Microbiological and Chemical Qualities of Marinated Anchovy Prepared with Different Vegetable Additives and Sauce

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SUMMARY

In this study, frozen anchovies (*Engraulis enchrasicholus*), which were going to be used for marination, were dissolved and cleared from heads, inner organs and fishbones, and then were ripened in marination solution with 4 % acidity and 7.5 % salt content at 4°C for 24 h. After marination, anchovies were processed with different vegetable additives and sauces including marinaded garlic, pasteurized hot pepper sauce, marinaded green olive with toothpick. Then, they were vacuum packed, stored at 4°C for 24 h. Samples were taken from these marinated anchovy fillets, and were examined microbiologically for total mesophilic aerobic bacteria, coliforms, coagulase positive staphylococci, *Salmonella* spp., sulfite reducing anaerobic bacteria, yeast and mold; and chemically for pH, total acidity and total salt content. In addition, ingredients and materials used for processing anchovy fillets after marination process such as vegetable additives, sauce and toothpick, were also examined microbiologically. Our results revealed that vegetable additives and sauces had no adverse effects on the microbiological quality of the final product and the anchovy fillets carried no pathogenic microorganisms that might danger public health. Therefore, we conclude that marinated anchovies are safe for consumption, if they are stored under proper conditions.

KEY-WORDS : Fish, anchovy - marinade - quality - vegetable additives.

RÉSUMÉ

Les qualités microbiologiques et chimiques des filets d'anchois marinés emballés avec différents additifs végétaux et sauces. Par M.K.C. SEN et S. TEMELLI.

Dans cette étude, les anchois congelés pour être utilisés en marinade, ont été décongelés et filtrés par lavage. Après avoir séparé les têtes, les organes intérieurs et les arêtes ont été extirpés, et les filets ont mariné pendant 24 heures à 4° C dans une solution de marinade, laquelle contenait du vinaigre à une acidité totale de 4 % et du sel en proportion totale de 7.5 %. Après marinage, ils ont été traités avec différents additifs végétaux et sauce (purs, avec ail marin, sauce au poivre pasteurisée et olives vertes marinées), puis emballés sous vide et conservés à 4° C pendant 24 heures. Les analyses microbiologiques (aérobies mésophiles totales, bactéries coliformes, staphylocoques coagulases (+), *Salmonella* spp., bactéries anaérobies sulfito-réductrices, levures et moisissures) et chimiques (pH, acidité et salinité totales) des filets d'anchois prêts à consommer ont été faites. En outre, les additifs et les sauces ont subi aussi des analyses microbiologiques. En résultat, on a prouvé que les différents additifs végétaux et sauces n'ont pas eu d'effet sur la qualité microbiologique du produit. Par conséquent, les filets d'anchois marinés préparés ne contiennent pas de bactéries pathogènes pouvant avoir un effet dangereux sur la santé publique et constituent un produit consommable lorsqu'il est conservé dans les conditions convenables.

MOTS-CLÉS : Poisson - anchois - marinage - qualité.

Introduction

Among all the animal-derived foods, fish meat is the one that can easily get spoiled. Therefore, mainly to protect public health, one must apply strict hygienic conditions from manufacture to consumption of this product [36]. In Turkey, where rich water sources exist, fish and other seafoods are widely consumed fresh in certain seasons. Several different methods have been developed to protect fresh fish and seafoods, from manufacture to consumption, such as cooking, cooling, smoke curing, dehumidifying, salting, canning and marination [1, 25].

Long cooking periods can cause the formation of tricyclic amines in the foodstuffs. These chemicals are considered as carcinogenic and could be dangerous for public health. In order to prevent the occurrence of this carcinogenic substances in fish meat, it can be processed with organic acids and salt either during cooking at reduced cooking temperatures or without any heat application. This widely accepted hygienic preservation method is known as marination [5, 27]. Fresh, frozen or salted fish can be used in marination technology. Food that is to be marinated must be kept in a marination solution for a certain period of time to have a complete and efficient marination. This process is known as cooking or ripening [25, 26, 34].

