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Influence of sous-vide cooking on physical, chemical, sensory and microbiological quality in deep water pink shrimp *(Parapenaeus longirostris)*

Einfluss des Vakuumgarens auf die physikalische, chemische, sensorische und mikrobiologische Qualität von Rosa Garnelen (Parapenaeus longirostris)

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Summary

The objective of the study was to determine the effect of sous-vide cooking on the physical, chemical, sensorial and microbiological quality of deep water pink shrimp *(Parapenaeus longirostris)* stored at 4 °C. Four different temperature-time combinations were studied (75 °C/10 min, 75 °C/15 min, 95 °C/10 min, 95 °C/15 min). According to sensory and microbiological results, the treatment at 95 °C for 15 minutes was the most effective treatment for shrimp to extend the shelf life. According to microbiological analysis, maximum shelf life of the groups processed at 75 °C/10 min, 75 °C/15 min, 95 °C/10 min, 95 °C/15 min were found to be 5 days, 9 days, 23 days and 26 days respectively. Overall acceptability scores of sensory analysis had high correlation with the microbiological results.

Keywords: Deep water pink shrimp, sous vide, microbiological quality, sensory, physical quality

Zusammenfassung

Ziel der vorliegenden Studie war es, den Einfluss des Vakuumgarens (Sous-vide) auf die physikalischen, chemischen, sensorischen und mikrobiologischen Eigenschaften der rosa Geißelgarnele *(Parapenaeus longirostris)* bei einer 4 °C Lagerung zu bestimmen. Es wurden vier verschiedene Temperatur-Zeit-Kombinationen untersucht (75 °C/10min, 75 °C/15min, 95 °C/10min, 95 °C/15min). Entsprechend der sensorischen und mikrobiologischen Analysen stellte sich die Kombination von 95 °C bei 15 minütiger Garzeit als die effektivste Behandlung zur Verbesserung der Lagerzeit der Garnelen heraus. Basierend auf den mikrobiologischen Analysen wurde die Haltbarkeit für die Gruppen 75 °C/10 min, 75 °C/15 min, 95 °C/10 min und 95 °C/15 min mit 5 Tagen, 9 Tagen, 23 Tagen und 26 Tagen bestimmt. Die sensorischen Analysen zeigten bezüglich der Gesamtnote eine hohe Korrelation mit den mikrobiologischen Ergebnissen.

Schlüsselwörter: Rosa Geißelgarnele, Vakuumgaren, mikrobiologische Qualität, physikalische Qualität, sensorische Qualität

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Introduction

The term "sous-vide" means "under vacuum" which describes a processing technique whereby freshly prepared foods are vacuum sealed in individual packages and then pasteurized at time-temperature combinations sufficient enough to destroy vegetative pathogens but mild enough to maximize the sensory characteristics of the product (Hyytia-Trees et al., 2000). Advantages associated with sous-vide processing include a superior flavour profile to frozen foods as the process seals in volatile flavour compounds within the package. There is increased tenderness and moisture, improved colour retention and reduced nutritional loss, since nutrients are not leached out in boiling water (Nyati, 2000). The shelf-life of sous-vide products may range from 6 to 42 days (Gonzalez-Fandos et al., 2004). There is very little information available on shrimps being processed by the sous-vide method. The main seafood species usually studied using the sous vide method are salmon, cod, mackerel and horse-mackerel. The safety of sous-vide products depends primarily on heat treatment and low temperature storage (Paik et al., 2006). In addition, the reduced needs for preservatives and flavour enhancers, better preservation of vitamins, and retention of most of the original food juices all contribute to higher quality of sous-vide foods over conventional meals (Hyytia-Treeset et al., 2000). The aim of the study was to investigate the influence of heating temperature and time of sous-vide cooking on the microbiological, sensorial, physical and chemical quality of the deep water pink shrimp which has a high commercial value in the world.

Materials and Methods

Frozen deep water pink shrimp *(Parapenaeus longirostris)* (peeled and gutted before frozen) with a storage period of three weeks was obtained from ASOS company (Pınarbaşı, İzmir, Turkey). Olive oil, lemon juice, salt and garlic powder used in the study were purchased from the market in İzmir.

