessive level of free water in the dough results in the domination of the viscous component of dough, with a decreased resistance to extension, increased extensibility and the development of sticky dough. The potential role of an aqueous liquid phase in doughs is to stabilize the surface active materials at the gas–liquid interface, to maintain the integrity of gas bubbles and to promote gas retention (Mastromatteo et al., 2013). Moreover,it is important for starch gelatinization during baking and contributes to oven spring through vaporization (Chavan and Chavan, 2011; Giannou et al., 2003; Chavan and Jana 2008).

Saccharomyces cerevisiae is the most common yeast used in bread making. Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose, and maltose) under anaerobic conditions producing carbon dioxide (CO_2) and ethanol (Chavan and Chavan, 2011; Giannou et al., 2003; Chavan and Jana 2008). The gases that result from that conversion acts as a leavening agent and enhances dough volume. In addition to its gas production, the yeast has a marked effect on the rheological properties of dough. Furthermore, yeast fermentation affects texture and sensorial characteristics of finished baked products (Sievert, 2012).

Salt is generally used at levels of about 1–2 % based on flour weight in breadmaking technology. Salt plays an important technological role in the bread making process as influences gluten behaviour, improving the strength of the dough, decreases yeast activity in the dough, thus retarding gas production, and enhances bread flavor and taste (Sievert, 2012; Lynch et al., 2009). Strengthening effect of salt on gluten ensures increasing dough development time, its resistance extensibility and elasticity (Noort et al., 2012).

Sugar is added to the bread formula as a source of fermentable carbohydrate for the yeast during early stages of fermentation. Later more sugars are released for gas production by the action of enzymes in the flour.In the ensuing baking phase, the sugars can undergo caramelisation, and/or the reducing sugars can react with the free amino acid groups of proteins in the Maillard reaction, a process strongly influenced by pH, water activity (a_w), type of reducing sugars and leavening agents. A wide range of different flavour compounds which are comprised during baking, give bread its appealing smell and taste (Hidalgo and Brandolini, 2011). Sugars also act as antiplasticizers retarding pasting of native starch or function as antistaling ingredients inhibiting starch recrystallization (Chavan and Chavan, 2011; Giannou et al., 2003; Chavan and Jana 2008).

Lipids are important components in bread making as they provide a variety of beneficial properties during processing and storage which reflects their overall diversity. In bread, lipids originate from multiple ingredients. The three main sources of lipids in a typical bread formula are wheat flour, shortening and surfactants (Pareyt et al., 2011). Lipids are an optional ingredient in bread but can improve dough handling and crumb appearance and contribute to product flavor. Lipids also improve the keeping quality, softness, and moistness and contribute to bread texture (Chavan and Chavan, 2011; Giannou et al., 2003; Chavan and Jana 2008). The binding of free lipids in wheat flour with gluten proteins may provide them with the ability to align at the interface of gas cells during the initial phases of dough mixing and increase gas cell stability throughout the bread making process. Bread formulations generally contain low levels (<5 %) of added shortening and oil. However, these ingredients affect both processing and sensory quality. Indeed, shortening/oil plasticizes and lubricates dough, increases dough rise, oven spring and loaf volume (Pareyt et al., 2011). In bread making, surfactants are generally divided into dough strengtheners that mainly interact with gluten, and crumb softeners or anti-firming agents that can complex gelatinised starch in bread making. surfactants are commonly used at a level of 0.3 %-1.0 % (Pareyt et al., 2011; Delcour and Hoseney, 2010). MAG and DAG, typical examples of crumb softeners and other well-known surfactants as dough strengtheners are diacetyl tartaric acid esters of mono- and diacylglycerols (DATEM), sodium/calcium stearoyl lactylate (SSL/CSL)

and ethoxylated monoglycerides (EMG) (Sievert, 2012; Pareyt et al., 2011; Belitz et al., 2009).

2.1.2 Bread-making procedure

Notwithstanding some differences among dough-making techniques, the standard bread-making flowsheet consists of several major steps. Every step targets specific objectives and induces several changes in the product. The bread-making process can be performed by the straight-dough method or the sponge-and-dough procedure. In the straight-dough method all the ingredients are combined and mixed together simultaneously to form a dough .The dough may be fermented in bulk before dividing, or go directly to dividing after a short resting periodour is the most important (Hidalgo and Brandolini, 2014). Fermentation could be carried out at least in two stages. The primary objective of first leavening is to induce changes in the rheological properties of the dough rather than volume increase. The first leavening provides a greater workability to the dough, which achieves the capacity to maintain shape during the second leavening (or proofing) where the volume increase occurs (Pagani et al., 2013). With the sponge-and-dough method, the ingredients are added at different times, during the refreshments of the dough. The sponge is produced first by mixing yeast-dispersed water with 60–70 % of the flour. After a leavening phase (from 3 to 4 h up to 10 h or more), depending on bread type, when the batter becomes spongy and foamy, other ingredients are added. The final dough is is divided, molded, leavened before baking (Hidalgo and Brandolini, 2014). The numerous other bread-making systems could be viewed as either modifications of one of these two procedures or as new procedures, depending upon one's point of view. The Chorleywood is a no-time dough process that requires considerable high-speed mechanical mixing in order to develop the dough structure within a short time. The Do-Maker process and the Am-flow process are based on the possibility of eliminating the long leavening times by using yeast cultures or pre-ferments propagated separately without or with small quantities of flour. The technological process of bread may be summarized in a sequence of operations that require long periods of time and which have the primary objective of aerating the dough and making it porous (Table 2.1.) (Pagani et al., 2013).

Table 2.1: Bread-making process: aim and modifications associated with the main operations (Pagani et al., 2013).

Step/Phase/Operation	Aim	Modifications
Mixing	Homogeneous distribution of ingredients (including minor components)	Hydration and solubilization of the water component
	Formation of uniform and coherent structure	Formation of soluble gluten
Leavening/proofing	Increase in volume of dough	Formation of gas (CO_2)
	Development of typical Flavour	Production of fermentation metabolites important for developing flavour and able to change the macromolecules solubility
Shaping	Division of dough into final pieces and giving shape to dough	Subdivision of gas bubbles and division of dough into final pieces inclusion of new air
Baking	Giving the product its typical aspect	Increase in volume due to evaporation of gases: 20–30% of the volume is obtained during baking (oven-spring)
		Formation of crust and crumb
	Decrease of water content	Protein denaturation
	Stabilization of leavened and shaped dough	Starch gelatinization
	Making product appetizing and digestible	Development of flavour
	Completing leavening of the dough	Evaporation of water and ethanol
Cooling	Product packaging	Change of solubility of sugars Hardening of fats

2.1.3 Quality assesment of bread

Breads are characterised by having a dry, thin layer named as crust,enclosing the soft, cellular structure of the crumb. Bread crust has considerably lower moisture content than that of the crumb; typically crust moisture contents are in the range 12-17 %, while for the crumb they will range from 35 to 42 %, depending on bread type.

The low moisture crust has a hard and brittle eating character which may be accentuated by the thickness of the crust. A fundamental requirement of bread crumb is that it should be relatively soft combined with a degree of resilience or springiness and a degree of chewiness (Cauvain and Young, 2011).

Bread quality is a very subjective term that greatly depends on individual consumer perception,which in turn is affected by social, demographic, and environmental factors.The global concept of bread quality could be integrated by instrumental attributes, those that can be objectively measured; sensory sensations including descriptive attributes related to consumer quality perceptions and nutritional aspects related to healthiness and functionality of the bread products (Rosell, 2011).

A wide range instrumental quality attributes have been defined and quantified to evaluate bread. Volume (rapeseed displacement), weight, specific volume, moisture content, water activity, color of crust and crumb, crust crispiness, crumb hardness, image analysis of the cell distribution within the loaf slice, and volatile composition are the instrumental methods for assessing quality. Among the different physical properties which can be considered as characterizing bread, morphology of is crucial not only for the mechanical properties of the crumb but also for moisture transfer within the product (Besbes et al., 2014).

The perceived quality of bread is a complex process associated with sensory sensations derived from product visual appearance, taste, odor, and tactile and oral texture (Rosell, 2011; Rosell and Collar, 2008; Cauvain, 2003). Generally, perceived quality of bread is intimately linked to freshness perception. In recent years, there has been a global trend towards the consume bakery products regarding to their nutritional and functional aspects with hopes of reaping additional health benefits that may reduce certain disease risks or promote optimal wellness (Bigliardi and Galati, 2013; Heenan et al., 2009). Hence, consumers are becoming aware while assessing to label information. Proximate composition(carbonhydrates, proteins, fat, dietary fibers), energy value, glycemic index, load index, percent daily value and other health claims (reduced salt, reduced energy, high fiber content,etc.) attributes that are significantly evaluated (Rosell, 2011; Lambert et al., 2009).

2.1.4 Shelf life assesment of bread

Perceived freshness of bread, involves interactions of sensory sensations attributed to product appearance, odour, taste, flavour and oral texture, is considered as one of the key determinates of acceptance and choice of bread (Heenan et al., 2009). Fresh bread products, unfortunately, have a relatively short shelf life since during their storage, a number of physical and chemical alterations occur known as staling. As a result of these changes, bread quality deteriorates gradually as it losses of freshness and crispness is paralleled by an increase in crumb hardness and rigidity. The pleasant aroma vanishes and flavor assumes a stale feeling (Chavan and Jana 2008), leading to loss of consumer acceptance (Chavan and Chavan, 2011; Heenan et al., 2009).

Bread staling involves changes in the properties of the crust and the crumb and in which water plays a significant role, in terms of moisture migration and starch retrogradation. Crust staling is associated with the migration of moisture from crumb to crust resulting in a soft and leathery texture, while crumb staling arises from both moisture migration and physicochemical changes in bread starch (Besbes et al., 2014; Bhatt and Nagaraju, 2009). Retrogradation is both time- and temperature-dependent, with the maximum staling rate for bread occurring around 4 °C. Water activity is a factor controlling moisture loss from breads, the lower the aw the lower the rate at which the product will lose moisture (Cauvain and Young, 2011).

Physical, chemical and textural quality characteristics and sensory attributes have been considered for evaluating bread staling. Moisture (crust and crumb), springiness and mouthful are the most sensitive quality attributes that deteriorate significantly after a few days of storage (Besbes et al., 2014). The firmness of the final product is most often measured because of the strong correlation between crumb firmness and consumer perception of bread freshness (Carr et al., 2006).

