Uludag Univ. J. Fac. Vet. Med.
24 (2005), 1-2-3-4: 89-93

Assessment of Microbiological Contamination Factors in Frozen Stuffed Snail Processing Stages

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Geliş Tarihi: 24.02.2006
Kabul Tarihi: 28.02.2006

Summary: In this study, six predetermined processing stages and six possible contamination sources in frozen stuffed snail production were examined for the enumeration of total aerobic mesophilic bacteria, coliforms, *Escherichia coli (E. coli)*, coagulase positive staphylococci and for the detection of *Listeria* spp. and *Salmonella* spp.

Results revealed that the cooling stage was a major contamination factor. Also, shells, although 'pasteurized', seemed to be one of the causes for the increases in the final microbial counts of the product. Another minor source for recontamination was determined as the cooling vat. Improvements were recommended for more effective technology applications, which would eliminate risks for contamination for the product.

Key Words: *Helix pomatia*, land snail, microbiological contamination.

Dondurulmuş Salyangoz Dolması Üretiminde Mikrobiyolojik Kontaminasyon Faktörlerinin Belirlenmesi

Özet: Bu çalışmada, dondurulmuş salyangoz dolması üretiminde daha önceden belirlenmiş altı işleme aşaması ve altı olası kontaminasyon kaynağı toplam aerobik mezofilik bakteri, koliform bakteriler, *Escherichia coli (E. coli)*, koagülaz pozitif stafilokokların sayısı ve *Listeria* spp. ve *Salmonella* spp. varlığı yönünden incelendi.

Sonuçlar soğutma aşamasının önemli bir kontaminasyon faktörü olduğunu göstermiştir. Ayrıca pastörize edilmesine rağmen kabukların da son üründeki mikrobiyal sayının artmasında bir etken olduğu düşünüldü. Diğer bir yan kontaminasyon kaynağı ise soğutma teknesi olarak tespit edildi. Ürünün kontaminasyon riskini ortadan kaldıracak daha etkili teknoloji uygulamaları için bir takım iyileştirmeler önerilmiştir.

Anahtar Sözcükler: *Helix pomatia*, kara salyangozu, mikrobiyolojik kontaminasyon.

Introduction

Land snail (classified in Gastropoda class of Mollusca pyhlum) is being produced and exported to many markets of European countries. It is a known fact that molluscan shellfish concentrate particulate matter including pathogenic bacteria during their filter-feeding metabolic process[1].

The increases observed in infections and intoxications due to the consumption of crustaceae and molluscan shellfish[4,8] drew our attention to investigate the contamination sources during the processing of frozen snail meat[11].

As a follow up study, we wanted to determine the factors leading to contamination sources in frozen stuffed snail, an alternative

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product in the market. Results of this study guided us to pinpoint the problems and propose elucidations on those points.

Materials and Methods

Materials

Samples were collected 10 times in different periods from a private fresh water produce processing plant (Kocaman Fisheries and Products Inc.) in Bursa, Turkey. Snail meat samples were collected from the following six predetermined processing stages (Figure 1): after receiving (frozen snail meat), after thawing, after boiling, after cooling, after storing and after freezing. Shell samples were taken from before pasteurization and after pasteurization.

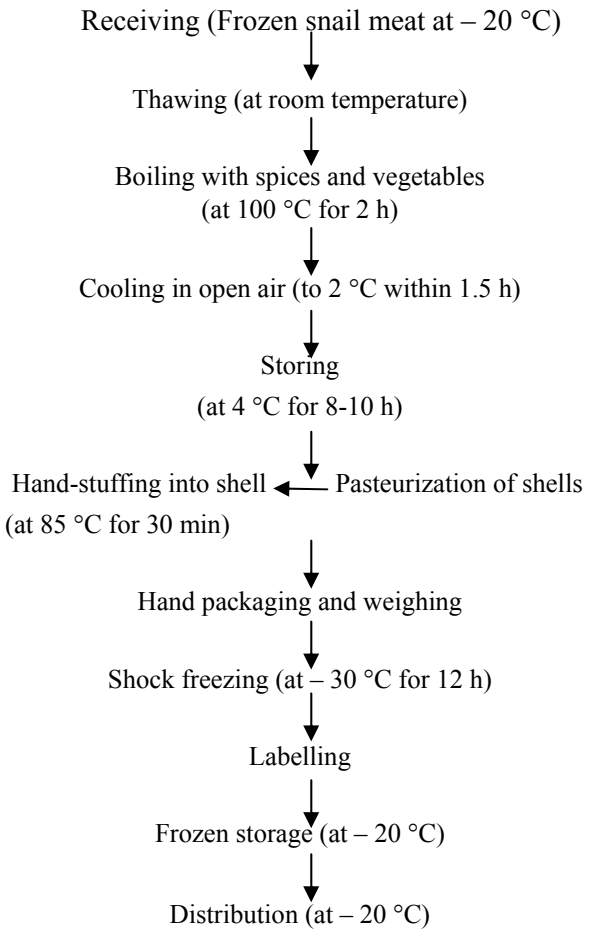

Figure 1.
Flow diagram of frozen stuffed snail processing stages in the plant.