Processing method

Frozen shrimps were thawed overnight in a refrigerator at 4 °C. For each portion 150 g shrimp, 10 g olive oil, 5 g lemon juice, 0.1 g salt, and 0.1 g garlic powder were added. Each portion was vacuum packaged in polyethylene bags (MULTIVAC, Wolfertschwenden, Germany). They were divided into 4 groups and immersed in a water bath (Memmert WB22) at desired temperatures according to thee groups. Temperature was measured by thermocouples, placed at the centers of 3 packs of each groups. 4 different temperature/ water bath time combinations were studied (75 ºC/10 min (A), 75 ºC/15 min (B), 95 ºC/10 min (C), 95 ºC/15 min(D)). After the thermal process each group was chilled with water/ice combination by immersion and then it was immediately stored at 4 ºC until analysis. Samples were submitted to analysis at 2-day intervals starting with day 0. Physical and chemical quality, instrumental, microbiological and sensory analyses were done within microbial acceptance duration. The study was duplicated.

Analytical Methods

Physical and Chemical Quality Analysis

Thiobarbituric acid, TBA, mg malonaldehide/kg was determined according to the method proposed by Tarladgis et al. (1960). Ten grams of shrimp was blended with 50 ml of distilled water in a Waring blender for 2 min. The mixture was transferred quantitatively into a Kjeldahl flask by washing with an additional 47.5 ml of distilled water. 2.5 ml of HCl solution was added. A small amount of antifoam was placed onto the lower neck of the flask and few saddle stones was added to prevent bumping. Apparatus assembled and flasks were heated at the highest heat obtainable on the Kjeldahl distillation apparatus. 50 ml of distillate was collected. 5 mL distillate and 5 mL of TBA reagent (0.02 M of the solution of 2-TBA in 90 % acetic acid) were blended and immersed in a boiling water bath for 35 min. After cooling under running tap water for 10 min, the absorbance was measured at 538 nm against a blank. The optical density of the diluted solution was measured in a Helios spectrophotometer (Thermo spectronic Rochester). The reading was multiplied by the factor 7.8 to convert to mg.malonaldehyde per 1,000 of shrimp.

pH values of raw and sous-vide cooked shrimps were recorded according to ASU (1980). For determing the pH, 5 g of homogenized shrimp sample was diluted with 5 ml of distilled water and pH value was measured using a Hanna 211 model pH meter (Cluj-Napoca, Romania). Analyses were done in triplicate.

Instrumental analysis

The colour was measured on homogenates prepared from shrimps by using a Kitchen Aid KPM5 Professional meat grinder (St. Joseph, MI, USA). The homogenate was placed in glass petri dishes (12 cm diameter) and the colour measurement was repeated ten times using different parts of the surface. Color measurements were carried out by using a Dr. Lange Spectro Pen®. This is a colorimeter operating on the spectral method described in DIN 5033 (Deutsches Institut für Normung, CIE 95, 2000) using the 45/0 ºC circular viewing geometry, the sample is illuminated with polychromatic light encircling it at an angle of 45º, with the optical unit observing the reflected light from a horizontal angle (0º) towards the sample surface. In the CIE L*a*b* system, L* denotes lightness on a scale from 0 to 100 from black to white; a* denotes (+) red or (–) green; and b* denotes (+) yellow or (–) blue (Schubring et al., 2003). Texture measurement was performed as instrumental texture profile analysis (TPA) using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a flat cylindrical plunger (5 cm diameter) according to (Cadun et al., 2009). The texture parameters hardness, chewiness were measured by double compression of the sample with a test speed of 0.8 mm/s using a cylindrical probe of 5.0 cm diameter. All TPA measurements were repeated 15 times using 15 different shrimps. In addition to these texture measurements on intact shrimps, the penetration force of homogenised shrimps was also determined using the TA.XT Plus Texture Analyser fitted with a spiked aeration plunger. Same test speed as mentioned above was used and measurements were repeated three times. Texture measurements were performed at room temperature.

Microbiological analysis

For all microbial counts, 10 g of shrimp were weighed and transferred into 90 ml of 0.1 % peptone water (Difco, 0118-17-0), and shrimps were homogenized in a Stomacher (IUL Instruments, Barcelona, Spain) for 60 seconds. From the prepared dilutions, total aerobic mesophilic bacteria counts

(TAMBC) were counted on PCA (Oxoid-CM144) incubated for 24/48 h at 30 ºC (Harrigan et al., 1976). Psychotropic bacteria counts (PBC) were counted on PCA (Oxoid-CM144) incubated for 7/10 days at 7 ºC (Ariyapitun et al.,1999). Anaerobic bacteria counts (ABC) were carried out in PCA (Oxoid-CM144) medium and then incubated in anaerobic jar at 35 ºC for 48 h (ICMSF, 1978). For *Enterobacteriaceae* e-numeration, duplicate 1ml pour plates of Violet Red Bile Glucose Agar (Merck) with overlay were prepared using appropriate dilutions. The plates were incubated at 30 ºC for 24 h (ICMSF, 1978). The numeration of *Micrococcaceae* was carried out on Baird-Parker (Merck) medium with incubation 35 ºC for 48 h (ICMSF, 1978).

