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Investigating the Shelf-Life of the Anchovy Dish Called '*Hamsikuşu*' in Frozen Storage at -18±1°C

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Abstract: '*Hamsikuşu*' is a popular anchovy dish of the Eastern Black Sea region of Turkey. In this study, the shelf-life of this product in frozen storage at -18±1°C was investigated. Four types of the product were packed and frozen at -35°C and stored at -18±1°C. The total bacteria counts, chemical analyses and sensory analysis of the samples were carried out before and at monthly intervals after freezing in order to test the shelf-life and quality.

The results showed that the product was edible after three months of frozen storage at -18±1°C. Therefore, it was concluded that anchovies can be marketed frozen out of season either cooked or uncooked if processed into '*Hamsikuşu*'.

Key Words: Anchovy, *hamsikuşu*, frozen storage, shelf-life

Dondurularak -18±1°C'de Muhafaza Edilen Hamsikuşu'nun Raf Ömrünün Belirlenmesi

Özet: '*Hamsikuşu*', Türkiye'nin Doğu Karadeniz Bölgesinde, hamsiden yapılan popüler bir yemektir. Bu çalışma ile, bu ürünün dondurulduktan sonra -18°C'de muhafazasındaki raf ömrünün belirlenmesi amaçlanmıştır. Dört farklı gruba ayrılan *hamsikuşu* örnekleri -35°C'de dondurularak -18°C'de muhafaza edilmiştir. Dondurma öncesi ve dondurulduktan sonra aylık periyotlarla yapılan duyusal testler, mikrobiyolojik ve kimyasal analizlerle '*Hamsikuşu*'nun kalitesi ve raf ömrü belirlenmiştir.

Elde edilen bulgulara göre dondurularak -18°C'de muhafaza edilen '*hamsikuşu*'nun 3 ay süreyle tüketilebilir özelliğini koruduğu saptanmıştır. Bunu göre, hamsinin "*hamsikuşu*" olarak işlenerek pişirilmiş ya da pişmemiş şekilde avlanma sezonu dışında da tüketime sunulabileceği bu çalışma ile ortaya konulmuştur.

Anahtar Sözcükler: Hamsi, *hamsikuşu*, donmuş depolama, raf ömrü

Introduction

Seafood is an important source of protein for humans. It is claimed to play an important role in a healthy diet. It contains high levels of omega-3 fatty acids and other long-chain unsaturated fatty acids that are effective in either preventing or decreasing the risk of several illnesses such as cardiovascular diseases (1, 2). Therefore, the consumption of seafood should be carried out effectively. However, Turkey lacks the expected effective consumption due to inadequate processing and utilisation techniques. Consumption is as low as around 9.7 kg/person/year (3). It has been reported that most of the seafood production in Turkey is consumed fresh, limiting the consumption to a short period of time and a small region (4).

Anchovies represent 60% of the total catch in Turkey (5). Seventy percent of this catch is provided from the Eastern Black Sea of Turkey (6). They are caught between November and March. In 1995, the production was reported to be 387 574 tonnes (5). It is either marketed fresh or processed into fish-meal and oil in Turkey because its catching season is short. Eleven percent of the total catch and 22% of anchovies go to fish-meal factories for the production of animal feed every year, while seafood consumption is very low in this country (3,6,7). There is only a small amount marketed out of season frozen and salted (4). Unfortunately, these ways are not sufficient to prevent anchovy protein from being wasted in animal consumption. Therefore, it is essential to try alternative ways of marketing anchovies for human consumption in Turkey.

The purpose of this study was to find an alternative way of marketing anchovies for human consumption in a form that will suit Turkish taste. Therefore, we investigated the shelf-life of a popular anchovy dish called "*hamsikuşu*" in frozen form in order to contribute to the ways anchovies are utilised in a wide range of areas in Turkey, and therefore stimulate more economic fish consumption.

Materials and Methods

Materials

The anchovies (*Engraulis* encrasicolus, Linneaus, 1758) used in making '*hamsikuşu*' were purchased within 6 hours of catching, from a market in Trabzon in December, 1997, and transported in ice. They were processed into "*hamsikuşu*" immediately upon arrival at the laboratory.

