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Production of Anchovy and Mussel Pastes as Appetizer

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Abstract

Anchovy (*Engraulis encrasicholus*) is a species which mostly caught from Black sea by seine catch. This species represents the majority of the marine species consumed in Turkey. Mussel (*Mytilus galloprovincialis*) is a bivalve that has long been used as a marine food by boiling and stuffing with rice. It is harvested from Mediterranean, Aegean and Marmara sea and is mainly consumed in coastal towns. In this study, anchovy and mussel were used for raw materials. Subsequent to filleting the anchovy and extracting the meat of mussel, these two species were boiled in salty water and grinded. Ultimately, they were introduced separately to the mix of tomato paste, red pepper paste, swatted garlic, cumin, fenugreek powder, ground pepper, salt and olive oil. On account of its consistence, this developed product can be conserved in a paste tube as well as it can be bottled in a glass jar to preserve. The paste is suitable specially for use as appetizer in every repasts and can also be used as consistent intensifier and flavouring supplement during the cooking process. Aside from the production, the shelf life of the product was appointed by routine microbiological test methods (total aerobic mesophilic, psychrotrophic, coliform, Enterobactericeae, Micrococcus, Staphylococcus bacteria, yeast and moulds counts) and also the species of microorganisms found in pastes were identified. This is the first report about anchovy and mussel pastes describing microbiological flora.

Keywords: Anchovy; Mussel; Paste; Shelf life; Microbiology

Practical Application

This novel products promise an easy method to prepare appetizers from anchovy and mussel. It is quite possible to produce this in domestic environment, using only simple kitchen materials. The products can be considered as fairly convenient for working class families with their relatively long shelf life duration (6-7 weeks). Although well sanitized utensils and use of latex gloves are highly recommended to minimize human-induced contamination such as Staphylococcus spp. Within better environmental conditions and with less human impact, these products promise new aquatic food products for the food industry.

Introduction

Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth. Low temperature storage and chemical techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are the most common in the industry today [1]. Spoilage of fresh and lightly preserved fish products is caused by microbial action. *Shewanella putrefaciens* and Pseudomonas spp. are the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish. Modified atmosphere stored marine fish from temperate waters are spoiled by the CO_2 resistant *Photobacterium phosphoreum* whereas Gram-positive bacteria are likely spoilers of CO_2 packed fish from fresh or tropical waters. Fish products with high salt contents may spoil due to growth of halophilic bacteria (salted fish) or growth of anaerobic bacteria and yeasts (barrel salted fish). Whilst the spoilage of fresh and highly salted fish is well understood, much less is known about spoilage of lightly preserved fish products. It is concluded that the spoilage is probably caused by lactic acid bacteria, certain psychrotrophic Enterobacteriaceae and/or *Photobacterium phosphoreum* [2]. The suggested process would address antimicrobial activity as well as destructive oxidation of the desired lipids and fats. However, more efforts are required to understand the role of proximate composition of fish, post-harvest history, environmental conditions, initial microbial load, type and nature of bacteria and their interaction in order to optimize the shelf-life of fish. Proper handling, pre-treatment and preservation techniques can improve the quality fish and fish products and increase their shelf life [3]. Fermentation is one of the preservation techniques. Fermented fish have, for many years, been considered as a Southeast Asian product. These products are highly salted and fermented until the fish flesh is transformed into simpler components [4]. The potential for improving the nutritional quality and shortening the processing time necessary to produce these sauces could be realized by developing controlled microbial fermentations using pure cultures of the appropriate microorganisms. To identify what these are, it would be necessary to survey the microflora of different fish sauces and determine microbial successions [5].

There are many studies related to fish and fishery products in literature [4-12]. However, production of anchovy and mussel pastes as appetizer are novel products for literature. The aim of this study was to produce new fishery products from minced anchovy and mussel, as well as determining the microbiological flora of these pastes.

Materials and Methods

Materials

Materials that are used respectively to produce anchovy and mussel pastes as appetizer are;

Filleted raw anchovy meat (300 g)

Sorted raw mussel meat (300 g)

2 × Canned tomato paste (250 g)

2 × Canned red pepper paste (250 g)

2 × 15 cloves of garlic (Allium cepa)

2 × Virgin olive oil (75 ml)

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2 × Common salt (1 teaspoon)

2 × Flaked red pepper (1 teaspoon)

2 × Black pepper (1 teaspoon)

2 × Cummin powder (2 teaspoons)

2 × Fenugreek powder (25 grams)

Ingredients composition is shown at Table 1 as percentages.

