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NIGDE OMER HALISDEMIR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES DEPARTMENT OF ANIMAL PRODUCTION AND TECHNOLOGIES

EFFECTS OF DIFFERENT MARINATION CONDITIONS ON THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITY OF ANCHOVY FILLETS INOCULATED WITH *Morganella psychrotolerans* DURING COLD STORAGE

OLUWATOSIN ABIDEMI OGUNKALU

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Doctoral Thesis

Supervisor

Assoc. Prof. Dr. İlknur BAĞDATLI

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Oluwatosin Abidemi OGUNKALU tarafından **Assoc. Prof. Dr. İlknur BAĞDATLI** danışmanlığında hazırlanan "**Effects of different marination conditions on the physico-chemical and microbiological quality of anchovy fillets inoculated with *Morganella psychrotolerans* during cold storage**." adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Hayvansal Üretim ve Teknolojileri Anabilim Dalı**'nda Doktora (İngilizce) tezi olarak kabul edilmiştir.

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THESIS CERTIFICATION

I certify that I have written the thesis to the best of my knowledge and belief. All information presented as part of this thesis is scientific and according to academic rules. I have acknowledged any help I received in preparing the thesis, and all sources used have been acknowledged in the thesis.

Oluwatosin Abidemi OGUNKALU

SUMMARY

EFFECTS OF DIFFERENT MARINATION CONDITIONS ON THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITY OF ANCHOVY FILLETS INOCULATED WITH *Morganella psychrotolerans* DURING COLD STORAGE

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The purpose of this research was to evaluate the impact of various marination conditions (1, 2, 3, 4% acetic and 6, 8, 10% NaCl) on the anchovy fillets inoculated with *Morganella psychrotolerans* during cold storage for three months. Samples were categorized into 14 groups. The research findings indicate that marination had a significant growth-retarding effect on *Morganella psychrotolerans*. Specifically, the groups treated with 3% and 4% acetic acid showed greater inhibitory effects on *Morganella psychrotolerans* and total psychrophilic bacteria since growth was not observed in these groups. Total Enterobacteriaceae count was not detected in the treated groups. In the groups treated with 2%, 3%, and 4% acetic acid total yeast and mold and lactic acid bacteria were inhibited. However, only the groups treated with 3% acetic acid showed suppression of total mesophilic bacteria. In terms of sensory analysis, the groups marinated with 2%, 3%, and 4% acetic acid had higher scores. The results obtained from the physicochemical analysis were as follows: For Thiobarbituric Acid Reactive Substances (TBARS) the results indicate that significant differences ($p<0.05$) exists between the groups as there was an increased in the value of control groups (CO), (CM) and the groups processed with lower concentration of acetic acid and sodium chloride (Grp 1, 5, & 9) and (Grp 2, 6, &10), while the lowest values were seen in the

groups marinated with high acetic acid and sodium chloride (Grp 3, 7, & 11) and (Grp 4, 8, and 12). Also, the peroxide value obtained showed that significant differences ($p<0.05$) exists between the groups. The value obtained was within the acceptable limit, while pH result decreased significantly among the groups after marination processes. From this research, it can be concluded that marination has the potential to extend the shelf life of anchovy fillets inoculated with *Morganella pychrotolerans*, inhibit bacterial growth, and render the seafood to be safe for consumption.

Keywords: Morganella psychrotolerans; Marination; Acetic acid; anchovy; fish quality; NaCl.

ÖZET

FARKLI MARİNASYON KOŞULLARININ *Morganella psychrotolerans* İLE İNOKÜLE EDİLMİŞ HAMSİ FİLETOLARININ SOĞUKTA DEPOLANMASI SÜRESİNCE FİZİKO-KİMYASAL VE MİKROBİYOLOJİK KALİTESİ ÜZERİNE ETKİLERİ

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Bu araştırmanın amacı, Morganella psikrotoleransları ile inokülasyon olmuş or aşılanmış hamsi filetolarının üç ay süreyle soğukta depolanması sırasında farklı marinasyon koşullarının (%1, 2, 3, 4 asetik asit ve %6, 8, 10 NaCl) etkisini değerlendirmektir. Örnekler 14 gruba ayrıldı. Araştırma bulguları, marine etmenin Morganella psikrotoleransları üzerinde önemli bir büyüme geciktirici etkiye sahip olduğunu göstermektedir. Özellikle, %3 ve %4 asetik asitle muamele edilen gruplar, bu gruplarda büyüme gözlenmediğinden Morganella psikrotoleransları ve toplam psikrofilik bakteriler üzerinde daha büyük inhibitör etkiler göstermiştir. Toplam Enterobacteriaceae sayısı marine edilen gruplarda tespit edilmedi. %2, %3 ve %4 asetik asitle marine edilen gruplarda toplam maya ve küf ve laktik asit bakterileri inhibe edildi. Ancak, yalnızca %3 asetik asitle marine edilen gruplar toplam mezofilik bakteri baskılanması gösterdi. Duyusal analiz açısından, %2, %3 ve %4 asetik asitle marine edilen gruplar daha yüksek puanlara sahipti. Fizikokimyasal analizlerden elde edilen sonuçlar şu şekildedir: Tiyobarbitürik Asit Reaktif Maddeleri (TBARS) için sonuçlar gruplar arasında önemli farklılıkların ($p<0,05$) bulunduğunu göstermektedir; kontrol gruplarının (CO), (CM) ve daha düşük konsantrasyonda asetik asit

ve sodyum klorür ile işlenen grupların (Grp 1, 5 ve 9) ve (Grp 2, 6 ve 10) değerlerinde artış vardır, en düşük değerler ise yüksek asetik asit ve sodyum klorür ile marine edilen gruplarda (Grp 3, 7 ve 11) ve (Grp 4, 8 ve 12) görülmüştür. Ayrıca, elde edilen peroksit değeri gruplar arasında önemli farklılıklar ($p<0,05$) olduğunu gösterdi. Elde edilen değer kabul edilebilir sınırlar içerisindeyken, pH sonucu marine işlemlerinden sonra gruplar arasında önemli ölçüde azalmışdı. Bu araştırmadan, marine etmenin Morganella pychrotolerans ile aşılanmış hamsi filetolarının raf ömrünü uzatma, bakteri büyümesini engelleme ve deniz ürünlerini tüketim için güvenli hale getirme potansiyeline sahip olduğu sonucuna varılabilir.

Anahtar Kelimeler: *Morganella psychrotolerans*; Marinasyon; Asetik asit; hamsi; balık kalitesi; NaCl.

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SYMBOLS AND ABBREVIATIONS

Symbols	Description
%	Percentage
°C	Degrees Celsius
g	Gram
H_2SO^4	Sulphuric acid
HCL	Hydrochloric acid
kg	Kilogram
KI	Potassium iodine
L	Liter
mg	Milligram
N	Nitrogen
ppm	Parts per million

Abbreviations	Descriptions
CFU	Colony Forming Unit
MRS	De Man, Rogosa and Sharpe Agar for (Lactic Acid Bacteria)
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PV	Peroxide Value
TAMB	Total Aerobic Mesophilic Bacteria
TBARS	Thiobarbituric acid reactive substances
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
VRBA	Violet Red Bile Agar

CHAPTER I

INTRODUCTION

Fish is a significant source of food consumed by humans; it is classified as an essential source of long-chain omega-3 polyunsaturated fatty acids (LC-PUFAs), which are Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) these kinds are not commonly supplied from many food sources. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) fatty acids are essential in human food because the human system cannot supply them for physiological purposes. Fish is also reported to be a source of nutrition for endocrine functions like iodine, selenium, and vitamin D (Utri-Khodadady and Głąbska, 2023). Additionally, a few studies showed a positive effect of PUFAs on life expectancy by preventing metabolic syndrome, cardiovascular disease, and obesity. (Speranza et al., 2021). Regardless of the several commendations and various health importance of fish consumption, the European Union has observed a reduction in its consumption trends since 2018 (Utri-Khodadady and Głąbska, 2023). The percentage of global fish produced and eaten directly has risen since the 1990s, and from 2012, according to (FAO, 2014b), about eighty-six percent (136 million tons) of global fishery produce are precisely exploited and eaten up by humans. Safe food remains the primary interest of food manufacturers, and the assurance of nutrients and secure food is essential worldwide (Fernandes, 2016). The critical qualities index of fishery products is freshness, which includes sensory, microbiology, chemical, and biochemical techniques that have been applied in the assessment of characteristics of fish and saltwater products when processing is carried out (Szymczak and Kołakowski, 2016). The FAO highlighted the essentials of food produced in all nations and affirmed that the population could be affected economically, socially, and environmentally (Fernandes, 2016). There are diverse ways fisheries produce could be preserved (FOA, 2014a). Salting, drying, and smoking of fish are examples of methods fish could be prepared, and these products are acknowledged worldwide. About 12% (16 million tons) of the total fish harvested globally for people to consume were exploited in drying, smoking, and several other preservation methods worldwide (FAO, 2014a). Along with smoking products, marinades are well-known goods in the European Union.

They are mostly made in Poland and the Baltic province in the east and middle of the continent (FAO, 2014a). The same is true for species that are used locally to prepare marinades, such as herring, and several fish species, including dogfish, shrimp, hake, mackerel, cod, sea salmon, and eels (Shenderyuk and Bykowsky, 1994). Several experiments were conducted on fish not commonly consumed in Ireland, Turkey, and Iceland to improve the preparation methods (Baygar et al., 2010; Fagan et al., 2006). About 1.8 million tons of fish were harvested in Turkey in 2013; anchovies accounted for approximately 80,000 tons (Tuik, 2015). Fresh anchovies collected in the Black Sea regions to the east and west are consumed. Fish flour and oil industries typically freeze or treat the extra. Expanding the available product range on the market, freezing and processing anchovies during the fishing season increases employment throughout the year and raises fish prices. In seafood consumption, turkey is ranked below the global average. There could be an increase in the consumption of fish by motivating people to be able to eat fish in the off-season. Marination is quick and much easier than other processing techniques like salting, preservation, and drying. Seafood can be processed using different salt and acid solutions, and the products can be closed and kept in airtight packaging or diverse oils (Kocatepe et al., 2019). The addition of sodium chloride and acetic acid preserved the fish samples from bacterial attacks and the growth of microbes. Records from several research revealed that pH ranges from 4 to 4.5, which causes fish cathepsin to be effective and, hence, leads to the denaturation of fibrous tissue protein to yield peptides and amino acids, and this process allows marinated samples to be tender texture and good aroma. The prohibition of microbes between liquid food is determined by the water activity, pH, temperature, and competing microbes and their population (Mandal and Basu, 2008). One of the oldest methods of preserving fish is marinating; adding marinades to fish improves its sensory qualities and extends the shelf life of fish and meat products (Meyer, 1963). In Turkey, marinades of fin fish are easily obtained from ready-to-eat fishery goods. Because of its high nutrient content, which allows it to be consumed raw or cooked without further preparation, the popularity of marinades is growing yearly (Symczak and Kolakowski, 2012). Fish produce, such as fresh, frozen, or salted fish, or a collection of fish treated with salt or another edible organic acid added to brines, sauces, or oils, can be referred to as marinated fish. Anchovy species are generally utilized for marinating because of their nutrients and great sensory characteristics. There are diverse techniques applied to carry out fish marinades; the preparation of fish marinades follows some routine process

that depends on the form of the marinades (i.e., cold, fried, and cooked, occasionally, pasteurization of marinades could be separated (Yeannes, 1991). Cold marination of fish is carried out in a marinating bath, which involves treatment with high acetic acid and salt components. The immersion period is based on the species characters (lipid contents, texture, size, and many more) (Cabrer et al., 2002; Kilinc and Cakli, 2005; Duyar and Eke, 2009; Fuentes et al., 2010). The retarding outcome of several mixtures of marinates from the impacts of microbial and the activity of enzymes elevated the percentage. The original features of fresh fish, such as how fresh the fish is, microbial load, and muscle spoilage, are essential elements that could impact the characteristics of the final product; in consideration of the choice of end users, a higher level of these components should be avoided to prevent viable deterioration (Harada, 2004). Marination carried out on defrosted fish material threatens fillet appearance, juice, and pigment nature; the threat is caused by the freezing effects due to storage, which leads to the alteration of muscle proteins. Anchovies are generally processed locally from fish fillets. In the marination process, fish fillets appeared glossy white, juicy, and gentle, with a slightly acidic taste. The effects of the freezing process result in blood stains at the abdomen section of fillets, and this appearance lowers the approval of the marinades. Thus, the quality of basic resources is crucial for tremendous-quality products (Šimat et al., 2019). Some essential components need to be weighed to achieve outstanding quality production. These components are as follows: constituents of the marination mixture, the proportion of fish to the solution, and the fish cure are critical factors that could alter the result of the product (Meyer, 1965). The characteristics of the fresh fish matter and the additives applied in the marination procedure were also considered crucial (Arason et al., 2014). Also, the fat constituents of fish meat, the harvesting techniques, and the approach carried out during practice on board could impact the fresh matter attributes and, therefore, the products of marination. More so, poor character additives could easily alter the end of the production. Hence, appropriate practice and speed freezing during boarding and preparation when landing is completed as outstanding products and appropriate constituents combined to produce a marinating mixture could yield outstanding results for marinades (Fernandes, 2016). In fish marination preservation, some compounded bacterial flora could be produced and linked with marinated fish deterioration. Bacteria identification is essential during scientific investigation (Vodnar et al., 2010).

This study aimed to assess the physical, chemical, and microbiological quality of anchovy fillets inoculated with *Morganella psychrotolerans* during cold storage, as well as the impact of marination conditions on the growth of *M. psychrotolerans.*

CHAPTER II

GENERAL INFORMATION

2.1 Importance of Marination

The process of marination causes a reduction in the rate at which bacteria and enzymatic activity occur, leading to alteration in the softness, texture, and composition of fish and extending the shelf-life (Sallam et al., 2007). When marination is carried out, microbial actions are restricted, pH reduction is attained, and ripening of the products occurs (Aksu et al., 1997; Dokuzlu, 1997). During ripening time, there is a reaction between salt and acid, the enzymes within fish materials, and the degradation of fish fats and proteins in fish muscles. The ripening process yields a pleasing aroma, and taste production is achieved in fish muscle (Erkan et al., 2000). Fish shelf-life is extended, and flavor characteristics are produced quickly due to the combination of acetic acid and salt that the fish are submerged. This process also reduces bacterial and enzymatic activity (Meyer, 1965; MC LAY, 1972). The primary goals of marination are to increase the value of the fish and prevent the degeneration of fish muscle caused by bacterial and enzymatic activity. Fish proteinase activities are enhanced because of the inclusion of acetic acid. Thereby, the release of amino acids through the activity of proteinases present in fish meat helps the release of energy baseline for acetic acid and salt conformity *lactobacilli*, which are amino acid–acid-decarboxylase bacteria (Fuselli et al., 1998). The inclusion of a marination solution led to the activeness of cathepsin tissues. This process, in turn, results in the breakdown of fish meat proteins, which yield peptides and amino acids. Marination procedures obtain adequate texture and flavor because salt and acetic acid act together (Shenderyk and Bykowski, 1989).

2.1.1 Spoilage bacteria of marinated fish

Many commercialized marinated products don’t undergo complete fermentation of products when vinegar is applied to produce fermented marinades, and few lactic acid bacteria (LAB) could develop in such products (Lyhs et al., 2002; Gokoglu et al., 2009). Lactic acid bacteria are a mostly distinct class of bacteria that comprises about six families and several classifications such as *Lactobacillus, Aerococcus, Alcalibacterium,*

Carnobacterium, Enterococcus, Lactococcus, Leuconostoc, and many others are defined class from this class of bacteria. They possess critical commercial assessments in food product fermentation. Based on the cooperation of several lactic acid bacteria genera, they are commonly activated through fermented food commodities. Yesilsu et al. (2018) examined the availability of LAB linked with the deterioration of the marination of anchovy (*Engraulis encrasicholus*) carried out with the use of vinegar. *Lactobacillus paracasei* subsp. *Paracasei* and *Lactobacillus curvatus* strains were identified from the produce by applying DNA sequencing of the 16S rRNA chromosomes. The first discovered lactic acid bacteria from deteriorated persimmon vinegar marinating anchovy fish. Twelve bacteria species were identified from deteriorated marinades of anchovy. As a result, the development of bacteria strains on Man Rogosa Sharpe agar dominated the colony, leading to the acknowledgment of the strains as lactic acid bacteria. Gram-positive, oxidase, and catalase-negative bacteria were also identified as responsible for deterioration and are referred to as protein swells that occur in cold fish marinades. Based on assumptions, proteolytic enzymes' actions result in protein deterioration because of the decarboxylated products and carbon dioxide being discharged. Lactic acid bacteria possess the potential to develop gas and could also cause the decomposition of glucose and homo-fermentation. Ammonia is the end point obtained from this procedure, and it revealed a reduction in the acid level of the product (Dijksterhuis and Samson, 2006; Lyhs and Björkroth, 2008). The primary species of lactic acid bacteria identified to be generally seen in fish muscle fermentation surroundings is *Lactobacillus curvatus*. This bacterium is usually linked with meat stored in the refrigerator, vacuum-packed meat, and garfish products (Hebert et al., 2012). According to the study of Yesilsu et al. (2018), the total lactic acid bacteria identified in deteriorated marinades of anchovy, L. paracasei subsp paracasei, were the highest-dominated bacteria. This study revealed *Lactobacillus spp*. was identified as a prevalent bacterial flora in the spoilage of vinegar-marinating fish products. One of the isolated specific spoilage bacteria of marinate herrings is *Lactobacillus spp.* (Meyer, 1956, 1962c; Kreuzer, 1957). From several types of deteriorated herring species, heterofermentative *Lactobacillus spp*. like *L. buchneri and L. fermentum* had been dominating (Meyer, 1956b; Kreuzer, 1957; Lerche, 1960; Sharpe and Pettipher, 1983). Moreover, *L. plantarum and L. delbruckii subsp. L. leichmannii maintained homofermentative glucose metabolism and* was identified in many deteriorations that occurred (Lerche, 1960; Sharpe and Pettipher, 1983).