Many analytical methods such as textural, thermal, thermomechanical, microscopic and molecular mobility analysis, have been used to investigate bread staling and the changes that accompany it. Characteristics of bread crumb that have been used as bases to determine the degree of staling are changes in taste and aroma, increased hardness, increased opacity, increased crumbliness, increased starch crystallinity and

decreased soluble strach content. Of the thermoanalytical methods, differential scanning calorimetry (DSC) and differential thermal analysis (DTA) have proven to be the most useful in providing basic information on starch retrogradation (Cauvain and Young, 2011; Gray and Bemiller, 2003). For textural assessment of bread staling, numerous instruments that measure compressibility such as Texture Analyzer, Compressimeter, Precision Penetrometer have been developed (Cauvain and Young, 2011). Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Near-infrared reflectance spectroscopy (NIRS), X-ray crystallography and Electrical impedance spectroscopy (EIS) instrumental techniques to investigate molecular mobility in model systems and bread, in an attempt to relate how water, starch and gluten are interacting at a molecular level and their relation to product properties (Curti et al., 2014; Fadda et al., 2014; Gray and Bemiller, 2003). As perceived freshness of bread is associated with moistness and firmness, quantifying moisture content and water activity is also required for assessment of shelf life. Also water activity could be considered as a parameter for identifiying and understanding potential microbial issues. In general term of microbial shelf life, although the a_w of bread crumb is high enough to support bacterial growth, this is not usually a problem and with bread products the main spoilage mechanisms involve mould growth (Cauvain and Young, 2011).

2.1.5 Enhancing shelf life of bread

Extending the shelf life without radically changing the eating qualities of the product can make all the difference to the size of a production batch, and affect economies of scale. There are several options for extending shelf life of bread. Controlling moisture and its migration, differentiating in formulation, adjusting storage temperature and packaging are commonly performed techniques for improving shelf life of bread (Cauvain and Young, 2010; Le-Bail et al., 2009). Moisture migration occurs by diffusion of water in the solid matrix and water vapor through the void spaces. In the macromolecular scale, water diffuses from the crumb to the crust and vaporizes to the ambiance due to a difference in partial vapor pressure. Control of the baking time and of the kinetics of baking, , hereof moisture migration , is an important parameter to consider in the staling (Besbes et al., 2013; Le-Bail et al., 2009). Changing the degree of starch gelatinization of the crust, by varying the degree of baking, may change permeability and diffusivity properties. Baking at

higher temperature condition resulting in intact and partially gelatinized starch granules in crust and that limit the diffusion of water vapor to ambiance. Similarly, the higher value of water diffusivity in crumb and crust could be obtained when baking at higher temperature due to ascending in porosity (Besbes et al., 2014; Besbes et al., 2013; Le-Bail et al., 2009). Degree of steaming may change the microstructure and water vapor permeability (WVP) of crust Increasing the amount of steam applied during baking resulted in lower WVP (Altamirano-Fortoul et al., 2011).

Since the structural architecture and the mechanical strength of bread is arised from mainly starch, protein and water, they are assumed to play an significant role in the change in cell wall properties during bread storage (Lagrain et al., 2012). Alteration in cell wall properties could be achieved by using hydrocolloids (Zannini et al., 2014), enzymes (Altamirano-Fortoul et al., 2014), emulsifiers (Nunes et al., 2009) and redox agents (Lagrain et al., 2012). These constituents influence bread shelf life quality through modification in water retention and structural network, thereof cell size and distribution (Lagrain et al., 2012). Adjusting the formula corresponding with the mobility of the water in the system and its availability is also preferred option (Ronda et al., 2014). Limitation to water mobility is favorable for bread stealing, nevertheless, retaining the appropriate moistness and mouthfeel of bread during storage is also crucial in perceived freshness (Bhise and Kaur; 2014; Cauvain and Young, 2011).

2.2 Sourdough

Recent trends in the bakery industry have included the desire for high-quality foods, which are minimally processed and do not contain chemical preservatives, thus increasing the interest toward natural preservation systems (Ryan, 2008). Among the natural preservation systems, the use of sourdough has a long tradition and still plays a significant role in nutritional properties and organoleptic properties of bread, besides extending shelf life (Gänzle, 2014).

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) and yeasts, either spontaneous or initiated through the addition of a sourdough starter culture, whether or not involving backslopping (Vuyst et al., 2009).

Sourdough microflora usually originates from flour, the environment, or something being used as inoculum (e.g., fruits and yogurt) (Catzeddu, 2011).

2.2.1 Properties of sourdough

A sourdough contains a variable number of lactic acid bacteria and yeasts, ranging from10^7 to 10^9 cfu/g and 10^5 to 10^7 cfu/g, respectively, with a ratio of about 100:1[42]. Although a large variety of lactic acid bacteria has been isolated from sourdoughs, only a few *Lactobacillus* species are highly adapted to the sourdough environment and usually dominate industrial and artisan fermentations. Especially, the species *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum*, *Lactobacillus brevis, Lactobacillus pontis, Lactobacillus rossiae*, are recognized as key organisms in sourdoughs (Gänzle, 2008). Obligately heterofermentative lactobacilli are characteristic for sourdough fermentation processes, because of their highly adapted carbohydrate metabolism, dedicated amino acid assimilation and stress responses (Vuyst et al., 2014).

Several species of yeast have been isolated from sourdough, but only a few of them are considered fundamental in the fermentation process (Catzeddu, 2011). Given the peculiarities of the sourdough ecosystem, only ascomyceteous yeasts are found, because of their fermentative ability in contrast to basidiomycetous yeasts and dimorphic ascomycetes (Vuyst et al., 2014; Huys et al., 2013). The most representative species belong to the genera *Saccharomyces* and *Candida*, with the species being *Saccharomyces exiguus, Candida humilis*, and *Candida krusei* (Catzeddu, 2011; Corsetti and Settanni, 2007). Endogenous factors in cereal products (carbohydrates, nitrogen sources, minerals, lipids, and free fatty acids, and enzyme activities) and process parameters (temperature, dough yield, oxygen, fermentation time, and number of sourdough propagation steps) markedly influence microflora composition (e.g., microbial species and LAB : yeast ratio) and hence the types of metabolites produced in the dough (Chavan and Chavan, 2011). The quality of sourdough is obviously affected by the presence of one species or another throughout the fermentation process.

Metabolic properties (production of lactic and acetic acids, synthesis of aroma substances, proteolytic and amylolytic activities) of sourdough are important for their selection as bread substance. The metabolic activites and stability of sourdough

depend on either environmental microbiata and its metabolic activities or specific technical process parameters. The sourdough fermentation can be performed as a firm dough or as a liquid suspension of flour in water (Decock and Cappelle, 2005). The ratio between water and flour in the dough is indicated as dough yield and it deals with the dough consistency. Considering that different flours have different capabilities to absorb water, doughs of various consistency are obtained having the same dough yield (Corsetti, 2013). In most of cases, traditional sourdoughs are firm doughs, characterized by a value of dough yield of ca. 150–160 (Minervini, 2014). A liquid sourdough shows values close to 200. The acidification rate and fermentation quotientis, indicates the molar ratio between lactic and acetic acids, influenced by the dough yield of a sourdough (Corsetti, 2013). The firmer the sourdough (lower dough yield value) amplifies the effect of the flour by providing the growing microbiota with a higher amount of carbohydrates available for fermentation and allowing a higher buffering capacity, thereby slowing down the rate of acidification (Vuyst et al., 2014). Also the firmer the sourdough (lower dough yield value), the more acetic acid is produced and the less lactic acid (Decock and Cappelle, 2005). Dough yield and temperature of fermentation, along with starter culture and ash content of flour, markedly influence the aroma of the sourdough and, especially, the molar ratio between lactic and acetic acids (Katina, 2006). More acetic acid is present in firm dough fermented at 25–30°C, while more lactic acid is found in soft dough fermented at 35–37°C (Decock and Cappelle, 2005; Brandt et al., 2004). Optimum temperatures for the growth of sourdough LAB 30 to 40° C depending on strain and for yeasts 25 to 27° C (Minervini, 2014).

The acidity of sourdough is measured as a function of both its pH and total titratable acidity values. This is in view of the fact that there is no direct correlation between the pH and total titratable acid value because of the presence of buffering substances in the dough system. Traditional sourdoughs have a pH range (3.5–4.3) that usually meets the growth requirements of the dominant sourdough microorganisms (Minervini, 2014; De Vuyst and Neysens, 2005). Overall, low values of initial pH favour yeasts over lactic acid bacteria (Minervini, 2014). Under sub-acid conditions (initial pH ranging from 5.0 to 5.5), *L. sanfranciscensis* showed a higher maximum growth rate whereas the growth of the *C. humilis* was not significantly affected in the pH range from 3.5 to 5.5. The opposite occurred at an initial pH value of 5.0 or lower, with lactobacilli being completely inhibited at pH 4 (Minervini, 2014; Brandt

et al., 2004). The ash content of flour influences the buffering capacity of the sourdough system that makes possible to reach a higher total titratable activity (Chavan and Chavan, 2011). Higher ash content of flour increased firmness in sourdough breads fermented with *Lactobacillus brevis*, *S. cerevisiae*, or a combination starter (Fadda et al., 2014). Total titratable acidity is a feature related to amount of organic acids produced during the sourdough fermentation (Decock and Cappelle, 2005). The lactic acid concentration produced in the sourdough is determined by its buffering capacity (Neysens and Vuyst, 2005).

2.2.2 Classification of sourdough

Different types of sourdough exist, on the basis of the processing conditions and/or technology used for production, with a specific microbiota occurring in each type listed in Tablo 2.2 (Vuyst and Neysens, 2005; Huys et al., 2013).

Table 2.2: Classification of sourdoughs and the corresponding characteristic microflora (Vuyst and Neysens, 2005).

