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Research Article

Microbiological and sensory evaluation of rainbow trout fillet processed by sous-vide method

Eshagh Zakipour Rahimabadi[a,b], Mohammad Reza Dezhand[a], Reza Nasehi[a], Masoud Sami[c]

[a] *Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran*
[b] *Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran*
[c] *Hygiene and Food Quality Department, Faculty of Veterinary Science, Shahid Bahonar University of Kerman, Kerman, Iran*

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* Direct inquiries to author:
e_zakipour@yahoo.com

ABSTRACT

This study evaluated the microbiological quality and sensory characteristics of rainbow trout (*Oncorhynchus mykiss*) fillets processed by the sous-vide method under chilled storage at 4 ºC. Two different heat treatments were studied (65 ºC for 15 min and 85 ºC for 5 min). For this, vacuum packed fillets of rainbow trout cooked in a water bath. After reaching a core temperature of 65 and 85 ºC, cooking was prolonged for 15 and 5 min, respectively for first and second treatment, then samples were stored in refrigerator (4 ºC) for 21 days. Results showed that microbial counts decreased by increasing the heating temperature and increased by increasing storage time. The heat treatment of 85 °C was the most effective one to ensure the safety and extend the shelf life of sous-vide trout fillets to preserve the sensory characteristics. Based on these results, sous-vide samples had an acceptable sensory quality after 21 days of storage at 4 ºC.

Keywords: Fish; Microbial quality; Shelf life; Sous-vide.

1. Introduction

The increase in consumer demand for minimally processed refrigerated convenience food with characteristics similar to that of the fresh product has led to a growth in the use of sous-vide processing technology to extend the shelf life and to keep the quality of fresh food (Schellekens, 1996).

Sous-vide processing involves application of a controlled cooking-pasteurization heat treatment to a raw food that is vacuum packaged in a heat-stable pouch or package (Schellekens, 1996). After this heat treatment, the product is rapidly cooled to temperatures around 0-3 °C, and stored under refrigeration for up to five weeks before reheating and serving. The products are stored, distributed and retailed under refrigeration to inhibit the growth of aerobic microorganisms.

Advantages of this treatment are greater convenience and improved sensory quality and nutrition retention over traditional thermally processed products (Creed, 1998). Heat treatment enhances fish and food taste, inactivates pathogenic microorganisms and increases shelf life, and yet influences the amounts of essential fatty acids (Merdzhanova, Stancheva, Dobreva, & Makedonski, 2013). Vacuum packaging prevents evaporative losses of flavor volatiles and moisture during cooking and inhibits off-flavors from oxidation (Church & Parsons, 2000). These processes result in especially flavorful and nutritious food (Church, 1998; García-Linares, González-Fandos, García-Fernández, & García-Arias, 2004; Ghazala, Aucoin, & Alkanani, 1996; Schellekens, 1996; Stea, Johansson, Jägerstad, & Frølich, 2006). Vacuum sealing also reduces aerobic bacterial growth and allows for the efficient transfer of thermal energy from the water (or steam) to the food.

The main concern of sous-vide processing as a preservation method is the microbiological safety, *ie.*, potential for the growth of pathogenic, anaerobic, bacteria under temperature abuse conditions during transportation, distribution and retailing of the food products (Simpson, 1993).

The shelf-life of sous-vide products ranges from 7 to 45 days and depends on the food composition, processing and legal regulations (Simpson, Smith, Simpson, Ramaswamy, Dodds, 1994). However, on the

other hand sous-vide processing might permit the survival of heat resistant and psychro-tolerant obligate and facultative anaerobes due to the low oxygen tension produced in foods. Moreover, the vacuum inhibits the aerobic spoilage microorganisms; thus it reduces the population of microbial competitors. For this reason, cook-chilled vacuum packaged food might exhibit health risks before noticeable spoilage (Church & Parsons, 2000).