2.2 Morganella Psychrotolerans

Morganella psychrotolerans is an innovation of histamine-forming bacteria which could develop at 0-2^{o}C. The features of its biological and chemical resemble *Morganella morganii* (Emborg and Ahrens, 2006). Many past reports have been written on the occurrence of poisoning by *Morganella morganii* (Emborg and Dalgaard, 2006; Emborg et al., 2005). The description was given to *Morganella* through Fulton in 1943, and in 1978, it was confirmed as a genus among the *Enterobacteriaceae* (Brenner et al., 1978). The bacteria strains were formally called *Proteus morganii, and presently, they are categorized as the genus morganella* because both species possess similar kinds of strains like *morganella morganii* with preference. *Morganella psychrotolerans*, such as bacteria, have been extracted from fresh fish, cold-smoked tuna, and garfish. The *psychrotolerans* that were isolated from the food exhibit a hazardous histamine aggregation at 0–5 ^{0}C, and they are shown to be essential to produce histamine in cold aquatic foods; the strains have been extracted from marine products, which is cross-linked in histamine fish poisoning formation (Emborg et al., 2005; Dalgaard et al., 2008). *Morganella psychrotolerans* are powerful bacteria that developed histamine; their isolation has recently been discovered. Additives must be added to fish to prevent spoilage since cold chilling alone is insufficient to stop *M. psychrotolerans* from producing histamine (Dalgaard et al., 2008). The cells of *Morganella psychrotolerans* are gram-negative, straight rods, 1Mm in breadth and 2-3Mm in length, having circle edges that occur in one pair and a shorter chain. Aerobic motility also has an anaerobic, facultative development. The development of *M. psychrotolerans* takes place between 2-35uC (77% growth in 0uC), in 0 close to 7.5% (w/v) NaCl (46% growth in 7.5% NaCl). Also, the growth occurs with pH 4.6-9.2 (77% growth in pH 4.6). The fermentation of glucose occurs in the presence of gas development and phenylalanine deamination. The fermentation happens to N-acetylglucosamine, potassium gluconate, xylitol, D-fructose, D-mannose, and D-ribosome. The following, including erythritol, xylose, arabinose, and glycerol, L-sorbose, L-rhamnose, dulcitol, inositol, D-adonitol, D-mannitol Sorbitol D and methylamethyla and -D-mannopyranoside-D-glycopyranoside, arbutin, aesculin, salicin, and amygdalin D-melibiose, D-sucrose, D-cellobiose, D-maltose, and D-lactose Inulin, D-trehalose, and gentiobiose, starch, glycogen, D-melezitose, and D-raffinose Fucose, arabitol, potassium, D-turanose, D-xylose, and D-tagatose 2. Potassium and ketogluconate 5. Ketogluconate was not

fermentable. The primary cellular fatty acids are 16:0 (31%) and 17:0 cyclo (30%). They are so tendered to apramycin, ceftiofur, chloramphenicol, *ciprofloxacin, florfenicol, gentamicin*, nalidixic acid, neomycin, spectinomy (Emborg et al., 2006).

2.3 Importance of *Morganella psychrotolerans* in Marinated Fish

Morganella psychrotolerans, such as bacteria, had been isolated from fresh tuna, dried tuna, and garfish. There is the development of a dangerous level of absorption of histamine at the rate of 0-5oC, and this is revealed to be crucial in the development of histamine in excellent marine food; the species has been discovered to cause fish poisoning from histamine sources (Emborg et al., 2005; Dalgaard et al., 2006). *Morganella psychrotolerans* are tough bacteria that develop histamine. The discovery of this bacteria is recent, current research of histamine food poisoning (HFP) *Morganella psychrotolerans*, which produces several HFP occurrences in contrast to the well-known mesophilic HPB, was shown to harbor *psychrotolerans* as histamine-producing bacteria (HPB) in Denmark and Japan (Dalgaard et al., 2008). The development of histamine by *Morganella psychrotolerans* could not be retarded just by using cooling marine foods; thereby, there is an urgency for supplementary preservation methods (Dalgaard et al., 2006). Thus, its existence in marinades fish would aid in allotting product shelf-life of fish through retardation of high levels of histamine development in marine foods under a reasonable storage situation. Also, it can operate as an instrument to expose appraisal research that identifies the level of histamine in seafood before it is consumed. The absorption of sodium chloride over 1-2% decreases the percentage of development and thereby inhibits histamine accumulation through Gram-negative producing bacteria (HPB) (Okuzumi et al., 1984a; Yamamoto et al., 1991; Morii et al., 1994; Aytac et al., 2000). The percentage of development of *Morganella psychrotolerans* is altered through the absorption of sodium chloride in the medium for development. Also, the absorption of sodium chloride altered the culture quantity to the highest. Furthermore, *Morganella psychrotolerans* development percentage was positively changed by switching pH ranges from 5 to 5.6. The reduction in the rate of development delay was formerly observed before the development of histamine in dangerous concentrations.

2.4 Anchovy

Anchovy (*Engraulis encrasicolus*) is identified as a species of nautical fish found in the Eugraulidae family. Anchovies are species harvested around the Mediterranean coast and are broadly spread in the Ataurique Ocean (Simat and Bogdanovic, 2012); these species are generally utilized as a material for the production of fish marination. Usually, anchovies are submerged in a solution that combines acetic acid and salt. Immediately when the ripening process is completed, they are kept and preserved in vegetable oil or sauce (Gokoglu and Ucak, 2020). They are distinguished by their tender appearance and fewer fat components than sardines (Simat and Bogdanovic, 2012). These attributes make them acceptable as essential resources for the minimum treatment of fish products.

Meanwhile, anchovies pose the danger of an outbreak of Anisakis species (Mladineo et al., 2012; Simat et al., 2015). As a result, anchovies should be frozen before processing is carried out. Anchovy fish are grouped as fatty fish with high quantities of polyunsaturated fatty acids (PUFAs). PUFAs which are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), generally called omega-3 fatty acids which, are crucial for the developmental stages of the nervous system of new-born during early life (Wang et al., 2011). Polyunsaturated fatty acids are vulnerable to lipid oxidation and chemical disintegration. During the process of lipid oxidation, there is a reduction in the fish's lipid nutrient level, leading to an aggregation of unwanted rancidity and possibly dangerous reaction products (Turhan et al., 2009). Anchovies are oily fish that possess less shelf life, and quality disintegration of fish generally occurs because of the speedy development of microorganisms and lipid oxidation. The rancidity that occurs from oily fish leads to off-odor and off-taste of the fish products and thereby reduces the acceptance to be reduced (Moini et al., 2009; Rostamzad et al., 2010). Marinate of anchovy species is carried out throughout the seasons, and they are crucial commodities that are usually shipped across borders; examination of native preservatives has been carried out to reduce the unfavorable impacts resulting from anchovy species while freezing and storing marinades anchovy (Fıçıcılar and Gencelep, 2020). Fish deterioration could be termed an ecological factor encompassing several changes in the available constituents like low molecular weight compound processes (chemical, enzymatic activity, autolytic process) and microbial accumulation (Bover-Cid et al.,

2003). Food deterioration occurs mainly through microbial proliferation and metabolism, which results in the development of amines, sulfides, alcohols, aldehydes, ketones, and organic acids with displeasing and undesirable off-flavors (Gram and Dalgaard, 2002).

2.5 Fish Freshness and Quality

Fish vulnerability to deterioration is high amongst several other animal sources consumed by humans. This results from special features found in the fish muscle like nutritional composition, phospholipid composition, increased water activity, availability of enzymes alongside destructive reactions inside muscles and guts, and a near-neutral pH (Soares and Goncalves, 2012). Huss (1995) identified three primary mechanisms by which raw fish spoilage and quality degradation: autolysis, bacterial activity, lipid oxidation, and action. Lipid oxidation usually happens quickly after autolytic and bacterial spoiling has occurred. This might happen due to elevated temperatures or exposure to light, which can accelerate spoilage (Quang, 2005). Fish that have only survived freezing at a temperature close to 0°C are often called unprocessed fish. However, the method used on fish during processing significantly impacts keeping raw fish at its optimal quality.

Similarly, the final state of the fish's completeness depends on the state of the fresh fish that will be processed (Fernandes, 2016). Therefore, methods of approach during processing should include all activities from harvesting to the point at which fish will be consumed to preserve quality attributes and fish nutrients. This might be accomplished by avoiding infection from bacteria and outside materials. As a result, there will be less rotting and less degradation of the portions that are fit for consumption (Huss, 1995). Fish completeness refers to a character's ability to maintain characteristics that suggest a living fish. The following methods, including sensory, microbiological, physical, and chemical examination, should be investigated to assess the wholeness and characteristics of fish (Olafsdottir et al., 1997).

Sensorial method analysis could be used to investigate how fresh fish handling practices affect fish quality (Huss, 1995). The shortcomings of the sensory approach were emphasized. For example, the method requires dependable and experienced human

resources, so it is not widely used. However, sensory analysis is the most utilized approach in the food sector compared to several exciting analysis techniques since it may yield a consistent and rapid result. Examining the external covering, cavity, and muscle of fresh fish, as well as the eyes, skin, gills, muscle structure (texture), and scent of the fish, or the smoothness, aroma, and smell of prepared fish are examples of sensory methods that provide a reliable assessment of the overall condition of marine food and the qualities of fish in every point assessment process (Martinsdottir et al., 2001). Despite the range of techniques used to preserve food, the infection and disintegration of food due to bacterial action is a significant issue in numerous nations across the globe (Leistner and Gorris, 1995). Preservation techniques are employed to stop spoiling from chemical and microbiological processes. Food preservation could extend its shelf life by approximately 200% by preventing or delaying chemical and biological deterioration. Antimicrobials could be used to avoid degradation brought on by microbiological processes; antioxidants, anti-browning agents, or anti-stalling techniques can be applied to delay chemical spoilage, for instance, brawling, staling, and lipid oxidation (Fernades, 2016).

CHAPTER III

LITERATURE REVIEW

3.1 Inoculation of Bacteria in Fish Marinade

Speranza et al. (2021) studied the sea bream fillets marinade and enhanced the product with *Bifidobacterium animalis subsp. lactis and Lactiplantibacillus plantarum:* Product design and brine optimization. Sea bream fillets infected with *Bifidobacterium animalis subsp*. Lactis or *Lactiplantibacillus plantarum* (strains 11, 68, 69). The brine comprises 55% vinegar, 3% sodium chloride, and 0.75% citric acid. Three (3) groups make up the studies: Group A is the control group (one that has not had probiotics injected), Group B is made up of probiotic-infused samples that are kept in sunflower oil, and Group C is made up of probiotic-infused samples that are packed and preserved in brine solution. Fish fillets were injected at a density of 8 log CFU/g, then stored for three weeks at 4^{o}C in high-barrier nylon/polythene bags filled with solutions, including oil and diluted brine, for 48 hours. According to the authors, both bacterial strains were active during the three-week processing time. According to sensory evaluations, samples treated with sunflower oil were favored over samples maintained in diluted brine because they produced superior outcomes. This suggests sunflower oil is a better conditioning solution than diluted brine—preference scores for samples treated with *Bifidobacterium animalis subsp*. Lactis were marginally higher than those for samples treated with *Lactiplantibacillus plantarum*.

Ucak and Gökoğlu (2020) investigated how high-pressure processing affected the development of biogenic amines and *Photobacterium phosphorus* proliferation in marinated herring. The marinate solution consists of 2% acetic acid with 8% sodium chloride and 4% acetic acid with 8% sodium chloride. Fish fillets were submerged in the marinating solution, and marinated fillets were inoculated with *Photobacterium. Phosphorus*, and then packaged in vacuum packaging with the treatment of HPP at varied pressure rates such as (100, 300, and 500 MPa), the period for the pressure to withhold was (5 and 10 minutes). The group of fillets that were without treatment is the control. All the groups were kept at 4±1^{o}C for 90 days. The authors reported the outcome of the analysis in which the combination of HPP and 4% acetic acid proved to

have significant impacts on the inhibition of the development of *Photobacterium phosphorus;* specifically, this impact was seen in the group treated with 300 and 500MPa pressure rate. The development of histamine-producing bacteria could not be identified in the batches treated with 500MPa pressure. Also, there was no growth of total psychrophilic bacteria in the group with treatment of 500MPa and 300MPa under 10 minutes in combination with 2% acetic acid within the processing period. Detection of histamine was not significant in the group processed with marinate solution along with 4% acetic acid in combination with HPP treatment, but with exemptions of the group without any treatment (control), which showed no presence of tyramine development in the group treated with 4% acetic acid. Likewise, putrescine could not be isolated in the group processed with 2% acetic acid and treated with HPP when the groups were initially stored. The cadaverine level was insignificant for the batches processed with 300 and 500MPa pressure rates; its development was inhibited by the 4% acetic acid and HPP treatment, compared to those processed with 2% acetic acid. The authors concluded that there are inhibition impacts on Photobacterium phosphorus development and suppression of histamine, tyramine, putrescine, and cadaverine occurrence in the samples treated with 4% acetic acid combined with HPP.

Ummul-Izzatul et al. (2020) investigated the survival of *Listeria monocytogenes* in short mackerel (*Rastrelliger brachysoma*) marinated with turmeric and salt at an isothermal storage temperature. The fish were immersed in 70% ethanol for one minute to lower the microbial burdens. Two batches of short mackerel, one for treatment and the other for control, were created. Group 2: For treatment, NaCl crystal was added with industrial turmeric powder in a sterilized bag packed with short mackerel marinated with turmeric-salt in a ratio of 1:1, and in which *L. monocytogenes* with a concentration of 108 CFU/mL, which was a applied by micropipettes for 100 µL. Group 1 (control): inoculated with a concentration of 10^8 CFU/mL of *L. monocytogenes* (six storage temperatures 0, 5, 10, 15, 20, and 25°C). Each bag was manually shaken for a minute after the inoculation process was complete to distribute the *L. monocytogenes* cells evenly across the fillet's surface. Six temperature ranges were then used to store the bags: 0°C, 5 °C, 10 °C, 15°C, 20°C, and 25°C. According to the authors, the average microflora load of bacteria has dropped to about 1 log CFU/g. In marinated mackerel, the growth of *L. monocytogenes* was found at 4.30 log CFU/g and grew to 5.22 log CFU/g between day zero (0 ˚C) and day twenty-five (0 ˚C). The overall *L.*

monocytogenes counts in the batch that was not marinated, temperature, and time showed a substantial increase in short mackerel, from 4.98 log CFU/g (0 days at 0 ˚C) to 6.22 log cfu/g (0 days at 25 ˚C). Though *L. monocytogenes* was seen to develop slowly over the study period (21 days of storage), this investigation showed that the development of L. monocytogenes marinated with turmeric salt was slowed down compared with the sample without marination (control). According to the results, the development of L. monocytogenes varied significantly ($p<0.05$) depending on the storage temperature. The authors concluded that short mackerel could have the growth of *L. monocytogenes* slowed to between 0.07 and 2.81 log CFU/g when turmeric and salt are combined for marinating.

Santoro et al. (2020) examined the antimicrobial activity of selected red and white wines against *Escherichia coli*: In vitro inhibition using fish as food matrix. There were five batches of fish fillets in which marinate solution prepared from mixed wine and sterilized distilled water (400ml) in (a 1:1) ratio and inoculated with E. coli 7 log CFU/25g of bacteria; the batch sample was preserved in sterilized distilled water only. After two hours, each batch of marinated fish fillet was strained from the solution and ground in a knife mill down 40-50mm particles; all samples were stored at room temperature for about 10 minutes; this is done for bacteria to be attached and treated samples were preserved at $4\pm1^{o}C$. The authors reported decreased development of bacteria on day 3 of processing at $4^{o}C$. The authors concluded that the different kinds of wine used for marination revealed the potential for reducing the number and development of bacteria in all samples of seabass fillets.

Ucak et al. (2019) examined the inhibitory effects of high-pressure processing (HPP) treatment on microbial growth and biogenic amine formation in marinated herring (*Clupea herangus*) inoculated with *Morganella psychrotolerans*. The authors investigate the inhibitory effects of HPP on biogenic amine development, such as histamine, tyramine, putrescine, and cadaverine, on herring muscle filleted and submerged in marinating solution with varied percentages of a mixture of both acetic acid and sodium chloride solution (2% acetic acid added with 8% sodium chloride; or 4% acetic acid added with 8% sodium chloride solution, herring fillets was submerged in the bacteria (*Morganella psychrotolerans*), then the treated sample was kept inside vacuum-packaging. HPP treatment was performed at 100, 300, and 500 MPa for five to

ten minutes. No treatment was added to one group; the rest was marinated at four °C for three months. The outcome of the investigations revealed that High-pressure processing with the addition of 4% acetic acid possessed a powerful barrier effect, which delayed the development of *psychrotolerans* because the group with HPP treatment of 300MPa within 10 minutes and 500MPa within 5 minutes and 10 minutes does not have any growth. No growth was seen in bacteria for the group submerged in marinate solution alongside 4% acetic acid, and the group was subjected to 500MPa. Histamine-producing bacteria showed no growth on herring marinated and stored. The authors also reported no histamine and cadaverine formation in the group treated with marinate of 4% acetic acid and stored. The authors' conclusion stated that the study's results could significantly help improve the preservation of marinated herring before it is consumed.

Giribldi et al. (2019) studied the quality of marinated ready-to-eat (RTE) swordfish fillets inoculated with *Lactobacillus paracasei* IMPC 2.1. The marination solution was prepared with NaCl, vinegar, and citric acid, with or without inoculation with the probiotic strain *Lactobacillus paracasei; two groups were analyzed:* Group 1: control-RTE group (without probiotics strains and brining), Group 2: RTE probiotic group (consists of probiotic strains and probiotics brining). The authors reported that the outcome of vaccination revealed that probiotic strain growth was at an increased level. Ready-to-eat products revealed significant differences in lipid profile and oxidation during the processing. The authors especially report an increase in the quantity of polyunsaturated fatty acids and a reduced amount of monounsaturated fatty acids, oleic acid, and lipid oxidation of *L. paracasei* IMPC 2.1. The authors concluded that probiotic strains inoculated with *L. paracasei* have potency in delaying lipid oxidation of fish fillets and could increase and retain polyunsaturated fatty acids.

Ibrahim et al. (2018) examined the effect of marination on *Vibrio parahaemolyticus* in tilapia fillets. There are five groups, which comprise two control groups and three groups with treatments (control 1 -damaging). Fish fillets were immersed in 300ml distilled water only. Group 2: control 2 (+positive) The fish fillets were inoculated with 107 CFU/ml of *V. parahaemolyticus* strains only and immersed in 300ml distilled water. Group 2: control 2 (+positive) The fish fillets were inoculated with 107 cfu/ml of *V. parahaemolyticus* strains only and immersed in 300ml distilled water. Group 3: the sample was inoculated with 107 cfu/ml of *V. parahaemolyticus* strains and immersed in

300ml of marinade (1) at a ratio of 1.5.1 (marinade: fish, ml/g). (Marinade consists of a mixture of (100 fresh lemon juice (50%), 5.9 g garlic powder, 9.23g table salt,0.5g turmeric, 0.15g hot chili powder, 0.5g black pepper and 200ml distilled water). Group 4: The samples were inoculated with 107 cfu/ml of *V. parahaemolyticus strains* and immersed in 300ml of marinade (2) at a ratio of 1.5.1 (marinade: fish fillet, ml/g). (Marinade 2 is like Marinade 1 but adds 4g/kg of thyme powder instead of lemon juice). Group 5 The samples were inoculated with 107 cfu/ml of *V. parahaemolyticus* strains and immersed in 300ml of marinade (3) at a ratio of 1.5.1 (marinade: fish fillet, ml/g). (Marinade 3 as Marinade 1 but the addition of pomegranate peel extract (PPE) (1% v/w). The authors reported a reduction in the counting of *V. parahaemolyticus* preserved with lemon from log 8.99 ±0.02 cfu/g to log ≤1 cfu/g on the 4th day as the count decreased by 100%. Meanwhile, there was also a reduction of 88.5% in the counting of *V. parahaemolyticus* in the group preserved with thyme powder from 4.86± 0.51 log cfu/g on the 5th day of processing. It was observed by the authors that there was also a reduction in the counts of the group preserved with pomegranate peel extract (PPE) (1% v/w) from 4.89± 0.38 log cfu/g in which the rate of reduction is 76.3% on the 5th day. The authors concluded that lemon juice proved more potent and viable in inhibiting the growth of *V. parahaemolyticus* at a 100% rate on the 4th day, which incubated.