Sensory analysis

Five trained panelists were asked to evaluate sensory attributes (appearance, odour, structure, flavour) of the samples by using the scoring test of Neuman et al., (1983). Sous-vide cooked shrimps were blind-coded by special codes; the panelists were not informed about the experimental approach. They were asked to give a score for each of appearance, odour, structure and flavour. And then groups were served to the panelists to complete the evaluation of the sensory attributes. The panelists were asked to wash their mouths with warm water between samples. According to the scoring table, a total score of sensory attributes of 20 as indicated as in excellent quality. Scores between 18.2 and 19.9 is indicated as (very good) quality, scores between 15.2 and 18.1 is indicated as (good) quality and scores between 11.2 and 15.1 is indicated as (middle) quality, scores between 7.2 and 15.1 is indicated as the limit of acceptability and scores between 4.0 and 7.1 is indicated as spoiled samples.

Statistical Analysis

The SPSS (SPSS, 1999, Version 9.0. Chicago, IL, USA) program was used to determine for significant differences between mean values. Differences between means values were analyzed by one-way analysis of variance (ANOVA) followed by Tukey and Duncan tests. The results are presented as means ± SD.

TABLE 1: *Changes in TBA (mg malonaldehyde/kg) values of groups during the storage period.*

Storage period (day)	A	B	C	D
0	0.60±0.03^{a1*}	0.59±0.10^{a1}	0.79±0.03^{a1}	0.76±0.03^{a1}
2	0.62±0.06^{a13}	0.94±0.07^{b2}	0.80±0.05^{a24}	0.76±0.05^{a34}
5	0.65±0.02^{a1}	1.02±0.07^{b2}	0.81±0.08^{a12}	0.83±0.13^{ab12}
7		1.13±0.09^{bc1}	0.85±0.06^{ab2}	0.87±0.09^{ab2}
9		1.34±0.09^{c1}	0.95±0.04^{bc2}	0.88±0.01^{ab2}
12			1.01±0.02^{cd1}	0.95±0.02^{abc2}
14			1.03±0.04^{cd1}	0.98±0.05^{bc1}
16			1.09±0.02^{d1}	1.13±0.05^{c1}
19			1.35±0.05^{e1}	1.35±0.12^{d1}
21			1.75±0.02^{f1}	1.79±0.06^{e1}
23			2.03±0.01^{g1}	2.15±0.03^{f2}
26				2.24±0.11^{g}

n: 3 (arithmetic mean ± SD); *: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; $^{abcdefg\ 1234}$:Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 ºC/10 min, B: group processed at 75 ºC/15 min, C: group processed at 95 ºC/10 min, D: group processed at 95 ºC/15 min

TABLE 2: *Changes in pH values of groups durIng the storage period.*

Storage period (day)	A	B	C	D
0	6.94±0.01^{a1*}	6.97±0.06^{a1}	6.92±0.03^{a1}	6.93±0.03^{a1}
2	6.97±0.01^{ab1}	6.97±0.01^{a1}	6.95±0.01^{a12}	6.94±0.01^{a2}
5	7.00±0.02^{b1}	7.01±0.01^{a1}	7.02±0.01^{b1}	7.02±0.01^{b1}
7		7.02±0.01^{a1}	7.02±0.01^{b1}	7.02±0.01^{b1}
9		7.04±0.01^{a1}	7.05±0.01^{bc1}	7.03±0.01^{b1}
12			7.06±0.02^{bc1}	7.04±0.01^{b1}
14			7.06±0.01^{bc1}	7.04±0.01^{b1}
16			7.07±0.02^{c1}	7.06±0.02^{bc1}
19			7.07±0.01^{c1}	7.06±0.01^{bc1}
21			7.09±0.02^{cd1}	7.09±0.01^{c1}
23			7.12±0.01^{d1}	7.10±0.02^{cd1}
26				7.14±0.02^{d}

n: 3 (arithmetic mean ± SD); *: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; $^{abcdefg\ 1234}$:Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 ºC/10 min, B: group processed at 75 ºC/15 min, C: group processed at 95 ºC/10 min, D: group processed at 95 ºC/15 min