Type I	Type II	Type III
Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative
L. sanfranciscensis	*L. brevis*	*L. brevis*
L. brevis	*L. fermentum*	
L. buchneri	*L. frumenti*	
L. fermentum	*L. pontis*	
L. fructivorans	*L. panis*	
L. pontis	*L. reuteri*	
L. reuteri	*L. sanfranciscensis*	
W. cibaria	*W. confuse*	
Facultative heterofermentative		Facultative heterofermentative
L. alimentarius		*L. plantarum*
L. casei		*P. pentosaceus*
L. paralimentarius		
L. plantarum		
Obligate homofermentative	Obligate homofermentative	
L. acidophilus	*L. acidophilus*	
L. delbrueckii	*L. delbrueckii*	
L. farciminis	*L. amylovorus*	
L. mindensis	*L. farciminis*	
L. amylovorus	*L. johnsonii*	
Yeasts		
Candida humilis		
Candida krusei		

Distinction can be made between type 0, I, II, and III sourdoughs and each type of sourdough is characterized by a specific sourdough LAB microflora. Type 0 sourdoughs are pre-doughs, in which LAB contaminating the faster growing baker's yeast development to flavor formation (Vuyst et al., 2014; Huys et al., 2013). Type I or traditional sourdoughs are usually firm doughs with a low dough yield that are characterized by continuous, daily refreshments at ambient temperature (<30 C) to keep the microorganisms in an active state, as indicated by a high metabolic activity, above all with regard to leavening, i.e. gas production (Vuyst et al., 2014; Vuyst and Neysens, 2005). Mother doughs, consist of flour, water and possibly other ingredients and fermented spontaneously, are used as an inoculum for subsequent doughs by addition of the desired amount of dough to a fresh flour-water batch according to defined cycles of preparation (Huys et al., 2013). When applied for a defined interval of time such a process provides a sourdough with constant and repeatable leavening and acidifying performances reliant on the growth of lactic acid bacteria and yeasts that are well adapted to the environment (Corsetti, 2013; De Vuyst and Vancanneyt, 2007). Due to the selective pressures that results from the environmental conditions of sourdough preparation, *L. sanfranciscensis* and *C. humilis/K. exigua* as prevalent LAB and yeast species, respectively in type I sourdough fermentations (Huys et al., 2013; Chavan and Chavan, 2011).

Type II sourdoughs are industrial semi-liquid sourdoughs that are usually performed as one-step propagation processes of long duration (typically 2–5 days) and with high water content at temperatures above 30 °C to enhance acidification (Vuyst et al., 2014). These large-scale sourdough productions result in semi fluid preparations, which are used as flavor ingredients or dough acidifiers ,exhibit high acid content at pH of <3.5 (Huys et al., 2013). Owing to higher dough yield, this type of sourdough is pumpable in an industrial bakery (Chavan and Chavan, 2011). The completely different process parameters of type II sourdough fermentations result in a different microbial ecosystem with respect to composition and population dynamics (Vuyst and Neysens, 2005). *Lb. amylovorus*, *Lb. fermentum*, *Lb. pontis* and *Lb. reuteri* are commonly found in type II sourdoughs (Huys et al., 2013). Most of these LAB strains have been selected mainly based on their capacity to rapidly acidify the flour-water mixture and/or their ability to produce specific flavors (Vuyst et al., 2014).

Type III sourdoughs can be either liquid or dried dough in powder form, which are initiated by defined starter cultures. They are used as acidifier supplements and

aroma carriers during breadmaking. They mostly contain LAB that are resistant to drying and are able to survive in that form, e.g.heterofermentative *L. brevis*, and facultative heterofermentative *P. pentosaceus* and *L. plantarum* strains (Vuyst and Neysens, 2005). Different drying techniques are used as well as liquid pasteurization, to achieve microbial stability. Spray-drying and drum-drying are the most commonly used drying techniques in type III sourdough production, contribute malted, caramelized flavor and toasted aroma to the end-sourdough. Keeping the sourdough in liquid form and stabilizing it by pasteurization or cooling, ensures volatile flavor compound existence and achieves more complete flavor properties (Chavan and Chavan, 2011). In contrast to type I preparations, doughs of types II and III require the addition of baker's yeast (S. cerevisiae) for leavening (Huys et al., 2013). During continuous propagation (type I) the temperature is lower and the rate of re-inoculation often exceeds 30%, resulting in a lower start pH that promotes yeast growth (Vuyst and Neysens, 2005).

2.2.3 Beneficial applications of sourdough

The use of sourdough in bread making influences all aspects of bread quality. Sourdough fermentation enhances sensory characteristics, such as aroma profile and texture (Guerzoni et al., 2007), retards bread staling during storage (Arendt et al., 2007) and protects bread from bacterial and fungal spoilage (Gerez, et al., 2009; Ryan, Dal Bello and Arendt, 2008) dependent on bioconversion of flour components at the dough stage (Choi et al., 2012; Gänzle, 2014).

The major contribution of sourdough to the quality of bread is accomplished through acidification and metabolite production by LAB (Choi et al., 2012). The acidification inhibits endogenous amylase, preventing excessive degradation of the starch during baking (De Vuyst and Vancanneyt, 2007). Moreover, acidification increases the gas-holding and water-binding abilities of pentosan and is a prerequisite for an acceptable bread volume (Choi et al., 2012; Loponen et al., 2009).

Lactic acid bacteria (LAB) produce a number of metabolites which have been shown to have a positive effect on the texture and staling of bread, e.g. organic acids, exopolysaccharides (EPS) and/or enzymes. EPS, produced by several food grade bacteria and also known to act as hydrocolloids, can improve the viscoelastic properties of dough, increase loaf volume, reduce crumb hardness and prolong shelf life (Torrieri, 2014; Rühmkorf et al., 2012; Poutanen et al., 2009; Tieking and

Gänzle, 2005). However, *in situ* production of exopolysaccharides during sourdough fermentation is challenged by simultaneous acidification due to metabolic activities of the bacteria, which may significantly diminish the positive technological impact of EPS (Torrieri, 2014; Katina et al., 2009). In particular, lactate and acetate have previously been identified to significantly affect dough rheology, bread volume and crumb hardness, and may counterbalance the positive effect of EPS (Torrieri, 2014; Kaditzky and Vogel, 2008).

Sourdough fermentation can modify the nutritional and healthiness of cereals in a number of ways, including improvement of texture and palatability of whole grain products, enrichment in fibre or reduction of gluten, stabilisation or increase of various bioactive compounds, improvement of mineral bioavailability, etc (Plessas et al., 2008; Katina et al., 2005). Organic acids produced in sourdough are responsible for a reduction of the glycemic index (Di Cagno et al., 2004). This seems to be associated with a delay in gastric empting in the case of acetic acid, whereas lactic acid induces interactions between starch and gluten during dough baking and reduces starch availability (Catzeddu, 2011; Björck and Elmståhl, 2003). Anti-nutritional effect of phytic acid is well documented as inducing a reduction of minerals bioavailability, particularly bivalent cations, such as iron, calcium, magnesium and zinc as well as the decrease of minerals absorption, including that of iron, in human and animals .Sourdough LAB could ensure the degradation of phytates by their hydrolysis and by the same way decrease the risk of reduced iron absorption and improve iron status (Chaoui, 2006).

Higher acidity due to organic acid formation seems to play the pivotal role in the enhancement of microbial shelf life of sourdough breads along with other antimicrobial substances produced by the sourdough microbiota such as, bacteriocins, ethanol, CO_2, hydrogen peroxide, peptides, diacetyl, phenyl lactic acid, reuterin, and fungicins (Plessas et al., 2012; Schnürer and Magnusson 2005; Katina et al., 2002; Messens and De Vuyst 2002). Among the organic acids, acetic and propionic acid produced by heterofermentative LAB are more effective than lactic acid (Schnürer and Magnusson, 2005).

In general, heterofermentative metabolism, by means of the fermentation quotient (i.e. the molar ratio between lactic acid and acetic acid), mainly influences the aroma profile. Acetic acid not only gives a strong aroma profile, but also increases the effectiveness of aroma substances present in bread (Şimşek et al., 2006; Göçmen,

2001). An enhanced proteolysis brought about by sourdough fermentation may account for the characteristic sensory properties of sourdough breads.

The transformation of amino acids or peptides to aroma compounds contributes substantially to food flavour. In particular, the conversion of glutamate by lactic acid bacteria enables the targeted optimization of food flavour (Torrieri, 2014; Gänzle, 2009; Plessas et al., 2011).

2.2.4 Sourdough preparation with non-cereals

The array of species of yeast and lactic acid bacteria that are available to serve as sourdough starter is vast. The initial source of the starter culture can have a large impact on how the organisms in the starter function in a dough system. Most sourdough cultures for bread production derive from sources associated with cereals (Hou and Hsu, 2013). Sourdough fermentation may have the potential to exploit the nutritional, functional and sensory features of legumes and derived flours. The microbiota of legume flours was poorly represented by lactic acid bacteria, whereas the number of moulds and yeasts was relatively higher (Curiel et al., 2015)

Yoghurt or Kefir could be used as inoculum in sourdough bread. Breads produced with kefir retained more moisture, had a firmer texture, lower acidity (pH 4.9–5.5) and retained their freshness for longer compared to baker's yeast bread (Plessas,2012; Plessas et al., 2005).

Lactic acid bacteria are a small part (2.0-4.0 log cfu g^{-1}) of the autochthonous microbiota of raw vegetables and fruits summarized in Tablo 2.3. *Weissella cibaria/Weissella confusa* and, especially, *Lactobacillus plantarum* were the most frequent species (Di Cagno et al., 2013).

Table 2.3: Species of lactic acid bacteria, which were isolated from raw or spontaneously fermented vegetables and fruits (Di Cagno et al., 2013).

Lactic acid bacteria species	Source
Lactobacillus plantarum	Tomatoes, marrows, carrots, cucumbers, eggplants, red-beets, capers, pineapple, plums, kiwi, papaya, fennels, cherries, cabbages
Lactobacillus rossiae	Pineapple
Lactobacillus fermentum	French beans, red beets, capers, eggplants, melon pod

Table 2.3 (contuining): Species of lactic acid bacteria, which were isolated from raw or spontaneously fermented vegetables and fruits (Di Cagno et al., 2013).

Lactobacillus curvatus	Peppers
Lactobacillus brevis	Tomatoes, capers, eggplants, cabbages, cucumbers, melon pod
Lactobacillus paraplantarum	Cabbages, capers
Leuconostoc mesenteroides subsp. mesenteroides	White cabbages, carrots, peppers, cucumbers, eggplants, lettuce, cherries
Weissella sol	Carrots
Weissella confusa, Weissella cibaria	Peppers, tomatoes, blackberries, papaya
Enterococcus faecalis *Enterococcus faecium*	French beans, tomatoes, capers, melon pod
Pediococcus pentosaceus	French beans, tomatoes, cucumbers, capers, cherries, cabbages

The intrinsic characteristics (content of sugar molecules, pH) of plants influences metabolism of lactic acid bacteria and yeast. Yeast and lactic acid bacteria of different strains from those associated with cereals are present on apples, grapes, peaches and other high-sugar fruits. Sourdough bread produced with cultures derived from fruit sources is less sour than that from cereal flour-derived starters (Di Cagno et al., 2013; Hou and Hsu, 2013). The apple-derived culture significantly out-performed the wheat-derived cultures in CO_2 gas production at every stage of culture elaboration and fermentation. Different sources of wild yeast and *Lactobacilli* could be used to manipulate the rate, timing and total gas production in sourdough bread-making to modify production parameters and the bread's end-use quality (Hou and Hsu, 2013).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

For total titratable acidity analysis sodium hydroxide (NaOH) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Peptone water buffered, dichloran-18-glycerol agar (DG18) and MRS broth were purchased from Merck KGaA (Darmstadt, Germany) for cell counts.

