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Determination Shelf Life and PAHs Content of Smoked Anchovy (*Engraulis encrasicolus*, Linneaus, 1758) Nugget with Different Level Liquid Smoke Flavors during Chilled Storage

Zayde Alcicek
Department of Fisheries Techniques and Fish Processing Technology,
Faculity of Aquaculture, Firat University, 23119 Elazig, Turkey

Abstract: This study evaluates the effect of different liquid smoking flavor solution on the shelf life of liquid smoked anchovy nuggets stored at 4°C. The fish were smoked in solutions of 0.4 and 0.7 mL with and without frying and then stored at 4°C. TVB-N, TVC and PVC showed increases with the storage time. As a consequence, the succession of these process, it has been determined that the liquid smoked anchovy nugget can be stored safely under chilled storage for 18 days and 0.7 mL solution and pre-cooking for nuggets was found to be optimal for this product.

Key words: Anchovy, nugget, liquid smoke flavoring, shelf life, socioeconomic, Turkey

INTRODUCTION

In recent years, fast food technology has been acquiring importance rapidly due to increase of civilization and socioeconomic factors. In the last two decades, fish consumptions have increased by awareness of consumers about essential fatty acids, mineral and vitamin content of fish. There is several kind of ready-to-eat seafood such as cakes, crackers, burgers, fish fingers, marinated products (Cakli *et al*., 2005; Boran and Kose, 2007). Beside of those, the anchovy is the most important trade fish on the coastal shelf of the Black sea because of processed fish oil and consumed fresh. Anchovy is caught between September and March by a commercial fishing vessel using a purse seine net. Anchovy is generally consumed fresh in Turkey due to its perishable meat (Alcicek *et al*., 2010). At the other hand, the smoking technique is an old fish preserving method for centuries. Smoking has gain to product some antimicrobial and antioxidant compounds as well as the unique organoleptic properties (Alcicek *et al*., 2010). Recently, the smoking industry is using as an alternative smoking method liquid smoked flavorings. By the way, the liquid smoking flavorings has some advantage on contrary to traditional flue gas smoking such as polycyclic aromatic hydrocarbons content which is cheaper and easier application (Alcicek *et al*., 2010; Alcicek, 2010; Cakli *et al*., 2005; Boran and Kose, 2007; AOAC, 1995; Hattula *et al*., 2001; Siskos *et al*., 2007). The effects of liquid smoking on minced fish and nugget prepared from anchovy is not well known. Present study is therefore designed to investigate the qualities of liquid smoked anchovy nuggets. The aim of this study was to determine whether anchovy can be used as a nugget and effect of liquid smoking of anchovy nuggets on the chemical, microbiological and sensory quality during chilled storage.

MATERIALS AND METHODS

Raw material: The anchovies (*Engraulis encrasicolus*, Linneaus, 1758) used in making anchovy nugget were purchased a market in Ankara, Turkey in February, 2010 and transported in ice. They were processed into anchovy nugget immediately upon arrival at the laboratory.

Preparation of samples: The anchovy were 1st headed and gutted, washed and filleted manually. Before anchovy minced, the anchovy fillets were cooked at boiled water for 10 min. The ingredients of anchovy nugget were 1000 g anchovy mince, 220 g potatoes, 1 egg, 100 mL milk, 30 g parsley, 20 g salt, 5 g cumin and 5 g black pepper. After preparation of anchovy nugget mince, the mince was separated two different groups. About 0.4 mL liquid smoke was added to the 1st group (Brennan and Gormley, 2002) while added 0.7 mL to the 2nd group. All ingredients were mixed in a large bowl. The 1st group was cooked by frying in sunflower oil (pre-cook) and the 2nd group was left uncooked. Two types of samples were produced and their shelf lives under chilled condition were compared each other. Each type of anchovy nugget stored with and without frying in sunflower oil. The samples were placed on polystyrene plates, wrapped in stretch film and stored. All samples were stored in +4°C for up to 18 days. Preparation of samples is shown in Fig. 1.