Speranza et al. (2012) examined the Shelf-life definition for Italian anchovies inoculated with *Lactobacillus plantarum* and *Bifidobacterium animalis subsp. lactis.* The constituents of the brining solution to marinate anchovies were 2% acetic acid, 10% sodium chloride, and 200 ppm of extract from citrus. The anchovies were inoculated with probiotics bacteria, followed by a marination process. Then, the treatments were stored in three varied storage tanks (air vacuum, oil, and mixed brine solution) and then preserved at 4°C. The authors carried out analyses to examine the quality characteristics such as (odor, color, texture, and overall) and the viability of the probiotics strains was also investigated. They observed restriction in the components of sensory grades (especially in odor). The outstanding treatment was stored at four °C in mixed brine solution because the sensory attributes were preserved to the highest level between 6-10 days, and the preservation was seen beyond 21 days of storage.

Cosansu et al. (2010) examined the effect of a *Pediococcus* on the sensory properties and ripening of anchovy marinade at 4^{o} C and 16^{o} C. The marinate mixture comprises

2% acetic acid and sodium chloride 10%. The fish was submerged in marinate, which was kept inside glass jars. The fish ratio to the marinated mixture was 1:1.5. There were two apportion groups for the samples. Group A (control) was submerged in marinate in brine mixt, but without inoculation of *Pediococcus sp*., sp.13 culture kept aside and preserved at four °C or 16°C. Also, Group B was submerged in a marinate mixture, which consisted of *pediococcus sp*.13 culture. Immediately, this process was concluded. Anchovies were ripened in 2% acetic acid and 10% NaCl solution at four °C (group A) and 16°C (group B). Inoculated samples were marinated similarly at 4°C (group C) and 16°C (group D). The authors reported pH values of all groups to be lower than 4.5, and the mesophilic and halophilic bacterial counts are usually seen as 2-3 log cfu/g while the research was ongoing; this revealed that products could still be consumed as it is safe for humans. The appearance and taste of group D treatments were the best.

3.2 Applications of Sodium Chloride and Acetic Acid to Fish

Yeşilsu et al. (2021) studied the effect of partial replacement of NaCl with KCl on the quality of marinated anchovies. Marinate solution was prepared with acetic acid and potassium chloride (KCI), which was used instead of sodium chloride (NaCl) at varied percentages, respectively (50%. 70%, and 100%), and marinades of anchovy fish were produced. Anchovy marinades were kept at 4 six months, and observation was made for any alteration in pH, acidity, total salt content, appearance, texture, lipid hydrolysis and oxidation (free fatty acid, peroxide value, thiobarbituric acid reactive substances), total volatile elemental nitrogen (TVB-N), histamine, microbiology, and sensory characteristics. Alteration was observed in samples with 100% KCI. Anchovy's fillet marinades were produced with 100% NaCl, and this percentage displayed a meager peroxide value.

Meanwhile, the lipid hydrolysis was moderately high compared to the second batch towards the end of the processing period. Total aerobic bacteria, lactic acid bacteria, psychrophilic bacteria, and yeast mold development were lowered by 2 log cfu/g in both batches for marinades. Histamine level in anchovy marinades was (7.72-10.23ppm) this value is lower than tolerable points. Anchovy marinades with 100% KCI displayed lower points for sensorial analysis almost at the end of the preservation period,

especially in taste. The authors established that replacing NaCl with potassium of about 50% could be applied to marinating.

Valerievna et al. (2021) worked on improving the technology of chilled semi-finished products from Japanese mackerel with an extended shelf life. Inoculation of Japanese mackerel was conducted with the aid of a multiphase salt applicator medium, which consists of food supplements that retard microorganism infection and a bacteriostatic impact and aid in the enhancement of sensory features of prepared samples. The recent acceptance of the salt multiphase applicator element allows rapid salt application and makes the treated fish reach maturation more quickly than the sample without treatment. The absorption of salt in the fish muscle, amine nitrogen, and peroxide value was conducted through titration techniques. Microbiological analysis (total mesophilic aerobic and facultative aerobic) (KMAFAnM) was identified in marinated samples by plate count agar in nutrient agar medium. The treated sample of Japanese mackerel along the new multiphase salt applicator medium, liquid retention ability, organic acids, and flavor supplements that consist of anti-bacterial features, aid in the enhancement of sensory features of the marinated products, stimulate increased quantity of salt and ripening process of mackerel and also, slow down the numeration of microorganism (KMAFAnM). Amine nitrogen components increased from 1.4g/kg initially when the salt was applied to 1.55g/kg for the sample without treatment of about 3.05g/kg in treated fish when the salt application was concluded. Absorption of salt in Japanese mackerel of marinated samples with the addition of control salt medium resulted in 1.19%.

Meanwhile, the sample with the addition of a multiphase salt applicator ranges between 2.84% - 3.17%. The total enumeration of microbes obtained from Japanese mackerel was 2.0 x 102 cfu/g, and immediately after salt was applied, there was a reduction of 0.1 to 3.0 x 101 cfu/g. This was determined according to the formula for the salt medium application. The result of the findings revealed there was a variation during the application of salt; the developed salt applicator was concluded around 4 to 5 hours and with temperatures around six °C to 8 °C. The author reported that the salt applicator they manufactured, with the new technology of food package container, promotes chilling of culinary semi-finished Japanese mackerel with an extended shelf life for

about 20 days preserved under temperatures 0 °C to 5 °C, with moistening of 95% - 98%.

Gokoglu and Ucak (2020) determined the effects of the freshness grade of anchovy (*Engraulis encrasicolus*) on the quality of marinated products stored at 4^{o}C. According to the authors, anchovy fish (*Engraulis encrasicolus*) was split into two groups, which were (group A) and (group B); the first (group A) was stored at an ambient temperature of (20^{o}C) for six hours. The second (group B) was stored at 0^{o}C for seventy-two hours. Then, the anchovy was soaked in the marinate solution of 3% acetic acid and 8% NaCl. The authors reported that the Total volatile elemental nitrogen (TVB-N), trimethylamine (TMA), thiobarbituric acid (TBA), and para-anisidine (p-Av) values in the two groups of samples marinated significantly increased during storage at 4^{o}C. The authors stated that the increase in quality variables was higher in raw anchovy samples stored at (20^{o}C) when compared to the samples stored at 0^{o}C. Though the sensory results of the two groups were reduced during storage, high results were attained for samples stored at 0^{o}C in contrast to samples stored at ambient temperature. The authors concluded that based on the result achieved in their studies, the quality of raw material significantly ($p<0.01$) impacts the quality of marinated anchovy.

Turan et al. (2017) studied the interaction between rancidity and organoleptic parameters of anchovy marinade (*Engraulis encrasicolus L.* 1758), including essential oils. The oxidative and sensory properties of anchovy marinades were produced with 10% NaCl, 4% alcohol vinegar, 0.2% citric acid solution, and 0.1% varied essential oils. Batch A, which stands for control, consists of sunflower seed oil. Batch is Batch B: sunflower seed oil with the addition of 0.1% rosemary oil, Batch C: sunflower seed oil with the addition of 0.1% coriander oil, Batch D: sunflower seed oil with the addition of 0.1% laurel oil, lastly Batch E: sunflower seed oil with the addition of 0.1% garlic oil. While preservation is ongoing, lipid oxidation was investigated, such as (2-thiobarbituric acid reactive substances (TBARS) content of batch A, which stands for control, showed a significant difference with a higher value than all the batches, which consists of necessary oils. The findings revealed that the essential oil possesses an inhibitory ability on lipid oxidation formation. This ability was observed to be high in laurel oil within the first 3 months; likewise, laurel oil and rosemary oil experienced like manner in the 4th month, and all the batches with essential oils for those six months of

preservation. L*(brightness) scores were almost the same for all batches in the 4th month, though by the 5th and 6th months, the batches with laurel oil displayed a tremendous result. Yellowness (b*) looks the same for all the batches for the first 3 months; meanwhile, a decreased level was recorded by adding laurel and rosemary oils. The authors established that the marinade process by adding 0.1% laurel oil of anchovy could inhibit lipid oxidation and enhance the sensory characteristics while fish were preserved in the fridge.

Szymczak et al. (2019) evaluated the reuses of brine to enhance the ripening of marine and freshwater fish resistant to marinating. Atlantic herrings, cod, salmon, and trout were marinated for seven days; the brine solution was first sieved in 50μm filtration material and later proceeded to 0.22 μm size. The maturation process for marinating products in fresh brine (FB), while the final combination of sodium chloride and acetic acid was at 6% and 5% separately, stands for control. The constituents of the brine solution include rock salt, tap water, and 80% of vinegar. Fish fillets (1000±10g) were poured in 2 dm3 in a glass jar containing fresh brine reused and brine. The procedures of marinating were carried out in three duplications at 7^{o}C. The proportion of fish to brine used was 1.5.1 (w: w). At the end of seven days, the fresh and reused brine procedures of marinating were concluded. Microfiltration was utilized through filters produced from glass fiber. NaCl and acetic acid were poured in percolate and separately measured at 6% and 5% absorption levels. Hygienic objects were applied at every phase of distillation procedures at a temperature of 5±1oC. The reused brine solution was demonstrated as the outstanding sample for the enhancement of maturation of fish high in starch (salmon and trout). Both are regarded as the fish that showed the highest level of defiance during marinating procedures.

Meanwhile, for cod, there is no significant level of enhancement in the maturation of the fish flesh. Hence, cod was recommended as the second level of defiance. The repetition of brine solution leads to a lowered result of lipid oxidative values, flesh firmness, high sensorial grades for the general organoleptic analysis, and a rising total volatile vases nitrogen value. But this does not imply cod fish.

Uçak and Gökoğlu (2017) determined the high hydrostatic pressure's impact on marinated herring's sensory quality (*Clupea herangus*). Fish fillets were dipped into

roughly 8% sodium chloride and 2% acetic acid marinade. The samples were loaded under vacuum at different weights of 100, 300, 500, and 600 MPa for five to ten minutes. Sensory panelists conducted the organoleptic test. Treatments were evaluated using a 9-point hedonic measure based on their overall acceptability, smell, color, taste, and texture. Comparing the results with the samples that were not treated, it was found that the organoleptic variables measured at high hydrostatic pressure and processed under 500 MPa did not change. The panels have not approved marinated fish with 500 and 600 MPa. For samples with 500 and 600 MPa, pressure impacts the results produced by taste, texture, and color. The results showed that 100 and 300 MPa were the ideal sample values for the variables.

Topuz (2016) studied the quality of fillets of small tunny fish (*Euthynnus alletteratus*), which is affected by the length of marinating, the amount of acetic acid, and the salt content. Studies were done on fish muscle to determine how long the marinating period, acetic acid, and salt affected the fish weight, shear force, amount of fatty acid (Omega-3), and organoleptic characteristics. Techniques utilizing response surfaces were used. Changes in the methods significantly affected the studied characteristics, including weight turnout grades, shear force, and total acceptability. The weight result, shear force, and total acceptability scores were all significantly changed by the acetic acid and salt absorption of the marinate mixture; however, the period or duration of marinating did not vary significantly. The densities of fish muscle marinades were increased when the absorption of acetic acid and sodium chloride was reduced over a stable 36-hour marinating period. There was a slight increase in the proportion of omega-3 fatty acids when NaCl and acetic acid absorption were reduced. Still, there was no discernible change in the proportion of omega-3 fatty acids in fish muscle marinades. The optimal marinating conditions included 30.28 hours; the absorbance of acetic acid was 1.1 ml/100 ml, and salt was 6.2 g/100 ml. Density resulted in 87.9±0.8% with this conditioning factor, and the data aligned with expected values.

Kindossi (2016) studied optimizing the marinating conditions of cassava fish (*Pseudotolithus sp.*) fillet for Lanhouin production through the application of Doehlert experimental design. The authors incorporated sodium chloride, citric acid concentration, and duration of marinate alongside the physicochemical and microbiological properties of the fish muscle sliced to produce Lanhouin, which was

investigated through the application of Doehlert experimental design, which was aimed at the conservation of fish quality and safety. Their findings revealed that marinate duration had a noticeable impact on the total viable and lactic acid bacteria counts and sodium chloride concentration of marinate fish muscle; the pH was significantly altered by adding citric acid content and marinate time with a high regression coefficient R 2 of 0.83. The salt concentration between 10g and 100g, with acid citric content of 2.5g/100g, proved to be the greatest and was processed for marination for six hours. The ideal marinate environment proved the perfect quality for fish muscle, producing a safe result for fermented fish.

Szymczak and Kołakowski (2016) study the total volatile elemental nitrogen (TVB-N) in meat and brine during marinating herring. The authors assessed the effects of raw materials on marinate herring quality. 5% salt was applied to 15% of the water, and acid contents were used to 3% to 8%. They observed an increased TVB-N level in herring fillets marinated. A lower level was obtained from carcasses and the herring freeze than the unfrozen herrings. High contents of sodium chloride decreased TVB-N levels; meanwhile, high levels of acid did not cause significant effects on the TVB-N of the fish flesh. The result that stands out for the fresh fish index was NH3-N, also tyrosine in brine; this could be regarded as the global index of fresh fish found in the marinate of herring muscle. The circulation from herring muscle to brine indicated a significant reduction in the muscle TVB-N of herring; this was achieved through the exchange of brining solution repeatedly; the author reported this action could be the best alternative instead of an increase in the level of NaCl and acetic acid of the brining solution use for marinades.

Kaya and Baştürk (2015) work on determining some quality properties of marinated sea bream (*Sparus aurata L.*, 1758) during cold storage. The authors submerged fish fillets inside a brine solution made up of 3.5% acetic acid and 11% salt, and the ratio ranged between 1:1.5 (fish: marinates brine) for the marination procedure. Immediately when the ripening was completed, the fish samples were divided into two batches and kept in plastic packets; one batch was mixed with only sunflower oil, while the second batch included sunflower oil and sauce that was produced. In the preservation period, the following analysis was carried out: sensory, crude protein, lipid, dry matter, crude ash, TBA, TVB-N, TMA-N, and peroxide. The authors reported the outcome of their studies

for each analysis of 200days of preservation; TVB-N value measured for sea bream marinates kept with only sunflower oil and sauced was 15.86/14.89 mg/100g, TBA 7.06/7.99 mg MA/kg, TMA-N 2.97/3.12, mg/100g, the value of peroxide was 7.23/7.45 meq/kg accordingly. Based on the analyses of the fish for chemical and sensory purposes, the outcome shows that sea bream marinates for only sunflower oil treatment, and the mixture of sunflower oil and sauce could be preserved at +4 °C for 200 days.

Çağlak and Karslı (2015) studied the shelf life of rainbow trout (*Oncorhynchus mykiss,* Walbaum 1792) marinated and brine-injected under refrigerator conditions was determined. Their results on the character change that occurred in rainbow trout (*Oncorhynchus mykiss*) after they were injected with a brine marinade and a salt brine solution during refrigeration storage (33 ±1ºC). The fish was separated into three batches: the fresh samples, the infected batches (4% acetic acid - 8% NACL), and the bringing batch (20% NACL). The approximate composition of the trout kept as a control was 16.83%, 4.18%, and 78.04%. Chemical examination revealed that the total volatile elemental nitrogen (TVB-N) and thiobarbituric acid (TBA) readings were below the maximum threshold while being stored. At the end of the preservation procedure, the TBA and TVB-N results from the fresh batches, treated samples with marinate inoculation, and brine inoculation batches indicated 20.42 mg/100g, 0.35 mg, MA/kg; 21.70 mg/100g, 0.30 mg/KG; and 17.60MG/100G, 0.30 mg ma/kg. Enumerations of total coliform were performed as follows: 4.34 cfu/g for total mesophilic aerobic bacteria (TMAB), 4.34 cfu/g for total psychrophilic aerobic bacteria (TPAB), and 3.07 cfu/g till the end of preservation. On day eleven of the analysis, the sensory results of the cooked and uncooked trout revealed lower values of quality for the fresh batches. Still, on day thirteen of the study, the treated group with batches inoculated with marinades and brine showed higher values of quality.

Erdem et al. (2015) determined the effects of different packaging methods on sensory quality and the chemical criteria of marinated shad (alosa I*mmaculata*, b., 1838). Fish marinating was carried out using many packaging techniques (submerged in brining solution, oil, and vacuum packaging) and was kept at 4±1°C. While the samples were stored, the dissemination of proximate constituents, acetic acid, and NaCl in fish was analyzed. When the preservation was concluded after seven months the outcome of analysis for parameters such as TVB-N was 8.05, 16.81 and 17.56 (mg/100g), TMA

2.28,2.53, and 2.73(mgl100g), TBA was 7.08,7.13 and 6.05 (mg malonaldehyde/kg) also, pH result seen was 4.42,4.72, and 4.77 for sample submerged in brine, oil solution, and vacuum packaging, independently. For the whole duration of preservation, there were significant varieties in each of the storage methods used on the TVB-N, TMA, TBA, pH, water activity, NaCl, and sensorial matters was significant ($p<0.05$).

Ahmed et al. (2015) examined the quality changes and microbial loads of marinated tilapia (*Oreochromis niloticus*) kept at refrigeration temperature. The varied concentration of acetic acid is applied differently to identify tilapia bacterial loads and its highest sensorial characters kept at 4^{o}C. The marinate type carried out was warm marination, and the solution was prepared with 2%, 3% acetic acid, and 3% NaCl, which was later moved and kept in the plastic jars, and 3% gelatin was used to cover it. Also, the cold marinate solution was produced with 5% acetic acid and 6% sodium chloride, and the mixture was kept for 30 days, which was later kept inside a packaging solution containing 2.5% acetic acid and 3% NaCl. The authors reported a higher concentration of bacterial loads shortly after day 7 of the processing in the warm marinades of filleted tilapia. An excellent sensory quality was observed within day 3 of storage with 2% acetic acid. Observation in cold marinade fillets revealed that there are decreased loads of bacteria after the 3rd day of inclusion of marinating solution till the end of the marinating procedure and packaging of filets with a solution of 2.5%acetic acid. The authors recorded a better sensorial quality for cold marinates than warm marinates. For the pH, the value recorded showed no significant difference (p >0:05) during tilapia preservation and processing.

Baygar et al. (2014) detected the specific parameters affecting the maturation of farmed sea bass (*Dicentrarchus labrax*) fillets stored in sunflower oil. Three batches of skinless, scaly, and scaleless anchovies, 2.5% acetic acid, and 11% NaCl were applied on sea bass fish in a pickling solution and incubated at four °C (±1). Anchovy flesh was divided into batches, and measurements such as pH, moisture %, acetic acid%, and sodium chloride % were subjected to the maturation pickling process with sunflower oil. For 90 days of storage, there were no significant changes in the anchovy meat produced. There was an observation on the skinless batch with low sodium chloride and acid concentration, while the batch with scale and scale less showed high values. This observation on the scaly and scale-less batch results from the anchovies' skin,

preventing the pickling solution and the oil from penetrating the flesh of the samples. The authors concluded that the batches of anchovies with a high percentage of sodium chloride and acid concentration are suggested to be suitable for the marinating procedure in determining shelf-life and quality.

Kurt Kaya (2014) examined the organoleptic and chemical changes while storing sea Bass marinades (*Dicentrarchus labrax L.*, 1758). Marinate constituents include 11% sodium chloride and 3.5% acetic acid. Shortly after the completion of the marinating process, the ratio of marinating solution to fish solution was 1:1.5 (fish: marinating brine); the fish fillets were divided into two batches; one batch was kept as plain, and the second batch was kept in sauced preserved in a closed plastic box for a period of 200days. The authors reported an acceptable sensory result for the two batches (plain and sauced) throughout the study period. However, the panels more accepted marinated fish fillets kept as plain. They also reported that the chemical outcomes, TVB-N, TBA, TMA-N, and Peroxide values, were all within acceptable limits for the whole period of research (200 days). Therefore, the authors concluded that Seabass fillets marinated and kept as plain and sauce could be preserved for 200 days and retain their qualities without spoilage.

