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Research Article

The Determination of Microbial Flora, Water Activity and Chemical Analyses in Smoked, Canned Mussels (*Mytilus galloprovincialis*, L.)

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Abstract: This study reports the water activity, moisture, sodium chloride, pH, acidity, vinegar, total plate count, fecal coliform, coliform and *Clostridium botulinum* of canned mussels processed by 2 different smoking techniques. The results indicated that the average measured water activity was 0.971 ± 0.001 for liquid smoked canned mussel with curry sauce and 0.982 ± 0.001 for traditional smoked mussel. The moisture of samples varied between 69.440% for traditional smoked mussel and 76.35% for salting before traditional smoking. The moisture, salt, pH, acidity and vinegar were significantly different in some of the groups ($P < 0.05$). Mussels smoked using liquid and oak wood sawdust, in tomato puree sauce, curry sauce and sunflower oil sauce, were sterilized at 120 ± 1 ºC and 5.2 ± 0.1 F_0. In none of the products was swelling or *C. botulinum* detected at 37 ° C or 55 ° C. Prior to canning, the mussels were investigated for fecal coliform, coliform and total plate count and found to carry no risk in terms of these parameters.

Key Words: Water activity, smoked canned mussel, salt, moisture, acidity, pH, vinegar, total plate count, coliform, fecal coliform

Tütsülenerek Konservelenen Midye'de (*Mytilus galloprovincialis*, L.) Mikrobiyal Flora, Su Aktivitesi ve Kimyasal Analizlerin Belirlenmesi

Özet: Bu çalışma, iki farklı tütsüleme tekniği uygulanan ve konservelenen midye'nin su aktivitesi, nem, tuz, pH, asitlik, sirke, toplam bakteri sayısı, koliform, fekal koliform ve *Clostridium botulinum* düzeyini içermektedir. Bulgulara göre; ortalama su aktivitesi (S_2L) 0,971 ± 0,001 ile 0,982 ± 0,001 (TSM) arasında değişmektedir. Örneklerin nem içeriği; % 69,440 (TSM) ile % 76, 35 (BTS) arasındadır. Grupların bazılarında pH, asitlik, tuz, nem ve sirke arasındaki değişim önemlidir ($P< 0,05$). Likid ve odun dumanı ile tütsülenmiş midye, köri sos, domates püreli sos ve ayçiçek yağlı sos içerisinde 120 ± 1 ºC ve 5.2 ± 0,1 F_0 değerinde sterilize edilmiştir. Konserve ürünlerin hiçbirinde 37 ve 55 ºC'deki inkübasyonda bombaj ve *C. botulinum*'a rastlanmamıştır. Konserve öncesi ön işlem uygulanmış midye, toplam bakteri sayısı, fekal koliform, koliform bakımından herhangi bir risk taşımamaktadır.

Anahtar Sözcükler: Su aktivitesi, tütsülenmiş konservelenmiş midye, tuz, nem, asitlik, pH, sirke, toplam bakteri sayısı, koliform, fekal koliform

Introduction

The aim of smoking seafood is, besides giving it a desirable taste and odor, to provide a longer shelf life through the antibacterial and antioxidant effects of smoking. Salted and smoked foods lose a large amount of the water that they bear in their structure. The water activity (a_w) value of fresh fish or meat is 0.98-0.99 (1, 2). Lee et al. (3) have reported that during storage there is only a small change in the water activity and color of dried mussels packed in lamina packaging with air inside. Water is one of the most important factors in foods affecting deterioration reactions. In particular, the progress of microbial development or deterioration is highly related to the free or dependent water content of the food (4). The reliability of canned foods is determined by the temperature and duration of heat applied. A pH of 4.9 of canned foods prevents the vegetation of *Clostridium botulinum* spores. Canned fish producers

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must apply sufficient thermal processing to minimize the risk from botulinal spores (5-7).

The purpose of this study was to examine the microbial stability of different smoked canned mussel products on the basis of their a_w, pH, moisture, acidity, vinegar and salt values and to compare them with steamed mussel.