Results and Discussion

Physical and chemical quality analysis

TBA values of groups are given in table 1. TBA value of raw shrimp was 0.30 ± 0.02 mg malonaldehyde/kg. Similar results for deep water pink shrimp were found by (Cadun et al., 2005). According to Schormüller (1969), the TBA value should be less than 1 mg malonaldehyde/kg for "excellent" quality, 3 mg for "very good" quality, and 3–5 mg for "good" quality. It has been proposed that the acceptability limit of TBA value for consumption is 8 mg malonaldehyde/kg. TBA values of all groups increased at the end of the storage period. Even, at the end of the storage period, all groups were found to be in perfect quality (Schormüller, 1968; Schormüller, 1969). Wang et al., (2004) reported that sous vide treated chicken wings had lower TBA values throughout the 7 weeks storage period, as compared to those of the non-vacuum packed controls. Jezek and Buchtova (2014) found that vacuum packing of rainbow trout slowed secondary oxidation and TBA values were low when compared with group packed with atmospheric oxygen. pH values of groups were given in table 2. pH values of groups increased significantly at the end of 26 days of storage period probably due to metabolism of microorganisms producing alkaline compounds like amines formed by deamination of amino acids (Huss, 1988; Jackson et al., 1997). These results were similar to those of Rashidi et al., (2014) who reported that pH value of vacuum packed jinga shrimp *(Metapenaeus affinis)* increased during the storage at 4 °C. Dhanapal (2012) reported that heating of muscle or isolated myofibrils usually results in an increase of pH. In the present study, differences in temperature and time of heating did not affect pH values significantly.

Instrumental analysis

Colour values of groups are given in table 3. In general lightness (L*) increased with elevated temperature (Schubring, 2009). In the present study, L* value (Lightness) increased as heating temperature and heating time increased. Similar increase in L* value of shrimps with increased

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TABLE 3: *Changes in colour values of groups during the storage period.*

Groups		Storage period (day)											
		0	2	5	7	9	12	14	16	19	21	23	26
L*	A	55.6^{a1}**	58.06^{b1}	57.63^{ab1}									
	B	58.32^{a2}	64.50^{b23}	61.69^{c2}	59.10^{a1}	59.38^{ac1}							
	C	62.01^{a3}	64.96^{b3}	65.10^{bd3}	65.22^{bd2}	65.61^{bd2}	66.35^{bde1}	66.55^{bde1}	66.66^{bde1}	67.24^{cd1}	68.58^{ce1}	69.34^{c1}	
	D	63.13^{a3}	67.88^{bde4}	66.95^{be3}	67.36^{be3}	66.65^{b2}	68.52^{bef2}	68.58^{eg2}	68.59^{eg2}	69.38^{cdg2}	70.29^{cfg2}	71.12^{c2}	70.25^{cfg}
a*	A	4.05^{a1}	3.24^{b1}	2.45^{c1}									
	B	2.74^{ab2}	3.55^{a1}	2.32^{b1}	2.67^{ab1}	2.61^{ab1}							
	C	3.30^{ab12}	3.40^{ab1}	3.05^{a12}	3.82^{abc2}	4.07^{abc2}	4.29^{abc1}	4.39^{bc1}	4.41^{bc1}	4.49^{bc1}	4.53^{bc1}	4.58^{bc1}	
	D	3.16^{a12}	3.47^{ac1}	3.67^{ab2}	3.79^{ab2}	3.84^{ab2}	3.89^{ab1}	3.9^{ab1}	3.95^{ab1}	4.26^{ab1}	4.56^{bc1}	4.81^{b1}	4.92^{b}
b*	A	9.70^{a1}	7.52^{b1}	6.56^{b1}									
	B	8.14^{ab2}	8.70^{a12}	6.92^{bc1}	5.97^{c1}	6.07^{c1}							
	C	9.82^{a1}	8.60^{ab12}	8.46^{ab12}	8.47^{ab2}	8.70^{ab2}	8.66^{ab1}	8.46^{ab1}	8.54^{ab1}	8.36^{b1}	8.19^{b1}	8.22^{b1}	
	D	10.34^{a1}	9.32^{ab2}	10.34^{a2}	9.63^{ab3}	8.83^{ab2}	8.49^{b1}	8.66^{ab1}	8.38^{b1}	8.30^{b1}	8.38^{b1}	8.28^{b1}	8.30^{b}