Turhan et al. (2013) reported the effect of ultrasonic marinating on the transport of acetic acid and salt in anchovy marinades. Anchovy fillets were submerged inside a marinate solution which consists of 4% acetic acid and 8% NaCl and was cured with the steady marinating procedure (20KHz ultrasound on varied ultrasonic magnitude (20, 25, and 30 W/cm2) and the process was carried out at varied duration (10, 20, and 30 minutes). Generally, water movement is altered through ultrasonic marinates in anchovy marinating, and the water level is reduced during the rise in ultrasonic magnitude. The ultrasonic marinate stimulates acetic acid and sodium movement, which is determined by the ultrasonic magnitude and duration of the marinate. The author established that their finding outcome showed that anchovy marinades were treated with 30W/cm2 of ultrasonic magnitude. This marination of ultrasonic magnitude showed significant changes while the pH value was significantly reduced. The authors established that 30W/cm2 of ultrasonic magnitude marination procedures can improve acetic acid and NaCl movement and reduce anchovy marinades.

Alpaslan et al. (2013) examined the effects of descaling and skin removal on chemical changes and sensory attributes of marinated sea bass fillets (*Dicentrarchus labrax*) in sunflower oil kept at 4ºC. Samples were separated into three batches; no treatment was added to a batch(control); the second batch was descaled and filleted, while the third batch was deskinned and filleted. The three groups of samples were submerged into an ice-water solution, with a ratio of (1:4v/v) for 1 hour. The marinate is made up of 2.5% acetic acid and 11% NACL; the solution was added to filleted seabass and put in 5000ml glass jars and covered and kept in the refrigerator at (4 ±1) °C for the marinating process, application of brine and marinate was concluded after 72 hours. The sensorial findings revealed that skinless sea bass fillet samples reached the eatable limit on the 70th day, but the batch scale and the scale less could not reach the limit at the end of the storing period of 90th days. Throughout preservation, the result of TVB-N and TMA-N was lower than the eatable limits; TBA got to the eatable limits of 8 mg malonaldehyde/kg on the 56th day for skinned fillets and at the 70th day for the batch that was descaled fillets. The TBA value for the batch with a scale never got to the control level within the 90th days of the preservation period. Based on their findings, the authors concluded that the batch with scale and descaling sea bass samples were suitable for marinate processing in brightness, juiciness, softness, acid and salt transportation, and sanitary. Some disadvantages regarding texture were also seen in the descaling batches; the findings also suggest that the scale impacts the shinning of the brining solution and results in an unwanted coloration the more the scales are attached to the external part of the fish.

Cappacioni et al. (2011) investigate the acid and salt uptake during the marinating process of (*Engraulis anchoita*) fillets, the influence of the solution: fish ratio, and agitation. The authors examined the period of subjecting the fish to solution and attributes of fish sensory; the fish ratio applied was 0.77:1, 3.1, and 10:1 were both carried out; one batch was done with agitation, and the second batch was not agitated. The results stated that the fish ratio facilitates an increased acid and salt absorption rate as the marination mixture rises. The batch of marination mixture with a 10:1 ratio yielded a dry and tough texture and was lightly salty when tasted. The result of salt absorption was revealed to be significantly lower than ($p<0.01$) in the batch that was agitated. The authors note that there is no effect of agitation on the acid uptake, and the rate of slat absorption was reduced, but there is rancidity of fish marinades. The batch

with a ratio of 3:1 minimizes the period of marination. It does not alter the sensory characteristics, and the authors concluded that this later batch can be applied to fish marination procedures.

Šimat et al. (2011) investigated the effect of different marinating baths on cold-marinated anchovies' sensory properties and shelf-life parameters (*Engraulis encrasicolus, L*). The combined impacts of acid and salt percentage perform crucial functions in marination procedures. The following factors are considered to ascertain the fish marinades' longevity, retain their qualities, and obtain great sensorial features from marinated products. They are as follows: appointment of effective methods for preparing raw fish material to carry out marination, the components, and the storing environment of marination. The authors examined twenty-two cold marinated fish samplings, which consisted of marination baths of varied constituents and (varied ratios of vinegar, alcohol vinegar, salt, and water); for the determination of the alterations in sensorial properties while the process of maturity was on; also, the shelf life factors. To achieve this, water activity, sodium chloride level, pH content, sensory characteristics of anchovy fillets, volatile amines level, and ratio of lipid oxidation were analyzed. The marination bath with 45% H20,30% vinegar, 5% alcohol vinegar, and 7% salt achieved the most excellent sensorial points, a pH level of 3.09, 3.60% sodium chloride, and water activities 0.84. The buildup of weaker volatile amines while storing components was (<20mg TVB-N / 100g; <1mgTMA/100g) and the TBA value was (6.41 to 7.36 malondialdehyde/kg) which indicates a rise in significant level; all these values were recorded during the process of maturation. Meanwhile, lipid oxidation was apportioned for sensorial characteristics in marinates of anchovy fillets.

Baygar et al. (2010) investigate the effects of pickling solution on the maturing and storage time of marinated sea bass (*Dicentrarchus labrax*) fillets. Sea bass fillets were kept inside a marination solution of 2.5% acetic acid and 11%NaCl at (4 ± 1) ºC for ten weeks. The following variables were examined on fish fillets: pH, moisture, acetic acid, and NaCl. In the cold storage, scaled fillets matured after 48hr and scales and skinless fillets matured in 30 hrs. The author noticed crucial changes after the fish samples had been kept in the marination solution. After that, fish fillets were kept in a marination solution, and acetic acid and NaCl levels in fish fillets increased within 1 hr—contradictory, moisture, and pH. Level reduced. The author stated that the quick

reduction in the pH was seen after 2hrs. The acetic acid of scaly fillets was decreased after 48 hours of the marination process. There is a reduction in the acetic acid of skinless and scale fillets after 30hrs it was marinated. NaCl has the lowest level of contents within the sea bass fillets, and storage time was achieved in skinless fillets.

3.3 Application of Herbs and Spices to Fish Marinade

Ochrem et al. (2021) studied the impact of marinated herring (*Clupea harengus L.*) meat on the addition of milk thistle (*Silybum marianum L.*). The samples were divided into three batches: batch C was the control, in which milk thistle was added to marinades after the herring fillets had been skinned. In the second sample, the milk thistle was applied to the fillets' surface before the marinade (4%w/w - wet tissue weight), and it was vacuum-packed for 16 hours to allow the milk thistle to penetrate the fish fillets. The last sample batch, which did not contain milk thistle, was vacuum-sealed and maintained at 48°C. After 16 hours, the process of marinating was completed. The control batch's marinades contain 5% acetic acid and 8% sodium chloride; the second batch, known as (MH), contains 5% acetic acid, 8% sodium chloride, and 4% milk thistle, and the third batch, known as (FH), contains 5% acetic acid and 8% sodium chloride. The results of their chemical analysis showed that the control batch's peroxide value was higher than the allowable limit and that, on the first day of the analyses, the control sample had a higher value of thiobarbituric acid reactive substances.

In contrast, the third batch (the FH batch) had the lowest values. The application of milk thistle achieved low peroxide values and a high concentration of components comprising polyunsaturated fatty acids. The authors concluded that adding milk thistle to the marinade solution improved the qualities of the items made by marinating.

Fıçıcılar and Genccelep (2020) researched the influence of garlic and hot pepper sauce on the quality of marinated anchovies during 7 months at four °C storage. They carried out the marination of anchovies with the inclusion of 3% garlic and 3% hot pepper sauce; the mixture of garlic and hot pepper sauce was poured into plastic jars and vacuum-packed to determine the impacts of garlic and hot pepper sauce on fish marinades shelf life was examined through the analysis of treatment, determination of physical, chemical and microbiological test. Total viable bacteria count (TVB-N), lactic

acid bacteria count (LAB), *Enterobacteriaceae* count, biogenic amines (histamine, tyramine, cadaverine, putrescine, tryptamine), pH, total volatile elemental nitrogen (TVB-N), Thiobarbituric Acid (TBARS) was carried out for seven (7) months while anchovies were stored at 4°C. The control batch was analyzed and compared with garlic and hot pepper sauce samples. The batch with the inclusion of garlic decreased the multiplication of microorganisms, and the physicochemical features (TBA, TVB-N) were maintained for a long duration. The authors also reported that the quantity of biogenic amines examined was reduced in the sample in which garlic was included.

Kocatepe et al. (2019) investigated the influence of different essential oils on marinated anchovy (*Engraulis encrasicolus L*. 1758) during refrigerated storage. The marinating process of anchovy was carried out for 24hrs at 4 ± 2 °C in a brine solution which was made up of (10%) salt, alcohol vinegar (4%) and citric acid (0.2%), and the solution was kept for 6 months at 4 ± 2 °C in sunflower oil, their studies are made up of five different groups of different oils that were used, which are group A – E, group A (sunflower oil), group B is (rosemary oil 0.1%), group C (0.1% coriander oil), group D (0.1%laurel oil), group E (0.1% garlic oil). Their result showed that for the 6 months storage period, TVB-N and microbiological analysis showed that for group A-E anchovy freshness was retained, and group E, which was preserved with garlic, showed the lowest TVB-N ratio and minimum total mesophilic bacteria count at the end of the period, they were stored.

Bilgin Fıçıcılar et al. (2018) searched the effects of green tea (*Camellia sinensis*) and bay leaf (*Laurus nobilis*) extracts on the physicochemical characteristics of vacuum-packed marinated anchovies. The effects of green tea and bay leaf extract on anchovy fish marinated in a solution were evaluated by looking at their microbiological, sensory, and physical properties. The wine vinegar (4.2%), NaCl combination (9%), and maturation time (24 hours) were combined to create the marinating solution that was applied to the anchovy fish. The fermentation process was then carried out in the processing area at 10°C. The components were mixed into the marinating mixture, and the anchovies were marinated after sieving. The products were vacuum-packed and stored at 4 °C for 240 days. According to the authors, adding bay leaf extract and green tea reduced the number of microorganisms, the total volatile base-nitrogen rate, and the amount of thiobarbituric acid reactive substances. They also found that green tea extracts functioned as a functional indicator of lipid peroxidation and produced a dark

coloration that deters consumers. The authors concluded that green tea and bay leaf extracts do not alter the aggregation of biogenic amines in anchovies with marinating mixtures. The biogenic amines recovered from the anchovy were significantly reduced due to lowered acid content and sound production.

Kaba et al. (2014) determined the fish ball's shelf life after marinating and frying. The anchovies were cooked, boiled for five minutes, and blended to produce minced fish. Other ingredients used were semolina, 0.60 percent crumb, 0.83% egg, 1% parsley, 1% onion, 0.10% garlic, 0.08% salt, 0.03% cumin, 0.03% red pepper, 0.03% thyme, and 0.03% ginger. A small portion was taken out of the meatball, combined with a fork, dipped into hot oil, and deep-fried. Subsequently, the fish ball was fried and cooled before being preserved for 150 days. Garlic, mustard seeds, white pepper, and parsley were added after the fish ball had been submerged in a solution of 7% NaCl and 1.5% vinegar. Fish balls stored at 4±°C underwent analysis. When the analysis was finished at the end of the preservation, the prepared fish's TVB-N, TBARS, TMA-N, and pH values were recorded as 13.66 mg/100g, 5.68 mg MA/kg, 5.63 mg/100g, and 3.42. The microbiology analysis's results were also within an acceptable range, and the fish ball's sensory result was approved, keeping it edible for 135 days at4±°C.

3.4 Application of Fruits and Vegetables as Additives to Fish Marinade

Gülderen et al. (2018) determined the sensory and chemical qualities of marinated African catfish (*Clarias gariepinus*, B., 1822) preserved in oil and tomato sauce. The authors carried out produced marinade fish by preparing a marination solution, and the catfish was filleted and kept inside ventilated plastic boxes; one sample consisted of only sunflower oil (batch A), and the second sample was sauced consisted of sunflower oil and tomato sauce (batch B) after that both samples were kept at +4°C for 200days.while the storing process, sensory and chemical qualities were determined. The findings revealed that TVB-N value was between 15.82/16.29mg/100g, TBA falls between 4.94/4.81mg MA/KG, the value 3.57/3.40 meq/kg, pH 4.35/4.36 in batch with marination solution preserve with only sunflower oil and the batch sauced .they reported that African catfish marinated and kept without sauce and with sauce (batch A and batch B)could be preserved at +4°C for duration of 200days and the chemical properties was retained throughout the storage time. The shelf-life of catfish marinades recorded was

110 days for batch A, and Batch B's shelf-life was 80 days based on the outcome of the sensory analysis.

Gokoglu et al. (2012) studied the effects of tomato and garlic extracts on oxidative stability in marinated anchovy. Tomato and garlic extract were applied to anchovy fish to see its impact on the formation of rancidity. The authors conducted a marination process of 2% acetic acid, 10% salt, and 6% plant extract for thirty -two hours. Anchovy oil was distilled from the fish after completion of marination, which was stored at 4^{o}C. Their findings revealed that peroxide, para-anisidine, conjugated diene, UV absorbance, and free fatty acids values analyzed from marinates of anchovy alongside plant extracts were less than those obtained from the untreated samples. The authors established that the extracts obtained from tomato and garlic have the potential to retard rancidity in anchovy marinades. Also, the effectiveness of tomato extracts was higher than garlic extract to inhibit rancidity.

Yesilsu et al. (2018) conducted the 16S rRNA gene sequence analysis to identify the lactic acid bacteria from spoiled marinated anchovy (*Engraulis encrasicolus*). The writer made a brine solution to separate the lactic acid bacteria by mixing 10% sodium chloride and 20% fresh persimmon vinegar. Soaked in the brine solution, 1500g of anchovy fillets were stored for 72 hours at 4°C. Sunflower oil was utilized to preserve the fish fillets and the plastic wraps that held them underwater. After that, the generated samples were kept for six months at four °C to allow the bacteria to proliferate. They discovered twelve different bacterial strains in the marinated anchovies. They found that the marinated anchovies they made included twelve different bacterial strains. 16S rRNA gene sequence analysis was used to extract the bacterial strains. Two main species were identified: *Lactobacillus curvatus* and *Lactobacillus paracasei subsp. paracasei*.

Fernandes (2016) studied the effect of different marination procedures on the quality of dried blue whiting (*Micromesistius potassium*). The authors examined the impacts of marination, which consisted of several constituents of soy sauce, sorbitol, sucrose, and citric acid, on blue whiting before and after drying. The effects on sensory characteristics such as (smell, structure or forms, and aroma), microbiological characters such as (Total viable counts, Listeria, and coliforms), Physical and chemical

composition (pH, water activity, TVB-N), and proximate constitutions (moisture protein, sodium chloride and fat) were analyzed. The authors compared crude (fish without marination) blue whiting applied as a control. The author reported an outcome that revealed several marination product additives and proportions utilized to distinguish the impacts on the sensory characteristics of dried blue whiting fish. The sweet aroma was significantly enhanced by sucrose in combination with soy sauce. At the same time, the citric acid improved the concentration of the sour aroma with a significant difference. The smell characters are likewise affected by the miniature control caused by soy sauce. Authoritarian structures or forms were discovered in the marinade product, while the group with marination solution resulted in a dried structure, aroma, and smell. Marination additives likewise caused alteration in the appearance; the addition of soy sauce influenced the red color, while the citric acid reduced the red appearance of the marinades. When drying was completed, the total volatile elemental-nitrogen concentration was decreased in the dried marinades compared to the sample without marination solution (dried sample). The batch without marination (control) revealed high numbers of bacteria when it was dried, and this was more than the results of the batch with marination solution; the author noted the marinating process caused the effect; likewise, cleaning could have led to the reduction in the number of bacteria. Their results revealed decreased pH in fish due to the impact of citric acid during the procedure of marinates, which yielded significantly reduced pH in the dried sample without marinates (control); the authors proposed that the sublimation of cellulose while the marinating process was carried out in several other kinds of fish samples restrained liquid.

Topuz et al. (2016) investigated the effects of olive oil and olive oil–pomegranate juice sauces on marinated anchovy's chemical, oxidative, and sensory quality. The olive oil and sauces made from emulsified olive oil and pomegranate mixed alongside gums were combined alongside fish anchovy fillets (*Engraulis encrasicholus*) already processed with marinate. The authors determined to make innovation on polyphenol-rich marinated sauces through emulsification of pomegranate juice alongside olive oil prepared with varied percentages, which are (25%, 35%, and 50% v:v). The authors expressed that to analyze the impacts of olive oil and olive oil- pomegranate sauces on characteristics of anchovy marinated, chemical analysis such as (TVB-N and TMA), oxidation such as (peroxides value, K230, thiobarbituric acid and K270 and sensorial

characteristics was determined while the products were stored at 4°C. The results of the investigators revealed that anchovy sauced alongside olive oil-pomegranate sauce could cause retardation of unwanted characters, alteration, delay rancidity and enhance the sensory characteristics.

Serdaroğlu et al. (2015) studied the quality changes of sardine fillets marinated with vinegar, grapefruit, and pomegranate marinades. The fillets of Sardine (*Sardina pilchardus*) were marinated using three different marinade solutions produced with vinegar (VM), grapefruit juice (GM), and pomegranate juice concentrate (PM). All marinade compositions comprise 4% salt and 0.1% black pepper. Samples were kept at 4ºC for 28 days. Proximate composition, pH, acidity, salt content, moisture content, penetrometer value, peroxide value, and TBARS value of marinated samples were examined during storage of 28th days. Sardines marinated with grapefruit juice had the highest pH value at each investigation time. On day 28, the highest acid value (0.94%) was obtained in VM samples, and the lowest (0.62%) was obtained in GM samples. No significant differences in salt content were found between marinade samples at each investigation time except day 28. GM samples had higher (softer in texture) at each evaluation period, and PM samples had lower penetrometer values (more complex in texture). PM samples had the lowest peroxide and TBARS values at each evaluation period. The authors established that pomegranate sauce could be applied as a functional marinade solution in sardine Marinates.

Maktabi et al. (2015) investigated the effects of traditional marinating on bacteria count and chemical characteristics in frozen rainbow trout fillets. The authors determined to assess chemical and microbiological alterations enhanced by applying conventional marinate procedures to rainbow trout flesh while stored in the freezer. Rainbow trout fillets were submerged in conventional marinates and kept at -18 °C for 56 days. The investigators did the following evaluation: analysis, such as chemical (TVB-N, TBARS), microbiological (mesophilic, psychrophilic bacteria counts), water holding capacity, and pH, was determined every seven days. Their examination revealed that the value of TVB-N, TBA, and water-holding capacity in the group with marinates showed higher values than the group without marinates; the authors recorded that the differences were insignificant ($p>0.05$). The values of bacteria in the group with marinates showed decreased numbers, while the group without marination showed higher values, and the

psychrophilic bacteria count was significantly different ($p<0.05$). Hence, they concluded that rainbow trout muscle has the potential for marinating, processing, and keeping at -18 °C for 56 days without significantly altering the qualities.