Depending on the seasonal variations, marination solutions can contain 5-8 % vinegar and 10-14 % salt. Fish are immersed into this solution in the ratio of 1.5/1 to obtain an optimum ripening in 18-24 h. At the end of the marination process, fish meat is expected to have total acidity of 2 % and total salt as 3 % [19, 29]. During marination, due to combined activity of salt and acid solutions, microbial activity is inhibited, pH is decreased and the product enters the period of ripening [1, 9]. In the ripening period, salt and vinegar act together with the enzymes originating from the fish, and start degrading the fats and proteins in the fish meat. As a result of this ripening, a nice aroma and a taste forms in the meat [10, 20, 34]. Increasing the vinegar content of the marination solution to achieve to a longer shelf life may cause defects in the taste and the odor of the final product. For this reason, marinades are known to have a limited shelf life and this period extends from a few weeks to a few months [1, 10, 33].

Although added in small amounts, addition of vegetable prepared under poor hygiene conditions, which possibly have high initial microbial counts, may have adverse effects in the aroma and the taste of the final product. In addition to this, these additives can cause spoilage and endanger public health [2, 4, 14, 21, 31, 33]. Vegetable additives, such as hot pepper sauce, garlic and olives are used in marinated fish manufacture, and are known to be effective on the shortening of the product's shelf life [25, 26]. Application of strict hygienic conditions and personel hygiene are of importance, since the addition of this kind of additives take place after marination. In this study, effect of vegetable additives and sauces prepared in various methods on the microbiological and chemical qualities of the end products after application of marination to frozen anchovies, have been examined.

Materials and Methods

MATERIALS

A total of 500 frozen anchovies (*Engraulis enchrasicholus*) with standard size (13-15 cm) were divided into 5 groups. The first group was thawed at 4°C before marination. The second, third and the fourth groups were mixed after marination, with marinade garlic, pasteurized hot pepper sauce and marinade olive with toothpick, respectively. The fifth group after marination without vegetable additives and sauces were used in this study. Frozen anchovies, which were going to be used for marination were ripened in marination solution with 4 % acidity and 7.5 % salt content at 4°C for 24 h after they were thawed, cleared from head, inner organs, fishbone and were filleted. At the end of this period, marinated anchovy fillets were filtered for 15 min and were processed with different vegetable additives and sauces as mentioned below and then were vacuum packed. After packaging, fillets were stored at 4°C for 24 h.

Unmixed anchovy fillets: Filtered and marinated anchovy fillets were set into the packing containers, covered with vegetable oil and then vacuum packed.

Anchovy fillets with marinade garlic: Garlic was marinated with the same marination solution used for fish marination. Filtered marinated anchovy fillets were set into the packing containers with 2 % marinated pressed garlic, were covered with vegetable oil and then vacuum packed.

Anchovy fillets with hot pepper sauce : 2 % pressed marinated garlic was added to filtered marinated anchovy fillets, covered with vegetable oil and were packed under vacuum.

Anchovy fillets with marinade olive with toothpick: Green olive ready to consume was marinated with the same marination solution used for fish marination. Filtered marinated anchovy fillets were rolled over the marinated, 30 % filled green olives and fixed with toothpicks. They were then placed into the packing containers and vacuum packed.

Samples of frozen anchovies and anchovy fillets prepared with different vegetable additives and sauces, which were stored at 4°C for 24 h, were examined microbiologically for total mesophilic aerobic bacteria, coliform bacteria, coagulase positive staphylococci, *Salmonella* spp., sulphite reducing anaerobic bacteria, yeast and mold; and chemically for pH, total acidity and total salt content. In addition, 15 samples from materials used for processing of anchovy fillets after marination process (vegetable additives, sauces and toothpicks) were also examined microbiologically.