3.1.2 Equipments

In this study, magnetic stirrer (Velp Scientifica, Milan, Italy), pH meter (Testo 230, Hampshire, UK), moisture analyzer (Sartorius MA 150, Germany), tristimulus colorimeter (Minolta Chroma Meter model CR 400, Milan, Italy), water activity meter (Novasina LabMaster-aw , Pfäffikon, Switzerland) and Texture Analyzer TA-XT Plus (Stable Micro Systems, Godalming, UK) were used.

3.1.3 Raw materials

For sourdough production, two flour types were used: wheat flour (0.59 % ash, 11.78 % protein and 13.9 % moisture) and rye flour (1.29 % ash, 13.84 % protein and 10.24 % moisture). Wheat flour and rye flour were obtained from Marmara Un, Tekirdağ and Ireks, Tekirdağ, respectively. Encapsulated sorbic acid 85 % and dough improver were supplied from Balchem Encapsulates, New Hampton, NY and Zeelandia Doruk, Tekirdağ. Strawberry, pomegranate, black carrot and red beet were purchased from local market. Water used for all analysis was distilled and purified with the ELGA Purelab Option DV 35 (Elga LabWater, Lane End, UK).

3.2 Methods

3.2.1 Sourdough preparation

The sourdough was developed using strawberry, pomegranate, black carrot and red beet samples as preferment substrate. The samples were crushed into small pieces and mixed with water in ratio 1:1 forming initial preferment (IP) and spontaneously fermented for 3 days at 28 °C for preparing preferment (P) samples. For the preparation of the sourdough, a three-stage technique derived from a traditional procedure was applied with using preferment samples. Dough I (DI) was prepared by mixing the 200 g preferment with 400 g wheat and rye flour mixture with the ratio (10:3) to achieve dough yield of 150. After 24 h incubation at 28 °C, Sourdough I (SDI) was formed. Then, 300 g of SD I was mixed with 600 g of wheat-rye flour (ratio in 10:3) and 300 ml of tap water to form Dough II (DII) with dough yield of 150. This refreshment step was performed once more and end of the second refreshment step Sourdough III (SDIII) was formed.

3.2.2 Determination of cell counts, pH and total titratable acidity (TTA) of dough and sourdough

During fermentation pH and total titratable acidity (TTA) were analyzed since these parameters are an important control for contaminations. The pH and the acidity were determined using a pH meter (Testo 230, Hampshire, UK). Preferment, dough and sourdough samples (10 g) were homogenized with 90 ml of sterile distilled water. The pH value was recorded and the acidity was titrated using 0.1 N NaOH to final pH 8.5 ± 0.1 (Paramithiotis et al., 2005). The TTA was expressed in ml 0.1 N NaOH needed for titrating 10 g of sample. Colony forming units of Dough I (DI) and Sourdough III (SDIII) were determined by plating serial dilutions on DG 18 agar for yeasts and on MRS agar for lactic acid bacteria with ISO 21527-2 and IFU standard methods, respectively.

3.2.3 Preparation of sourdough bread

Sourdough bread samples were prepared using three different level of sourdough with the % 10-20-30 based on flour weight. The recipe for the wheat bread (control bread) (C) and nomenclature of sourdough breads according to sourdough usage level and preferment source are given in Table 3.1. and Table 3.2., respectively.

Sourdough bread sample was prepared by mixing flours, salt, yeast (baker's yeast, Özmaya, Turkey), sourdough, water and other ingredients. The amount of water to be added was determined so as to have always dough with a consistency of 500 Brabender units (BU). All the ingredients were mixed for 2 minutes slow and 8 minutes high speed with a spiral mixer (Diosna SP 12 F, Dierks and Söhne GmbH, Osnabrück, Germany). Mixing of the ingredients was followed by a first proofing stage (10 min, room temperature). After dividing and shaping of the loaves into 450 g pieces, the main proofing stage took place (60 min, 37 °C, 80% relative humidity). Baking of the loaves carried out at 220 °C for 35 min with 200 ml steaming application. After cooling, loaves were characterized and then packed in orientated polypropylene bags (OPP) and stored at room temperature for 7 days.

Table 3.1: Wheat bread (control bread) recipe.

Ingredients	Amount (g)
Wheat flour	800
Rye flour	240
Water	640
Salt	13
Yeast	31
Gluten	31
Dough improver	6
Encapsulated sorbic acid	4

Table 3.2: Nomenclature of sourdough bread.

Preferment Source	% 10 Sourdough	% 20 Sourdough	% 30 Sourdough
Strawberry	S-10	S-20	S-30
Pomegranate	P-10	P-20	P-30
Black Carrot	BC-10	BC-20	BC-30
Red Beet	RB-10	RB-20	RB-30

3.2.4 Measurement of moisture loss, specific volume and color of bread

After cooling, the bread was weighed and bread volume was measured by the rapeseed displacement method (AACC Method 10-05.01). Each bread was put in a container and covered with rapeseed to totally fill the container. After the removal of the bread, the volume of the rapeseed was noted. Bread volumes were calculated by deducting the rapeseed volume from the container volume. Specific bread volume

was calculated as ml/g (bread volume/bread weight). Moisture loss (%) was determined according to deducting of the weight of the bread from the initial weight of the dough before baking. In formulation:
Moisture loss= [(final bread weight-initial dough weight)/initial dough weight]*100

Color of bread crust and crumb samples was measured with a tristimulus colorimeter (Minolta Chroma Meter model CR 400, Milan, Italy) with a circular measurement area (D = 8 mm). The colorimeter was calibrated using a white standard plate (L = 100). Chromatic coordinates (L*, a*, b*) were reported as the average of six measurements on each sample.

3.2.5 Determination of moisture content, water acitivity and pH of bread

The pH values of bread samples were determined using a Testo pH meter (Testo 230, Hampshire, UK). Ten grams of bread were blended with 90 mL of distilled water and the pH value was recorded. The moisture content of samples was analyzed by gravimetric method using a moisture analyzer (Sartorius MA 150). Water activity was measured using a electrical hygrometer (Novasina LabMaster-aw). These analyses were conducted after 7 days for investigating storage effect.

3.2.6 Determination of firmness of bread

The texture of the bread crumb was determined by texture profile analysis (TPA), which was done with a Texture Analyzer TA-XT Plus (Stable Micro Systems, Godalming, UK) 2.5-cm thick piece was placed on the flat stage, and the texture was determined using the (AACC 74-09) standard method. The sample was compressed with a 36-mm diameter cylinder probe. The test settings were as follows: pre-test speed, 1.0 mm/s; test speed, 1.7 mm/s; post-test speed, 10.0 mm/s; strain: 40%; distance, 6.2 mm; time, 5.0 s; trigger force, 20.0 g. The height of the first compression curve measured the resistance of the bread crumb to the compressing cylinder probe and represented the bread hardness effect.

3.2.7 Sensory evaluation of bread

A panel of 10 bakery experienced specialists was used to evaluate the sensory characteristics of the sourdough breads produced. They were asked to evaluate the overall acceptance of each bread concerning taste, odour, texture, color, appearance

and general acceptance. A 9-point hedonic scale was used where 1 = dislike extremely to 9 = like extremely.

3.2.8 Statistical analysis

All experimental analyses were conducted in triplicate and results are reported as mean value ± standard deviation. Data were subjected to statistical analysis using SPSS software (version 16.0 for Windows, SPSS Inc.) for the analysis of variance (ANOVA). Duncan's new multiple range test was used to analyze differences between samples ($p<0.05$).

4. RESULTS AND DISCUSSION

4.1 Cell counts, pH and Total Titratable Acidity of Dough and Sourdough

Preferment preparation for sourdough was conducted with fruits and vegetables having two distinct pH-range medium: acidic andmneutral. Preferment origin significantly (<0.05) effect pH and total titratable acidity (TTA) of initial and final preferment as given in Table 4.1. Initial preferment pH and total titratable acidity value ranged from 3.70 (P) to 6.44 (RB); 0.33 (RB) to 4.63 (P), respectively. Total titratable acidity was measured significantly lowest in initial preferment of black carrot and red beet initial preferment, which have neutral pH medium. In consequence of spontaneous fermentation of preferment samples, pH and TTA value of samples was found to 3.58 (P) - 5.08 ml (RB); 4.53 (RB) - 7.73 (P) ml (RB), respectively. Although pomegranate and black carrot final preferments were significantly different in terms of pH value, the highest TTA was obtained in these samples.

Table 4.1: pH and total titratable acidity of preferment samples throught spontaneous fermentation[a].

Substrate Type	pH 0^{th}.day	pH 3^{th}day	TTA 0^{th}day	TTA 3^{th}day
Strawberry	3.95±0.03 c	3.75±0.08 c	3.83±0.32 b	5.30±0.30 b
Pomegranate	3.70±0.04 d	3.58±0.03 d	4.63±0.40 a	7.73±0.31 a
Black carrot	6.31±0.10 b	4.02±0.01 b	0.50±0.10 c	7.93±0.35 a
Red beet	6.44±0.05 a	5.08±0.06 a	0.33±0.06 c	4.53±0.06 c

[a]Data represent average values ± standard deviation of three independent sample.Total titratable acidity expressed in ml. Means within a column with different letters are significantly different ($p < 0.05$).

As preferment fermentation steps within each refreshment step, origin of substrate had a significant impact on pH and TTA of dough and sourdough samples except TTA of DII and SDIII and pH of SD III (<0.05) (Table 4.2 and Table 4.3). According to our results, titratable acidity of final preferment was concurning with pH and TTA value of Dough I samples. As observed in final preferment, the highest TTA value was measured in black carrot and pomegranate originated Dough I samples and similar findings was obtained in pH measurement as exhibiting the lowest value. The pH and total titratable acidity of dough and sourdough samples

through refreshment steps are shown in Table 4.2 and 4.3, respectively. Initially, as considering in Dough I attributes, varied pH and total titratable acidity values were obtained in connection of substrate type. However with refreshment steps, these attributes became resembling each other. Refreshment step was terminated when stabilised pH was achieved with a range 3.90 - 4.08. Total titratable acitiy of Sourdough III was measured between 14.07 (ml) to 14.83 (ml). The pH and titratable acidity of sourdough III, which was used in bread preparation, were concurring with existing literature. Wolter et al. (2014) investigated usage of Lactobacillus plantarum as a starter culture with several flour type in sourdough preparation and demostrated that pH and TTA of sourdough ranged from 3.6 to 4.4 and 4.8 to 35.3 (ml), respectively. Wheat flour sourdough, which was main flour component in our sourdough, possessed of 3.8 pH and 12.8 ml TTA. Sanz-Penella et al. (2015) reported that pH and TTA of whole wheat sourdough, using Bifidobacterium pseudocatenulatum as a starter culture in preparation, was found as 4.17 and 17.24 (ml/g), respectively.