Fish is one of the most perishable foods. During storage, endogenous chemical and enzymatic reactions occur in the lipids and proteins, which lead to the appearance of off-odors and off-flavors (Gram & Huss, 1996; Rodríguez, Barros-Velázquez, Piñeiro, Gallardo, & Aubourg, 2006). The sensory spoilage in fish is manifested by changes in color, odor, taste, and texture as a result of dehydration and the development of off-flavors and off-odors due to rancidity and the formation of trimethylamine and total volatile base nitrogen during cold storage (Richards & Hultin, 2002; Sallam, Ahmed, Elgazzar, & Eldaly, 2007). The application of high cooking temperatures diminishes the sensory quality of fish (González-Fandos, Villarino-Rodríguez, García-Linares, García-Arias, & García- Fernández, 2005). Sous-vide preparation, on the other hand, combines low temperatures and long cooking times with vacuum packaging, delaying the appearance of off-odors and off-flavors during storage (Church & Parson, 1993, 2000). Hence, sous-vide cooked foods have received a lot of attention by researchers over the past two decades.

The aim of this work was to evaluate the microbiological quality and sensorial characteristics of rainbow trout (*O. mykiss*) fillets processed by the sous-vide method under chilled storage at 4 ºC.

2. Materials and Methods

2.1 Sample preparation

For this study, 90 whole fresh fish of rainbow trout (*O. mykiss*) with an average weight of 250-300 g were purchased from a fish farm in Jushan (Kerman province, Iran). Fish were placed in insulated boxes with ice powder and immediately transported to fish processing and packaging plant (Kerman, Iran). Then fish were washed, cleaned and filleted. Fillets (with the mean weight of 100 g) were randomly divided in 3 groups:

- Control: without sous-vide processing)
- Treatment1: processed at 65 ºC for 15 min
- Treatment 2: processed at 85 ºC for 5 min

Afterward, fillets for each treatment were packaged in polyethylene pouch and were heat sealed using a vacuum sealing machine (DZQ400-2D, Shanghai Kebao packaging machinery Co. Ltd., Shanghai, China). Heat process was carried out in a water bath (Model 1, Padideh Nojen Pars, Mashhad, Iran). The internal temperature was obtained using a thermometer (IP 65, ALLA, France). Once the cooking processed had finished, the pouches were removed from the water bath and immediately chilled using iced cold water (2 °C). After chilling, all treatments were stored in refrigerator at 4 ºC for 21 days.

2.2 Microbial analyses

Microbial analyses were performed on control and treated samples at 0, 3, 7, 14 and 21 days of storage at 4 ºC. With the aid of a sterile scalpel, 5 g of sample was aseptically removed and placed in a stomacher bag containing 45 mL of 0.85% (w/v) sterile peptone water. Samples were homogenized using a stomacher (Seward, England) for 2 min. Subsequent dilutions were obtained by mixing 1 ml aliquots with 9 ml of buffered peptone water.

The total viable counts were determined on plate count agar (PCA, Oxoid, England) followed by incubation at 37 ºC for 48 h (AOAC, 2005). Psychrotrophs were determined on PCA with an incubation temperature of 4 ºC for 10 days (McFaddin, 2000). In Enterobacteriaceae count, violet red bile glucose agar (VRBGA, Merck) was used (Sallam, 2007). Lactic acid bacteria were determined on Man-Rogosa-Sharp Agar and incubated at 30 ºC for 72h (Sallam, 2007).

2.3 Sensory analysis

The sensory analysis was carried out using a panel of 10 judges selected and trained. The quality of each sample was classified using characteristics describing: color, odor, taste and texture. The samples were reheated in their bags in a water bath at 75 ºC and were stored at 4 °C until analysis. Sensory evaluation was performed at days 0, 3, 7, 14 and 21. All the results were discussed and the most suitable attributes and scales were selected. A scale of 1 (very soft) to 5 (firm) was used for texture; 1 (soapy shape) to 5 (white) for color; 0 (off odor) to 15 (fresh) for odor; 1 (undesirable) to 10 (fresh) for flavor (Ranken & Kill, 1993).

2.4 Statistical analysis

The data were analyzed using the one way analysis of variance test (one way ANOVA). The least significant difference ($p<0.05$) and Mann-Whitney U test were performed to evaluate the significance of differences among mean values for microbial and sensory quality, using the SPSS 13.0 for windows, SPSS Inc.