Materials and Methods

Processing Method

Mussels 6-8 cm long and of average weight 25-30 g obtained under culture conditions by a private enterprise were used. Cleaned and block-frozen mussel meat was transported to the laboratory in a styrofoam box and left to defrost at 5 ± 1 °C overnight. Defrosted mussels were dipped into 2 different solutions. The first group of mussel meat (BTS) was exposed to a 3% salt, 1% monosodium glutamate (MSG) solution for 1 h, with a brine proportion of 1:1. The other group (BLS) was left in a liquid smoke solution of 0.4% called Aromsa Smoke Aroma TR 6442, in addition to salt and MSG in the same ratios, for 13.5 h at 5 ± 1 °C. After draining, the first group of mussels (BTS) were dried at 30 °C for 15 min and smoked with oak wood sawdust smoke for 45 min in an AFOS semi-control mechanical smoking kiln. Because of the moistness of the mussel kiln temperature did not exceed 55 °C and the inner temperature of the mussel was 35 °C throughout smoking. Following the smoking process all mussel groups were processed for canning. Before canning, all mussel groups were heated up to 75 °C in order to provide a vacuum during the can sealing stage. The heated mussels with liquid and traditional smoke were canned with 3 different sauces (tomato puree sauce: S_1, curry sauce: S_2 and sunflower oil sauce: S_3) in 200 g cans with a bay leaf in each. All of the sauces contained 4% acetic acid. After the closed cans were placed in the autoclave, they were sterilized at 120 ± 1 °C for F_0 5.2 ± 0.1 min.

Product Group Codes:

SM: Steamed mussel

TSM: Traditional smoked mussel.

BTS: Group kept in brine solution prior to traditional smoking

BLS: Group kept in brine solution containing liquid smoke

S_1M: Traditional smoked canned mussel with tomato sauce

S_1L: Liquid smoked canned mussel with tomato sauce

S_2M: Traditional smoked canned mussel with curry sauce

S_2L: Liquid smoked canned mussel with curry sauce

S_3M: Traditional smoked canned mussel with sunflower oil sauce

S_3L: Liquid smoked canned mussel with sunflower oil sauce

Methods of Analysis

Mussels that had undergone different technological processes were analyzed in comparison with steamed mussels (SM). Moisture was determined according to the AOAC (8). The vinegar and acid levels of the food and sauce groups were determined according to Varlık et al. (9) and salt content according to the TSE. (10). The pH of the samples was measured in a distilled water solution, prepared with a 1/10 meat/water ratio in a Metrohm 692 pH-meter with an electrode connection.

Total plate count was determined using plate count agar (PCA) after incubation for 24 h at 37 °C (11,12). Colonies were counted and data reported as colony forming units (cfu/g). Total and fecal coliforms were determined according to methods previously described (13,14). Sample dilutions of 10^{-1}, 10^{-2} and 10^{-3} with buffered peptone water were transferred to 3 series of test tubes each containing 10 ml of Modified Lauryl Sulphate Tryptose Broth. Following 24–48 h of incubation at 37 ºC, positive tubes were transferred to tubes containing Brilliant Green Bile Broth (BGLB) and incubated for 24–48 h at 37 ºC. The number of test tubes giving positive results with the BGLB was noted.

Canned mussels were left to incube for 10 days at 37 °C and 55 °C, and their swelling status was examined. In samples left at room temperature (22-24 °C) for 1 day, after 10 days of incubation aerobic and anaerobic mesophil bacteria (*C. botulinum*) were investigated. Anaerobic bacteria were determined by Differential Reinforced Clostridial Medium. The occurrence of reproduction was checked after 48 h of incubation at 37 and 55 °C (15).

The a_w values of the foods were determined in a Novasina EEJA-3. For the calibration of the apparatus, 16.20 g of NaCl equal to $a_w = 0.880$ was used. After, the food was put in the measuring chamber, it was left there for 24 h until balance was provided in the atmosphere that circulates in the chamber and then the direct reading was verified. The a_w values of the samples were measured by an electrode consisting of Li salt, on the sensor-top in the heat-controlled chamber. The system's sensitivity was $\pm$ 0.02 a_w / $\pm$ 0.5 °C and the repeatability of the measurements was $\pm$ 0.005 a_w / $\pm$ 0.2 °C.