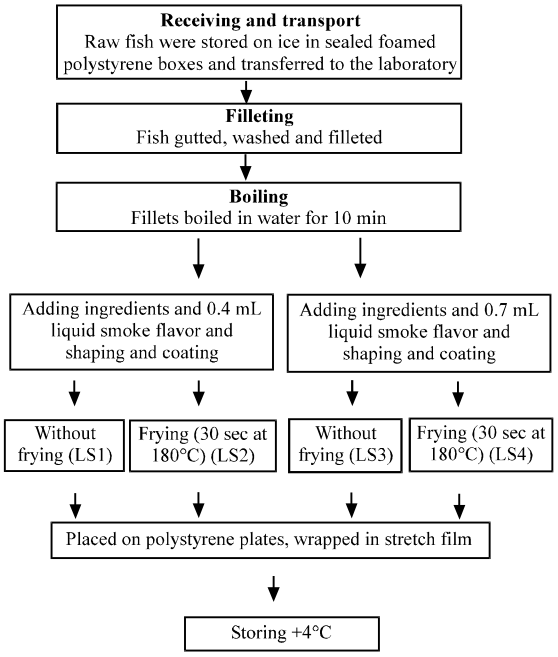

Fig. 1: Flow diagram of the production of liquid smoked anchovy nugget either with or without frying

Chemical analyses: Chemical composition of fish samples was analyzed by standard methods following AOAC protocols (AOAC, 1995). Dry matter content was analyzed by oven drying 3 g of liquid-smoked anchovy samples at 105°C until a constant weight was reached. The flesh was diluted 5 times with distilled water and the pH was measured using a pH meter (Mettler-Toledo, England). Total fat content was analyzed by hexane extraction. Percent protein (Kjeldahl N×6.25) was determined from a 1 g sample for each treatment. TVB-N analysis was performed using the method of Goulas and Kontaminas (2005). NaCl content in fish samples was determined by the Mohr method (Treadwell and Hall, 1928). Water Phase Salt (WPS) content calculated according to Kose (2010). All analyses were carried out in duplicate.

Microbiological analyses: Total Viable Counts (TVCs) and Psychrotrophic Viable Counts (PVCs) were investigated. Anchovy nugget samples were homogenized in Tryptone water for 30 sec in a stomacher. Serial dilutions were prepared until 10^{-7} g mL^{-1} samples were obtained.

TVCs were performed on pure plates with Plate-Count Agar (Merck KGaA, Darmstadt, Germany) after 48 h at 37°C. The same plates were used for the determination of PVCs. The plates were incubated for 7 days at 4°C. After incubations, all colonies were counted following the rules recommended by Gilliland *et al*. (1976).

PAH analyse: Benzo (a) pyrene compound in fish samples were determined using the method of Alcicek *et al*. (2010). The limit of detection was found as 1 ppb. For quality assurance, spiked samples were studied with the samples. The blank fish samples were spiked with benzo (a) pyrene standard at 1/2 MRL (Maximum Residue Level) and MRL levels each in duplicate. The mean recovery was found as 90±0.91% for the levels studied.

Sensory analyses: Five experienced panelists who were members of academic staff and trained in sensory descriptors for smoked seafood were used to evaluate the quality of anchovy nuggets during storage. Anchovy nuggets were assessed on the basis of appearance, odor, taste and texture characteristics using a nine point descriptive scale. A score of 7-9 indicated very good quality; a score of 4.0-6.9 good quality; a score of 1.0-3.9 denoted as spoiled. All the numbers representing the type of samples were always remained unknown to the panelists.

Statistic analyze: The significance of effects of liquid smoked with and without frying sunflower oil was determined by one-way Analysis of Variance (ANOVA) and the multiple Duncan test using the statistical program SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL) at $p \leq 0.05$.