Methods

Two types of "*Hamsikuşu*" were prepared using unsalted and salted anchovies (brined in 25% salt solution and matured for a month at 4 ±1ºC). This product is made commonly in the Eastern Black Sea region of Turkey. However, the proportions of the ingredients are not clear. Therefore, the proportion of each ingredient of typical "*hamsikuşu*" was identified and tested before the experiments. The ingredients of "*Hamsikuşu*" made from unsalted anchovies were 27% anchovy flesh, 35.1% corn flour, 6.3% wheat flour, 3.1% chard, 7.8% green onions, 1.8% parsley, 0.6% dry mint, 0.6% dry red pepper, 0.6% salt and 16.6% hot water. The ingredients of "*hamsikuşu*" made from salted anchovies were almost the same except that no salt was added and green onions were 8.1%, parsley 1.9%, dry mint 0.7% and chard 3.2% . The anchovy were first headed and gutted. After washing, they were filleted manually. The vegetables were first washed in potable water and dried. Secondly, they were chopped finely. Then all the ingredients were mixed well in a large bowl while hot water was added slowly. Finally, around 20-25 g of the mixture was shaped into meatballs by hand by a person wearing sterile gloves. Then both types of product were divided into two further groups. One group was cooked by frying the product in sunflower oil and the other was left uncooked. All samples were frozen at -35ºC and stored at -18ºC for up to 150 days. The samples were analysed at monthly intervals after thawing at 4ºC for 24 h. The following analyses were carried out.

Microbiological Analysis

The counts of total aerobic mesophilic and psychrotrophic microorganisms were carried out using plate count agar (PCA), incubating at 37ºC for 72 h for mesophilic microorganisms and at 6ºC for 10 days for psychrotrophic ones (8,9). Twenty-five gram samples were aseptically weighed and put into a sterile blender containing 225 ml of sterile peptone water (PW) (0.1% w/v) and blended for 2 min at low speed. Further decimal dilutions were prepared in PW (9,10).

Chemical Analysis

Histamine analysis was carried out by the colorimetric method according to Hardy and Smith (11,12). Total volatile basic nitrogen (TVB-N) content was determined by the method of Lücke and Geidel (13). Thiobarbutaric acid (TBA) values, expressed as mg malonaldehyde/kg, were estimated using the method described by Smith et al. (14).

Sensory Analysis

For sensory analysis, the samples were first thawed. The uncooked products were fried in sunflower oil. Then they were cooled before being submitted to the panellists for assessment. During the sensory analysis, two different methods were used to test the suitability of the panellists even though all of them were familiar with the product. Firstly, a control sample was prepared freshly and the panellists were expected to give the highest score for this product. Secondly, one of the samples was submitted twice to the panellists under different numbers. All the numbers representing the type of samples were unknown to the panellists.

Eight to 10 trained panellists were selected from the 30 that attended. For each analysis they gave scores for the overall acceptability of the cooked samples on a 5-point quality scale: excellent, 5; good, 4; moderately good (acceptable), 3; poor, 2; very poor, 1.

Statistical Analysis

The data obtained were tested using analysis of variance (ANOVA) (15).

Results and Discussion

Table 1 shows the results of psychrotrophic bacteria counts for both cooked and uncooked samples. The highest psychrotrophic bacteria count was observed with the uncooked samples prepared with the salted and unsalted anchovy (2.09×10^4 and 2.45×10^4 cfu/g respectively), before freezing. According to Tukey's test, there was a significant difference during frozen storage ($p<0.001$) for uncooked samples, except on days 0-30, 60-90 and 90-120 for salted samples, and on days 120-150 for unsalted samples. In comparison with the cooked samples, the count was below 30 cfu/g.

Similar results were observed with the mesophilic bacterial counts except that the level was slightly higher (Table 2). According to Tukey's test, there was also a significant difference during frozen storage ($p<0.001$) for uncooked samples, except on days 60-90 and 120-150 for salted samples, and on days 120-150 for unsalted samples.

The acceptability limits for total viable counts (TVC) are between 10^4 and 5×10^6 cfu/g in several countries, according to varying types of fish products. In Japan, a microbiological standard for aerobic TVC of 10^5/g was given for frozen foods including fish products which are heated before being frozen and need to be cooked before being eaten, and 3×10^6/g for foods that need to be cooked before being eaten (16). Our results were found to be within the acceptable quality with regard to this recommendation concerning total aerobic psychrotrophic bacteria.