Methods

MICROBIOLOGICAL ANALYSIS

All samples were inoculated as double parallels, to Plate Count Agar (PCA-OXOID CM325) by poured plate method for total mesophilic aerobic bacterial count; to Violet Red Bile Agar (VRBA-OXOID CM107) by double layer poured plate method for coliform bacterial count; to Differential Reinforced Clostridial Medium (DRCM-OXOID CM149) by poured plate method for sulphite reducing anaerobic bacterial count; and to Potato Dextrose Agar (PDA-OXOID CM139) by spread plate method for yeast and mold counts. Baird Parker Agar (BPA-OXOID CM275) was used for the detection and enumeration of coagulase positive staphylococci, and suspicious colonies were examined with coagulase test. For *Salmonella* spp. counts, nonselective pre-enrichment, selective enrichment, solid media inoculations were applied. Biochemical and serological tests were performed for *Salmonella* suspicious colonies [3, 7, 24]

CHEMICAL ANALYSIS

pH value was determined by Orion Research Model pHmeter as total acidity from acetic acid type. Salt content determination was done according to Varlik et al. [35].

Statistical Analysis

Total mesophilic aerobic bacterial counts were evaluated with Instat program [15], and Kruskal-Wallis nonparametric variance analysis. When any difference between groups were encountered Dunnet's multiple comparison test was performed. For pH, salt and acidity values SPSS program [30] was used with single way of variance analysis and Tukey's Honestly Significant Difference Test as a post test.

Results

Microbiological analyses of frozen anchovies, which were used for marination, revealed 2.7 x 10^5 cfu/g of total mesophilic aerobic bacteria, 7.1 x 10^1 cfu/g of coliform bacteria, and 1.0 x 10^2 cfu/g of coagulase positive staphylococci. Yeast and mold examinations yielded values under the detection limits. No *Salmonella* spp. or sulphite reducing anaerobic bacteria were detected. The mean pH value of fish was determined as 6.45.

The results of microbiological and chemical analysis of marinated anchovy fillets prepared with different vegetable additives and sauces are presented in Table I and Table II, respectively. The microbiological analysis of the other ingredients used in the processing of anchovy fillets (vegetable additives, sauce and toothpick) are presented in Table III.

Statistical analysis of the results from the microbiological analysis revealed that only total mesophilic aerobic bacteria with statistically significant p value of less than 0.001. Results of statistical analysis of chemical examinations showed that percent salt content ended with statistically significant p value of less than 0.01 and pH and acidity values revealed statistically insignificant results.

Discussion

Generally, microbial flora of fish varies due to fish species, season, nutritional conditions, developmental stage of the fish and the type of water it exists. Although in general a fresh and healthy fish meat is accepted to be sterile, it is known that skin, gills, and intestinal contents include 10^2-10^7 cfu/cm^2 , 10^3-10^6 cfu/cm^2 , 10^3-10^8 cfu/cm^2 microorganism, respectively [1, 9, 17, 33]. During the cold conservation per-

Sample	n	Total mesophilic aerobic bacteria		Coliform bacteria		Coagulase (+) staphylococci		Yeast and mold		*Salmonella spp.*	Sulphite reducing anaerobic bacteria
		x	Sx	x	Sx	x	Sx	x	Sx		
Unmixed	100	$3.7x10^2$	$2.0x10^1{}_d$	$<1.0x10^1$	0	$<1.0x10^2$	0	$<1.0x10^2$	0	-	-
Marinade garlic	100	$6.0x10^2$	$3.6x10^1{}_b$	$<1.0x10^1$	0	$<1.0x10^2$	0	$<1.0x10^2$	0	-	-
Pasteurized hot pepper sauce	100	$4.6x10^2$	$2.3x10^1{}_c$	$<1.0x10^1$	0	$<1.0x10^2$	0	$<1.0x10^2$	0	-	-
Marinade olive with toothpick	100	$1.9x10^3$	$7.3x10^1{}_a$	$<1.0x10^1$	0	$<1.0x10^2$	0	$<1.0x10^2$	0	-	-

a, b, c, d : The differences between the groups demonstrated with different capitals in the same column are significant ($P < 0.05$)

TABLE I. — The results of microbiological analysis of marinated anchovy fillets prepared with different vegetable additives and sauces (cfu/g).