RESULTS AND DISCUSSION

The percentage of total crude protein, crude fat, pH, ash, moisture, salt and WPS content of raw anchovy and liquid smoked anchovy nuggets are shown in Table 1. The crude protein ratios of LS1 and LS2 did not changed significantly due to adding like potato and the other additive matters in the mince. Since, the anchovy is a fat fish the crude fat content of LS1, LS2 and raw anchovy were found to be 14.31±0.07, 14.21±0.21 and 38.4±0.28, respectively. This dramatic decrease can also be explained by additive matters. The pH value of LS1 and LS2 did not change significantly. The mean ash content increased which can be explained by adding high starch content of matters such as potato and salt content. The moisture ratio of all anchovy nugget groups decreased in comparison with that of the raw material. These decrease can be explain with the raw fish has more water content than processed anchovy nuggets. Similar findings were reported by Yanar *et al*. (2006). Level of WPS accepted 3.5% by FDA (2010). This study concluded over those limits that can be eliminated by using lower salt level. The results showed that different level of the liquid smoke flavorings did not affect of the proximate composition of

Table 1: Proximate composition of raw and different liquid smoked processed anchovy nuggets

	Components						
Groups	Crude protein	Crude fat	pH	Ash	Moisture	Salt	WPS (%)
0,4 mL LS group	35.45±0.07	14.31±0.07	6.70±0.14	3.45±0.12	46.08±0.02	2.15±0.01	4.45
0,7 mL LS group	35.50±0.14	14.21±0.21	6.65±0.09	3.35±0.03	46.03±0.04	2.1	4.36
Raw	33.35±0.07	38.40±0.28	6.15±0.01	2.40±0.20	68.44±0.01	0.2	2.91

raw and different liquid smoked anchovy nuggets. As a consequence all of those results are quite agree with the results proposed by Alcicek *et al.* (2010).

Figure 2 shows that changing TVB-N value of the samples during chilled storage. TVB-N value of the samples ranged from 4.01±0.01 to 4.42 mg N/100 g after process. TVB-N amounted to 6.27 mg/100 g in the raw fish and increased in the treated samples, ranging 4.42±0.0, 4.41±0.05, 4.27±0.02 and 4.01±0.01 for LS1-LS4 samples, respectively. These increases can be explained by water loss in the samples during processing. Similar TVB-N values were reported by Kolsarici and Ozkaya (1998). However, there was not a significant increase in the TVB-N values for all the samples until 4th day of storage. In contrary, significant increase was observed after 4th day and this increase continued for all samples at the end of the storage. The increase TVB-N values of LS1 and LS2 can be explained with storage processes without pre-cooking. But the lowest value for the samples of LS3 and LS4 was determined as 25.89±0.06 and 24.85±0.06 mg N/100 g end of the storage. Similar results were found in cooked anchovy dish called Hamsikusu under frozen storage by Kose *et al.* (2001). These results clearly evident that pre-cooked and using high liquid smoke flavoring provide lower TVB-N value than without pre-cooking under chilled storage for anchovy nuggets. Notably in Fig. 2, the increased liquid smoke concentration and pre-cooking had a positive effect on extending the shelf life of the anchovy nugget when TVB-N value was a criterion for quality. According to Huss (1998) when TVB-N concentrations of samples reach 30 mg/100 g the panelists would consider that the samples were spoiled. In similar way, some researchers also reported a positive correlation between microbiological growth and TVB-N (Kose *et al.*, 2001). The results of this study are strongly agree with the results presented by those earlier researchers. The average TVC of fresh samples was 4.7 log cfu g^{-1}. After processing, a TVC reduction was observed in all groups. This reduction was due to the liquid-smoking process as discussed in recent studies (Dimitridau *et al.*, 2008; Siskos *et al.*, 2007). Figure 3 shows that TVC value of the samples. LS1 and LS2 have increase level than LS3 and LS4 after processing. These result evident that both pre-cooking and the antimicrobial effect of the liquid smoke flavoring affected TVC values of samples. According to