Şekil 1.
İşletmedeki dondurulmuş salyangoz dolması üretim akış şeması.

Samples of drinking water used at the plant, personnel hands, from packaging material (styrofoam plates) and cooling vat surfaces used during processing were also collected. All samples were kept in chillers, and were processed within 2 h of collection.

Samples from the personnel hands were taken as indicated in the literature[5]. Swab method was used to sample from packaging material and cooling vat surfaces as indicated[6].

Packaging room air samples were collected by placing open-lid agar plates on specific areas without air circulation for 15 min and were incubated under appropriate conditions[9].

Two hundred milliliters of water samples were collected at different intervals from drinking water used at the plant in sterile conditions[12].

Methods

Twentyfive and 10 grams of snail meat and shell samples were taken to determine *Listeria* spp. and S*almonella* spp., and for the determination of other bacteria, respectively. Ten fold dilutions of the samples were made by using 0.1% sterile peptone water and previously reported methods as indicated in Temelli et al.[11] have been applied for the enumeration of total aerobic mesophilic bacteria, coliforms, *E. coli* and coagulase positive staphylococci; and for the determination of the presence of *Listeria* spp. and *Salmonella* spp. Water samples were analysed as denoted by Andrews[2].

Statistical Analysis

Statistical analyses were performed by statistical software SPSS for Windows. Microbial levels at processing stages were examined by Friedman nonparametric repeated measures test. Dunn's multiple comparisons test was used as post-test when significant differences were determined[10].

Results

Microbiological analyses of the samples collected from six predetermined processing stages and of the samples suspected as contamination sources are given in Table I and Table II, respectively.

Discussion

In this study, average total aerobic mesophilic bacteria count of frozen snail meat in receiving was 3.69 log cfu/g. This number in-

creased up to 4.22 log cfu/g after thawing at room temperature, reduced to 1.92 log cfu/g after boiling, and rised to 3.68 log cfu/g during cooling. Counts differed significantly between the samples taken from these three stages ($p<0.001$) (Table I). The increase observed in the total aerobic mesophilic bacteria counts from samples at cooling might possibly be due to the insufficiency of the cooling system used. After boiling, snail meat (with spices and vegetables) was transferred to open air vats, which was surrounded by water cooling pipes and was kept at room temperature of the proccessing plant. With this type of a cooling system, meats had to stay open air at danger zone temperatures for 1.5 h, which would have allowed the recovery of the heat-injured bacteria, germination of the spores and also post-contamination from the cooling vat. In the final product, frozen stuffed snails' total aerobic mesophilic bacteria counts were 3.54 log cfu/g. This value was below the indicated levels of cooked crustacea and shellfish European Council Directive 93/493 EEC, 93/51 EEC values (10^5 cfu/g)[7], and The Turkish Manual of Seafood Quality Control Limits (10^6 cfu/g)[3].

Table I. Results of the microbiological analyses of the samples (n=10) collected during the processing stages of frozen stuffed snail.

Tablo I. Dondurulmuş salyangoz dolmasının üretim aşamalarından alınan örneklerin (n=10) mikrobiyolojik analiz sonuçları

Microorganisms	Total aerobic mesophilic bacteria		Coliforms		Coagulase positive staphylococci	
Samples	Mean[a]	Sx[b]	Mean[c]	Sx	Mean[a]	Sx
After receiving	3.69 C[e]	2.85	0.66 C	0.48	< 10 B	0
After thawing	4.22 A	3.14	1.41 A	0.76	0.84 AB	0.67
After boiling	1.92 D	0.65	< 3 D	0	1[d] B	1.01
After cooling	3.68 C	2.99	0.41CD	0.05	0.60 AB	0.34
After storing	3.98 B	3.46	1[d] B	0.68	0.95 A	0.78
After freezing	3.54CD	2.98	0.67 C	0.63	0.60 AB	0.34

[a]Mean, log cfu/g; [b]Sx, standart error; [c]Mean, log MPN/g; [d]Mean, cfu/g;
[e]A-D: Differences between the processing stages demonstrated with different capital letters in the same column are significant ($p<0.05$)