Methods

Production of minced anchovy and mussel: The process of producing anchovy and mussel pastes has not been conducted in an aseptic laboratory, instead, a regular kitchen has been used to prepare and mix the ingredients. The purpose was to see the shelf life of these products in refrigerated conditions (+4°C) when processed by ordinary people in domestic environment. None of the equipment that used to prepare the anchovy and mussel pastes were sterilized, whilst they were sanitized by commercial cleansings. For the supply of raw anchovy and mussel meat (already sorted, ready to process), a regular wholesale market hall is used. Subsequent to filleting operation of anchovy, the meat of mussel and boneless anchovy were boiled in preheated, distilled, salty (4%) water for 5 minutes. Afterwards, the fish and mussel meats were minced by a knife separately. The ultimate weight of boiled and minced meats was 300 ± 10 grams each.

Production of paste: On this phase, two separated paste mixtures were prepared using exactly the same amounts of ingredients on the same conditions. Each one of them includes 250 ± 10 grams of canned tomato paste, 250 ± 10 grams of canned red pepper paste, 15 smashed cloves of garlic (70 grams), 75 ml of ordinary virgin olive oil, a teaspoon of common salt, a teaspoon of flaked red pepper, two teaspoon of cumin powder, a teaspoon of black pepper and 20 ml of fenugreek powder. All these ingredients were introduced into a big bowl to homogenize after the meats were added. Ultimately, the anchovy paste and the mussel paste were build (950 ± 50 grams each).

Refrigerated storage of finished products: Petri dishes were selected as container to piling the final products in the refrigerator. Approximately 12-20 grams of each paste were placed in petri dishes in aseptic environment. Once all the pastes were placed in petri dishes, they were stocked in the refrigerator to be used only for microbiological controls on a regular basis.

Microbiological analysis: Ten grams of anchovy and mussel pastes were homogenized in 90 ml of peptone water separately. Other decimal dilutions were prepared from them. Anchovy and mussel pastes were homogenized in a stomacher (IUL, Barcelona, Spain) for 1 minute. Total mesophilic and psychrotrophic bacteria counts analysis was made by using pour plate method. Plate Count Agar (PCA, Oxoid, CM0325) was used for these analysis. Inoculated petri dishes were incubated 48 hours at 30°C for total mesophilic bacteria count [13] and 14 days at 4°C for psychrotrophic bacteria count [14]. For determining yeast and mould counts; Yeast Extract Glucose Chloromphenicol Agar (YGC, Merck) was used. Inoculated petri dishes were incubated 5 days at 25°C [15]. Enterobactericeae analyse was done by using Violet Red Bile Glucose Agar (VRBD, Merck) according to the method of Vanderzant and Splittstoesser [16]. Inoculated petri dishes were incubated 24 hours at 37°C. Violet Red Bile Agar (VRB, LABM, LAB031) was used for determining coliform bacteria count. Inoculated petri dishes were incubated 24 hours at 30-32°C according to the method of DeMan et al. [15]. Staphylococcus bacteria count analyse was done by using Baird Parker Agar (BPA, Merck). Inoculated petri dishes were incubated 30 hours at 37°C [16]. For determining Micrococcus bacteria count; Mannitol Salt Phenol Red Agar (MSPRA, Merck) was used. Inoculated petri dishes were incubated 48 hours at 30°C [17].

Bacterial identification: The isolated all bacteria species were identified according to method of API system (biomerieux, France). Isolated Micrococcus bacteria was identified by using API E, isolated yeast and mould counts were identified by using API CAUX. Staphylococcus bacteria count was identified by using API Staph test kits.

Statistical analyses: SPSS version 11 statistical package program for social science was used for statistical analyses of anchovy and mussel pastes. One-way analysis of variance was done for determining significant differences with groups. No significant differences indicated as ($p>0.05$). Significant differences indicated as ($p<0.05$).