Table 4.2: pH of dough and sourdough samples throught refreshment steps[a]**.**

	Strawberry	Pomegranate	Black carrot	Red beet
Dough				
DI	5.55±0.13 b*	5.15±0.12 c**	5.29±0.07 c*	6.11±0.12 a*
DII	5.49±0.09 b*	5.62±0.04 a*	5.38±0.07 b*	5.49±0.02 b**
DIII	5.05±0.06 ba**	5.14±0.06 a**	4.99±0.06 bc**	4.93±0.02 c***
Sourdough				
SDI	4.60±0.08 a*	4.65±0.11 a*	4.33±0.09 b*	4.57±0.15 a*
SDII	4.00±0.12 b**	4.15±0.03 a**	4.04±0.08 ab**	3.95±0.03 b**
SDIII	3.95±0.13 a**	4.08±0.15 a**	3.99±0.10 a**	3.90±0.10 a**

[a]Data represent average values ± standard deviation of three independent sample. Means within a row with different letters are significantly different ($p < 0.05$). Means within a column different number of asterisk are significantly different ($p < 0.05$).

Table 4.3: Total titratable acidity of dough and sourdough samples throught refreshment steps[a].

	Strawberry	Pomegranate	Black carrot	Red beet
Dough				
DI	3.13±0.31 b*	4.97±0.42 a*	4.67±0.35 a*	2.37±0.21 c*
DII	3.10±0.36 a*	3.50±0.40 a**	3.23±0.55 a**	2.87±0.91 a*
DIII	3.93±0.59 bc*	4.73±0.50 a*	4.53±0.21 ab*	3.43±0.15 c*
Sourdough				
SDI	7.73±0.15 b*	7.60±0.30 b*	10.20±0.40 a*	7.87±0.58 b*
SDII	13.33±0.35 ab**	11.90±0.30 c**	12.90±0.62 b**	13.87±0.12 a**
SDIII	14.07±0.49 a***	14.07±0.25 a***	14.67±0.42 a***	14.83±0.45 a***

[a]Data represent average values ± standard deviation of three independent sample. Total titratable acidity expressed in ml. Means within a row with different letters are significantly different ($p < 0.05$). Means within a column different number of asterisks are significantly different ($p < 0.05$).

Microbial counts of Dough I and Sourdough III are shown in Table 4.4. Cell counts of $4.9*10^4$ (P) to $7.8*10^4$ (BC) (cfu/g) were obtained for lactic acid bacteria and of $2.5*10^4$ (P) to $7.6*10^4$ (RB) (cfu/g) for yeast in Dough I samples. Pomegranate originated dough samples had the lowest cell counts. By means of refreshment steps, increased cell counts were observed for both lactic acid bacteria and yeast for each sourdough samples. In this study, growth of lactic acid bacteria was more exceessive than yeast; increase in lactic acid bacteria and yeast was observed at least in black carrot samples. As a consequence of this black carrot sourdough has the mimimum cell counts. Cell counts of $5.8*10^7$ (BC) to $1.4*10^8$ (RB) (cfu/g) were obtained for lactic acid and of $6.9*10^4$ (BC) to $1.8*10^6$ (S) (cfu/ g) for yeast in Sourdough III. Sourdoughs mainly contained yeasts and lactic acid bacteria in ratio which varied from about 1:100 to 1:1000. In existing literature lactic acid bacteria and yeast counts could differentiate according to starter culture, flour type and fermentation process. Aplevicz et al. (2014) reported that in grape sourdough, counting of aerobic lactic acid bacteria was 7.52 ± 0.07 log cfu/g and yeasts was 7.62 ± 0.29 log cfu/g which was concuring with our study. Corsetti et al. (2001) investigated microbial counts of different wheat sourdough and demonstrated that cell number of lactic acid bacteria vary from 7.5 to 9.3 log cfu/g and yeast ranged from 5.8 to 8.4 log cfu/g.

Table 4.4: Cell counts for Dough I and Sourdough III[a].

	Strawberry	Pomegranate	Black carrot	Red beet
Yeast				
DI	$6.0*10^5$	$2.5*10^4$	$6.3*10^4$	$7.6*10^4$
SD III	$1.8*10^6$	$8.4*10^4$	$6.9*10^4$	$3.1*10^5$
Lactic acid bacteria				
DI	$6.9*10^4$	$4.9*10^4$	$7.8*10^4$	$7.2*10^4$
SDIII	$1.3*10^8$	$9.0*10^7$	$5.8*10^7$	$1.4*10^8$

[a]Data expressed as cfu/g.

4.2 Bake Loss (%), Specific Volume and Color of Bread

Loss of moisture during baking in bread samples ranged from 14.00 % (S-20) to 16.07 % (C and S-10) and is given in Table 4.5. The addition level of sourdough and type of substrate, used as a starter culture in sourdough preparation, did not led to significantly variation in bake loss (<0.05). The addition of sourdough could effect distinctly bake loss depend on sourdough type. Wolter et al., (2014) also investigated impact of sourdough fermented with *L. plantarum FST 1.7* on baking of wheat and gluten-free breads and did not observe significant decrease in bake loss. In

sourdough breads prepared with buckwheat, oat, quinoa and sorghum; the addition of sourdough induced a decrease in bake loss. Highest bake loss was observed in control bread (16.07 %) as similar this study. Decrease in bake loss was observed in bread samples with the increase in usage of sourdough; % 30 addition of sourdough bring about the decrease in bake loss significantly different from control bread (<0.05) (Table 4.7).

Table 4.5: Bake loss (%) of control bread and sourdough bread samples[a].

Control	16.07±1.14				
S-10	16.07±2.02	S-20	14.00±0.59	S-30	15.63±0.68
P-10	15.85±1.28	P-20	14.96±0.71	P-30	14.07±0.56
BC-10	14.74±0.46	BC-20	15.41±0.90	BC-30	14.44±0.77
RB-10	15.19±2.00	RB-20	15.33±0.59	RB-30	14.22±0.59

[a]Data represent average values ± standard deviation of three independent sample.

The effect of substrate type and addition level of sourdough was observed significantly different in specific volume given in Table 4.7 (<0.05). The specific volume of sourdough bread samples ranged from 6.19 to 7.24 (ml/g) and shown in Table 4.6. Addition of sourdough significantly (<0.05) cause decrease in specific volume as observed that control bread exhibiting the highest value, 7.53 (ml/g) (Table 4.7). Sourdough bread samples prepared with %10 and %20 sourdough addition did not significantly vary in specific volume on the basis of fruits and vegetables. Nevertheless, increase in sourdough addition led to significantly differantion within the preferment origin as S-30 and BC-30 (<0.05). Black carrot originated sourdough bread with %30 sourdough exhibited lowest value (6.19 ml/g) (Table 4.6).

Table 4.6: Specific volume of control and sourdough bread samples[a].

Control	7.58±0.08 a				
S-10	7.24±0.08 b	S-20	6.75±0.14 c	S-30	6.54±0.07 cde
P-10	7.06±0.15 b	P-20	6.64±0.12 cd	P-30	6.38±0.05 ef
BC-10	7.12±0.19 b	BC-20	6.71±0.06 c	BC-30	6.19±0.05 f
RB-10	7.08±0.21 b	RB-20	6.59±0.11cde	RB-30	6.44±0.10de

[a]Data represent average values ± standard deviation of three independent sample and expressed as ml/g.
Means with different letters are significantly different (p< 0.05).

There is considerable consensus with regard to the positive effects of the addition of sourdough on bread volume and crumb structure (Arendt et al., 2007). Despite this, there are several studies reported that addition of sourdough resulted in specific volume decrease depending on the proteolytic activity and aciditification properties

of sourdough (Wolter et al., 2014; Sanz-Penella et al., 2012; Gül at al., 2005; Plessas et al., 2005).

Table 4.7: Multi-comparision analysis of bake loss (%) and specific volume parameters for control and sourdough bread samples[a].

		Bake loss (%)	Specific volume
Type of substrate	Control	16.07 a	7.58 a
	Strawberry	15.23 a	6.84 b
	Pomegranate	14.96 a	6.69 bc
	Black carrot	14.86 a	6.67 c
	Red beet	14.91 a	6.70 bc
Sourdough concentration (%)	Control	16.07 a	7.58 a
	10	15.46 ab	7.13 b
	20	14.93 ab	6.67 c
	30	14.59 b	6.39 d

[a] Means within a colum with different letters are significantly different for each factor ($p < 0.05$).Specific volume expressed as ml/g.

The color of the bread crust and crumb in terms of chromatic coordinates is reported in Table 4.8. Control bread sample had a lighter crust and crumb than sourdough bread sample ones. ANOVA results highlighted a significant effect of sourdough concentration on L*(<0.05), of fruits and vegetables on L* and b* (<0.05) in bread crumb; of sourdough concentration on a* (<0.05), of their interaction on L* and b* (<0.05) in bread crust. The parameter L* (lightness) value ranged from 44.41 (S-10) to 54.89 (S-20) and the parameter b* (yellowness) ranged from 23.69 (S-10) to 30.91 (RB-10 and S-20) in crust. The crust lightness (L*) of sourdough bread samples exhibited slightly decline concurring with existing literature. Rinaldi et al. (2015) and Crowley et al. (2002) found a darker crust for sourdough breads prepared with a straight-dough procedure. First phases of Maillard reactions, which was more consistent under sourdough bread-making could clarify this results.The addition of sourdough led to significantly increase in parameter a* (redness). The highest a* was observed in the sample prepared with %30 addition of sourdough originated from black carrot (BC-30) (17.97). Regardless of sourdough strain, Torrieri et al. (2014) reported that higher parameter a* was observed in samples with higher sourdough concentration. L* of bread crumb obtained from samples with %30 sourdough addition, significantly lower than other sourdough bread samples as control bread (<0.05). Pomegranate originated sourdough breads, possessed of lowest L* and b* , were also significantly different in crumb color than control bread (<0.05) (Table 4.8). The parameter L* (lightness) value ranged from 71.17 (P-30) to 76.20 (C); the

parameter a* value ranged from 1.06 (C) to 3.01 (P-10) and the parameter b* (yellowness) ranged from 12.25 (P-20) to 13.52 (P-20) in crumb.