All analyses were performed in triplicate and the data analysis was carried out with Student's t-test in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Differences were studied at the $P < 0.05$ level.

Results

The chemical analyses before and after canning are summarized in Table 1. The application of salt to the food before the technological process enables the development of flavor. As observed in Table 1, the salt content of SM was initially 0.92% and increased significantly after brining and canning. The starting pH value of the analyzed SM was approximately 6.63. A decrease was observed in the pH values of all the canned foods with 4% acetic acid. When the moisture contents of the pre-processed groups (SM, BTS, BLS, TSM) were compared with each other, it was seen that the moisture level of sample SM at 73% reached 76.35% in BTS as a result of being left in brine and that there was no significant change in BLS. The most important changes were in TSM, with an average rate of 69.44%, and in BTS, with an average rate of 76.35% ($P < 0.05$).

A statistical difference in the pH, salt, moisture and vinegar values was seen in some of the groups before canning ($P < 0.05$) but there was no difference in acidity, salt or water activity ($P > 0.05$).

In all brining processes, salt was used in equal amounts but the amounts used in liquid and traditional smoking differed. In this research, the established a_w values were 0.97-0.98. Sauce content was clearly responsible for the high water activity.

The microbial analysis of the pre-processed mussel is summarized in Table 2. According to the results, there was no risk in terms of total plate count, coliform and fecal coliform. The coliform and fecal coliform levels determined in BLS and TSM appear to be caused by the employees' not obeying the sanitation rules during processing, but these products do not create a problem when consumed provided that they are canned at a high sterilization temperature. Furthermore, *C. botulinum* was not determined at 37 or 55 °C in any of the canned foods.

Table 1. The chemical analyses of mussels (*Mytilus galloprovincialis*, L.) after and before canning.

Product Code	pH[a]	Acidity[a] (%)	Salt[a] (%)	Vinegar[a] (%)	a_w[a]	Moisture[a] (%)
SM	6.65 ± 0.071	0.075 ± 0.016	0.920 ± 0.014	0.042 ± 0.003	0.979 ± 0.002	72.980 ± 0.071
BTS	6.605 ± 0.007	0.081 ± 0.003	0.970 ± 0.042	0.039 ± 0.001	0.975 ± 0.001	76.350 ± 0.453*
BLS	6.325 ± 0.035*	0.085 ± 0.005	1.495 ± 0.007	0.040 ± 0.001	0.975 ± 0.001	72.795 ± 0.799
TSM	6.555 ± 0.007*	0.074 ± 0.012	1.295 ± 0.007	0.029 ± 0.003*	0.982 ± 0.001	69.440 ± 0.226*
S_1M	4.985 ± 0.007*	0.034 ± 0.001	2.510 ± 0.042*	0.070 ± 0.001*	0.977 ± 0.001	70.240 ± 0.622*
S_1L	5.045 ± 0.007*	0.035 ± 0.001	3.020 ± 0.042*	0.076 ± 0.003	0.980 ± 0.001	73.430 ± 0.311
S_2M	4.995 ± 0.007*	0.033 ± 0.000*	1.730 ± 0.014	0.075 ± 0.004*	0.981 ± 0.001	71.135 ± 0.29*
S_2L	5.055 ± 0.007*	0.032 ± 0.001*	1.730 ± 0.014	0.068 ± 0.005*	0.971 ± 0.001	73.800 ± 0.042*
S_3M	4.675 ± 0.007*	0.061 ± 0.000*	2.370 ± 0.057*	0.014 ± 0.001*	0.973 ± 0.001	77.065 ± 0.106
S_3L	4.980 ± 0.014*	0.031 ± 0.000*	2.260 ± 0.000	0.069 ± 0.001*	0.973 ± 0.001	67.585 ± 0.021

[a] Values are reported as means ± standard deviation (n = 3) *Significance level for Student's t-test ($P < 0.05$) compared to the steamed mussel.