Giarratana et al. (2015) studied the effect of allyl isothiocyanate against anisakis larvae during the anchovy marinating process. Allyl isothiocyanate is an organic synthesis occurring from vegetation that originated from Cruciferae and possesses decisive antimicrobial and biocidal actions against vegetation parasites. Anisakidosis is a disease that can be transferred from animal to human; it occurs through the consumption of larvae of nematodes in fresh, half-cooked, brined, or seafood marinades. The authors investigate the impacts of the plants on the parasite larvae and how effective it is to be applied when carrying out marinate procedures. The experimental design was carried out through 3 different techniques; the first one was invitro with the inclusion of 3 media solutions, the second was with semisolid media with the inclusion of blended anchovy fillets, and the last one was an imitation of 2 types of anchovy muscles to produce marinades products. Their findings revealed that the Allyl isothiocyanate levels in all the tested samples were 0, 0.01, 0.05, and 0.1%. The action of Allyl isothiocyanate over parasitic nematode larvae was noticeable in the second technique (semisolid media) in a high proportion. Initially, anchovy muscle was submerged in phosphate buffer solvent (1.5% NaCl, pH 6.8) alongside 0.1% *Allyl isothiocyanate* marinated in an ideal environment, leading to a higher level of larvae unstimulated. The authors concluded that *Allyl isothiocyanate* is an excellent choice for examining the effectiveness of biocidal activity on Anisakis larvae while carrying out marinated procedures in commercial places.

Demirok et al. (2014) determined the proteolytic and sensory changes during marinating rainbow trout (*Oncorhynchus mykiss*) flesh in pomegranate juice. Marinate of rainbow trout sliced muscles was conducted within 12 days and was stored at four °C inside three varied marinate solutions proportions: 100% acetic acid solution (AAS), 50% acetic acid mixed with 50% pomegranate juice mixture (AAPJS), and 100% pomegranate juice solution (PJS). The authors' investigations revealed that the level of the moisture and pH of rainbow trout muscle reduced; meanwhile, the proximate analysis (ash, protein, fat, and salt content rose towards the end of the marinate procedure in all the samples. The sample with 100% pomegranate solution (PJS) was

seen to be dark, reddish, and decreased in yellow color quality; this is a result of the movement of color pigments from the material (pomegranate juice) ($p < 0.01$). Generally, fish fillets with marinate solution usually have a high total free amino acid quantity ($p < 0.01$) as a result of the high content of proteolytic disintegration lower than the lowest pH solution; this situation can also be observed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis image. The sample revealed high organoleptic grades compared to other fish muscle samples with 50% or 100% Pomegranate juice marinate. Additionally, the fish sample with acetic acid pomegranate juice solution (AAPJS) and pomegranate juice solution (PJS) presented to be firmer in texture and sweeter in taste than the samples incorporated with the marinating solution of acetic acid.

Topuz et al. 2014 examined the effects of olive and olive oil–pomegranate juice sauces on marinated anchovy's chemical, oxidative, and sensory quality. The authors mixed olive oil and pomegranate juice sauce with gums and added the mixture to anchovy (*Engraulis encrasicholus*) fillets already submerged into a marinated solution. Polyphenol-rich marinate mixture was carried out through emulsification of pomegranate juice and olive oil alongside varied percentages such as (25%,35%, and 50% v/v). Chemical analyses like (TMA and Total volatile elemental Nitrogen), PV, K230 Thiobarbituric acid, and K270) also conducted the sensorial assessment while the samples were stored at 4^{o}C. Their result proved that a mixture of olive oil and pomegranate sauce applied on anchovy to produce marinates can inhibit unwanted character alterations, extend oxidative degradation, and enhance sensory characteristics.

Adeyemi et al. (2013) examined how a marinade made of moringa oleifera affected the microbiological stability of African catfish that had been smoke-dried (*Clarias gariepinus*). Marinade solution was prepared from three concentrations of *Moringa oleifera* (MOM) which are 1%, 2%, and 3% (W/V), and brine(w/v) solution of 5% into each of the concentrations, and there is another group which was the control. The fish were rinsed with water degutted and assigned to each marination group. Then, the fish were submerged in the marination solution for two hrs. and then removed, and hot smoking of the fish was carried out for 12 hrs. Subsequently, the smoked salmon was stored on a laboratory shelf at room temperature (37±2oC) in a netted box for two months. Six fungal species—*Cladosporium sp, Neurospora crassa, Candida sp,*

Aspergillus niger, and *Saccharomyces cerevisiae*—and seven bacterial species—*Staphylococcus sp, Bacillus sp, Klebsiella sp, Corynebacterium sp, Pseudomonas sp, Escherichia coli*, and *streptococcus sp*—as well as six fungal species—were isolated in the study, according to the authors. During the processing period, there was a continuous growth in the population of microbes, and the highest microbial load was observed in the control group, which was processed with brine—reduction in microbial loads. Fungi counts were seen in all groups of MOM and 5% brine than what was observed in the control group. The result of Antibacterial was high in the group with 3% MOM, while 5% brine showed high counts of antifungal. The authors concluded that *Moringa oleifera* could be used to preserve smoked-dried catfish to prevent spoilage, thereby reducing economic wastage and protecting humans from diseases.

Baygar et al. (2012) determined sea bass marinades' chemical and sensory quality changes stored at (4 ±1) ^{o}C in marinating solution. Sea bass fillets, including scale, skinned, and descale, were submerged into a marinating mixture of 2.5% acetic acid and 11% sodium chloride and kept at (4±1) C. The sensory and chemically analyzed characteristics for marinating fish fillets were controlled while the samples were maintained for 90 days. The outcome of the sensory findings revealed that fillets from the skin could not yield the general acceptance points for the whole duration of the kept samples. While the outcome generated from chemical characteristics of the fish fillets such as TVB-N, TMA, and pH checked for marinate samples of fillet with scale and fillet without scale, their measures for acceptance fluctuated throughout the period fish fillets were kept. Meanwhile, the outcome for the TBA measurement revealed that its value was higher than the acceptance values at 70 days from the marinate samples without scale and skinned fillets, and the value recorded was 56. Based on their findings, the authors concluded that sea bass could be processed and preserved in marinate solution.

Samples et al. (2010) examine berry marinades to enhance the oxidative stability of herring fillets. In this study, 50g/L powder of elderberry, cranberry, or black currant was used to prepare the marinating of herring fillets, and 5 % sodium chloride solution was prepared and inoculated into the herring fillets. The author reported that cranberry and blackberry have more potency in the retardation of tocopherol and the occurrence of ammonium. The marinating solution prepared with elderberry yields a significantly high

rate of carbonyls, ammonium, and biogenic amines. Meanwhile, volatile lipid components of propanal, hexanal, 2-penten-1-ol, and 1-penten-3-ol are lower in samples with black currant after the injection of the NaCl mixture. All the marinating samples yielded significantly reduced water retention capacity simultaneously with a reduced flesh pH. The authors concluded that herring fillets in a substance of berry powder could improve the characteristics and extend the shelf life of herrings and subsequently act as the origin of antioxidant benefits for the end users.

Nilgun et al. (2012) studied the shelf life of Anchovy (*Engraulis encrasicholus, L*.1758) Patties Stored at 4°C. The authors investigated the shelf life of anchovy patties during storage at 4°C. The sensory characteristics, pH, TVB-N, thiobarbituric acid (TBA), TMA, total mesophilic aerobic bacteria, total yeast mold, and total coliform bacteria counts of anchovy patties were analyzed daily, and on the eighth day of storing anchovy, the sensory and microbiological properties revealed that the fish could not be consumed. The findings showed that the duration of storing the fish altered the sensorial value, pH level, TVB-N, TBARS, TMA, total yield-mold, total coliform bacteria count, and TMAB of anchovy patties showed a significant difference ($p<0.05$).

Inanli et al. (2010) studied the chemical composition of marinated anchovy (*Engraulis encrasicolus L*., 1758) and sensory evaluation in different sauces. Marinate of anchovy was produced with a salt content of 10% and acetic acid of 6% and was kept for 7 days at 0-4°C immediately when the proximate analysis was concluded (crude fat, crude ash, crude protein, moisture, and pH). There were four batches of treatment in which two varied sauces were produced; olive oil was included in one batch, and the other did not contain olive oil; sensorial investigation was conducted through ten (10) panelists. The findings of the sensory analysis revealed that marinates produced with sauce A (sunflower oil, pepper paste, tomato paste, salt, had the most excellent taste compared with the marinates produced with sauce B (sunflower oil, pepper paste, tomato paste, salt, red pepper, bay leaf, sugar).

Ozogul et al. (2010) determined the effects of combining smoking and marinating on the shelf life of anchovy stored at 4°C. The authors examined smoked and anchovy that have undergone marinate procedures for sensory, chemical such as (TVB-N, thiobarbituric acid, TBARS, PV, FFA, and pH), microbiological analysis such as (total

aerobic count, coliform, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*) was conducted. At the same time, the samples were stored for seven months. Their findings showed that the sensory properties were reduced slowly while the samples were stored. The chemical analysis outcome follows the same trends as the sensory properties, except for the TVB-N value. There was a significant rise in the value of TBA ($p<0.05$) from 1.9 to 4.25 MA/kg when the storage was completed for six months. There was a variation in the value of peroxide, which varied significantly ($p<0.05$) while the fish was in the storage facility. The former TVC of 3.8 log CFU/g rose to 6.2 log CFU/g towards the end of the string duration. Based on the outcomes of all the parameters, the authors then concluded that marinated and smoked anchovy could be preserved for six months.

Domiszewski et al. (2011) studied the quality of lipids in marinated herring. The authors examine the obtainable marinated herring in the industries as the origin of LC n-3UFA and can identify the concentration of lipid degradation in the products. There are sixteen varieties of oil marinates obtainable in the local market, and these sixteen varieties are supplied through 5 leading suppliers; these products were analyzed through the use of Bligh and Dyer techniques, analysis such as constituents of fatty acids, and oxidative concentration (PV, AV and TOTOX) The type of fatty acids that dominate was MUFA, and the percentage is approximately around 48% overall, then polyunsaturated fatty acids, approximately 28%, and Saturated fatty acids SFA,24%. The result from peroxide value of 8-17meqO2/kg, AV IS 4-19, and TOTOX ranges within 24-53. The concentration of lipid oxidative revealed excellent reserve qualities, and none of the treatments were higher than TOTOX Value 10. Based on the type of fatty acids, about 100g of fish muscle consists of omega-3(n-3) PUFA ranging from 2.7-3.8g and 2-2.7g /100g of EPA added to DHA. Even with various determinants like hauling season, techniques employed in the storage of fresh fish, which could be due to variations in the technological procedures, could impact the fat constituents of fish fillets; there is no basis for controversy about the contents of lipids obtainable from the local market which are the robust origin of essential long-chain unsaturated fatty acids (LC-n3 PUFA). Nevertheless, the product in this category could have high oxidative lipids, leading to rancidity in the fish's taste.

Šimat et al. (2019) investigated the impact of lemon juice on the marination of anchovy (*Engraulis encrasicolus*): chemical, microbiological, and sensory changes. Their studies revealed qualitative changes (physical, biochemical, microbiological, and sensory) in anchovy fillets during the maturation of a marinade produced from fresh lemon juice and olive oil with acetic acid. The researcher carried out the marinating process at four °C left for 7 days for the sensory of the product to be acceptable, and at the of the acceptance when the marinating process was completed, the result showed the pH value to be 4.2, aw to be 0.85 and NaCl components to be 4% when analyzed. The authors stated that volatile and non-volatile amines were isolated from the marinated anchovy fillets. Their concentration in the fillets reveals that the accumulation of the volatile and non-volatile compounds during processing was weak; the result of the (TVB-N/100g value was <14mg, and TMA/100g value was <0.5mg, <30mg biogenic amines/kg). The authors stated that lemon juice has good potency when applied as a preservative because it lowers the bacteria counts in the products, and the inclusion of 0.5%acetic acid enhances the sensory properties of the products.

CHAPTER IV

MATERIAL AND METHODS

4.1 Materials

After being purchased from a Nigde local market, anchovy fillets were preserved in Styrofoam boxes and delivered to the Animal Production and Technologies Department, Faculty of Agricultural Sciences and Technologies. The fresh anchovy fillets purchased were kept at -80 °C until the marinating procedure began.

Anchovy with ice (a) Anchovy after thawing (b)

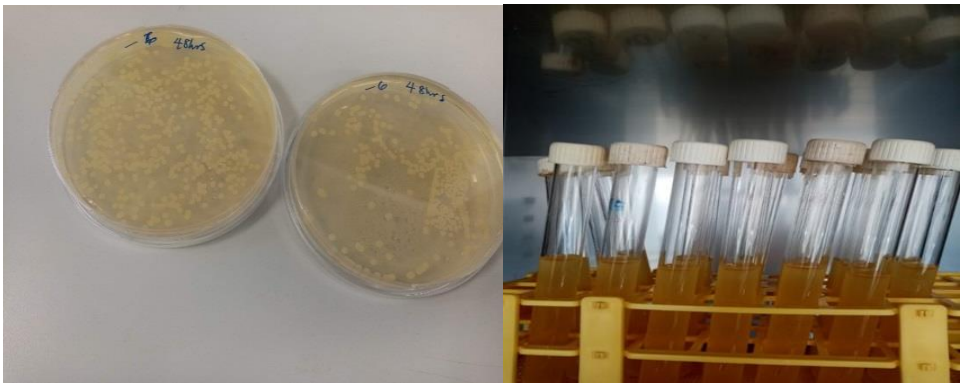
Photo 4.1. Anchovy fillets (a, b).

4.2 Methods

4.2.1 Bacterial strain

*Morganella psychrotolerant (DSM, 1786) was acquired from the G*erman group or company of microorganisms and cell culture (DSMZ, Braunschweig, Germany). Bacteria were cultivated in Trypticase soy Broth (TSB, Oxoid) at 25^{o}C for 48 hours. Cells were preserved with glycerol in Eppendorf at 0.5 glycerol and 0.5 bacterial cells and kept at -80 C. Later, the process was repeated when the cells were re-submerged in the solution of tryptic soy broth and plated in Tryptic Soy Agar Petri dishes, and serial dilution was made from and cultured at 10^{1} to 10^{6}. Cells were re-submerged in Tryptic

Soy Broth's solution at a level of 10^6. The fish and bacteria solution percentage applied for the inoculation process was 100g/ml.



Bacterial strains (10^6) (a) Cultured bacteria (10^6) (b)

Photo 4.2. Preparation of bacterial strains (a, b)

4.2.2 Inoculation process

Anchovy fillets were submerged in a bacterial culture solution for five (5) minutes to inoculate bacteria on the surface of the fish fillets. The fish and bacteria solution proportion for the inoculation process was 100/1 g/ml.

(a) (b)

Photo 4.3. Inoculation of *M. psychrotolerans* in anchovy (a, b)

4.2.3 Marination process

Frozen anchovy fillets could thaw overnight at 4±1 ^{o}C. Two marinating solutions were processed alongside 1%, 2%, 3%, and 4% acetic acid (v/v) and 6%, 8%, and 10%

sodium chloride (w/v). The percentage of fish to solution was 1:1.5 (w/v). The marination procedure was completed at four °C for three days. Afterward, anchovy fillets were withdrawn from the solution and seeped on sterile steel wire mesh for 30 minutes. There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

Photo 4.4. Anchovy marinated in jars and kept in the refrigerator for 72hrs

Photo 4.5. Anchovy is distributed into plates after completion of the marination process (72 hours) in the refrigerator.

4.2.4 Microbiological analyses

Morganella psychrotolerans

The fish sample (10 g) was carefully withdrawn from polythene bags. It was blended for proper mix and homogenized in a laboratory blender containing a 90 mL pre-chilled sterile ringer solution. Further decimal serial dilutions were processed from the homogenized mixture in the same bag of chilled dilution used from this homogenate. The adequate dilution was afterward employed for the isolation of bacteria *Morganella psychrotolerans* through the spread of diluted samples on Tryptic soy Agar plates (Oxoid) and incubated at 25 °C for 48 h (Emborg and Dalgaard, 2008).

Total aerobic mesophilic bacteria count.

The total aerobic mesophilic bacterial count was enumerated using petri dish spreading techniques (ICMSF). 10g of fish sample was blended and homogenized in two minutes with stomacher equipment after 90ml of ringer solution was added to the fish sample; a process of serial dilutions followed this, then 0.1mL of dilution was pipetted into the petri dishes which were already contained with PCA (Plate Count Agar) for spreading on its surface, after the conclusion of spreading process, the petri dish was incubated at 37 ^{o}C, total aerobic mesophilic bacterial count for 24-48hrs.

Total psychrophilic bacteria count.

Plate Count Agar (PCA) was employed to enumerate the total psychrophilic bacteria count. A 10g fish sample was blended and homogenized with 90 mL of pre-chilled sterile ringer solution in a stomacher device for 60 secs. Further dilution was made with a 9 mL sterile ringer solution; serial dilution was carried out. The total number of psychrophilic bacteria was analyzed using petri dish surface spreading techniques (ICMSF, 1982), and the bacteria were incubated at 8 C for 7 days.

Lactic Acid Bacteria Count.

Spreading was conducted on Man Rogosa and Sharpe (MRS) to count lactic acid bacteria. A 10g fish sample was blended and homogenized with 90 mL of pre-chilled sterile ringer solution in a stomacher device for 60 secs. Further dilution was made with a 9 mL sterile ringer solution; serial dilution was carried out. Then, the plates were incubated in anaerobic jars for 48 hours at 30^{o}C (Anonymous,1998).

Total yeast and mold count

The yeast and mold were determined through the medium of Potato Dextrose Agar (PDA); to adjust the pH to 3.5, citric acids were added to the agar; the enumeration was carried out with the use of a petri dish in which 0.1 is pipetted into the surface of the agar in the petri dish and spread on the entire surface (ICMSF,1982). 10g of fish sample was blended and homogenized with 90mL of pre-chilled sterile ringer solution in a stomacher device for 60 secs. Further dilution was made with a 9mL sterile ringer solution; serial dilution was carried out. The total number of psychrophilic bacteria was analyzed using petri dish surface spreading techniques (ICMSF, 1982), and the bacteria were incubated at 25 ^{o}C for 5 days.

Total Enterobacteriaceae count

For the Enterobacteriaceae determination, the pour plating method was used in Violet Red Bile Agar (VRBA) medium, Petri surface spreading (ICMSF, 1982), and the plates were incubated at 37 C for 36-48 hours (Anonymous, 1998). The enumeration was carried out using a petri dish in which 0.1 is pipetted into the surface of the agar in the petri dish and spread on the entire surface (ICMSF, 1982). 10g of fish sample was blended and homogenized with 90mL of pre-chilled sterile ringer solution in a stomacher device for 60 secs. Further dilution was made with a 9mL sterile ringer solution; serial dilution was carried out.

4.2.5 Physicochemical analysis

4.2.7 Determination of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were determined based on the AOCS Official Method Cd 19-90 (1998) techniques, which is determined by the colorization of malondaldehyde (MDA) with thiobarbituric acid TBA reagent. A lipid sample dissolved in 5ml of butanol was mixed with 5ml of TBA reagent. The absorbance was determined through a 530 nm UV-VIS spectrophotometer after incubation at 95°C for 120 min in a water bath. TBARS were expressed as mg of malondialdehyde (MDA) per kg fish sample.

TBA = 50 x (The absorbance of lipid- The absorbance of blank) / sample weight (mg).

4.2.8 Peroxide value (PV)

Peroxide value (PV) was examined using the AOAC method (1990). When the chloroform / acetic acid solution was added to the anchovy oil sample, Potassium iodine was added to the mixture and then kept in the dark cupboard for the reaction. After that, sodium thiosulphate was added to titrate-free iodine. The result was expressed in terms of active oxygen (meq/kg) equivalents per kilogram of fish oil.