Table 4.8: Multi-comparision analysis of crust and crumb colorimetric parameters for control and sourdough bread samples[a].

		Crust		
		L*	a *	b *
Type of substrate	Control	50.33±1.59 a	16.21±0.26 b	27.67±1.12 a
	Strawberry	49.21±5.15 a	17.03±0.84 a	27.63±3.39 a
	Pomegranate	46.85±2.32 a	17.24±0.37 a	26.30±2.06 a
	Black carrot	49.61±2.25 a	17.54±0.55 a	28.56±1.69 a
	Red beet	49.45±3.59a	17.34±0.49 a	28.62±2.69 a
Sourdough concentration (%)	Control	50.33±1.59 a	16.21±0.26 c	27.67±1.12 a
	10	48.28±3.59 a	16.90±0.43 b	27.17±3.07 a
	20	49.78±4.19 a	17.28±0.71 ab	28.24±2.60 a
	30	48.29±2.88 a	17.68±0.32 a	27.93±2.19a
		Crumb		
		L *	a *	b *
Type of subsrate	Control	76.20±0.89 a	1.06±0.45 a	12.98±0.23 a
	Strawberry	74.47±2.10 ab	1.26±0.08 a	13.04±0.56 a
	Pomegranate	73.08±1.69 b	1.46±0.14 a	12.42±0.33 b
	Black carrot	74.94±2.11 a	1.51±0.33 a	13.05±0.56 a
	Red beet	75.04±1.69a	1.39±0.12 a	12.79±0.20 ab
Sourdough concentration (%)	Control	76.20±0.89 a	1.06±0.45 a	12.98±0.23 a
	10	75.49±1.73 a	1.32±0.17 a	12.70±0.33 a
	20	75.20±1.23 a	1.39±0.21 a	12.93±0.69 a
	30	72.47±1.43 b	1.51±0.22 a	12.84±0.41 a

[a] Means within a column with different letters are significantly different for each factor($p < 0.05$).

4.3 Moisture content, Water Activity (a_w) and pH of Bread Samples

The moisture content of samples on first day ranged from 33.61 % (S-30) to 34.46 % (P-10) given in Table 4.9. As observed in bake loss, preferment origin used in sourdough and addition of sourdough did not significantly effect the moisture content of bread samples on first day (<0.05) while end of the storage both parameters led to significanlty difference in moisture content (Table 4.10). The moisture content of bread samples on the 7th (end of the storage) ranged from 32.48 % (BC-30) to 34.19 % (RB-10) as given in Table 4.9. End of the storage black carrot originated sourdough samples exhibited lowest moisture content (33.07 %) in which maximum moisture loss occurred throught storage (Table 4.10). This case was also observed in sourdough bread samples prepared with % 30 addition. Nevertheless, the moisture content of bread samples either on first day or end of the storage did not significantly vary in terms of preferment origin and sourdough addition (<0.05) (Table 4.3.1). Still, results of moisture content on 7th day highlighted the significantly

difference between the sourdough bread samples originated from black carrot and red beet which are admitted as same vegetable category (rooted vegetable) (<0.05) (Table 4.10). As obtained results did not show significant trend in terms of sourdough addition, it could be concluded as sourdough addition did not impact on bread moisture content and moisture loss during storage. There are several studies reported that minimizing moisture loss and thereby enhancing shelf life could be achieved by sourdough application in bread during storage (Plessas et al., 2005).

Table 4.9: Moisture content of control and sourdough bread samples during storage[a].

1^{st}day					
Control	33.52±0.54 a				
S-10	34.69±0.66 a	S-20	34.33±0.59 a	S-30	33.31±0.86 a
P-10	34.46±0.52 a	P-20	33.69±0.71 a	P-30	34.27±0.85 a
BC-10	33.63±0.71 a	BC-20	33.51±0.90 a	BC-30	34.62±1.59 a
RB-10	34.26±0.37 a	RB-20	34.29±0.81 a	RB-30	34.16±1.42 a
7^{th}day					
Control	33.52±0.88 abc				
S-10	34.06±0.21 ab	S-20	33.59±0.53 abc	S-30	32.95±0.32 c
P-10	33.98±0.53 a	P-20	33.65±0.91 abc	P-30	33.60±0.62 abc
BC-10	33.59±0.55 abc	BC-20	33.13±0.69 abc	BC-30	32.48±0.85 c
RB-10	34.19±0.72 a	RB-20	33.90±0.32 ab	RB-30	33.61±1.25 abc

[a] Data represent average values ± standard deviation of three independent sample. Means with different letters are significantly different within each day ($p < 0.05$).

Table 4.10: Multi-comparision analysis of moisture content for control and sourdough bread samples during storage[a].

		1^{st} day	7^{th} day	% change 1^{st} day vs 7^{th} day
Type of substrate	Control	33.69 a	33.52 ab	-0.44 a
	Strawberry	34.11 a	33.53 ab	-1.63 a
	Pomegranate	33.99 a	33.81 ab	-0.50 a
	Black carrot	33.92 a	33.07 b	-2.41 a
	Red beet	34.23 a	33.90 a	-0.95 a
Sourdough concentration (%)	Control	33.69 a	33.52 ab	-0.44 a
	10	34.15 a	34.01 a	-0.38 a
	20	33.96 a	33.57 ab	-1.09 a
	30	34.09 a	33.16 b	-2.64 a

[a] Means within a row with different letters are significantly different for each factor($p < 0.05$).

The water activity (aw) of control bread and sourdough bread was found to 0.94 (0.937-0.944) in all samples and did not change throught storage (0.935-0.944) (Table 4.11). Neither sourdough addition nor sourdough source significantly impact on the water activity (<0.05). In existing literature, effect of sourdough on water activity was conflicting. Belz et al. (2012) demostrated that the breads containing sourdough in combination with the different NaCl concentrations showed slightly lower a_w than the breads containing only NaCl which was not significantly different

from control as general. On the other hand, Cevoli et al. (2015) reported that average water activity of control sample is significantly lower compared with that of the samples containing yeasts and lactic acid bacteria which provides water retention by means of metabolite (e.g. exopolysaccharides) production.

Table 4.11: Multi-comparision analysis of water activity for control and sourdough bread samples during storage[a].

		1st day	7th day
Type of subsrate	Control	0.940 a	0.937 a
	Strawberry	0.941 a	0.940 a
	Pomegranate	0.940 a	0.944 a
	Black carrot	0.941 a	0.940 b
	Red beet	0.941 a	0.941 a
Sourdough concentration (%)	Control	0.940 a	0.937 a
	10	0.939 a	0.938 a
	20	0.943 a	0.943 a
	30	0.941 a	0.943 a

[a] Means within a row with different letters are significantly different for each factor($p < 0.05$).sample.

The pH of control and sourdough bread samples on first day ranged from 5.05 (RB-30) to 5.58 (C) shown in Table 4.12. Preferment origin and usage rate of sourdough significantly impact on pH values of control and sourdough bread samples (<0.05) (Table 4.13). Through the acidification properties of sourdough, the addition of increased amounts of sourdough resulted in a progressive decrease of bread pH compared to control bread exhibiting the highest value, 5.58 (Table 4.13). During storage pH value of control and sourdough bread exhibited a decrease. Neither preferment type nor addition level of sourdough leaded to significantly variation in pH change throught storage (<0.05) (Table 4.13). Nevertheless, maximum change (%) was observed within the sourdough breads prepared by % 30 application. Red beet originated sourdough bread samples significantly differ from pomegranate and strawberry originated bread samples (<0.05) (4.13). In basis of same sourdough concentration usage, pH of sourdough bread samples did not to vary according to preferment origin.The differentiation was observed within the strawberry and red beet originated sourdough bread samples, which was in evidence along with incremental sourdough application and shelf life (Table 4.12). End of the shelf life, the pH of control and sourdough samples was found to 4.97 (RB-30) to 5.53 (C) given in Table 4.12. As distinct from our study, Cevoli et al. (2015) reported that throught storage pH was unchanged because of inactivation and injured of lactic acid bacteria and yeast during baking.

Table 4.12: pH of control and sourdough bread samples during storage[a].

1st day					
Control	5.58±0.07 a				
S-10	5.39±0.02 b	S-20	5.24±0. 05 def	S-30	5.18±0. 07 efg
P-10	5.39±0.03 b	P-20	5.27±0.06 cd	P-30	5.17±0.04 fg
BC-10	5.35±0. 04 bc	BC-20	5.27±0.03 cde	BC-30	5.11±0.06 gh
RB-10	5.40±0. 04 b	RB-20	5.19±0.03 defg	RB-30	5.05±0.01 i
7th day					
Control	5.53±0.06 a				
S-10	5.30±0.06 b	S-20	5.20±0.02 c	S-30	5.06±0.02 de
P-10	5.39±0.02 b	P-20	5.13±0.07 cd	P-30	5.05±0.02 de
BC-10	5.31±0.09 b	BC-20	5.13±0.03 cd	BC-30	5.00±0.05 ef
RB-10	5.32±0.03 b	RB-20	5.09±0.02 d	RB-30	4.97±0.03 f

[a] Data represent average values ± standard deviation of three independent sample. Means with different letters are significantly different within each day ($p< 0.05$).

Table 4.13: Multi-comparision analysis of pH for control and sourdough bread samples during storage[a].

		1st day	7th day	% change 1st day vs 7th day
Type of substrate	Control	5.58 a	5.53 a	-0.85 a
	Strawberry	5.27 b	5.19 b	-1.64 a
	Pomegranate	5.27 b	5.19 b	-1.54 a
	Black carrot	5.24 bc	5.15 bc	-1.88 a
	Red beet	5.21 c	5.13 c	-1.65 a
Sourdough concentration (%)	Control	5.58 a	5.53 a	-.0.85 a
	10	5.38 b	5.33 b	-0.86 a
	20	5.24 c	5.14 c	-2.05 a
	30	5.13 d	5.02 d	-2.13 a

[a] Means within a column with different letters are significantly different within each factor ($p< 0.05$).