Table 2. The microbial flora prior to canning mussels (*Mytilus galloprovincialis*, L).

Product Code	Total Aerobic Plate Count* cfu/g	Coliform* cfu/g	Fecal Coliform* cfu/g
SM	5.4×10^4	23	Absent
BTS	9×10^2	11	Absent
BLS	6.5×10^4	≥ 2400	9
TSM	4.9×10^4	≥ 2400	250

*Determined from 5 samples (n = 5).

Discussion

The quality of the acid used and its proportion in the sauce is very important of canned food. The vinegar used is usually diluted acetic acid in canned products. In this study the pH value of all canned foods was altered by acetic acid. The pH range of canned foods was 4.6 to 5.0. Minor (16) reported that pH 4 might be achieved using vinegar or citric acid and other organic acids to preserve foods. The significance of differences in the pH, salt and acid levels was determined with Student's t-test. According to Student's t-test the difference in pH, salt, acidity and vinegar was due to the sauce content. Water activity (a_w), pH and moisture content are recognized as important indices for controlling the stability of foods. The present study revealed that the moisture content was 68-77% in all mussel groups. The moisture content of SM was 72.9%. Chellappan (17) reported that fresh oyster meat moisture was 80.09%, but steamed oyster moisture was 71.36%. The results obtained for SM meat agreed with those of Chellappan (17).

The accumulations of salt differed among the processed mussel groups. It is assumed that the reason for this is that the mussels were left in brine for different durations and because of the sauce contents in the cans. A decrease in the moisture percentage was observed in TSM and S_3L, due to the sauce content of the food and the smoking technique applied.

Knowing the a_w value of seafood is important for following microbial development. It is possible to control the a_w partly after pre-drying as a result of smoking, whereas it is not possible to keep it stable with the number of total bacteria after canning. In this research, the water activity of the processed mussels was 0.971-0.982. Lee et al. (18) reported in their research on the quality differences of *M. edulis* that the water capacity of the meat changes due to its salt content and that with a salt content of more than 2.8% the water comes out but is absorbed if the salt content is lower. Fernandez-Salguero et al. (19) have reported that some smoked fish products have an a_w of 0.935-0.993 and canned fish products have an a_w of 0.968-0.974. In this research, the microbial spoilage prior to canning was not determined. The bacterial load of the mussels decreased during the shell removal by heating (20). According to Minor (16), the limit values of the total bacteria count of shelled meat are 1×10^5 cfu/g in uncooked meat and 5×10^5 cfu/g in cooked meat. The absence of swelling and *C. botulinum* at 37 and 55 ºC in all of the canned foods is a sign of a safe product in microbial terms. Schantz and Sugiyama (21) reported that *C. botulinum* is eradicated by heating up to 100 ºC. Eklund (22) reported that sodium chloride, sodium nitrite, liquid smoke and other preservatives prevented *C. botulinum* survival.

The microbial control of mussels was guaranteed by the technological processes applied. Furthermore, the samples prepared by using BLS and TSM smoking methods had similar bacterial loads. The results emphasize the importance of hygiene during processing. The moisture level of mussel was decreased by traditional smoking. The power of movement of bivalve forms is generally limited and they feed on the organic substances that the sea brings. They can reflect bacterial changes around them (23). Providing the quality and safety of these forms from the area where they are caught to reaching consumers is important in terms of human health. The details should be investigated while choosing suitable smoking methods for bivalves.