n: 10 (arithmetic mean ± SD); ** :Means in the same row with the same letter in the same attribute do not differ significantly at the level of 0.05 significance; abcdefg $^{123\,abcdefg}$: Means in the same column with the same number in the same attribute do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D: group processed at 95 °C/ 15 min

heating temperature were determined by Niamnuy et al., (2007) and Schubring (2009). L* values of all groups increased at the end of the storage period. In group C and D this increase was significant ($p<0.05$). b* values of all groups decreased significantly at the end of the storage period ($p<0.05$). The increase in b*(yellowness) value could be due to the oxidation of lipids and proteins and the subsequent formation of yellow pigments (Thanonkaew et al., 2007). Gokoglu and Yerlikaya (2008) reported an increase in b* values in shrimps *(Parapenaeus longirostris)* during refrigerated storage at 4 °C for 3 days. a* (redness) values of groups with a heating temperature at 75 °C, decreased at the end of the storage period while a* values of groups with a heating temperature 95 °C, increased at the end of the storage period. Martinez-Alvarez et al., (2009) reported that the cooking process significantly changed the colour of shrimps. According to TPA measurements, hardness and chewiness values of group A increased ($p<0.05$) significantly, whereas hardness and chewiness values of group processed at D decreased ($p<0.05$) significantly at the end of the storage period. Hardness value and chewiness value of groups are given in table 4 and 5, respectively. Penetration force values of groups are given in table 6. Penetration force of all groups decreased at the end of the storage period ($p<0.05$). Decrease in penetration force can possibly be caused due to increasing denaturation of proteins. Penetration value of group D had significantly higher values than the other groups until day 14. After day 14, no significant differences were determined between group C and group D. Physicochemical changes occurring during thermal processing strongly depend on the denaturation of meat protein, moisture loss composition and characteristics of the muscles, the heating method, as well as the time/temperature evolution during cooking (Christensen et al, 2000; Mora et al., 2011).

Microbiological analysis

The initial microbiological loads of frozen shrimp were found to be 5.78 ± 0.02, 5.41 ± 0.04, 5.30 ± 0.02, 1.14 ± 0.57, 1.95 ± 0.02 for log TAMBC/g, log PBC/g, log ABC/g, log *Enterobacteriaceae*/g, log *Micrococcaceae*/g, respectively. The total aerobic mesophilic bacteria count (TAMB, log CFU/g) did not exceed the maximum limit (7 log APC/g) of microbiological criteria for frozen shrimp given by ICMSF (1978) and FDA (2013). Microbiological quality of the shrimps is given in table 7. Microbial load decreased

TABLE 4: *Changes in hardness values (N) of groups during the storage period.*

Storage period (day)	A	B	C	D
0	11.90±9.28^{a1}*	14.23±6.77^{a1}	16.01±8.79^{ab1}	21.69±8.74^{a1}
2	16.23±10.39^{ab1}	15.49±5.21^{a1}	13.54±8.22^{a1}	25.73±5.15^{a2}
5	14.42±10.49^{ab1}	13.82±8.95^{a1}	15.15±8.13^{ab1}	23.86±12.71^{a1}
7	26.38±8.73^{b1}	23.87±13.42^{a1}	19.44±6.41^{ab1}	21.03±11.03^{a1}
9		15.66±11.85^{a12}	11.62±6.18^{a1}	25.31±13.34^{a2}
12			26.63±9.28^{b1}	20.42±13.17^{a1}
14			14.23±8.76^{a1}	12.72±8.80^{a1}
16			13.49±9.25^{a1}	12.63±8.45^{a1}
19			13.58±8.87^{a1}	11.87±9.42^{a1}
21			12.87±8.91^{a1}	11.68±8.95^{a1}
23			12.43±6.82^{a1}	11.78±7.63^{a1}
26				09.67±8.89^{a}

n: 15 (arithmetic mean ± SD); *: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; $^{abcdefg\ 1234}$:Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D: group processed at 95 °C/15 min