Average coliform count, which was 0.66 log MPN/g in receiving, increased up to 1.41 log MPN/g during thawing. Then, while the count was < 3 MPN/g in boiling, a 0.41 log MPN/g count was determined after cooling. Here, we suspect the cooling vat as a possible source for recontamination (Table II). *E. coli* was not detected from any of the processing stages. In statistical analysis, significant differences were observed between frozen snail meats' coliform counts in receiving and counts obtained from the following important stages of processing ($p<0.001$) (Table I). Coliform counts obtained in the end-product were below the indicated limits for coliforms defined in the Microbiological Criteria of Frozen Land Snails by the Turkish Manual of Seafood Quality Control Values (160 MPN/g)[3].

Table II. Results of the microbiological analyses of the samples (n=10) collected from shells, air, water, packaging material, personnel hands and cooling vat during frozen stuffed snail processing.

Tablo II. Dondurulmuş salyangoz dolmasının üretimi sırasında kabuklar, hava, su, paketleme materyali, personel elleri ve soğutma teknesinden alınan örneklerin (n=10) mikrobiyolojik analiz sonuçları

Microorganisms	Total aerobic mesophilic bacteria		Coliforms		Coagulase positive staphylococci	
Samples	Mean[a]	Sx[b]	Mean[c]	Sx	Mean[a]	Sx
Shell (pre-pasteurization)	3.98	3.46	1[d]	0.69	0.95	0.78
Shell (pasteurized)	2.70	2.13	< 3	-	< 10	-
Packaging room air	0.91[e]	0.09	ND[f]		ND	
Water	1.02[g]	0.06	< 3[h]	-	ND	
Packaging material	< 10	-	< 3	-	< 10	-
Personnel hands	0.81	0.45	0.67	0.63	< 10	-
Cooling vat	0.76	0.23	0.30[d]	0.30	< 10	-

[a]Mean, log cfu/cm^2; [b]Sx, standart error; [c]Mean, log MPN/cm^2; [d]Mean, cfu/g; [e]Mean, log cfu/plate;
[f]ND, not determined; [g]Mean, log cfu/ml; [h]Mean, log MPN/ml

In our study, we did not detect any coagulase positive staphylococci in frozen snail meat samples of receiving. Mean coagulase positive staphylococci in thawed snail meats were found as 0.84 log cfu/g ($p<0.001$) (Table I), and than increased to 0.60 log cfu/g during cooling for 1.5 h. This long and ineffective cooling time might be one of the possible causes for this increase. Still, frozen stuffed snails', coagulase positive staphylococci counts were below the indicated Turkish Manual of Seafood Quality Control Limits (10^3 cfu/g)[3].

We did not detect either *Listeria* spp. or *Salmonella* spp. from any of the samples. These results are in accordance with the related European Directives' criteria of zero tolerance for *Listeria* and *Salmonella* in 25 g of live and cooked crustaceae and molluscan shellfish[7], and with the Turkish Manual of Seafood Quality Control Values[3].

After pasteurization of the shells, average total aerobic mesophilic bacteria count was still found as 2.70 log cfu/g (Table II), with no coliforms, *E. coli* and coagulase positive staphylococci. With this total load, shells seemed to be another contributing factor to increase total counts of the final product.

The packaging room air, water used in the plant and the packaging material were determined to pose no contamination risk for the process (Table II).

Personnel hands with low total aerobic mesophilic bacteria (0.81 log cfu/ml) and coliform counts (0.67 log MPN/ml), particularly with the absence of *E.coli* and coagulase positive staphylococci indicate that the personnel working at this part of the plant applied proper hygiene practices (Table II).

Results of this study show that frozen stuffed snail with its final microbial counts is an acceptable product when evaluated by the current related regulations. However we found the cooling stage as the major factor significantly increasing total aerobic mesophilic bacteria, coliforms and coagulase positive staphylococci during the process. Another source for contamination seemed to be the shells, although pasteurized, effecting the total aerobic mesophilic bacteria counts in the final product. The cooling vat was another suspect source in the cooling stage. With this type of a production flow, the system can easily lead to unsafe end product. Therefore, we recommend the following improvements to eliminate possible fluctuations during processing: Effective technology should be used during cooling (e.g. cooling in a cold room and in a closed system) in order to shorten the time lapse in danger zone. Proper heat-time combination should be applied to obtain shells with lower total aerobic mesophilic bacteria counts.

Acknowledgements

We would like to thank Kocaman Fisheries and Product Inc., for contributions in the realization of the study, and to Aysegul Eyigor for critical comments and revision on English.

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