4.2.9 pH

To determine pH, anchovy fillet samples were blended and put in distillation bottle water with a 1:1 (w/v) ratio, and a pH electrode was dipped into homogenates (Manthey et al.,1988). All parameters were conducted at room temperature (24±1°C) using a pH meter (Thermo Scientific Orion 2-star, Germany).

4.2.10 Sensory analysis

The method of Amerina et al. (1965) was used for the sensory evaluation. Anchovies fillets kept in the refrigerator were analyzed according to the odor, texture (tight structure, elasticity, and water drop), color, appearance (color, gloss), and general

acceptance. Eight experienced panelists were evaluated, a Coding system of 3- 3-digit values was carried out on the different specimens to be analyzed by the panelists under room temperature, and an uncooked sensory evaluation was carried out using a nine-point hedonic scale. A score of 9-7 indicated ‘‘very good’’, a score of 6.9–4.0 ‘‘good’’, and a score of 3.9-1.0 was denoted as spoiled.

4.2.11 Statistical analysis

All measurements were performed in triplicate, and analyses were carried out using the SPSS software (Statistical Analysis System, Cary, NC, USA). Duncan multiple comparison test One-way ANOVA was conducted to find the significant difference between the storage days and groups during analyses.

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 Morganella Psychrotolerans

Table 5.1 presents the effects of marinate solution on the growth of *Morganella psychrotolerans* when applied to anchovy fillets. Bacterial growth was observed during the study period in control samples and in samples treated with 1% and 2% acetic acid and different concentrations of NaCl (6%, 8%, and 10%, respectively).

Table 5.1. The effects of marination conditions on *M. psychrotolerans* (log CFU/g) growth in marinated anchovy during storage

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
M. Psychrotolerans	0	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	3	2.30±0.00^{Ab}	2.45±0.01^{Ab}	2.04±0.00^{Ac}	2.24±0.02^{Be}	1.88±0.01^{Ce}	1.61±0.01^{Dc}	1.61±0.02^{CDc}	0.00±0.70^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}
	15	2.46±0.01^{Ba}	2.48±0.00^{Aa}	2.36±0.03^{Cb}	2.29±0.01^{Dd}	2.09±0.01^{DEd}	0.74±1.04^{Ec}	1.83±0.07^{Ec}	1.57±0.04^{Fa}	0.00±0.00^{Fa}	1.56±0.02^{Fa}	0.50±0.71^{Fa}	0.00±0.00^{Fa}	0.00±0.00^{Fa}	0.00±0.00^{Fa}
	30	*	*	2.48±0.00^{Aa}	2.43±0.00^{Bc}	2.33±0.02^{Cc}	0.00±0.00^{Dbc}	0.00±0.00^{Ebc}	1.68±0.03^{Fa}	0.00±0.00^{Fa}	1.83±0.07^{Fa}	1.00±0.00^{Fa}	0.00±0.00^{Fa}	0.00±0.00^{Fa}	0.00±0.00^{Fa}
	45	*	*	3.48±0.00^{Aa}	3.46±0.00^{Ab}	3.44±0.01^{Ab}	0.00±0.00^{Bc}	0.30±0.43^{Bc}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}
	60	*	*	*	4.48±0.00^{Aa}	4.48±0.00^{Ba}	2.38±0.03^{Cab}	2.46±0.00^{Dbc}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	2.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}
	75	*	*	*	*	*	3.20±0.28^{Aab}	0.00±0.00^{Aab}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	1.00±0.90^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}
	90	*	*	*	*	*	3.33±0.21^{Aa}	0.00±0.00^{Aa}	1.00±0.70^{Ba}	0.00±0.00^{Ba}	1.20±0.85^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}	0.00±0.00^{Ba}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably $p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (Morganella psychrotolerans) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

Also, on day 15th, the control samples (CM) of *M. psychrotolerans* reached 2.48 log CFU/g. In the samples treated with 1% acetic acid with their respective salt (6%, 8%, 10) concentration(Grp 1; Grp 5, and Grp 9), bacteria growth detected on the 60th day of the processing time was 4.48 log CFU/g. Likewise, for the sample treated with 2% acetic acid with NaCl concentration(6%, 8%, 10) respectively, (Grp 2; Grp 6 and Grp 10) Grp 2 (2% acetic acid with 6% NaCl) Bacterial cell was not seen from the beginning of storage period until day 60th in which the growth of *Morganella psychrotolerans* was seen. In comparison, Grp 6 (2% acetic acid with 8% NaCl) showed a slight fluctuation in the growth of *M. psychrotolerans*, in which no bacterial cell was observed at the beginning of storage. However, on day 45th, there was an observation of bacterial cells. Also, on day 60, bacterial growth was observed until the processing time ended. For Grp 10(2% acetic acid with 10% NaCl), bacterial cells were observed from the beginning of the storage period until the end. This delay in the growth of *M. psychrotolerans* for samples processed with 2% acetic acid with NaCl (6%, 8%, 10) could be a result of the concentration of acetic acid and high concentration of salt, especially in Grp 10 (2% acetic acid with 10% NaCl) that does not have any bacterial cell throughout the study period. Additionally, samples treated with 3% and 4% acetic acid and the salt solution showed the combined effect of high acetic acid and NaCl, as there was no *M. psychrotolerans* development in these samples. Our results are consistent with a report by Ucak et al. (2019), which found that a marinade solution consisting of salt solution and 4% acetic acid might inhibit the growth of *M. psychrotolerans* bacteria in herring fillets by blocking bacterial multiplication. Since no bacteria were isolated during the study period, we saw that a high concentration of acetic acids, such as 3% or 4%, and a high salt concentration could prevent and hinder the multiplication of *M. psychrotolerans. T*hus, no bacterial isolations were observed throughout the research period. One of the most common spoilage microorganisms found in lumpfish roe stored at five °C (pH 5.4 and 3.5–4.8% sodium chloride in the H2O phase) has been identified as *M. psychrotolerant* (Emborg et al., 2006). Moreover, it has been discovered that Psychrotolerans produces hazardous histamine concentrations that are present in unprocessed vacuum-packed (VP) and VP cold-smoked tuna, which can lead to the development of Histamine Fish Poisoning (HFP) (Emborg et al., 2006) Emborg et al., 2005).

Table 5.2. The effects of marination conditions on growth of total psychrophilic bacteria count (log CFU/g) in marinated anchovy during storage

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Psychrophilic	0	1.48±0.00 Ac	1.48±0.00 Ab	1.48±0.00 Ac	1.48±0.00 Af	1.48±0.00^{A} f	1.48±0.00 Ac	1.48±0.00^{A} d	1.48±0.00 Ab	1.48±0.00 Aa	1.48±0.00 Aa	1.48±0.00 Aa	1.48±0.00 Aa	1.48±0.00 Aa	1.48±0.00 Aa
	3	2.46±0.00 Ab	2.48±0.00 Ab	2.41±0.03 Ab	2.28±0.02 Be	1.75±0.08^{C} e	1.57±0.14 Dc	1.67±0.06^{C} Dd	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea
	15	4.19±0.06 Ba	4.78±0.72 Aa	3.29±0.02 Cb	2.48±0.00 Dd	2.27±0.02^{D} Ed	1.83±0.03^{E} c	1.85±0.00^{E} d	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Ea
	30	*	*	437±0.04 Aa	3.48±0.00 Bc	2.48±0.00^{C} c	2.29±0.02 Dbc	2.11±0.16^{E} bc	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa	0.00±0.00 Fa
	45	*	*	4.44±0.05 Aa	4.35±0.07 Ab	3.47±0.01^{A} b	1.24±1.75 Bc	1.15±1.63^{B} d	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba
	60	*	*	*	5.48±0.00 Aa	5.42±0.00^{B} a	2.97±0.72 Cab	2.48±0.00^{D} bc	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea	0.00±0.00 Ea
	75	*	*	*	*	*	5.53±0.10 Aab	3.48±0.00^{B} ab	0.00±0.00 Ca	0.00±0.00 Ca	0.00±0.00 Ca	0.00±0.00 Ca	0.00±0.00 Ca	0.00±0.00 Ca	0.00±0.00 Ca
	90	*	*	*	*	*	4.06±0.62 Aa	4.47±0.01^{A} a	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba	0.00±0.00 Ba

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably $p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (Morganella psychrotolerans) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.2 Total Psychrophilic Bacteria Count

The results of psychrophilic bacteria in anchovy fish with the marinating solution with acetic acid and NaCl are presented in Table 2. The initial cell count observed in raw material was 1.48 log CFU/g. In the control sample with the injection of *Morganella psychrotolerans* (CM), the multiplication of psychrophilic was detected on day 15^{th}, and the cell counts were 4.78 log CFU/g. Also, in the control sample without bacteria inoculation (CO), the cell count was 4.19 log CFU/g. Likewise, In the groups with the addition of marinating solution which consists 1%, 2%, 3%, and 4% acetic acid with NaCl (6%,8%, 10%), bacterial growth was seen to exhibit exponential growth during the study time in groups treated with 1% acetic acid with the inclusion of NaCl (6%, 8%, 10%)(Grp 1; Grp 5, and Grp 9), on day 60th bacterial growth, reached 5.42 log CFU/g in the group processed with 1% acetic acid and 8% of salt and the group treated with 1% acetic acid and 10% salt with a value of 5.48 log CFU/g, this value does not reach or exceed 7.0 log CFU/g which is termed as the acceptable maximum value in fish products/variety, ICMSF (ICMSF, 1986). The observation for the groups treated with 2% acetic acid with the inclusion of NaCl (6%, 8%, 10%) (Grp 2; Grp 6, and Grp 10) Showed that there was a reproduction of bacteria growth on third day (1.57 log CFU/g and 1 .67 log CFU/g) in GRP 2 and Grp 6 of the sample treated with 2 % acetic acid and salt(6%, 8%,) which expresses an exponential increase till the end of the processing period with a value of 4.04 log CFU/g and 4.47 log CFU/g respectively. The groups were treated with a marinating solution of 3% and 4% acetic acid with NaCl (6%, 8%, 10%); accordingly, bacterial cells could not be isolated from the beginning of the processing period till the end. The results of our investigation are consistent with the research of Ucak et al. (2019), which found that no bacterial cells were observed in the groups treated with 4% acetic acid. Furthermore, Fuseli et al. (1994) observed that no psychrophilic bacterium was isolated from marinated anchovies (*Engraulis anchoita*).

5.3 Lactic Acid Bacteria (LAB)

Marinating promotes the growth of specific lactic acid bacteria (LAB) rarely identified in unmarinated food. The acidic environment that acetic acid provides is favorable for activating the proteolytic enzymes found in fish muscle. The energy source for lactic acid bacteria (LAB) proliferation comes from proteolysis products. As a result, when

fish were marinated, many LAB species were isolated (Bjorkroth, 2005). Additionally, the initial lactic acid bacteria (LAB) isolation from the raw fish in our study was 1.08 log CFU/g; the lactic acid bacteria (LAB) results are shown in Table 5.3. On day 15, the CO value of the LAB isolated from the control samples (CO and CM) reached 2.48 log CFU/g, while the CM value reached 3.48 log CFU/g. A LAB value of 2 log CFU/g was extracted from anchovies' marinades, according to Cosansu et al. (2010). Their study findings concur with our study's findings. Moreover, Kilinc and Cakli's results (Kilinc & Cakli, 2004). 1.30 log CFU/g of LAB isolated from marinated sardine was found to have a lower value.

Table 5.3. The effects of marination conditions on growth of Lactic Acid Bacteria (LAB) (log CFU/g) in marinated anchovy during storage.

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Lactic Acid Bacteria	0	1.08±0.43 ^{Ab}	1.08±0.43 ^{Ac}	1.08±0.43 ^{Ac}	1.08±0.43 ^{Ad}	1.08±0.43 ^{Ad}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Ab}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}	1.08±0.43 ^{Aa}
	3	1.85±0.11 ^{Bab}	2.47±0.01 ^{Ab}	0.93±0.04 ^{Cc}	1.16±0.65 ^{Cd}	0.84±0.34 ^{Cd}	0.00±0.00 ^{Db}	0.00±0.00 ^{Ca}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}
	15	2.48±0.08 ^{Ba}	3.48±0.00 ^{Aa}	2.00±0.02 ^{Cb}	1.98±0.12 ^{Cc}	1.63±0.04 ^{Cc}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Dab}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Ca}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Eb}	0.00±0.00 ^{Eb}
	30	*	*	2.47±0.01 ^{Ab}	2.47±0.01 ^{Ac}	2.12±0.01 ^{Bbc}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cc}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}
	45	*	*	3.48±0.00 ^{Aa}	3.47±0.01 ^{Bb}	2.48±0.00 ^{Cb}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Dc}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}	0.00±0.00 ^{Db}
	60	*	*	*	4.41±0.02 ^{Ba}	4.48±0.00 ^{Aa}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cc}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}	0.00±0.00 ^{Cb}
	75	*	*	*	*	*	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ac}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}
	90	*	*	*	*	*	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ac}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}	0.00±0.00 ^{Ab}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably $p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.4 Total Aerobic Mesophilic Bacteria Count

Total aerobic mesophilic bacteria enumerated from fresh anchovies was (0.00) log CFU/g on day 0, Table 5.4. After the treatment was applied, mesophilic counts were determined in control groups CO (4.32) log CFU/g and CM (4.43) log CFU/g on the 15th day, during the storing time. In the samples processed with 1% acetic acid with their respective salt (6%, 8%, 10) concentration (Grp 1; Grp 5, and Grp 9), bacteria growth was observed on day 45th of storage time as 5.48 log CFU/g, meanwhile for Grp 5 &Grp 9 bacterial value determined on day 60th of the processing time was 4.48 log CFU/g. Likewise, the sample treated with 2% acetic acid including NaCl concentration(6%, 8%, 10)respectively, (Grp 2; Grp 6 and Grp 10) Grp 2 There was a proliferation of bacterial cells on third day in Grp 2 & Grp 6, The growth continues and showed an exponential growth throughout the study period and the value determined are (Grp 2) 5.7 log CFU/g and (Grp 6) 5.2 log CFU/g. For Grp 10, no bacterial cells were observed at the start of the processing period until it ended. There was inhibition in the growth of total aerobic mesophilic in Grp 10, which has no bacterial cells throughout the study period, due to the incorporation of high acetic acid and NaCl. There was no isolation in samples processed with 3% and 4% acetic acid and the salt solution, respectively, as there was no growth of total aerobic mesophilic bacteria. Sen and Temelli's (2003) study on the microbiological and chemical properties of marinated anchovies found that the total aerobic mesophilic bacterial value in marinades of anchovies was 2.6 log CFU/g. 6log CFU/g ICMSF is the highest permissible limit for TAMB value (ICMSF, 1986). The total aerobic mesophilic bacteria (TAMB) value in our investigation does not exceed Table 1's regulatory level. Like our findings, Cosansu et al. (2010) found that adding pediococcus culture to marinated anchovy increased observation growth while staying within the permitted limit. The addition of acetic acid and sodium chloride to fish-marinated anchovies showed its ability to inhibit bacteria. (Sen and Temelli, 2003) noted that acetic acid selectively inhibited the growth of bacteria and yeast. Previous studies have shown that while marinating fish, it is uncommon for microorganisms to isolate themselves during the processing phase (Erkan et al., 2000; Fuselli et al., 1994).

Table 5.4. The effects of marination conditions on the growth of aerobic mesophilic bacteria count (log CFU/g) in marinated anchovy during storage

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Mesophilic	0	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Ae}	0.00±0.00^{Af}	0.00 ± 0.00^{Af}	0.00 ±0.00^{Af}	0.00 ±0.00^{Ad}	0.00 ±0.00^{Ab}	0.00±0.00^{Aa}	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Aa}	0.00±0.00^{Ad}	0.00±0.00^{Ab}
	3	2.45±0.04^{Ab}	2.47±0.00^{Ab}	2.14±0.09^{Ad}	2.08±0.01^{ABe}	2.07±0.03^{ABe}	1.45 ±0.21^{Be}	0.74±1.04^{Ccd}	0.00±0.00^{Db}	0.00±0.00^{Da}	0.00±0.00^{Db}	0.00±0.00^{Db}	0.00±0.00^{Da}	0.00±0.00^{Dd}	0.00±0.00^{Db}
	15	4.32±0.03^{Aa}	4.43±0.01^{Aa}	3.21±0.03^{Bc}	3.14±0.04^{Bd}	2.48±0.00^{Cd}	1.57±0.04^{De}	0.83±1.17^{Ecd}	0.00 ±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Fb}	0.00±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Fd}	0.00±0.00^{Fb}
	30	*	*	5.27±0.02^{Ab}	4.22±0.02^{Bc}	3.35±0.04^{Cc}	2.27± 0.03^{Dd}	1.83 ±0.18^{Ec}	0.00 ±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Fb}	0.00±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Fd}	0.50±0.71^{Fb}
	45	*	*	5.48±0.00^{Aa}	4.45±0.00^{Bb}	4.42±0.01^{Bb}	3.48 ±0.00^{Cc}	1.97±0.10^{Cc}	0.00 ±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Da}	0.00±0.00^{Ba}	0.00±0.00^{Fa}	2.33±0.21^{Dc}	1.84±0.27^{Ea}
	60	*	*	*	5.32±0.03^{Aa}	5.29± 0.02^{Aa}	4.47± 0.01^{Bb}	3.48 ±0.00^{Cb}	0.00 ±0.00^{Fb}	0.00±0.00^{Fa}	0.00±0.00^{Fb}	0.00±0.00^{Fb}	0.00±0.00^{Fa}	2.33±0.21^{Dc}	1.84±0.27^{Ea}
	75	*	*	*	*	*	5.42±0.00^{Aa}	4.48±0.00^{Bab}	0.00 ±0.00^{Eb}	0.00±0.00^{Ea}	0.50±0.28^{Eb}	0.00±0.00^{Eb}	0.00±0.00^{Ea}	2.43±0.02^{Eb}	2.07±0.09^{Da}
	90	*	*	*	*	*	5.70 ±0.72^{Aa}	5.21±0.02^{Aa}	1.48 ±0.00^{Ca}	0.00±0.00^{Da}	0.00±0.00^{Db}	0.00±0.00^{Db}	0.00±0.00^{Da}	2.46±0.02^{Ba}	2.15± 0.06^{Ba}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably $p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (Morganella psychrotolerans) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.5 Total Enterobacteriaceae Count

The initial coliform count for fresh anchovy was 0.98 log CFU/g Table 5.5. There was an increase in the observation of coliform bacteria in the control groups; CO reached 2.8 log CFU/g, and CM value was 2.42 log CFU/g. For groups processed with 1%,2%,3%, and 4% acetic acid with the inclusion of NaCl (6%,8%,&10%) and inoculated with *M. psychrotolerans*, there is no observation for isolation of coliform throughout the processing period; the bacterial are inhibited as a result of marinating solution treatment applied. Our research findings are consistent with a study by Kocatepe et al. (2019) that marinates anchovies with various essential oils and found no coliform growth in the marinated fish. Additionally, Fuselli et al. (1994) observed that no coliform cells were isolated from cold marinades of anchovies. Similarly, anchovies marinated in marinade and kept at 4^{o}C for eleven months did not show any signs of total coliform bacteria, according to a study by Gunsen et al. (2011). These results are consistent with the current research and demonstrate that acetic acid added to marinades inhibited the growth of coliforms.

Table 5.5. The effects of marination conditions on growth total Enterobacteriaceae count (log CFU/g) in marinated anchovy during storage.