TABLE 5: *Changes in chewiness values of groups during the storage period.*

Storage period (day)	A	B	C	D
0	1.56±1.05^{a1}*	2.91±1.60^{a1}	3.19±2.38^{a1}	5.07±1.41^{a1}
2	2.31±1.64^{a12}	3.16±1.55^{a12}	2.01±0.89^{a1}	5.90±1.46^{a2}
5	2.67±1.46^{a1}	2.87±1.25^{a1}	3.13±1.78^{a1}	6.51±3.76^{a1}
7	2.49±0.51^{b1}	2.91±1.21^{a1}	4.35±2.11^{a1}	4.90±3.84^{a1}
9		2.98±2.48^{a1}	1.86±1.07^{a1}	4.35±2.67^{a1}
12			5.91±2.57^{a1}	4.15±2.62^{a1}
14			3.02±2.11^{a1}	2.81±2.70^{a1}
16			2.90±1.99^{a1}	2.70±1.67^{a1}
19			2.87±2.05^{a1}	2.65±2.20^{a1}
21			2.88±1.97^{a1}	2.45±1.45^{a1}
23			2.45±2.04^{a1}	2.40±2.20^{a1}
26				1.38±1.67^{b}

n: 15 (arithmetic mean ± SD); *: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; $^{abcdefg\ 1234}$:Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D: group processed at 95 °C/15 min

TABLE 6: *Changes in penetration force (N) of groups during storage period.*

Storage period (day)	A	B	C	D
0	0.84±0.05^{a1}*	1.20±0.22^{a1}	1.10±0.40^{a1}	2.30±0.23^{a1}
2	0.83±0.06^{a1}	1.18±0.15^{a1}	1.06±0.32^{ad1}	2.26±0.14^{a2}
5	0.81±0.08^{a1}	0.97±0.03^{b1}	1.33±0.08^{ac2}	2.12±0.10^{a3}
7	0.61±0.07^{b1}	0.68±0.06^{c1}	0.71±0.03^{b1}	0.87±0.05^{b2}
9		0.55±0.03^{c1}	0.71±0.02^{b2}	0.76±0.02^{b3}
12			0.72±0.07^{b1}	0.99±0.09^{b2}
14			0.77±0.04^{db1}	0.98±0.64^{b1}
16			0.70±0.051^{b}	0.88±0.07^{b2}
19			0.69±0.061^{b}	0.76±0.06^{bc1}
21			0.67±0.081^{b}	0.75±0.05^{c1}
23			0.61±0.05^{b}	0.72±0.04^{c2}
26				0.69±0.07^{c}

n: 3 (arithmetic mean ± SD); *: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; $^{abcdefg\ 1234}$:Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D: group processed at 95 °C/15 min

authors, reported that *Enterobacteriaceae* were detected in raw trout and after the sous-vide treatment, *Enterobacteriaceae* were only detected in batches processed at 70 °C after 45 days of storage at 10 °C. Gonzalez-Fandos et al. (2005) reported that *Enterobacteriaceae* were detected in raw salmon (2.66 ± 0.91 log CFU/g) whereas no growth was observed after the sous vide treatment. Nyati (Nyati, 2000) reported that *Enterobacteriaceae* were not isolated from any product after thermal process despite their presence in the raw materials. Just after the sous vide process, the initial total aerobic mesophilic bacteria counts of group A, B, C, D were 5.25 log CFU/g, 3.78 log CFU/g, 3.41 log CFU/g and 3.25log CFU/g respectively. The most important microorganisms at refrigeration temperatures are psychrotrophic microorganisms (Paik, 2006). Psychrotrophic bacteria count was lower when the heating temperature and heating time were higher ($p<0.05$). Psychrotrophic bacteria count increased significantly at the end of the storage period ($p<0.05$). Similar findings were reported by Gonzalez-Fandos et al., (2004), who reported that the psychrotroph growth in sous-vide processed rainbow trout was lower when the heat temperature was more severe and

TABLE 7: *Changes in total mesophilic bacteria count, psychrotrophic bacteria count and anaerobic bacteria count (log cfu/g) of groups during storage period.*