TVB-N, TBA and histamine contents are used in the determination of the quality and the spoilage degree in fish products. The highest acceptable level for TVB-N was recommended as 30-35 mg/100 g for fish products (17). In this study, the lowest TVB-N values were detected in the samples before freezing (Table 3). There was a significant increase ($p<0.001$) in the TVB-N values for all the samples during frozen storage up to the 60^{th} day. This significant increase continued until the 90^{th} day of storage for the samples made from unsalted anchovies and until the end of the storage period for the samples made from salted anchovies. However, the change was not found to be significant until the 120^{th} day for uncooked samples, and then a significant increase occurred ($p<0.001$). At the end of the storage period, the highest value (14.51 mg/100g) was detected in the uncooked samples prepared with salted anchovies. In comparison with the unsalted anchovies, the highest TVB-N value was also observed with the uncooked samples (12.72 mg/100 g). The lowest value for the samples prepared with unsalted anchovies was 4.24 mg/100 g. A slightly higher value (4.54 mg/100 g) was found with the samples prepared with salted anchovies. The anchovies used in the salted '*hamsikuşu*' samples were kept in salt solution for a month, and then processed. Their storage time was longer than that of the unsalted anchovy samples and, therefore, a higher TVB-N value can be expected.

The oil in oily fish such as anchovies can oxidise. TBA values represent the degree of rancidity in the products and values above 3-4 mg malonaldehyde/kg fish meat

Table 1. Psychrotrophic bacteria counts log (cfu*/g) for '*hamsikuşu*' samples.

Days	Salted Anchovy		Unsalted Anchovy	
	Uncooked	Cooked	Uncooked	Cooked
0	4.32	<1.48	4.39	<1.48
30	4.31	<1.48	4.05	<1.48
60	3.71	<1.48	3.66	<1.48
90	3.58	<1.48	3.92	<1.48
120	3.53	<1.48	3.68	<1.48
150	3.20	<1.48	3.60	<1.48

*colony forming units

Table 2. Mesophilic bacteria counts log (cfu/g) for '*hamsikuşu*' samples..

Days	Salted Anchovy		Unsalted Anchovy	
	Uncooked	Cooked	Uncooked	Cooked
0	5.41	<1.48	5.29	<1.48
30	4.88	<1.48	4.92	<1.48
60	4.67	<1.48	4.76	<1.48
90	4.68	<1.48	4.77	<1.48
120	4.18	<1.48	4.90	<1.48
150	4.28	<1.48	4.74	<1.48

Table 3. The results of TVB-N (mg/100g) for both anchovy and mixed "*hamsikuşu*" samples.

Days	Salted Anchovy		Unsalted Anchovy	
	Uncooked	Cooked	Uncooked	Cooked
0	4.54±0.1 (1.25)	4.54±0.1 (1.25)	4.24±0.09 (1.16)	4.24±0.09 (1.16)
30	5.45±0.3 (1.50)	5.45±0.3 (1.50)	5.05±0.4 (1.39)	4.50 ±0.3 (1.24)
60	9.31±0.5 (2.56)	6.36±0.2 (1.75)	9.31±0.2 (2.56)	6.36±0.2 (1.75)
90	9.31±0.3 (2.56)	7.57±0.4 (2.08)	9.31±0.2 (2.56)	9.31±0.2 (2.56)
120	9.39±0.4 (2.57)	9.31±0.5 (2.56)	9.39±0.4 (2.58)	9.31±0.6 (2.56)
150	14.51±0.4 (3.97)	12.72±0.3 (3.49)	12.72±0.2 (3.49)	9.37±0.06 (2.57)

The numbers in parentheses represent the values of the final products (mixed) containing all the ingredients. n = 6 for all types of samples.

Table 4. TBA The results of TBA (mg malonaldehyde/kg) for both anchovy and mixed "*hamsikuşu*" samples.

Days	Salted Anchovy		Unsalted Anchovy	
	Uncooked	Cooked	Uncooked	Cooked
Initial	0.55±0.07 (0.151)	0.14±0.04 (0.038)	0.81±0.02 (0.222)	0.81±0.04 (0.222)
30	1.20±0.06 (0.330)	0.55±0.07 (0.151)	2.84±0.20 (0.780)	1.67±0.08 (0.459)
60	1.63±0.02 (0.448)	1.20±0.02 (0.329)	2.67±0.07 (0.734)	1.50±0.04 (0.412)
90	2.37±0.10 (0.275)	1.46±0.20 (0.401)	2.63±0.40 (0.723)	1.88±0.07 (0.516)
120	2.62±0.04 (0.720)	2.52±0.10 (0.692)	2.57±0.30 (0.706)	1.88±0.07 (0.516)
150	1.88±0.10 (0.516)	1.89±0.40 (0.519)	3.89±0.20 (1.069)	3.31±0.10 (0.909)

The numbers in parentheses represent the values of the final products (mixed) containing all the ingredients. n = 6 for all types of samples.