3. Results and Discussion

3.1 Microbiological quality

Changes in the total viable counts (TVC) are shown in Table1. Initial value of TVC in control, cooked treatment at 65 °C and cooked treatment at 85 °C were 5.57 ± 0.07, 4.74 ± 0.05 and 0 (zero) log CFU g^{-1}, respectively. TVC values in the different treatments were significantly ($p<0.05$) different with each other in all days. TVC values was increased from day 0 to 21 in all treatments, but in cooked treatment at 85 °C, the levels of TVC, up to day 7 were zero and were only detectable after 14 days of storage.

Table 1
Total viable count (log CFU g^{-1}) of rainbow trout (*Oncorhynchus mykiss*) fillet processed by different sous-vide methods[a]

Day	Control samples	Treatment 1	Treatment 2
0	5.57 ± 0.07^{Ae}	4.74 ± 0.05^{Be}	0.00 ± 0.00^{Cc}
3	6.69 ± 0.09^{Ad}	5.12 ± 0.03^{Bd}	0.00 ± 0.00^{Cc}
7	7.69 ± 0.09^{Ac}	6.06 ± 0.20^{Bc}	0.00 ± 0.00^{Cc}
14	8.01 ± 0.12^{Ab}	6.80 ± 0.08^{Bb}	3.63 ± 0.05^{Cb}
21	8.83 ± 0.02^{Aa}	7.75 ± 0.05^{Ba}	3.76 ± 0.06^{Ca}

[a] Values are expressed as means ± SD of triplicates; Different capital letters superscripts indicate significant ($p<0.05$) differences between treatments within the same storage period. Different small letters superscripts indicate significant ($p<0.05$) differences between same treatments within the different storage period.

Considering that recommended limit for TVC in fish is 7 log CFU g^{-1} (ICMSF, 1986), samples processed at 65 ºC after 15 days of storage reached to recommended limit, but in samples processed at 85 ºC after 21 days of storage TVC value were 3.76 log CFU g^{-1}, that indicating the microbial quality was acceptable. In this study shelf life of processed samples was higher than those reported by Pantazi, Papavergou, Pournis, Kontominas and Savvaidis (2008) and Savvaidis, Skandamis, Riganakos, Panagiotakis and Kontominas (2002). Pantazi et al. (2008) studied the shelf life of chilled fresh Mediterranean swordfish (*Xiphias gladius*) stored under various packaging condition. They observed that samples packed in vacuum condition, after 9 days of storage at 4 ºC reached to recommended limit. Savvaidis et al. (2002) reported that trout fillets processed under vacuum packaging reached to recommended limit after 8 days of storage at 4 ºC. The higher shelf life for present study were related to the influence of applied heat, that heat treatments of 85 ºC were more effective than heat treatments of 65 ºC. With regard to microbial levels of raw fish, it must be considered that they vary according to water conditions, temperature and handling (González-Fandos et al., 2005).

Schellekens (1996) stated that the nature of food (fat content, pH, water activity and essential amino acids) is an important determining factor of the lethality of a heat treatment and also of the possibility of pathogen growth. This author pointed outs that it would be important to study the influence that each factor has on the growth and inactivation of the microorganisms with the aim of establishing additional hurdles when the first barrier (refrigeration at adequate temperature) in sous-vide foods is not fulfilled.

Changes in the psychrotrophic counts (PTC), were similar to TVC. Psychrotroph growth was lower when the heat treatment was more severe (Table 2). PTC were 0.00 log CFU g^{-1} in samples processed at 85 ºC after 14 days of storage at 4 ºC. However, the PTC increased by 2.36-7.33 log units between day 0 and 21 in samples processed at 65 ºC, whereas in samples processed at 85 ºC the increase was of 0.00-4.44 log units during the same period. Significant differences ($p<0.05$) were found between samples processed at different temperatures after 21 days of storage. Also storage time had a significant effect ($p<0.05$) on PTC. These low PTC have been also observed by other authors. González-Fandos et al. (2005) studied microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. Their result showed that in treatment processed at 90 ºC psychrotrophic counts were under the detection limit (1 log CFU g^{-1}) after 45 days and in treatment processed at 65 ºC their level were 4.75-6.50 log CFU g^{-1} during the same period. The psychrotrophic nature of many of the indigenous bacteria found in cold water fish could result in rapid growth even at low temperature. However, microorganisms may be injured by milder treatments, being this injury characterized by increased nutritional requirements of microorganisms to grow (Montville, 1997). Moreover, under unfavorable values of other environmental factors, which include oxygen concentration, the minimal growth temperature increases