Sample	n	PH		% Salt		% Acidity	
		x	Sx	x	Sx	x	Sx
Unmixed	100	3.95	0.0057	3.38	0.0518_b	2.00	0.0349
Pasteurized hot pepper sauce	100	3.95	0.0066	3.22	0.0879_b	1.97	0.0466
Marinade garlic	100	3.96	0.0071	3.45	0.0529_{ab}	1.93	0.0696
Marinade olive with toothpick	100	3.98	0.0097	3.76	0.0199_a	2.02	0.0633

a, b : The differences between the groups demonstrated with different capitals in the same column are significant ($P < 0.05$).

TABLE II. — The results of chemical analysis of marinated anchovy fillets prepared with different vegetable additives and sauces.

Sample	n	Total mesophilic aerobic bacteria	Coliform bacteria	Coagulase (+) staphylococci	Yeast and mold	*Salmonella spp.*	Sulphite reducing anaerobic bacteria
Vegetable liquid oil	15	$<1.0x10^1$	$<1.0x10^1$	$<1.0x10^2$	$<1.0x10^1$	-	-
Marinade garlic	15	$<1.0x10^1$	$<1.0x10^1$	$<1.0x10^2$	$<1.0x10^1$	-	-
Pasteurized hot pepper sauce	15	$2.0x10^1$	$<1.0x10^1$	$<1.0x10^2$	$<1.0x10^1$	-	-
Marinade green olive	15	$7.0x10^1$	$<1.0x10^1$	$<1.0x10^2$	$<1.0x10^1$	-	-
Toothpick	15	$1.0x10^1$	$<1.0x10^1$	$<1.0x10^2$	$1.0x10^1$	-	-

TABLE III. — The results of microbiological analysis of materials used for processing of anchovy fillets (vegetable additives, sauce and toothpick) (cfu/g-ml).

iod of fish, initial bacterial counts showed an increase in psychrothrophic bacteria and a steady level or decrease in mesophilic bacteria [22, 23]. Psychrotrophic bacteria like *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., *Alcaligenes* spp., *Flavobacterium* spp., *Vibrio* spp., *Achromobacter* spp. and spores of E type *Clostridium botulinum* keep their viability in frozen canned fish and at the same time enterococci, coliform bacteria and staphylococci can also be detected [11, 16, 23]. It is reported that the number of microorganisms decreased, even inhibited during marination process, depending on the acid and salt concentration, however *Lactobacillus* spp. and *Micrococcus* spp. could still be isolated [9, 12,18].

In our study, microbiological analyses of frozen anchovies which were used for marination yielded $2.7x10^5$ cfu/g total mesophilic aerobic bacteria, 7.1x101 cfu/g coliform bacteria, $1.0x10^2$ cfu/g coagulase positive staphylococci. Yeast and mold examinations revealed a value under the detection limits. *Salmonella* spp. and sulphite reducing anaerobic bacteria were not detected. These results are parallel with the results of different scientists who stated that high microbial counts had decreased with the process of freezing [6, 11, 23]. The reason for this reduction in marinated products can be due to salt and acidity. It was reported that acetic acid had antibacterial effects on bacteria, yeast and molds and this effect was more prominent on bacteria and yeast [32]. Therefore, in our study it was shown that marination solution with 4 % total acidity and 7.5 % total salt content decreased or even inhibited the total bacterial count of fish.