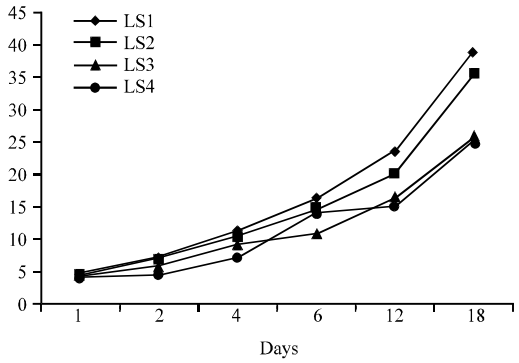

Fig. 2: TVB-N value of the samples

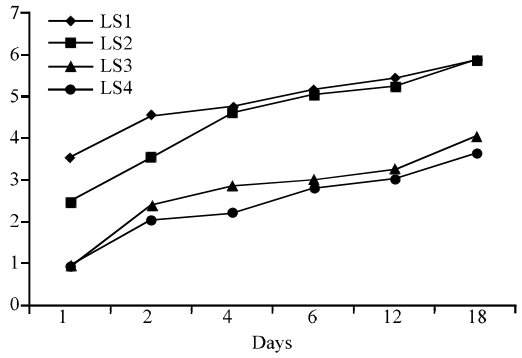

Fig. 3: TVC value of the samples (log cfu g^{-1})

Gennadios *et al.* (1997), the edible coating could reduce the load of spoilage and pathogenic micro-organisms when heated. The TVC values in the LS3 and LS4 samples were still low during storage time. This result can also be explained by pre-cooking effect as well as to friying. Differences between the TVC values were statistically significant during storage time ($p<0.05$). Maximum micro-biological growth had already been reached in LS1 and LS2 samples by the 18th day while LS3 and LS4 samples were still found acceptable by the panelists. The increasing of TVC values during storage period was similar found in sardine fingers stored at -18°C by Cakli *et al.* (2005). Psychrotrophic pathogens can grow in refrigerated foods and may cause some food borne illnesses. A number of researchers have been reported that these kinds of pathogens could be frequently isolated from smoked products (Sunen *et al.*, 2001). PVC values of LS1 and LS2 gradually increase until 4th day of storage (Fig. 4). However, LS1 and LS2

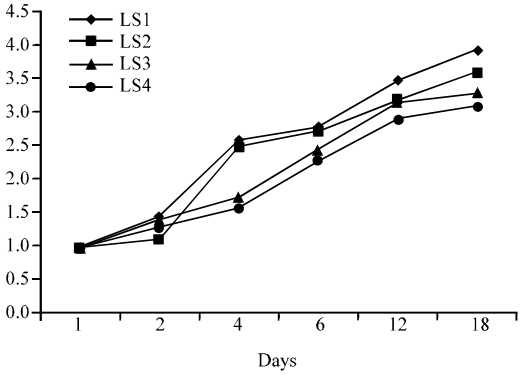

Fig. 4: PVC value of the samples (log cfu g^{-1})

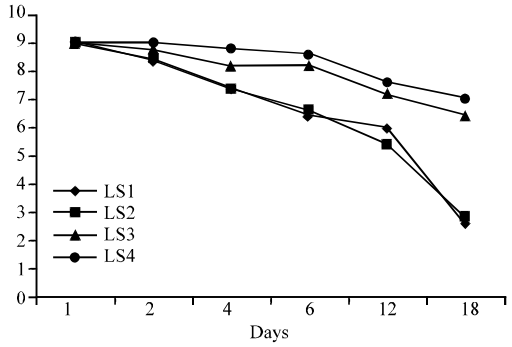

Fig. 5: The changes in sensory analysis total scores during the storage time

dramatic increase in the 4th day and all of the samples were gradually increased during storage time. It must be mentioned that no detectable amounts of benzo (a) pyrene which is an indicator for PAHs (Dimitridau *et al*., 2008) were found in any samples.

Figure 5 shows the changes in sensory analysis scores during storage time. LS1 and LS2 were found good quality until 12th day of storage while were found spoiled 18th day by the panelists. In contrary, LS3 and LS4 were found good quality until the end of the storage. This situation implies a good relationship between sensory and chemical properties and also microbiological analyses.

CONCLUSION

The results of all of mentioned investigation show clearly that the liquid smoked anchovy nuggets can be safely stored at chilled storage for 18 days. This study also points out that anchovy (*Engraulis encrasicolus*, Linneaus, 1758) is a good candidate to market as nugget with using 0.7 mL liquid smoke concentration and pre-cooking.

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