Results and Discussion

Microbiological analysis

Microbiological changes of anchovy and mussel pastes are shown in Table 2. Enumeration of total mesophilic bacteria load of pastes showed that bacteria levels increased during fermentation period. Numbers of total mesophilic bacteria in the anchovy pastes ranged from 4.48 log cfu/g to 6.92 log cfu/g after 8 weeks of fermentation. Total mesophilic bacteria count of mussel pastes increased from 4.86 log cfu/g to 6.89 log cfu/g at the end of 8 weeks. No significant ($p<0.05$) changes in total mesophilic bacteria counts between the groups occurred during fermentation. But according to time of fermentation, these differences were significant ($p>0.05$) for two groups. Psychrotrophic bacteria counts were determined after 6 weeks of fermentation. On week 8; psychrotrophic bacteria count increased to 4.78 log cfu/g and 4.66 log cfu/g for anchovy and mussel pastes, respectively. Enterobactericeae and coliform bacteria could not be isolated throughout the period of fermentation for two groups due to slowing-down effect on microbiological growth of chopped garlic depending on the garlic concentration, along with some other spices such as black and red pepper [18,19]. Yeast and mould counts of anchovy and mussel pastes ranged between 2.58 log cfu/g (week 2, anchovy paste) and 1 >log cfu/g. No harmful growth ($p <0.05$) was detected for yeast and mould count during the fermentation for both products. The reason why there was no excessive yeast and mould growth is similar with the reason why there was no Enterobactericeae and coliform bacteria growth; it is due to the inhibiting effect of garlic [20]. Micrococcus spp. growth showed little difference in terms of storage. It started with 4.28 log cfu/g for anchovy paste and 5.00 log cfu/g for mussel paste on first week. On the week 8; the numbers were not much different; 5.04 log cfu/g for anchovy paste and 5.48 log cfu/g for mussel paste. Staphylococcus spp. counts showed fairly little difference for either weeks and groups of anchovy and mussel pastes. On the first week; 4.02 cfu/g for anchovy paste and 3.99 cfu/g for mussel paste. For the 8th week; the Staphylococcus spp. loads were 4.52 cfu/g and 4.74 cfu/g for anchovy and mussel pastes, respectively. Although Staphylococcus spp. are commonly found in most kinds of food, they are not in natural microflora of aquatic species. It is only possible to find Staphylococcus spp. in aquatic products when there is human impact or there is a non-aquatic food supplementation [9]. Therefore, it is recommended to produce this kind of products in more sanitized conditions and with less human impact.

Findings of a study about the pastramis which were prepared experimentally have been mixed with the various cemen pastes are

	Percentage					
	30%	**25%**	**7%**	**2,5%**	**1%**	**<1%**
Materials	◦Meat	◦Tomato pure	◦Garlic	◦Fenugreek powder	◦Cummin powder	◦Common salt
		◦Red pepper pure	◦Virgin olive oil			◦Black pepper
						◦Flaked red pepper