indicate quality loss in the product (18,19). Table 4 shows the TBA values of the samples analysed. The changes in the TBA values during frozen storage were found to be significant ($p<0.001$) for each month for salted samples. However, for unsalted samples, the change was not found to be significant from the 30th day up to the 120th day, and then TBA values changed significantly up to the 150th day for uncooked samples. In comparison with cooked samples, the changes in values were found to be significant ($p<0.001$), except on days 90-120. All the values were within the recommended range for TBA. However, the values of the samples prepared with unsalted anchovies were close to the maximum recommended level on the 150th day of storage. The highest values were observed with both uncooked and cooked samples prepared with unsalted anchovies (3.89 and 3.31mg malonaldehyde/kg, respectively) at the end of the storage period. The lowest TBA values for all types of samples were also detected in the initial samples. It was observed that the uncooked samples prepared with the unsalted raw material showed a sudden increase in TBA level from 0.81 to 2.84 mg malonaldehyde/kg on the 30th day of storage. Then this level decreased gradually to 2.57 mg malonaldehyde/kg, and increased sharply up to 3.89 mg malonaldehyde/kg, from the 120th day to the 150th day of storage. A sharp increase also occurred from 1.88 to 3.31 mg malonaldehyde/kg with the cooked samples made from the same type of raw material.

Histamine, which is a derivative compound of histidine mainly formed by bacterial decarboxylation, can cause poisoning if high levels are ingested (20). The foods commonly involved in histamine poisoning of humans are routinely analysed for histamine and many countries have regulations on histamine content in certain foods such as scombroid fish, e.g. tuna and mackerel (21), or non-scombroid fish, e.g. anchovies (22). There have been several discussions about whether or not dietary histamine is the only causative agent of histamine poisoning (23-25). However, Morrow et al. (26) found evidence that it is dietary histamine which causes poisoning. Although other histamine-like amines such as cadaverine, putresine and spermidine play an important role in histamine poisoning (27), the presence of histamine in foods is still used an indicator in this type of poisoning.

It was pointed out that the causative levels differ from different foods as well as different types of fish species. The limit of 10 mg/100 g is considered by some experts to be a suitable maximum limit (28). WHO claims that more than 20 mg/100 histamine can cause poisoning (29). No histamine was detected in the samples analysed in our study.

Twelve out of the 30 people attending (40%) were found to be suitable for the task of sensory analysis. As can be seen from Table 5, all the products were found to be of good quality up to 90 days of frozen storage. The cooked products were of acceptable quality up to the 120th day of frozen storage, according to sensory analysis. In comparison, the uncooked samples were only found to be edible until the 90th day of storage. All the products were rejected at the end of the 150th day.

According to all chemical, microbiological and sensory parameters used to detect the deterioration level, cooking has a retarding effect on the deterioration of the products. Although the results of chemical and microbiological analysis show that the products can be edible for 5 months in frozen storage, they were rejected by the panellists after the 4th month for the cooked samples and the 3rd month for the uncooked samples. Several factors can affect the shelf-life and the consumer acceptability of the products. These factors require further investigation.

Conclusion

It was found that '*Hamsikuşu*' can be stored frozen either cooked or uncooked, and can be of marketable quality for up to 3 months providing it is processed from good quality fresh raw materials. Although the products can be consumed safely for up to 5 months according to chemical and microbiological analyses, sensory analysis showed that acceptable quality only lasted three and four months for uncooked and cooked samples, respectively.

This study shows that this species of anchovy can be marketed as ready-made foods such as '*hamsikuşu*'. This product will increase the anchovy's variety of usage. Since this product is only known locally, determining its shelf-life will be useful in any attempts to introduce it to other parts of Turkey and other countries, possibly as a delicatessen food. This study is also important in that it shows an alternative way of utilising anchovies more economically, for human rather than animal consumption.

Table 5. The results of sensory analysis for overall acceptability of the samples scored by 8-10 trained panellists.

Samples	30th day	60th day	90th day	120th day	150th day
Control	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0
Uncooked Samples With Salted Anchovies	5.0±0.0	4.2±0.4	3.5±0.5	2.7±0.5	1.0±0.0
Cooked Samples With Salted Anchovies	4.5±0.5	4.2±0.4	4.0±0.7	3.2±0.4	1.0±0.0
Uncooked Samples With Unsalted Anchovies	4.2±0.4	3.9±0.3	3.7±0.5	2.2±0.4	1.0±0.0
Cooked Samples With Unsalted Anchovies	4.7±0.5	4.5±0.5	4.0±0.0	3.0±0.0	1.0±0.0

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