Analysis	Groups	Storage period (day)											
		0	2	5	7	9	12	14	16	19	21	23	26
TAMBC	A	5.25^{a1}*	5.30^{a1}	6.23^{b1}									
	B	3.78^{a2}	3.80^{a2}	3.85^{a2}	5.44^{b1}	6.34^{c1}							
	C	3.41^{a3}	3.57^{b3}	3.60^{b3}	4.32^{cd2}	4.23^{c2}	4.41^{d1}	4.70^{e1}	4.96^{f1}	5.59^{g1}	5.91^{h1}	6.05^{i1}	
	D	3.25^{a4}	3.38^{b4}	3.66^{c4}	4.00^{d3}	4.04^{d3}	4.38^{e1}	4.40^{e2}	4.45^{e2}	4.93^{f2}	5.30^{g2}	5.83^{i2}	6.10^{j}
PBC	A	4.66^{a1}	4.71^{a1}	6.34^{a1}									
	B	2.08^{a2}	3.43^{b2}	3.70^{c2}	5.51^{d1}	6.38^{e1}							
	C	2.32^{a3}	2.48^{b3}	2.48^{b3}	3.29^{c2}	3.82^{d2}	3.89^{d1}	3.91^{d1}	3.97^{d1}	4.71^{e1}	4.94^{f1}	5.94^{g1}	
	D	1.70^{a4}	2.30^{b4}	3.34^{c4}	3.30^{c2}	3.30^{c3}	3.34^{c2}	3.46^{d2}	3.57^{e2}	3.80^{f2}	4.57^{g2}	4.89^{h2}	5.62^{i}
ABC	A	3.53^{a1}	4.67^{a1}	5.19^{a1}									
	B	3.48^{a1}	3.54^{b2}	3.59^{b2}	3.70^{c1}	4.62^{d1}							
	C	3.11^{a2}	3.49^{b3}	3.50^{b2}	3.70^{c1}	3.52^{b2}	4.20^{d1}	4.23^{de1}	4.27^{de1}	4.36^{e1}	4.56^{f1}	4.89^{g1}	
	D	3.14^{a2}	3.11^{a4}	3.20^{a3}	3.60^{b2}	3.68^{b3}	4.17^{c1}	4.18^{c1}	4.23^{c1}	4.40^{d1}	4.58^{e1}	4.71^{f1}	4.84^{g1}

n: 3 (arithmetic mean ± SD); *: Means in the same row with the same letter in the same attribute do not differ significantly at the level of 0.05 significance; abcdefg $^{123\ abcdefgij}$: Means in the same column with the same number in the same attribute do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D: group processed at 95 °C/ 15 min

just after the shrimps were sous-vide cooked in all groups. The heat treatment had a significant effect on the microbiological counts of the shrimp ($p<0.05$). Microbiological counts of all groups increased during the storage period. Similar results were found by Martinez-Alvarez et al., (2009) who reported that heat treatment (in sous-vide cooking) was effective in eliminating the microflora of the raw shrimp. Using high temperature and extended time of cooking slowed down or stopped (especially numerations of *Enterobacteriaceae* and *Micrococcaceae*) the microbiological growth. *Enterobacteriaceae* and *Micrococcaceae*-counts of the raw shrimp were detected as 1.14 ± 0.57 log CFU/g and 1.95 ± 0.02 log CFU/g, respectively. Despite their presence in the raw material, *Enterobacteriaceae* were detected only at the end of storages of group A and B, (2.60 log CFU/g and 2.78 log CFU/g, respectively). After vacuuming of 'sous-vide' products, there is usually 1–5 % oxygen left in the package at the beginning of the process, this allows facultative anaerobic bacteria to grow (Gonzalez et al., 2005), this explains why *Enterobacteriaceae* can only be found at the end of the storage period. Our results were similar with those of Gonzalez-Fandos et al., (2004). These

TABLE 8: *Overall sensory quality of groups during storage period.*

Storage period (day)	A	B	C	D
0	14.33±2.89^{a1}*	15.67±1.53^{a2}	17.33±1.53^{a3}	18.33±0.58^{a4}
2	10.67±3.21^{ab1}	12.33±0.58^{b2}	17.00±1.73^{ab23}	18.67±0.58^{a3}
5	5.33±1.53^{b1}	12.00±0.00^{b2}	16.67±1.15^{ab3}	18.33±0.58^{a3}
7		11.00±1.00^{b1}	16.67±1.15^{ab2}	18.33±0.58^{a2}
9		5.00±2.00^{c1}	16.3±30.58^{abc2}	17.33±0.58^{a2}
12			15.33±1.53^{abc1}	17.00±1.00^{ab1}
14			13.33±0.58^{bcd1}	16.67±2.08^{ab1}
16			12.67±1.53^{cd1}	14.67±0.58^{b1}
19			11.00±1.00^{de1}	12.00±0.00^{c1}
21			8.33±1.53^{ef1}	9.67±0.58^{cd1}
23			6.00±1.00^{f1}	7.33±0.58^{d1}
26				5.33±0.58^{e1}