Table 2
Psychrotrophic counts (log CFU g^{-1}) of rainbow trout (*Oncorhynchus mykiss*) fillet processed by different sous-vide methods[a]

Day	Control samples	Treatment 1	Treatment 2
0	4.90 ± 0.31^{Ae}	2.36 ± 0.20^{Be}	0.00 ± 0.00^{Cb}
3	6.42 ± 0.26^{Ad}	5.14 ± 0.09^{Bd}	0.00 ± 0.00^{Cb}
7	6.94 ± 0.18^{Ac}	6.10 ± 0.25^{Bc}	0.00 ± 0.00^{Cb}
14	7.73 ± 0.13^{Ab}	6.72 ± 0.08^{Bb}	0.00 ± 0.00^{Cb}
21	8.12 ± 0.10^{Aa}	7.33 ± 0.09^{Ba}	4.44 ± 0.14^{Ca}

[a] Values are expressed as means ± SD of triplicates; Different capital letters superscripts indicate significant ($p<0.05$) differences between treatments within the same storage period. Different small letters superscripts indicate significant ($p<0.05$) differences between same treatments within the different storage period.

Table 3
Enterobacteriaceae counts (EBC) (log CFU g^{-1}) of rainbow trout (*Oncorhynchus mykiss*) fillet processed by different sous-vide methods[a]

Day	Control samples	Treatment 1	Treatment 2
0	5.07 ± 0.15^{Ae}	0.00 ± 0.00^{Be}	0.00 ± 0.00^{Cb}
3	6.16 ± 0.09^{Ad}	4.75 ± 0.06^{Bd}	0.00 ± 0.00^{Cb}
7	6.53 ± 0.05^{Ac}	5.10 ± 0.12^{Bc}	0.00 ± 0.00^{Cb}
14	7.07 ± 0.06^{Ab}	6.53 ± 0.06^{Bb}	0.00 ± 0.00^{Cb}
21	8.06 ± 0.13^{Aa}	7.31 ± 0.08^{Ba}	3.54 ± 0.06^{Ca}

[a] Values are expressed as means ± SD of triplicates; Different capital letters superscripts indicate significant ($p<0.05$) differences between treatments within the same storage period. Different small letters superscripts indicate significant ($p<0.05$) differences between same treatments within the different storage period.

Table 4
Lactic acid bacteria count (log CFU g^{-1}) of rainbow trout (*Oncorhynchus mykiss*) fillet processed by different sous-vide methods[a]

Day	Control samples	Treatment 1	Treatment 2
0	2.56 ± 0.39^{Ad}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
3	3.66 ± 0.47^{Ac}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
7	4.50 ± 0.13^{Ab}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
14	5.03 ± 0.13^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
21	5.54 ± 0.03^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}

[a] Values are expressed as means ± SD of triplicates; Different capital letters superscripts indicate significant ($p<0.05$) differences between treatments within the same storage period. Different small letters superscripts indicate significant ($p<0.05$) differences between same treatments within the different storage period.

considerably. In addition, injured cells are still viable, though their recovery by normal enumeration procedures is negatively affected in three ways. Firstly, lag times increase considerably, even when optimal recovery media are used. Secondly, sometimes generation times increase as well in comparison to fully viable cells under the same intrinsic and extrinsic conditions (Mossel, Corry, Struijk, & Baird, 1995). Finally, the incubation temperature may affect the recovery of debilitated populations, and note that heat injured non-spore-forming bacteria grow over a much narrower range than fully vital ones (Thomas, Reinbold, & Nelson, 1963).

Enterobacteriaceae contamination of raw samples ranged from 5.07 to 8.06 log CFU g^{-1} (Table 3). After heat treatment, at day 0 their levels were under the detection limit (1 log CFU g^{-1}) (Table 3). Enterobacteriaceae were only detectable after 21 days of storage in samples processed at 85 ºC. In samples processed at 65 ºC they were detectable after 3 days of storage and were able to multiply and reach to7.31 log CFU/g after 21 days. Both processing conditions and storage time influenced the Enterobacteriaceae bacteria counts. Enterobacteriaceae counts increased with increasing storage time and decreased with increasing applied heat. The final Enterobacteriaceae counts were significantly lower ($p<0.05$) in heat treatments than control samples.