ERKAN et al. [10] demonstrated that microorganisms present in fresh trout fillets were inhibited by the marination process with 2 % acidity and 10 % salt content and even could not be isolated during storage. AKSU et al. [1], in the study which they examined the effects of different acid and salt concentrations on the shelf life of marinated anchovies, found out that brine containing 4 % acetic acid and 12 % salt degraded the 10^6 cfu/g mesophilic bacteria, 10^4 cfu/g coliform bacteria, 10^3 cfu/g *Staphylococcus-Micrococcus* group bacteria and 10^3 cfu/g yeast and mold at the first day of marination process. FUSELLI et al. [13] reported that they could not isolate *Staphylococcus* spp., coliform bacteria, *E. coli*, *Clostridium* spp. and yeast and mold in anchovies marinated with 2 % acetic acid. DOKUZLU [9] reported that 50 cfu/g coliform bacteria had been isolated in the products marinated wit 2 % acetic acid and 12 % salt solution. NEYTS and DEBEVERE [28] inoculated *Listeria Monocytogenes* and *Salmonella* spp. in different doses to the marinated products and stated that the rise in the amount of inoculated microorganism increased the viability period of the microorganism in the product. Our results are similar with the results of other researchers who reported the absence of bacteria in the end product [9, 12, 17, 37]. Different processing methods applied to anchovy fillets after marination changes only the total mesophilic bacterial counts. Therefore, analysis of marinade garlic, pasteurized hot pepper sauce, marinade green olive with toothpick and vegetable oil used before packing showed that they had no prominent effect. In a related study, it was shown that filling materials used in marination had no effect on the microflora [12]. In another study, it was determined that adding different vegetables, spices and onion to the marinade caused *Lactobacillus, Streptococcus* and yeast at the level of 10^3-10^7 cfu/g in the end product [8].

In our study, the reason why unmixed anchovies packed only with vegetable oil to have a comparatively lower microbial counts can be explained with the use of vegetable oil alone and the short duration of preparing the product, thus leading to a shorter exposure with the environmental contamination.

It was thought that prolonged period of preparation could explain the high microbial load encountered in products prepared with sauce and garlic. The highest count of total mesophilic bacteria is seen in the products prepared with marinade olive and toothpick, where a rather intense handlind the food is required, and 30 % of the product is made up of olive. The reason for this relatively higher microbial count could be explained with the fact that olive is a fermented product and it could contain the bacteria that took part in fermentation process. In packs containing green olives rolled with anchovy fillets, there is an increase in lactobacilli counts, probably due to the decrease in the acidity of olive brine which eventually causes spoiling by the effects of proteolytic enzymes [16]. Results we obtained in this study indicated that vegetable additives and sauces used during packing after marination did not affect the hygienic quality of the end product since they were also marinated or pasteurized. However, microbiological quality of the processed marinated anchovy fillets could change depending on handling and environmental conditions [12, 13, 16].

pH value that greatly effects the microbiological and enzymatic activity is greatly reduced during marination [1, 32]. The mean pH value of frozen anchovies was 6.45 and this value decreased significantly after marination down to 3.95-3.98. AKSU et al. [1] showed that pH value changes between 4.10 -4.25 in the first day depending on the acetic acid concentration used in marination. VARLIK et al. [33] stated that pH value must be between 4.1-4.5 in marinated products. DOKUZLU [9] determined the pH value of anchovy fillets being 3.87 after marination. ERKAN et al. [10] determined the starting pH value of trout marinades as 4.33.

In our study, total acidity value of the anchovy fillets after marination ranged between 1.9-2.3 %. These values are in parallel with the results of other researchers who reported that total acidity must be between 2.2 -2.5 % [1, 25, 26, 29]. Increase in acidity as a protective factor becomes important in the vinegar and salt combination which has a conserving effect and this in turn plays an important role in a decrease in pH and inhibition of microorganisms. Total salt content was found between 3.4-3.7 %, which has an effect on the shelf life of the product. Our results on this issue are also in agreement with the results of others, who stated that total salt content must be at least 3.5 % in the end product [9, 25, 26].

As a result, different vegetable additives and sauces used for preparing the anchovy fillets had no adverse effect on the microbiological quality of the product. Marinated and processed anchovy fillets do not include any pathogenic bacteria that may cause public health problems. These products, if stored under proper conditions, can be consumed safely.

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