4.4 Firmness of Bread Samples

The initial firmness of samples ranged from 217.90 (g) (C) to 449.12 (g) (RB-30) and is given in Table 4.14. Substrate type used as preferment source and addition level of sourdough did significantly led to difference in firmness as shown in Table 4.15 (<0.05). Incremental sourdough usage rate eventuated in firmer bread crumb irrespective of preferment origin. This outcome was at least partly due to the lower specific volume found in these samples. In accordance with our results, Plessas et al. (2005) reported that with increase in kefir grains concentration, which have extensive microbiota, led to decline in specific volume and thereby firmer bread samples were obtained.Similar results were obtained in a study related with using bifidobacteria as starter culture in wheat sourdough (Sanz-Penella et al., 2012). Notwithstanding, there several studies existing in literature demostrated that sourdough application could provide increase in volume and so the softer crumb with considering usage rate (Moroni et al., 2012; Crowley, 2002). Among the preferment origin used in

sourdough preparation, black carrot and pomegranate exhibited firmer feature throught storage. Black carrot sourdough bread prepared with %30 application significantly different from other breads and represented the highest firmness value, 449.12 (g) and 838.18 (g). As shown in Table 4.14, end of the storage firmness value was found to 339.09 (g) (C) to 838.18 (g) (BC-30). Throught to storage, change in firmness did not significantly vary in terms of utilization ratio or substrate type; yet black carrot-originated sourdough breads exhibited higher firming rate and significantly different from control bread (<0.05) (Table 4.15). According to results obtained in this study, sourdough application did not led to differentiate in firming rate, which are conflicting with existing literature. Rinaldi et al. (2015) reported that bread samples prepared with sourdough addition exhibited significantly higher hardness in comparison with standard bread through storage. Crowley et al. (2002) found that crumb firmness of 40 % sourdough used in soft wheat bread became significantly higher than standard sample after 2 days of storage.

Table 4.14: Firmness of control and sourdough bread samples during storage[a].

1^{st} day					
Control	217.90±8.97 g				
S-10	242.92±13.19 fg	S-20	316.66±0. 64 d	S-30	397.51±23.28 b
P-10	269.10±24.04 ef	P-20	359.84±29.92 c	P-30	409.34±39.01 b
BC-10	247.04±10.02 fg	BC-20	297.61±14.62 de	BC-30	449.12±17.16 a
RB-10	246.65±2.42 fg	RB-20	314.33±14.25 d	RB-30	380.35±33.21 bc
7^{th}day					
Control	339.09±19.94 de				
S-10	391.61±14.55 b	S-20	521.17±24.21 d	S-30	628.04±4.70 c
P-10	449.67±39.55 d	P-20	558.59±14.98 d	P-30	697.62±13.59 b
BC-10	425.49±27.36 d	BC-20	530.07±22.49 d	BC-30	838.18±71.23 a
RB-10	393.74±3.73 de	RB-20	571.23±57.05 d	RB-30	674.56±13.59 bc

[a] Data represent average values ± standard deviation of three independent sample and firmess expressed as g.. Means with different letters are significantly different within each day ($p< 0.05$).

Table 4.15: Multi-comparision analysis of firmness for control and sourdough bread samples during storage[a].

		1^{st}day	7^{th} day	% change 1^{st} day vs 7^{th}day
Type of substrate	Control	217.90 c	339.09 d	55.75 b
	Strawberry	319.03 b	513.61 c	61.51 ab
	Pomegranate	346.10 a	568.63 ab	65.38 ab
	Black carrot	331.26 ab	597.91 a	79.39 a
	Red beet	313.78 b	546.51 bc	73.37 ab
Sourdough concentration (%)	Control	217.90 d	339.09 d	55.75 a
	10	251.43 c	415.13 c	65.61 a
	20	322.11 b	545.27 b	70.24 a
	30	409.08 a	709.60 a	73.38 a

[a] Data represent average values ± standard deviation of three independent sample and firmness expressed as g..Means within a column with different superscript letters are significantly different ($p< 0.05$).

4.5 Sensory Evaluation of Sourdough Bread Samples

The sensory evaluation results of control and sourdough breads are given in Table 4.16 Among the all bread samples strawberry sourdough bread prepared with 10 % addition (S-10) exhibited the highest score (41.10). In respect of sourdough addition 10 % sourdough application resulted in higher score (40.10) than control bread while 30 % addition represented lower score than (34.55) control bread (37.50) as well 20 % addition (37.45). The sourdough addition provided an increase of the score several attributes such as taste, odour and color whereas control bread was preferred mainly in respect to texture and apperance. Considering fruits and vegetables, strawberry sourdough breads had a highest score (38.30); followed by pomegranate (37.50), red beet (37.50) and black carrot (36.37). The prominent features of strawberry sourdough breads were taste and odour owing to its exquisite flavour.

Table 4.16: Sensory evaluation of control and sourdough bread samples[a].

Sample	Taste	Odour	Color	Texture	Apperance	General Acceptance	Overall Score
Control	5.50±1.43	5.90±1.37	6.20±1.03	7.00±1.05	6.40±0.70	6.50±0.71	37.50
S-10	6.40±1.35	6.60±1.26	6.60±1.53	7.20±1.23	7.10±0.99	7.20±1.03	41.10
P-10	5.40±1.90	6.80±0.92	6.70±1.06	6.40±1.43	6.70±0.95	7.10±1.29	39.10
BC-10	6.50±1.43	6.50±1.08	6.60±1.71	6.60±1.90	6.60±0.70	6.70±1.06	39.50
RB-10	6.40±1.35	6.50±1.08	7.00±0.94	7.20±0.63	7.20±1.03	6.40±1.07	40.70
10 % Sourdough average	6.18	6.60	6.73	6.85	6.90	6.85	40.10
S-20	6.60±1.43	6.70±0.82	6.70±1.34	6.10±0.99	6.50±1.27	5.70±1.34	38.30
P-20	6.00±2.00	6.20±1.55	7.10±0.99	6.50±0.85	6.40±0.97	6.50±1.08	38.70
BC-20	5.50±1.43	5.70±1.77	6.90±1.52	5.40±1.17	6.30±1.06	5.70±1.16	35.50
RB-20	7.10±1.52	5.80±1.81	6.40±1.35	5.60±1.26	6.30±0.67	6.10±1.29	37.30
20 % Sourdough average	6.30	6.10	6.78	5.90	6.38	6.00	37.45
S-30	5.70±1.34	6.20±1.32	6.30±0.82	5.50±1.18	5.90±0.88	5.90±1.10	35.50
P-30	5.60±2.22	5.40±1.65	6.50±1.08	5.90±0.88	5.50±0.85	5.80±1.14	34.70
BC-30	5.30±1.42	6.10±1.52	6.30±1.16	5.10±0.74	5.70±0.95	5.60±1.35	34.10
RB-30	5.00±2.00	5.50±0.97	6.30±0.82	5.60±0.97	5.90±0.88	5.60±1.17	33.90
30 % Sourdough average	5.40	5.80	6.35	5.53	5.75	5.73	34.55

[a] Data represent average values ± standard deviation of ten independent sample .

Concurring with our results, Plessas et al. (2005) reported that a bigger preferrence for bread made with kefir as far as flavour and overall quality concerned but nevertheless there was no siginificantly difference between two types of bread. In existing literature, studies represented that greater addition of sourdough increased

the acidity of bread samples. Rizzello et al. (2012) investigated the application of wheat germ sourdough in white bread and reported that acidity of taste and flavour and salty taste increased with wheat germ sourdough usage.

5. CONCLUSION AND RECOMMENDATIONS

Characteristics of sourdoughs such as pH, total titratable acidity, and cell counts and of breads such as bake loss, specific volume, colorimetric parameters, water activity, moisture content, pH and firmness were determined in the scope of this study. Furthermore, sensory analysis carried out for identifying the consumers' preference. The results of this study indicate that the use of different substrates in the preparation of sourdoughs provides breads with different sensory characteristics. Future studies are required to examine the effect of sourdough and bread making application on bioactive compounds of substrates. Also, in vivo and in vitro studies are required to understand the bioavailability of nutritive compounds of sourdough breads.

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APPENDICES

APPENDIX A: Pictures
APPENDIX B: ANOVA Tables

APPENDIX A

Figure A.1: Representative Sourdough I.

Figure A.2: Representative Sourdough III.

Figure A.3: Sourdough breads with 30% sourdough application.

Figure A.4: Sourdough breads with 20% sourdough application.

Figure A.5: Sourdough breads with 30% sourdough application.

APPENDIX B

Table B.1: Statistical analysis results of preferments, doughs and sourdoughs throughout sourdough preparation steps.

		Sum of Squares	df	Mean Square	F	Sig.
pH Preferment 0^{th} day	Between Groups	19.656	3	6.552	1845.62	0
	Within Groups	0.028	8	0.004		
	Total	19.684	11			
pH Preferment 3^{rd} day	Between Groups	4.103	3	1.368	636.181	0
	Within Groups	0.017	8	0.002		
	Total	4.121	11			
TTA Preferment 0th day	Between Groups	44.703	3	14.901	212.869	0
	Within Groups	0.56	8	0.07		
	Total	45.263	11			
TTA Preferment 3rd day	Between Groups	26.463	3	8.821	113.817	0
	Within Groups	0.62	8	0.077		
	Total	27.083	11			
pH Dough I	Between Groups	1.636	3	0.545	46.017	0
	Within Groups	0.095	8	0.012		
	Total	1.731	11			
pH Dough II	Between Groups	0.089	3	0.03	7.856	0.01
	Within Groups	0.03	8	0.004		
	Total	0.12	11			
pH Dough III	Between Groups	0.073	3	0.024	8.587	0.01
	Within Groups	0.023	8	0.003		
	Total	0.095	11			
pH Sourdough I	Between Groups	0.181	3	0.06	5.066	0.03
	Within Groups	0.095	8	0.012		
	Total	0.276	11			
pH Sourdough II	Between Groups	0.064	3	0.021	3.956	0.05
	Within Groups	0.043	8	0.005		
	Total	0.107	11			
pH Sourdough III	Between Groups	0.051	3	0.017	1.153	0.39
	Within Groups	0.117	8	0.015		
	Total	0.168	11			
TTA Dough I	Between Groups	13.83	3	4.61	42.554	0
	Within Groups	0.867	8	0.108		
	Total	14.697	11			
TTA Dough II	Between Groups	0.629	3	0.21	0.592	0.64
	Within Groups	2.833	8	0.354		
	Total	3.462	11			
TTA Dough III	Between Groups	3.142	3	1.047	6.317	0.02
	Within Groups	1.327	8	0.166		
	Total	4.469	11			
TTA Sourdough I	Between Groups	13.797	3	4.599	30.322	0
	Within Groups	1.213	8	0.152		
	Total	15.01	11			
TTA Sourdough II	Between Groups	6.247	3	2.082	13.506	0
	Within Groups	1.233	8	0.154		
	Total	7.48	11			
TTA Sourdough III	Between Groups	1.442	3	0.481	2.815	0.11
	Within Groups	1.367	8	0.171		
	Total	2.809	11			

Table B.2: Statistical analysis results of doughs and sourdoughs within substrate.