Materials on the same column indicates same amount of percentage in products

Table 1: The percentages of Ingredients for both products

Microorganism Types								
Storage (Week)	**Groups**	**Total Mesophilic Bacteria Count (log cfu/g)**	**Micrococcus Bacteria Count (log cfu/g)**	**Staphylococcus Bacteria Count (log cfu/g)**	**Yeast & Mould Counts (log cfu/g)**	**Psychrotrophic Bacteria Count (log cfu/g)**	**Coliform Bacteria Count (log cfu/g)**	**Enterobacteriaceae BacteriaCount (logcfu/g)**
1.	A	4.48 ± 0.04^{aA}	4.28 ± 0.04^{bA}	4.02 ± 0.14^{aA}	$<1^{bA}$	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	M	4.86 ± 0.52^{aA}	5.00 ± 0.15^{aA}	3.99 ± 0.10^{aA}	1.89 ± 0.11^{aA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
2.	A	5.26 ± 0.40^{aB}	4.74 ± 0.06^{aA}	4.20 ± 0.06^{aA}	2.58 ± 0.19^{aB}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	M	5.32 ± 0.08^{aA}	5.00 ± 0.11^{aA}	4.02 ± 0.05^{aA}	2.02 ± 0.70^{bA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
3.	A	5.17 ± 0.05^{aB}	5.28 ± 0.10^{aB}	4.43 ± 0.06^{aA}	$<1^{bA}$	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	M	5.33 ± 0.11^{aA}	5.32 ± 0.16^{aA}	3.96 ± 0.17^{aA}	1.35 ± 0.37^{aA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
4.	A	5.42 ± 0.05^{aB}	4.78 ± 0.02^{aB}	4.21 ± 0.04^{aA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	M	5.44 ± 0.04^{aB}	5.13 ± 0.10^{aA}	3.96 ± 0.16^{aA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
5.	A	5.40 ± 0.07^{aB}	4.97 ± 0.04^{aB}	4.56 ± 0.04^{aB}	1.56 ± 0.07^{aB}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	M	5.55 ± 0.14^{aB}	5.06 ± 0.09^{aA}	4.34 ± 0.07^{aA}	1.35 ± 0.37^{aA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
6.	A	5.10 ± 0.62^{bB}	4.96 ± 0.05^{aB}	4.64 ± 0.13^{aB}	$<1^{aA}$	4.48 ± 0.07^{aB}	$<1^{aA}$	$<1^{aA}$
	M	5.83 ± 0.07^{aC}	5.11 ± 0.07^{aA}	4.44 ± 0.06^{aA}	1.39 ± 0.55^{aA}	4.39 ± 0.15^{aB}	$<1^{aA}$	$<1^{aA}$
7.	A	5.69 ± 0.10^{bC}	5.11 ± 0.06^{aB}	5.65 ± 0.16^{aB}	$<1^{aA}$	4.64 ± 0.15^{aB}	$<1^{aA}$	$<1^{aA}$
	M	6.29 ± 0.12^{aC}	5.41 ± 0.51^{aA}	4.44 ± 0.11^{aA}	1.36 ± 0.39^{aA}	4.65 ± 0.05^{aB}	$<1^{aA}$	$<1^{aA}$
8.	A	6.92 ± 0.04^{aD}	5.04 ± 0.16^{aB}	4.52 ± 0.08^{aA}	1.20 ± 0.17^{aA}	4.78 ± 0.05^{aB}	$<1^{aA}$	$<1^{aA}$
	M	6.89 ± 0.11^{aD}	5.48 ± 0.22^{aA}	4.74 ± 0.17^{aB}	1.42 ± 0.48^{aA}	4.66 ± 0.06^{aB}	$<1^{aA}$	$<1^{aA}$

A stands for anchovy paste M stands for mussel paste
Different capital letters (A,B,C,D) stand for significant differences ($p > 0.05$) dependent on storage
Different small letters (a, b) stand for significant differences ($p > 0.05$) between groups for each week

Table 2: Microbiological changes of anchovy and mussel pastes.

shown [21]. The count of total microorganisms was between 2.8×10^7 and 7.0×10^7/g on the first day, and between 2.2×10^6-3.4×10^6/g on the 60th day. The number of yeast was between 8.2×10^4 and 1.4×10^7/g on the first day, and between 3.8×10^3 and 1.2×10^4/g on the 60th day. The number of mould was between 1.2×10^6 and 2.5×10^6/g on the first day, and between 7.6×10^4 and 5.8×10^8/g on the 60th day. The number of Staphylococcus-Micrococcus microorganisms was between 5.1×10^6-2.1×10^8/g on the first day, and between 1.4×10^5 and 3.9×10^5/g on the 60th day. Enterobactericeae microorganisms were not cultivated in all phases of first stage of pastramis samples. Similar results were determined with this study about inhibiting Enterobactericeae. Another study about production of sardine fermented fish sauce was done by Kilinc [8]. In this study, authors reported that the fish sauces with spices were determined lower bacteria counts than fish sauces without spices. *Staphylococcus aureus* and yeast-mould counts were not detected during fermentation period. The use of spices in fermentation of sardine enhanced good colour, aroma and taste. Verluyten et al. [22] studied the effect of different spices relevant for the production of fermented sausages. In this study; addition of spices to the sausage mixture is clearly a factor that will influence the effectiveness of bacteriocinogenic starter cultures in fermented-sausage manufacturing. In another study, Microorganisms involved in the natural fermentation of Sardinella sp. were enumerated and characterized. Total heterotrophic bacterial counts obtained after six days of fermentation were 6.23×10^5 cfu/g and 5.65×10^5 cfu/g for fish treated with 10 and 15% (w/v) sodium chloride, respectively. Staphylococcus species were the predominant organisms isolated from the fermenting fish [5]. Similar results were determined about high number of Staphylococcus species found in pastes with this investigation. Lactic acid fermented fish were done by using sardine (*Sardina pilchardus*) and anchovy (*Engraulis engrasicholus*). In this study it was determined that only lactic acid, only lactic acid bacteria or lactic acid and lactic acid bacteria together can be used for extending the shelf-life of fish products [23]. Tayar [11] studied the effect of various heating processes on Turkish sausages. This author reported that from microbiological point of view during the heat treatment in which core temperature was 62°C, coliform bacteria was destroyed and in important decline in the total bacteria counts were observed.