References

1. Yiğit,V.: Gıdalarda Su Etkinliği Ve Önemi, TÜBİTAK Marmara Bilimsel ve Endüstriyel Araştırma Enstitüsü, Beslenme ve Gıda Teknolojisi Bölümü, 1983; Yayın No: 73, 17 sayfa.
2. Temiz, A.: Gıdalarda Mikrobiyal Gelişmeyi Etkileyen Faktörler, sayfa 56. Editör: A. Ünlütürk, F. Turantaş Gıda Mikrobiyolojisi, 1998; Mengi Tan Basımevi, Çınarlı-İzmir.
3. Lee, E. H., Ha, J. H., Oh, K. S., Kown, C. S.: Preparation of powdered sea mussel and anchovy for instant soup. Bull. Korean Fisher. Soc., 1984; 17: 208-212.
4. Certel, M., Ertugay, M.F.: Gıdalarda su aktivitesi termodinamiği. Gıda, 1996; 21: 193-199.
5. Naczk, M., Artyukova, A. S.: Canning in Seafoods. In: Sikorski, Z.E., Ed. Resources, Nutritional Composition and Preservation, CRC Press, Boca Raton, Florida, 1990; 181-198.
6. Regenstein, J. M., Regenstein, C. E.: Introduction to Fish Technology, Van Nostrand Reinhold, New York, 1991; 120-127.
7. Ababouch, L.: Safety of canned seafoods. Quality Assurance in the Fish Industry, Proceedings of An International Conference, Copenhagen, Denmark, Elsevier, 1992; pp 259-267.
8. AOAC: Official Methods of Analysis (13th edn) Association of Official Analytical Chemists, Washington, D.C., 1980.
9. Varlık, C., Uğur, M., Gökoğlu, N., Gün, H.: Su Ürünlerinde Kalite Kontrol ve Yöntemleri, Gıda Teknolojisi Derneği Yayınları, 1993; No.17.
10. Türk Standartları Enstitüsü: Bitkisel Sıvı Yağlı Barbunya Pilaki Konservesi, 1990; TSE-2664, Ankara, Türkiye.
11. Borcaklı, M., Kalafatoğlu, H., Aran, N., Topal, Ş., Karakuş, M.: Gıdalarda Temel Mikrobiyolojik Analiz Yöntemleri, 1994; Marmara Araştırma Merkezi Gıda ve Soğutma Teknolojileri Bölümü, Kocaeli-Gebze, Türkiye, Yayın No: 128.
12. Refai, M. F.: Manuals of Food Quality Control, 4. Microbiological Analysis, 1979; Food and Agriculture Organization of the United Nations, Rome.
13. American Public Healt Association- American Water Works Association: Standard Methods for the Examination of Water and Waste Water 14th. Edition WPCF Publication Office, New York, 1976; 1193 p.
14. Harrigan, W. F., McCance, M.: Laboratory Methods in Food and Dairy Microbiology, Academic Press, London, 1976; 452 p.
15. Hersom, A.C., Hulland, E. D.: Canned Foods Thermal Processing and Microbiology, Churchill Livingtone, London 1980; 380 p.
16. Minor, L. J.: Sanitation. Safety Environmental Standards, Avi Publishing Company, Westport, CT, 1983; 83 p.
17. Chellappan, N. J.: Processing of oyster meat for freezing, Fish. Technol., 1991; 28: 122-124.
18. Lee, B.H., Lee, J. G., Choe, H.Y.: Quality changes in shucked sea mussel, *Mytilus edulis.* Bull. Korean Fisher. Soc., 1975; 8: 208-212.
19. Fernandez-Salguero, J., Gomez, R., Carmona, A.: Water activity in selected high-moisture foods. J. Food Comp. Anal., 1993; 6: 364-369.
20. Bauchriti, N., El.- Marrakchia, A., Goyal, S. M., Boutaib, R.: Bacterial loads in moroccan mussels from harvest to sale. J. Food Prot., 1995; 58: 509-512.
21. Schantz, E. J., Sugiyama, H.: Toxic proteins produced by *Clostridium botulinum.* J. Agr. Food Chem., 1974; 22: 26-30.
22. Eklund, M. W.: Significance of *Clostridium botulinum* in fishery products preserved short of sterilisation. Food Technol., 1982; 36: 107-112.
23. Altuğ, G., Bayrak, Y.: Determination of the levels of indicator bacteria and Salmonella spp. in *Chamelea gallina*, L. and seawater on the coastline of Şile, Turkey. J. Mar. Biol. Ass. U.K. 2002; 82, 673-674.