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Coliform	0	0.98 ± 0.03^{Ac}	0.98 ± 0.03^{Ac}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}	0.98 ± 0.03^{Aa}
	3	1.97 ± 0.01^{Bb}	2.06 ± 0.03^{Ab}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}
	15	2.38 ± 0.02^{Ba}	2.42 ± 0.02^{Aa}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cb}
	30	*	*	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}
	45	*	*	*	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}
	60	*	*	*	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}
	75	*	*	*	*	*	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}
	90	*	*	*	*	*	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably $p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.6 Total Yeast and Mold

Table 5.6 shows that the anchovies' initial total yeast and mold count was 0.00 log CFU/kg due to the absence of any bacterial cells on the first and third days of storage. On the fifteenth day, bacterial growth was observed in the groups treated with 1% acetic acid and the control group. However, no bacterial cells were found in the groups treated with 2%, 3%, and 4% acetic acid of yeast and mold. The yeast and mold isolated from anchovies in the control groups and those marinated with 1% acetic acid on day 15 showed significantly different outcomes ($p<0.05$). Grp 5 & Grp 9 marinated in 1% acetic acid attained 2.99 log CFU/g and 2.85 log CFU/g on day 60, respectively, while the group marinated in 1% acetic acid as of day 45 reached (Grp 1: 2.97 log CFU/g). According to the study findings, acetic acid inhibited the microbiological spread of anchovies.

Table 5.6. The effects of marination conditions on growth of total yeast and mold (log CFU/g) in marinated anchovy during storage

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Yeast and mold	0	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Ad}	0.00±0.00^{Ad}	0.00±0.00^{Ae}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}
	3	0.00±0.00^{Ab}	0.00±0.00^{Ab}	0.00±0.00^{Ad}	0.00±0.00^{Ad}	0.00±0.00^{Ae}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}
	15	2.15±0.04^{Ba}	2.30±0.14^{Aa}	1.66±0.06^{Cc}	1.57±0.12^{CDc}	1.46±0.02^{Dd}	0.00±0.00^{Ea}	0.00±0.00^{DEa}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}	0.00±0.00^{Ea}
	30	*	*	2.08±0.03^{Bb}	2.12±0.04^{Ab}	1.97±0.03^{Cc}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}
	45	*	*	2.97±0.02^{Aa}	2.86±0.02^{Ba}	2.63±0.04^{Cb}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}	0.00±0.00^{Da}
	60	*	*	*	2.99±0.02^{Aa}	2.83±0.02^{Ba}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}	0.00±0.00^{Ca}
	75	*	*	*	*	*	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}
	90	*	*	*	*	*	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}	0.00±0.00^{Aa}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably ($p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.7 Physico-Chemical Parameters

5.7.1 Peroxide values

Peroxide values have been determined to evaluate lipid oxidation using prehistoric techniques. Variation in the absorption of elementary lipid oxidation outcome represented milliequivalent functional oxygen per kilograms of lipid (meqO2 /kg) (Ochrem et al., 2021). From the start of processing, the peroxide concentration measured was 1.50 meq O2 /kg, and as the storage continued, an increase was observed in all the groups (Table 5.7). The control groups had the highest PV assessed on day 15 of the processing phase (CO:17.00, CM:19.00 meq O2/kg of lipids) (Table 7). Compared to all the groups marinated with acetic acid and NaCl, the peroxide values (PV) measured in the control group on day 15 were considerably higher. All the marinating groups showed this rise in peroxide value throughout the trial. The group treated with 4% acetic acid (Grp 4, Grp 8, and Grp 12) had the lowest PV as of day 90, measuring 33.50, 35.50, and 38.5 meq O2/kg, respectively. The acetic acid used in the marinade at a higher concentration caused the value to decrease. The present findings are consistent with a study conducted by Gokoglu et al. (2012) on marinated fish enhanced with garlic or tomato, which also showed higher peroxide values (PV).

Similarly, the Ochrem et al., 2021 investigation on marinated herring with milk thistle added showed an increasing trend in the peroxide value test. Furthermore, compared to the control groups, the groups that included olive oil-lemon (25, 35, and 50%) had a reduced PV, according to Topuz et al. (2014). The current findings demonstrated that low acetic acid concentrations could not stop lipid oxidation; high acetic acid concentrations had more significant inhibitions.

Table 5.7. The results of peroxide analysis of marinated anchovy.

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
PV	0	1.50±0.71^{Ac}	1.50±0.71^{Ac}	1.50±0.71^{Ae}	1.50±0.71^{Af}	1.50±0.71^{Ae}	1.50±0.71^{Ag}	1.50±0.71^{Ag}	1.50±0.71^{Ag}	1.50±0.71^{Ah}	1.50±0.71^{Ag}	1.50±0.71^{Ag}	1.50±0.71^{Ad}	1.50±0.71^{Af}	1.50±0.71^{Af}
	3	7.00±0.00^{Ab}	8.00±0.00^{Ab}	5.99±0.00^{Bd}	6.00±0.00^{Be}	6.00±0.00^{Bd}	6.00±0.00^{Bf}	6.00±0.00^{Bf}	6.00±0.00^{Bf}	5.99±0.00^{Bg}	6.00±0.00^{Bf}	6.00±0.00^{Bfg}	6.00±0.00^{Bcd}	6.00±0.00^{Be}	6.00±0.00^{Be}
	15	17.00±0.00^{Aa}	19.00±0.71^{Aa}	7.98±0.00^{Bc}	8.50±0.71^{Bd}	5.00±0.00^{BCd}	8.00±5.66^{Bf}	7.50±0.71^{BCef}	7.50±2.12^{BCef}	8.98±0.00^{Bf}	6.50±0.71^{BCf}	3.00±2.83^{Cef}	5.00±5.66^{BCcd}	7.00±1.41^{BCe}	6.50±2.12^{BCe}
	30	*	*	18.50±0.71^{Ab}	15.98±0.00^{Bc}	15.50±0.71^{Bc}	12.00±0.00^{Ce}	10.99±0.00^{Dde}	10.50±0.71^{DEde}	9.98±0.00^{Ee}	9.99±0.00^{Ee}	10.00±0.00^{Ede}	10.00±0.00^{Ec}	10.50±0.71^{Ed}	10.00±0.00^{Ed}
	45	*	*	20.50±0.71^{Aa}	20.98±1.41^{Ab}	20.50±0.71^{Ab}	15.50±0.71^{Bd}	14.49±0.71^{BCd}	13.47±0.71^{BCDd}	14.96±0.00^{BCd}	14.99±1.41^{BCd}	14.50±0.71^{BCd}	13.00±.141^{CDc}	12.00±0.00^{Dd}	12.00±0.00^{Dd}
	60	*	*	*	42.50±0.71^{EFGa}	28.50±0.71^{FGHa}	25.50±0.71^{Bc}	25.95±0.00^{Ac}	50.50±0.71^{Cc}	26.95±1.41^{Dc}	24.50±0.71^{DEFc}	25.00±1.41^{DEc}	40.46±0.71^{EFGb}	27.50±0.71^{GHc}	23.00±1.41^{Hc}
	75	*	*	*	*	*	52.500±3.54^{ABb}	56.00±4.26^{Ab}	51.90±2.82^{ABb}	49.95±0.00^{BCb}	44.82±0.00^{Ab}	44.00±5.66^{Cb}	30.00±1.41^{Da}	30.91±1.41^{Db}	29.44±0.71^{Db}
	90	*	*	*	*	*	60.38±0.71^{Aa}	60.00±0.00^{Aa}	56.50±0.71^{Ba}	52.40±0.71^{Ca}	47.36±0.70^{Da}	49.50±0.71^{Da}	33.50±21.2^{Fab}	35.50±2.12^{EFa}	38.35±0.70^{Ea}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably ($p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.8 Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid **(**TBARS) is commonly employed to indicate lipid oxidation activity; it is used for the initial effect of oxidation development and responsiveness of the analytical techniques employed (Shahidi & Zhong, 2005). The initial thiobarbituric acid (TBA) value measured in fish raw material was estimated as (0.15 mg MDA/kg) Table 5.8. The TBA values calculated for the control groups CO and CM (6.09 mg MDA/kg and 7.18 mg MDA/kg) were significantly higher ($P<0.05$) than the groups treated with marinate solution consist acetic acid; the control groups could not be consumed based on the TBA results. Meanwhile, the result obtained for TBA value for the groups marinated showed an increase in TBA values as the storage processing continued to increase with the lowest values and towards the time the processing ended. The thiobarbituric acid **(**TBA) values in the group process with 1% acetic acid also had high values following the control groups. However, they are still below the limit value for TBA (5.53, 5.93, and 5.93 MDA/kg) on days 45th and 60th. The group treated with 2% acetic acid had increased value but fell below the acceptable limit of 2% acetic (4.09, 4.23, and 4.56 mg MDA/kg), respectively.

Meanwhile, the group treated with 3% and 4% acetic acid has the lowest TBA values 3% (3.43, 3.10, and 3.72 mg MDA/kg) and 4% (3.60, 3.70, and 3.65 mg MDA/kg) on day 90th. High concentrations of acetic acid and NaCl caused preventive effects on the lipid oxidation of anchovy. According to Schromuller (1968), the TBA value in the excellent sample should be lower than 3 mg and not higher than 5 mg malonaldehyde per kilogram. The acceptable limit was recorded to be 7-8 mg/kg. The increase in TBA value was recorded for marinated anchovies in the study (Gokoglu & Ucak, 2020; Günsen et al., 2011). Also, there is an increase in the TBA of marinated rainbow trout (Maktabi et al., 2015). TBA value also increased in marinated anchovy treated with pomegranate in the study of (Gokoglu et al., 2009). A similar increase was reported by Šimat et al. (2019) for TBA value greater than 3 MDA/kg in the brining and marination of anchovy marinated with lemon juice. The high value observed in the control groups (CO and CM) on day 15th of our study could be associated with the reaction of 2-TBA acid with varied byproducts resulting from the disintegration of protein (Ghani et al., 2017). Fish marinate causes the penetration of NaCl into the innermost of muscle and melts more protein in a further phase, which leads to alteration in protein contents

(Szymczak et al., 2012). Statistical variations were seen among the groups in terms of TBA value.

Table 5.8. The results of TBARS analysis of marinated anchovy

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
TBA	0	0.15±0.01^{Ac}	0.15±0.01^{Ac}	0.15±0.01^{Ae}	0.15±0.01^{Af}	0.15±0.01^{Ae}	0.15±0.01 Ag	0.15±0.01 Ae	0.15±0.01^{Ad}	0.15±0.01 Ae	0.15±0.01^{Af}	0.15±0.01 Af	0.15±0.01 Af	0.15±0.01 Af	0.15±0.01 Ag
	3	1.58±0.37^{Bb}	2.06±0.01^{Ab}	1.41±0.03^{BCd}	1.43±0.12^{BCc}	1.50±0.01^{BCd}	1.35±0.05 BCe	1.35±0.09 BCe	1.29±0.40^{BCd}	1.34±0.07 BCg	1.141±0.02^{Cf}	1.21±0.02 BCb	1.42.0.17^{B} Cd	1.18±0.00 BCd	1.25±0.05 BCd
	15	6.09±0.51^{Ba}	7.18±0.00^{Aa}	3.36±0.21^{Cc}	1.97±0.06^{Dd}	1.99±0.17^{Dd}	1.82±0.09 De	1.78±0.11 Dcd	1.84±0.08^{Dc}	1.81±0.07 Dc	1.80±0.13^{Dd}	1.69±0.13 Dde	1.68±0.0.11^{De}	1.76±0.03 Dd	1.76±0.03 De
	30	*	*	4.18 ±0.20^{Ab}	2.63±0.11^{BCc}	2.79±0.58^{Bc}	2.01±0.03 Ce	2.15±0.23 Cc	2.01±0.04^{Cc}	2.20±0.14 Cc	2.14±0.12^{Cc}	2.22±0.17 Ccd	2.10±0.16 Cd	2.09± 0.07^{Cc}	2.08±0.11 Cd
	45	*	*	5.53±0.02^{Aa}	5.38±0.16^{Ab}	5.00±0.02^{Bb}	2.94±0.07 Dd	3.32±0.30 Cb	3.38±0.23^{Cb}	2.77±0.07 Db	2.70±0.07^{Db}	2.75±0.34 DEbc	2.40±0.00 Eb	2.48±0.04 Eb	2.40±0.08 Eb
	60	*	*	*	5.93±0.04^{Ab}	5.93±0.08^{Ab}	3.22±0.14 CDAb	3.66±0.30 BCbc	3.99± 0.56^{Bc}	3.10±0.19 CDe	2.19±0.16^{Dd}	3.10±0.60 CDab	3.00±0.01 CDa	2.99±0.02 CDa	3.09±0.04 CDa
	75	*	*	*	*	*	3.84±0.07 ABCb	3.95±0.06 ABa	4.32±0.40^{Aa}	3.22±0.47 CDab	2.94±0.07^{Dab}	3.54±0.36 BCDa	3.57±0.06 BCDa	3.20±0.04 CDb	3.32±0.12 BCDb
	90	*	*	*	*	*	4.09±0.17 ABCa	4.23±0.47 ABa	4.56 ±0.43^{Aa}	3.43±0.04 CDa	3.10±0.16^{Da}	3.72±0.37 BCDa	3.66±0.01 BCDa	3.70±0.07 BCDa	3.75±0.30 BCDd

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably ($p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.9 pH

The variations in pH readings of anchovies marinated in an acetic acid and NaCl solution with *M. psychrotolerans* inoculation throughout storage (Table 5.9). Shortly after being obtained from the laboratory, the average value of fish raw material utilized for pH analysis was determined to be 6.02± 0.16. On day 15, the pH values for CO and CM in the control groups were 6.8 and 7.43, respectively. 1%, 2%, 3%, and 4% of the groups receiving acetic acid and NaCl (marination) were treated with 6%, 8%, and 10% of the mixture. Following the application of salt and acetic acid (marination), the pH value fell in all groups that received treatment; this value reduction is significant ($p<0.05$) Table 5.9, and stayed lower, which is a result of the flesh's raw substance being perforated by acetic acid. This decrease in pH during the marinating process was documented in the research of (Makabi et al., 2015; Gokoglu & Ucak, 2020). When Aksu et al. (1997) marinated anchovy fish by immersing it in a marinade mixture containing 2% acetic acid, they found that the pH values were 4.50 and 4.25. The pH values of 4.33 and 4.37 were obtained in the studies conducted by Varlik et al. (2000) and Sallam et al. (2007) using 2% acetic acid as a marinate solution on trout fish and Pacific saury. These results are comparable to the ones we obtained in our investigations. Sardine treated with a marinade that included 7% acetic acid was measured by Kilinc and Cakli (2004), who found that the pH of the fish was 3.80. The pH measurement of 4.11 was also recorded by Erdem et al. (2005), who applied a 4% concentration of acetic acid to anchovies. These earlier investigations yielded lower pH values than those obtained from the current study, likely due to the higher concentration of acetic acid used in their work.

Furthermore, seafood products treated with 2% and 4% acetic acid mixtures showed a substantially different pH value ($p<0.05$), according to Gokoglu et al. (2004). Additionally, Guiffrida et al. (2007) reported a fluctuating flow in the pH values of marinated seabass, rising from 4.4 to 4.9 and then decreasing to 4.5. This pH value fluctuation is consistent with the outcome of our research. The pH value measured on day 75 increased in value on day 90 of the storage period, causing a fluctuation to occur on that day. According to Bilgin et al. (2018), the pH value derived from marinade products typically decreases during the first three months of processing before rising during the next three and five months. They clarified that the pH value's rise following

the pattern's decline may have been caused by the fish muscles' dissipation of hydrogen ions and carbon dioxide, which decreased the pH value. However, developing protein degradation compositions like trimethylamine and ammonia by the deterioration bacteria raises the pH value. A possible side effect of fish processing is the growth of lactic acid bacteria, which can cause amino acids to break down. As a result, carbon dioxide was found in the products, and multiple carboxyl group deletions were noted. These chemical reactions linked the marinating product's pH and acidity (Shenderyuk & Bykowski, 1989).

Table 5.9. The results of pH analysis of marinated anchovy

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
PH	0	6.02±0.16^{Ab}	6.02±0.16^{Ab}	6.02±0.16^{Aa}	6.02±0.16^{Aa}	6.02±0.16^{Aa}	6.02±0.16^{Aa}	6.02±0.16 Ab	6.02±0.16 Aa	6.02±0.16 Aa	6.02±0.16^{A} a	6.02±0.16 Aa	6.02±0.16 Aa	6.02±0.16^{Aa}	6.02±0.16 Aa
	3	5.75±0.05^{Ac}	5.87±0.09^{Ab}	5.52±0.31^{Aa}	5.18±0.59^{BCc}	4.74±0.58^{CDb}	4.62±0.47^{DEb}	4.32±0.35 DEFd	4.17±0.27 EFGb	425±0.36^{E} FGHb	4.76±1.05^{D} EFGb	3.96±0.14^{F} GHd	3.74±0.19 GHIc	3.40±0.16^{Ic}	3.65±0.22 HIcd
	15	6.86±0.06^{Ba}	7.43±0.32^{Aa}	4.90±1.08^{Ec}	6.38±1.25^{Ca}	4.58±0.41^{Fc}	4.51±0.21^{Fb}	4.34±0.91 Dc	4.25 ±0.17 ^{Gb}	3.81±0.39 HIJcd	3.89±0.23^{H} Ibc	3.92±0.14 Hd	3.55±0.08 Kd	3.70±0.08^{Jb}	3.76±0.11 IJbc
	30	*	*	5.11±2.29^{Ac}	5.17±0.73^{Ae}	4.70±0.53^{Fd}	4.07±0.42^{Hd}	4.14±0.46 Ac	4.00±0.33 Cb	3.71±0.17 Fcd	3.74±0.16^{H} c	3.73±0.12 ABc	3.52± 0.09^{Eb}	3.52±0.09^{Fb}	3.62±0.05 Gcd
	45	*	*	6.17 ±2.76^{Aa}	5.61±0.94^{Dd}	5.01±0.63^{Fd}	4.53±0.84^{Fd}	4.16±0.75 Bb	4.14±0.39 E^{b}	3.70±0.55 Fd	3.64±0.24^{F} bc	3.76±0.29 Cb	3.50 ±0.13^{Eb}	3.62±0.01^{Fb}	3.73±0.05 Fbc
	60	*	*	*	5.03±0.71^{De}	4.65±0.46^{Ed}	3.90±1.74^{Fd}	4.13±0.49 Ac	4.14±0.30 Cb	3.74±0.06 Ed	3.71±0.22^{D} Ebc	3.71±0.22 Bc	3.45±0.12 Cb	3.77±0.08^{Eb}	3.54±0.11 Fd
	75	*	*	*	*	*	3.98±1.78^{ABc}	4.05±1.81 Ae	3.94±1.77 CDc	3.73±0.12 B^{Ccd}	3.71±0.16^{C} Dbc	3.83±0.08 ABCde	3.45±0.16 Ed	3.48±0.14^{DEc}	3.67±0.09 BCbc
	90	*	*	*	*	*	4.07±.1.82^{Bc}	4.07±1.82 Ae	4.14±0.04 ABb	3.95±0.08 Cbc	3.84±0.12^{D} Ebc	3.72±0.05^{E} Fe	3.61±0.17 Gcd	3.67±0.09^{FG}	3.81±0.01 Db

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably ($p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

5.10 Sensory Analysis

Sensory analysis is quick and simple and provides instant information about the fish's characteristics. The unique sensory qualities of seafood products impact the public's satisfaction (Reineccius, 1991). Measurements of colors, odors, textures, appearances, and overalls are all part of the sensory analysis, and they are often made by humans utilizing their sense receptors. It is advised not to eat seafood that has been rejected based on sensorium characteristics, even though its acceptance and analysis are determined by chemical quality. (Turan et al., 2017). This results from instability in product color due to the alteration that reduces its fresh form. The forms in which seafood products appear immediately after they are harvested are indistinct of many attractive colors that captivate one to desire the fish. When processing fish with a marinade solution, sensory analysis is a crucial criterion to determine the durability of the fish. (Kilinc and Cakli, 2004). The sensory results showed that anchovies marinated with acetic acid and refrigerated for 3 months had shelf life preserved throughout the processing. There was a notable disparity ($P<0.05$) among the groups see Table 5.10. The most often used general method for evaluating seafood is compared to the control groups (CO & CM); the groups treated with 1%, 2%, 3%, and 4% acetic acid have higher sensory values. The marinating process significantly influenced the texture, color, look and odor, and general appearance of anchovies. The groups with high acetic acid processing thus obtained the highest sensory scores. On the fifteenth day of storage, the control groups (CO & CM) were eliminated due to poor scores. In the meantime, the panelists rejected the sample treated with 1% acetic acid on days 45 and 60 (Grp 1, Grp 5, and Grp 9, respectively). Following that, the groups marinated in 2%, 3%, and 4% acetic acid had favorable sensory evaluations and could remain in the storage period until the very end. This result is consistent with a study by Gokoglu et al. (2004) that found marinated sardines kept at $4^{o}C$ for three months with 2% and 4% acetic acid still had excellent qualities. Proteolytic enzymes, which break down proteins when exposed to acidic environments, are present in seafood products marinated in solution. Therefore, the deterioration of proteins led to some unique flavorings in marinates (Yapar, 1998).