		Sum of Squares	df	Mean Square	F	Sig.
pH Strawberry Dough	Between Groups	0.797	2	0.399	32.876	0.001
	Within Groups	0.073	6	0.012		
	Total	0.87	8			
pH Pomegranate Dough	Between Groups	0.573	2	0.286	24.381	0.001
	Within Groups	0.07	6	0.012		
	Total	0.643	8			
pH Black Carrot Dough	Between Groups	0.204	2	0.102	13.216	0.006
	Within Groups	0.046	6	0.008		
	Total	0.25	8			
pH Red Beet Dough	Between Groups	0.827	2	0.414	37.525	0
	Within Groups	0.066	6	0.011		
	Total	0.893	8			
pH Strawberry Sourdough	Between Groups	0.448	2	0.224	24.054	0.001
	Within Groups	0.056	6	0.009		
	Total	0.504	8			
pH Pomegranate Sourdough	Between Groups	0.458	2	0.229	36.708	0
	Within Groups	0.037	6	0.006		
	Total	0.495	8			
pH Black Carrot Sourdough	Between Groups	0.25	2	0.125	29.093	0.001
	Within Groups	0.026	6	0.004		
	Total	0.276	8			
pH Red Beet Sourdough	Between Groups	2.091	2	1.045	219.83	0
	Within Groups	0.029	6	0.005		
	Total	2.119	8			
TTA Strawberry Dough	Between Groups	0.682	2	0.341	3.886	0.083
	Within Groups	0.527	6	0.088		
	Total	1.209	8			
TTA Pomegranate Dough	Between Groups	3.762	2	1.881	12.007	0.008
	Within Groups	0.94	6	0.157		
	Total	4.702	8			
TTA Black Carrot Dough	Between Groups	1.709	2	0.854	2.88	0.133
	Within Groups	1.78	6	0.297		
	Total	3.489	8			
TTA Red Beet Dough	Between Groups	3.727	2	1.863	9.528	0.014
	Within Groups	1.173	6	0.196		
	Total	4.9	8			
TTA Strawberry Sourdough	Between Groups	72.009	2	36.004	276.96	0
	Within Groups	0.78	6	0.13		
	Total	72.789	8			
TTA Black Carrot Sourdough	Between Groups	30.362	2	15.181	62.963	0
	Within Groups	1.447	6	0.241		
	Total	31.809	8			
TTA Pomegranate Sourdough	Between Groups	65.002	2	32.501	400.7	0
	Within Groups	0.487	6	0.081		
	Total	65.489	8			
TTA Red Beet Sourdough	Between Groups	85.469	2	42.734	233.1	0
	Within Groups	1.1	6	0.183		
	Total	86.569	8			

Table B.3: Statistical analysis results of control and sourdough breads.

Dependent Variable	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Bake Loss	Model	8884.753[a]	13	683.443	593.927	0
	Sourdough Concentration	4.616	2	2.308	2.006	0.155
	Substrate Type	0.739	3	0.246	0.214	0.886
	Sourdough Concentration * Substrate Type	10.94	6	1.823	1.584	0.191
	Error	29.919	26	1.151		
	Total	8914.671	39			
Specific Volume	Model	1805.504[a]	13	138.885	8642.911	0
	Sourdough Concentration	3.349	2	1.675	104.209	0
	Substrate Type	0.166	3	0.055	3.436	0.031
	Sourdough Concentration * Substrate Type	0.135	6	0.023	1.404	0.251
	Error	0.418	26	0.016		
	Total	1805.922	39			
Crust L*	Model	93536.962[a]	13	7195.151	943.908	0
	Sourdough Concentration	17.892	2	8.946	1.174	0.325
	Substrate Type	45.437	3	15.146	1.987	0.141
	Sourdough Concentration * Substrate Type	206.839	6	34.473	4.522	0.003
	Error	198.191	26	7.623		
	Total	93735.153	39			
Crust a*	Model	11555.862[a]	13	888.912	4304.928	0
	Sourdough Concentration	3.652	2	1.826	8.843	0.001
	Substrate Type	1.248	3	0.416	2.015	0.137
	Sourdough Concentration * Substrate Type	2.066	6	0.344	1.667	0.169
	Error	5.369	26	0.206		
	Total	11561.231	39			
Crust b*	Model	30221.605[a]	13	2324.739	618.87	0
	Sourdough Concentration	7.311	2	3.656	0.973	0.391
	Substrate Type	31.556	3	10.519	2.8	0.06
	Sourdough Concentration * Substrate Type	111.959	6	18.66	4.967	0.002
	Error	97.667	26	3.756		
	Total	30319.272	39			
Crumb L*	Model	216699.192[a]	13	16669.17	7027.056	0
	Sourdough Concentration	66.603	2	33.301	14.039	0
	Substrate Type	22.149	3	7.383	3.112	0.044

Table B.3 (contuining): Statistical analysis results of control and sourdough breads.

Crumb L*	Sourdough Concentration * Substrate Type	1.479	6	0.247	0.104	0.995
	Error	61.676	26	2.372		
	Total	216760.867	39			
Crumb a*	Model	96.769[a]	13	7.444	12.021	0
	Sourdough Concentration	0.679	2	0.339	0.548	0.585
	Substrate Type	2.772	3	0.924	1.492	0.24
	Sourdough Concentration * Substrate Type	4.591	6	0.765	1.236	0.321
	Error	16.1	26	0.619		
	Total	112.869	39			
Crumb b*	Model	6429.930[a]	13	494.61	3098.513	0
	Sourdough Concentration	0.338	2	0.169	1.059	0.361
	Substrate Type	2.398	3	0.799	5.007	0.007
	Sourdough Concentration * Substrate Type	2.249	6	0.375	2.348	0.06
	Error	4.15	26	0.16		
	Total	6434.081	39			
Moisture Content 1st day	Model	45185.995[a]	13	3475.846	3582.551	0
	Sourdough Concentration	0.238	2	0.119	0.123	0.885
	Substrate Type	0.504	3	0.168	0.173	0.914
	Sourdough Concentration * Substrate Type	5.606	6	0.934	0.963	0.469
	Error	25.226	26	0.97		
	Total	45211.22	39			
Moisture Content 7th day	Model	43967.597[a]	13	3382.123	8596.544	0
	Sourdough Concentration	4.311	2	2.156	5.479	0.01
	Substrate Type	3.795	3	1.265	3.216	0.039
	Sourdough Concentration * Substrate Type	0.574	6	0.096	0.243	0.958
	Error	10.229	26	0.393		
	Total	43977.826	39			
Moisture Content Change 1st day vs 7th day	Model	162.432[a]	13	12.495	1.289	0.28
	Sourdough Concentration	32.136	2	16.068	1.657	0.21
	Substrate Type	18.741	3	6.247	0.644	0.593
	Sourdough Concentration * Substrate Type	43.233	6	7.206	0.743	0.62
	Error	252.046	26	9.694		
	Total	414.479	39			

Table B.3 (contuining): Statistical analysis results of control and sourdough breads.

pH 1st day	Model	1052.245[a]	13	80.942	37714.88	0
	Sourdough Concentration	0.582	2	0.291	135.514	0
	Substrate Type	0.027	3	0.009	4.2	0.015
	Sourdough Concentration * Substrate Type	0.023	6	0.004	1.767	0.145
	Error	0.056	26	0.002		
	Total	1052.301	39			
pH 7th day	Model	1085.660a	13	83.512	38272.38	0
	Sourdough Concentration	0.366	2	0.183	83.779	0
	Substrate Type	0.022	3	0.007	3.303	0.036
	Sourdough Concentration * Substrate Type	0.033	6	0.006	2.549	0.045
	Error	0.057	26	0.002		
	Total	1085.717	39			
pH Change 1st day vs 7th day	Model	133.000[a]	13	10.231	5.717	0
	Sourdough Concentration	12.195	2	6.098	3.407	0.049
	Substrate Type	0.566	3	0.189	0.106	0.956
	Sourdough Concentration * Substrate Type	16.616	6	2.769	1.547	0.202
	Error	46.53	26	1.79		
	Total	179.53	39			
a_w 1st day	Model	34.517[a]	13	2.655	38352.56	0
	Sourdough Concentration	0.00006667	2	3.33E-05	0.481	0.623
	Substrate Type	0.000008333	3	2.78E-06	0.04	0.989
	Sourdough Concentration * Substrate Type	0	6	6.67E-05	0.963	0.469
	Error	0.002	26	6.92E-05		
	Total	34.519	39			
a_w 7th day	Model	34.536[a]	13	2.657	41443.6	0
	Sourdough Concentration	0	2	8.61E-05	1.343	0.278
	Substrate Type	0	3	3.98E-05	0.621	0.608
	Sourdough Concentration * Substrate Type	0	6	5.65E-05	0.881	0.522
	Error	0.002	26	6.41E-05		
	Total	34.538	39			
Firmness 1st day	Model	4169574.790[a]	13	320736.5	727.146	0
	Sourdough Concentration	149651.537	2	74825.77	169.639	0
	Substrate Type	5579.331	3	1859.777	4.216	0.015
	Sourdough Concentration * Substrate Type	9730.076	6	1621.679	3.677	0.009
	Error	11468.326	26	441.089		
	Total	4181043.116	39			

Table B.3 (contuining): Statistical analysis results of control and sourdough breads.

Firmness 7th day	Model	12108702.845[a]	13	931438.7	893.736	0
	Sourdough Concentration	522629.147	2	261314.6	250.737	0
	Substrate Type	34216.231	3	11405.41	10.944	0
	Sourdough Concentration * Substrate Type	51378.183	6	8563.03	8.216	0
	Error	27096.823	26	1042.186		
	Total	12135799.67	39			
Firmness 1st day vs 7th day	Model	188672.465[a]	13	14513.27	55.64	0
	Sourdough Concentration	412.523	2	206.262	0.791	0.464
	Substrate Type	1736.355	3	578.785	2.219	0.11
	Sourdough Concentration * Substrate Type	1246	6	207.667	0.796	0.582
	Error	6781.958	26	260.845		
	Total	195454.423	39			

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List of Publications:

- Kamiloglu, S., **Pasli, A. A.,** Ozcelik, B., Van Camp, J., and Capanoglu, E., 2015: Colour retention, anthocyanin stability and antioxidant capacity in black carrot (*Daucus carota)* jams and marmalades: Effect of processing, storage conditions and in vitro gastrointestinal digestion. *Journal of Functional Foods*, 13, 1-10.

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