According to Turkish Food Codex [24]; for fermented meat products, mould counts must not exceed the limiting value of 3.0 cfu/g and Staphylococcus aureus must be limited to 3.0 cfu/g. There is no specific limitation for total aerobic mesophilic bacterial load for fermented meat derivatives. However, the limit value for total aerobic mesophilic bacterial load of refrigerated meat products is 6.0 log cfu/g. In our products, upper limits for yeast and mould count was 2.58 cfu/g for anchovy paste (on second week) and 2.02 log cfu/g for mussel paste (on second week). Amongst the Staphylococcus spp. isolates of our products, no Stahphylococcus aureus were detected. As for the total mesophilic bacteria count, while the anchovy paste was due on the 8th week, the due date of mussel paste was found as 7th week. On week 7; total mesophilic bacteria counts for anchovy paste was 5.69 log cfu/g and for mussel paste, it was 6.29 log cfu/g. On week 8; this counts were 6.29 log cfu/g and 6.89 log cfu/g for anchovy paste and mussel paste, respectively.

Bacterial identification

In this study, identified bacteria species showed no difference

depending on groups. Identified bacteria species and ratios were the same for both anchovy and mussel pastes. For Enterobactericeae spp., only determined bacteria species was Erwinia spp with a ratio of 80%. Staphylococcus bacteria species were determined as Staphylococcus sciuri and Staphylococcus auricularis with 49.9% and 42.9% ratios, respectively. For the yeasts and moulds identification, determined species throughout the fermentation are identified as Cryptococcus spp. with a ratio of 80%, Cryptococcus humicola with a ratio of 68.8% and Trichosporon mucoides with a ratio of 30.8%.

Kuda et al. [25] studied fish nukazuke is salted and long-fermented fish with rice bran. Histamine (Hm) forming bacteria were isolated from fish nukazuke. They screened bacteria that can inhibit the Hm forming (Hm suppressive) bacteria. Both of the isolates were identified to *Tetragenococcus halophilus*. Paluda Müller et al. [26] reported that Thai fermented fish product prepared from snakehead fish, salt, palm syrup and roasted rice. In fermented fish product 95% of the yeasts as *Zygosaccharo mycesrouxii* was identified as well as lactic acid bacteria. Jeotgal or jeot, a traditional Korean salted and fermented food, is made by adding 20–30% (w/w) salt to various types of sea food worked by Guan et al. [27]. Eleven genera were isolated from both jeotgal samples, including species in the genera Staphylococcus, Bacillus, Halomonas, and Kocuria, with Staphylococcus spp. constituting the highest number. Staphylococcus spp. may not be hugely involved in proteolysis, but they may play a significant role in the ripening of jeotgal. Bacteria of the genus Bacillus and its relative sand of the genus Staphylococcus may be the major organisms involved in jeotgal fermentation. These studies show resemblance with our study in terms of Staphylococcus spp. and yeast loads.

Conclusion

In this study, shelf life of anchovy paste was detected as 7 weeks, whereas the shelf life of mussel paste was detected as 6 weeks according to microbiological analysis. Bacteria species identification showed resemblance for both products. Identified bacteria species were; Erwinia spp., Staphylococcus spp., Cryptococcus spp. and Trichosporon spp. Anchovy and mussel pastes are new products for literature. This is the first report about anchovy and mussel pastes describing microbiological flora and shelf life. For this reason, the results of this study will be very helpful for food industry and readers.

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