Table 5.10. The Sensory scores of marinated anchovy

	Storage period (days)	CO 1	CM 2	GR1 3	GR5 4	GR9 5	GR2 6	GR6 7	GR10 8	GR3 9	GR7 10	GR11 11	GR4 12	GR8 13	GR12 14
Odor	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00 Aa	9.00±0.00 Aa	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	3	8.38±0.52^{Bb}	6.88±0.35^{Cb}	8.63±0.52^{ABb}	8.88±0.35 ABa	8.50±0.53 Bb	8.75±0.46A^{Ba}	8.50±0.53^{ABb}	8.50±0.53^{ABb}	8.50±0.53^{Bb}	9.00±0.00^{Aa}	8.38±0.52^{Bb}	8.50±0.53^{ABb}	8.75±0.46^{ABa}	8.63±0.53^{ABa}
	15	1.50±0.53^{Gc}	1.00±0.00^{Ic}	4.63±0.53^{Fc}	5.63±0.52 Eb	7.00±0.00 CDc	7.00±0.00^{CDb}	6.63±0.52^{Dc}	7.88±0.35^{Ac}	7.38±0.52^{BCc}	7.25±0.46^{Cb}	7.00±0.00^{Cc}	8.00±0.00^{Ac}	7.63±0.52^{ABb}	8.00±0.00^{Ab}
	30	*	*	4.00±0.00^{Ed}	4.13±0.35 Ec	4.24±0.46 Ed	5.00±0.76^{Dc}	6.00±0.76^{BCd}	6.25±0.46^{BCd}	5.75±0.71^{Cd}	6.38±0.52^{ABc}	6.38±0.52^{ABd}	6.88±0.35^{Ad}	6.50±0.53^{ABc}	6.50±0.53^{ABc}
	45	*	*	1.13±0.35^{Fe}	4.00±0.00 Ed	4.25±0.46 Ed	5.25±0.46^{Dc}	6.25±0.46^{Ccd}	6.25±0.46^{Cd}	6.13±0.35^{Cd}	6.25±0.46^{Ccd}	6.38±0.52^{BCd}	6.50±0.53^{ABCe}	6.75±0.46^{ABc}	6.88±0.35^{Ac}
	60	*	*	*	1.13±0.35 Dd	1.25±0.46 De	5.00±0.00^{Cc}	6.00±0.00^{Ad}	5.00±0.00^{Cf}	5.00±0.00^{Ce}	6.00±0.00^{Ad}	6.00±0.00^{Ae}	6.00±0.00^{Af}	5.25±0.46^{Be}	6.00±0.00^{Ad}
	75	*	*	*	*	*	5.00±0.00^{Bc}	5.00±0.00^{Be}	5.75±0.46^{Ae}	6.00±0.00^{Ad}	6.00±0.00^{Ad}	6.00±0.00^{Ae}	5.00±0.00^{Bg}	6.00±0.00^{Ad}	5.75±0.46^{Ade}
	90	*	*	*	*	*	5.00±0.00^{Bc}	4.63±0.52Ce	5.00±0.00^{Bf}	5.00±0.00^{Be}	5.00±0.00^{Be}	5.00±0.00^{Bf}	5.13±0.00^{Bg}	5.38±0.00^{Be}	5.38±0.53^{Ae}
Texture	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00 Aa	9.00±0.00 Aa	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	3	8.13±0.64^{Cb}	6.63±0.52^{Db}	8.38±0.52B^{Cb}	8.75±0.46 ABa	8.50±0.53 Ba	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	15	1.00±0.00^{Ec}	1.00±0.00^{EDc}	5.00±0.00^{Cc}	6.50±0.53 Bb	7.00±0.00 Bb	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.25±0.46^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ab}
	30	*	*	4.38±0.52^{Dd}	4.63±0.52 Dd	5.25±0.46 Cc	6.13±0.64^{Bd}	6.38±0.52^{Ac}	6.88±0.35^{Ac}	6.75±0.46^{Ac}	6.88±0.35^{Ac}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	7.00±0.00^{Ac}
	45	*	*	1.63±1.13^{Ce}	4.13±0.35 Bc	4.75±0.89 Ab	5.88±0.83^{Bc}	6.75±0.46^{Ac}	6.88±0.35^{Ac}	7.00±0.00^{Ac}	6.75±0.46^{Ac}	6.88±0.35^{Ac}	7.00±0.58^{Ac}	7.13±0.35^{Ac}	7.25±0.71^{Ac}
	60	*	*	*	1.88±1.13 De	1.88±0.99 Dd	6.00±0.00^{Bd}	6.00±0.00^{Bd}	5.00±0.00^{Ce}	6.00±0.00^{Bd}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	6.00±0.00^{Be}	6.00±0.00^{Be}	7.00±0.00^{Ac}
	75	*	*	*	*	*	6.00±0.00^{Cd}	6.00±0.00^{Cd}	6.00±0.00^{Cd}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	7.00±0.00^{Ac}	6.50±0.53^{Bd}	6.50±0.53^{Bd}
	90	*	*	*	*	*	6.00±0.0	6.00±0.0	6.00±0.0	6.00±0.0	6.00±0.0	6.00±0.0	6.38±0.5	6.25±0.4	6.00±0.0

							0^{Bd}	0^{Bd}	0^{Bd}	0^{Bd}	0^{Be}	0^{Bd}	2^{Ad}	6^{ABde}	0^{Be}
Color	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00 Aa	9.00±0.00 Aa	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	3	8.13±0.35^{Db}	6.25±0.46^{Eb}	8.25±0.46^{CDb}	8.38±0.52 BCDb	8.63±0.52 ABCa	8.38±0.52^{BCDb}	9.00±0.00^{Aa}	5.92±8.50^{Aa}	9.00±0.00^{Aa}	8.75±0.46^{ABa}	8.63±0.52^{ABCa}	8.75±0.46^{ABCa}	8.13±0.35^{CDb}	8.63±0.52^{ABCb}
	15	2.00±0.00^{Fc}	1.00±0.00^{Gc}	5.38±0.74^{Ec}	6.00±0.00 Dc	6.00±0.00 Db	7.00±0.00^{Cc}	7.13±0.35^{Cb}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	7.50±0.53^{Bb}	7.50±0.53^{Bb}	8.00±0.00^{Ab}	7.00±0.00^{Cc}	7.00±0.00^{Cc}
	30	*	*	4.00±0.00^{Ed}	4.13±0.35 Ed	4.00±0.00 Ec	5.00±0.00^{Dd}	5.50±0.53^{Cc}	5.88±0.35^{Bc}	5.38±0.52^{Cd}	6.00±0.00^{Bc}	6.13±0.35^{Bc}	6.75±0.46^{Ac}	6.25±0.46^{Be}	6.25±0.46^{Be}
	45	*	*	1.13±0.35^{Ge}	4.13±0.35 Fd	4.25±0.46 Fc	5.00±0.00^{Ed}	5.50±0.53^{Dc}	5.88±0.35^{CDc}	5.88±0.35^{CDc}	6.75±0.46^{BCc}	6.25±0.46^{ABCc}	6.38±0.52^{ABd}	6.63±0.52^{Ad}	6.63±0.52^{Ad}
	60	*	*	*	1.13±0.35 Ee	1.50±0.53 Dc	4.25±0.71^{Cd}	5.00±0.00^{Cd}	5.00±0.00^{Ac}	5.00±0.00^{Ce}	6.00±0.00^{Ac}	6.25±0.53^{Ad}	5.00±0.00^{Ce}	5.25±0.00^{Cf}	6.00±0.00^{Ce}
	75	*	*	*	*	*	5.00±0.00^{Cd}	5.00±0.00^{Cd}	6.00±0.00^{Bc}	6.00±0.00^{Bc}	6.00±0.00^{Bc}	6.00±0.00^{Ac}	5.00±0.00^{Ce}	5.00±0.00^{Cf}	5.00±0.00^{Cf}
	90	*	*	*	*	*	5.00±0.00^{Bd}	5.00±0.00^{Bd}	5.00±0.00^{Bd}	5.00±0.00^{Be}	5.00±0.00^{Bd}	6.00±0.00^{Ac}	4.00±0.00^{Cf}	5.00±0.00^{Bf}	5.00±0.00^{Af}
Appearance	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00 Aa	9.00±0.00 Aa	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	3	8.00±0.53^{Db}	6.00±0.00^{Eb}	8.38±0.52^{CDb}	8.50±0.53 ABCDb	8.50±0.53 ABCDa	8.38±0.52^{BCDb}	9.00±0.00^{Aa}	8.38±0.52^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Ab}	9.00±0.00^{Aa}	8.63±0.53^{CDb}	8.74±0.56^{BCDb}	8.13±0.99^{BCDb}
	15	2.00±0.00^{Ic}	1.00±0.00^{Jc}	5.00±0.00^{Hc}	5.38±0.52 Gc	6.00±0.00 Fb	6.50±0.53^{Ec}	7.25±0.46^{CDb}	6.50±0.53^{BCb}	7.25±0.46^{Ab}	7.38±0.52^{Bc}	8.00±0.00^{CDb}	7.63±0.52^{Dc}	7.00±0.00^{Dc}	7.00±0.00^{Ac}
	30	*	*	4.00±0.00^{Fd}	4.00±0.00 Fd	4.00±0.00 Fc	5.00±0.00^{Ee}	6.25±0.52^{CDc}	5.00±0.00^{BCc}	6.25±0.52^{Dd}	5.88±0.35^{Bd}	5.38±0.52^{Bc}	6.13±0.35^{Acd}	6.13±0.35^{ABe}	6.63±0.52^{ABde}
	45	*	*	1.13±0.35^{Fe}	4.00±0.00 Ed	4.25±0.46 Ec	5.00±0.00^{De}	5.38±0.52^{Dcd}	5.00±0.00^{Cc}	5.38±0.52^{Cc}	5.88±0.35^{BCd}	5.88±0.35^{ABCc}	6.13±0.35^{ABd}	6.25±0.46^{Ad}	6.38±0.52^{ABd}
	60	*	*	*	1.13±0.33 Fe	1.50±0.53 Ed	4.25±0.71^{De}	5.00±0.00^{Cde}	4.25±0.71^{Cd}	5.00±0.00^{Ce}	5.00±0.00^{Ad}	5.00±0.00^{Bd}	6.00±0.00^{Ce}	5.50±0.53^{Cf}	5.00±0.00^{Ae}
	75	*	*	*	*	*	6.00±0.00^{Ad}	6.00±0.00^{Ac}	6.00±0.00^{Ac}	6.00±0.00^{Ac}	6.00±0.00^{Ad}	6.00±0.00^{Ac}	6.00±0.00^{Ae}	6.00±0.00^{Af}	6.00±0.00^{Ae}
	90	*	*	*	*	*	*	5.00±0.00^{Ae}	5.00±0.00^{Ad}	5.00±0.00^{Ae}	5.00±0.00^{Ae}	5.00±0.00^{Ae}	5.00±0.00^{Af}	5.00±0.00^{Af}	5.00±0.00^{Af}
Overall Ap	0	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00 Aa	9.00±0.00 Aa	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}
	3	7.75±0.89^{Cb}	6.13±0.35^{Dbc}	8.38±0.52^{ABb}	8.88±0.35 Aa	8.88±0.35 Aa	8.75±0.46^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	9.00±0.00^{Aa}	8.88±0.35^{Aa}	8.88±0.35^{Aa}	8.63±0.53^{ABb}	8.38±0.52^{Bb}
	15	1.25±0.46^{Ec}	1.00±0.00^{Fc}	6.00±0.00^{Dc}	6.00±0.00 Db	7.00±0.00 Cb	7.00±0.00^{Cb}	7.00±0.00^{Cb}	7.81±0.53^{Bb}	8.00±0.00^{Ab}	7.50±0.53^{Bb}	8.00±0.00^{Ab}	8.00±0.00^{Ab}	8.00±0.00^{Ac}	8.00±0.00^{Ac}
	30	*	*	4.38±0.5	4.50±0.35	4.38±0.53	5.63±0.5	6.25±0.4	6.21±0.3	5.63±0.9	6.38±0.5	6.50±0.5	7.00±0.0	6.64±0.5	7.00±0.0

			2^{Fd}	Fc	Fc	2^{DEc}	6^{CDc}	5^{ABc}	2^{Ed}	2^{CDcd}	3^{ABCc}	0^{Ac}	2^{BCDe}	0^{Ad}
45	*	*	1.25±0.46^{Ee}	4.06±0.35 Dc	4.25±0.46 Dc	5.38±0.52^{Cd}	6.25±0.71^{ABc}	6.50±0.53^{ABd}	6.13±0.64^{Bc}	6.38±0.52^{ABc}	6.50±0.53^{ABc}	6.38±0.52^{ABd}	6.75±0.46^{Ad}	7.00±0.52^{ABe}
60	*	*	*	1.25±0.46 Dd	1.50±0.53 Dd	4.25±0.71^{Cf}	5.00±0.00^{Bd}	5.00±0.00^{Bf}	6.00±0.00^{Acd}	6.00±0.00^{Ad}	6.00±0.00^{Ad}	6.00±0.00^{Ae}	6.00±0.00^{Af}	6.00±0.00^{Af}
75	*	*	*	*	*	6.00±0.00^{Ac}	6.00±0.00^{Ac}	6.00±0.00^{Ae}	6.00±0.00^{Acd}	6.00±0.00^{Ad}	6.00±0.00^{Ad}	6.00±0.00^{Ae}	6.00±0.00^{Af}	6.00±0.00^{Af}
90	*	*	*	*	*	6.22±0.00^{Ae}	5.00±0.00^{Ad}	5.00±0.00^{Af}	5.00±0.00^{Ae}	5.00±0.00^{Ae}	5.00±0.00^{Ae}	5.00±0.00^{Af}	5.00±0.00^{Ag}	5.00±0.00^{Ag}

Note: Means denoted by varied capital letters in the same row differ remarkably ($p < .05$). Means denoted by varied lowercase letters in the same column differ remarkably ($p < .05$).

There were 14 groups, two control groups (CO) fish without marination and bacteria and (CM) fish samples inoculated with bacteria without marination and 12 treatments which were inoculated with bacteria (*Morganella psychrotolerans*) and marinated with a solution of acetic acid and sodium chloride. (Grp 1) 1% acetic acid + % 6 NaCl, (Grp 5) 1% acetic acid + % 8 NaCl, (Grp 9) 1% acetic acid + % 10 NaCl, (Grp 2) 2% acetic acid + % 6 NaCl, (Grp 6) 2% acetic acid + % 8 NaCl, (Grp 10) 2% acetic acid + % 10 NaCl, (Grp 3) 3% acetic acid + % 6 NaCl, (Grp 7) 3% acetic acid + % 8 NaCl, (Grp 11) 3 % acetic acid + % 10 NaCl, (Grp 4) 4 % acetic acid + % 6 NaCl, (Grp 8) 4% acetic acid + % 8 NaCl, (Grp 12) 4% acetic acid + % 10 NaCl.

CHAPTER VI

CONCLUSION

Fishery products greatly benefit human well-being, yet how they are consumed is related to the dangers of infections and histamine fish poisoning (HFP). Also, more reports and data on incidents need to be collected due to inadequate reports and incorrect diagnoses. HFP is among the significant recurrent illnesses resulting from the consumption of seafood. Emborg isolated *psychrotolerans* as a strong histamine producer, a new psychrotolerant bacteria. The isolated *psychrotolerans* show a harmful histamine aggregation at 0–5^{o}C, and they are considered necessary in the production of histamine in chilled aquatic foods. The strains have been extracted from marine products cross-linked in histamine fish poisoning formation. Marination with the inclusion of acetic acid and salt proved to have sound inhibitory effects on *M. psychrotolerans*, psychrophilic bacteria, total mesophilic bacteria, total yeast and mold, lactic acid bacteria, and coliform) as all these bacteria decreased. Also, all the Physico-chemical parameter values fall below the acceptable limit. The inclusion of acetic acid also enhanced the overall acceptability of the samples. This research's findings would be necessary to the aquaculture and seafood industries. The groups treated with 3 % and 4 % acetic acid with the inclusion of NaCl (6%, 8%, 10) had retarding effects on the reproduction of *Morganella psychrotolerans*, as there was no observation of bacterial cells in the groups with the addition of this treatment from the inception of the study till it ends. There was a continuous increase in the growth of bacteria in the control groups, and the group processed with 1% and 2% acetic acid and their varied NaCl concentration (6%, 8%, 10). Meanwhile, the value isolated for *Morganella psychrotolerans* in groups treated with 1 % acetic acid and NaCl 6%, 8% (5 and 9) was 4.48 log CFU/g on day 60th of the storing time. There was no observation in the bacterial growth for total psychrophilic in groups treated with a marinating solution of 3% and 4% acetic acid includes NaCl (6%, 8%, and 10%) from the inception of the processing till the end. This research shows that acetic acids incorporated with a salt solution to produce marinate can extend the life span of seafood production and render it safe for consumption. There is a general understanding that microorganisms' activities could be hindered during the marinating process because of the combination of sodium chloride and acetic acid solutions. Hence, marinating has a higher percentage to be

applied as sterilization. A low concentration of acetic acid and sodium chloride could not retard the growth of *Morganella psychrotolerans* for a more extended period; this means acetic acid quantity should not be too low. The outcome of this study will improve microbiology attributes and ensure the safe consumption of marinated anchovy fish.

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PUBLICATION FROM THE THESIS WORK

Effects of different marination conditions on the physico-chemical… This study is aimed to determine the effects of different margination conditions (1,2,3,.. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0302038 (13-11-2024).