*: Means in the same row with the same number do not differ significantly at the level of 0.05 significance; : Means in the same column with the same letter do not differ significantly at the level of 0.05 significance; A: group processed at 75 °C/10 min, B: group processed at 75 °C/15 min, C: group processed at 95 °C/10 min, D:group processed at 95 °C/15 min

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these authors found significant differences ($p<0.05$) between the trout processed at different temperatures after 14 day of storage (70 ºC and 90 ºC). However in the present study, significant differences between the shrimps processed at different temperatures were seen from the very beginning. Gonzalez-Fandos (2005) reported that psychrotropic bacteria counts were below 1log CFU/g in salmon processed at 90 ºC for 15 minutes after 45 days of storage at 2 or 10 ºC whereas, the psychrotrophic bacteria counts increased by 4.75–6.5 log units between day 0 and 45 in salmon slices processed at 65 ºC depending on temperature of storage whereas in salmon processed at 90 ºC for 5 minutes the increase was 1–2.6log units during the same period. According to TAMB counts, groups A, B, C, D exceed the acceptable limit (< 6 log CFU/g) on day 5, 9, 23, 26 respectively (FDA, 2013). During the storage period significant differences were determined between the groups. Our findings were similar with those reported by Gonzalez-Fandos et al., (2005). The mesophilic and anaerobic populations in salmon slices processed at 90 ºC for 5 minutes were above 3 log CFU/g after 14 days of storage at 10 ºC, whereas in salmon processed at 65 ºC for 10 minutes, populations were found to be above 3log CFU/g in all the samples stored at 10 ºC. The heat treatment had a significant effect on mesophilic and anaerobic cell count. In the present study anaerobic counts showed the same trend as aerobic mesophilic bacteria count and psychrotrophic bacteria count with a significant increase throughout the storage period. The initial anaerobic bacteria count of group A, B, C, D were 3.53 ± 0.01, 3.48 ± 0.03, 3.11 ± 0.04, 3.14 ± 0.06, respectively and increased to5.19 ± 0.11, 4.62 ± 0.03, 4.89 ± 0.00, 4.84 ± 0.03, respectively at the end of their shelf life. Anaerobic bacteria counts were lower than the other microbiological counts (total aerobic mesophilic and pschrotrophic). Nyati (2000) reported that reduced shelf life was attributed to the lower heat treatment (67 ºC) applied to the rack of lamb as a sous vide treatment during 5 weeks storage at 3 ºC. Vacuum cooking was shown to be the most effective system for preventing microbial growth whilst avoiding further contamination when compared with the traditional cooking process in boiling water (Martinez-Alvarez et al. 2009).

Sensory analysis

Overall sensory quality of the groups was given in table 8. Sous-vide technology has the capability to satisfy consumer demands for acceptable sensory quality beyond that of other cook-chill technology (Resurreccion, 2003). Overall quality of all groups decreased significantly during the storage period ($p<0.05$). On the first day of the storage, group D had the significantly highest scores in the overall quality of the sous-vide cooked shrimps whereas group A had the significantly lowest scores in the overall quality. After day two, no significant differences were determined between group C and group D during the storage period. Group A had significantly the lowest value of the overall quality during the shelf life. It could be related to microbiological and chemical changes during the storage. Decrease in the overall quality of sensory determination of the groups is comparable to the increase in the microbiological quality of the groups. Gonzalez-Fandos et al.(2004) reported that trout processed at 90 ºC and stored at 2 ºC preserved a reasonable acceptability until the end of the storage period (45 days). Resurreccion (2003) reported that improved flavour, due to vacuum packaging which prevents the development of oxidative off-flavours, and texture particularly meat tenderness and juiciness was obtained. Jang and Lee (2005) reported that sous-vide packaging appeared to produce better initial sensory quality when compared to the conventional processing and also sensory quality was better retained in sous vide processed Korean seasoned beef products.

Conclusion

Sous-vide cooking at temperatures of 95 ºC result in better storage abilities compared to sous-vide cooking at 75 ºC. Sous-vide treatment of shrimps at higher temperatures is therefore recommended to achieve the desired sensory and microbiological quality over storage times.

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Conflict of interest

The authors declare that no conflict of interest exits.

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