Although lactic acid bacteria were detected in raw samples (2.56 ± 0.39 log CFU g^{-1}), no growth was observed after sous-vide treatment (Table 4). Our results agree with those reported by Rosnes, Kleiberg, Bergslein and Vidvei (1999). These authors studied the microbiological quality of sous-vide salmon processed at 70 ºC for 15 min and storage at 4 and 10 ºC. They did not detect viable lactic bacteria in sous-vide treatments during the storage time of 42 days.

3.2 Sensory quality

The results obtained from the color and texture is shown in Table 5. Significant differences ($p>0.05$) were not found between treatment processed at 65 ºC and those processed at 85 ºC on all days. The mean values of these 2 parameters for treatments processed at 65 ºC were not significant ($p>0.05$) between days 0, 3, 7 and 14 but there was a statistically significant ($p<0.05$) between day 21 and previous days. The ANOVA results for treatments processed at 85 ºC showed statistically significant ($p<0.05$) differences between days 0, 14 and 21. As storage time lengthens, muscle tissue might be expected to soften and become doughier as muscle fibers deteriorate. However, sous-vide cooking has been found to maintain the initial texture of fish (Schafheitle, 1990). Flavor showed significant difference between treatment processed at 65 ºC and those processed at 85 ºC only on day 0 and the lowest scores in taste were reached by the samples processed at 85 ºC (Table 5). Changes in these factors for treatments processed at 85 ºC were not significant ($p>0.05$) between all days but batches processed at 65 ºC showed significant ($p<0.05$) decrease from day 0 to 21.

As regards to odor, significant differences ($p<0.05$) were found between batches processed at 65 ºC and those processed at 85 ºC on all days and samples processed at 65 ºC had a score lower than those processed at 85 ºC. The lowest scores in odor were

Table 5
Sensory analysis of rainbow trout (*Oncorhynchus mykiss*) fillet processed by different sous-vide methods[a]

Sensory factor	Day	Treatments: Samples processed at 65 ºC	Treatments: Samples processed at 85 ºC
Color	0	5.00 ± 0.00^{Ab}	5.00 ± 0.00^{Ac}
	3	4.80 ± 0.63^{Ab}	4.60 ± 0.84^{Abc}
	7	4.60 ± 0.84^{Ab}	4.40 ± 1.26^{Abc}
	14	4.40 ± 0.96^{Ab}	4.20 ± 1.03^{Ab}
	21	3.00 ± 0.00^{Aa}	2.80 ± 0.42^{Aa}
Texture	0	5.00 ± 0.00^{Ac}	5.00 ± 0.00^{Ac}
	3	4.60 ± 0.84^{Abc}	4.60 ± 0.84^{Abc}
	7	4.50 ± 1.08^{Abc}	4.50 ± 1.08^{Abc}
	14	4.20 ± 1.03^{Ab}	4.20 ± 1.03^{Ab}
	21	2.90 ± 0.31^{Aa}	2.90 ± 0.31^{Aa}
Odor	0	13.10 ± 3.07^{Bb}	14.40 ± 1.89^{Ab}
	3	12.60 ± 3.09^{Bb}	13.20 ± 2.89^{Aab}
	7	11.40 ± 3.09^{Bb}	12.60 ± 3.09^{Aab}
	14	10.20 ± 2.53^{Bab}	12.00 ± 3.16^{Aab}
	21	8.80 ± 0.42^{Ba}	11.20 ± 3.29^{Aa}
Flavor	0	10.00 ± 0.00^{Ab}	9.60 ± 0.51^{Ba}
	3	9.70 ± 0.48^{Aab}	9.70 ± 0.48^{Aa}
	7	9.40 ± 0.51^{Aa}	9.40 ± 0.96^{Aa}
	14	9.40 ± 0.51^{Aa}	9.20 ± 1.03^{Aa}
	21	9.30 ± 0.67^{Aa}	9.00 ± 1.24^{Aa}

[a] Different capital letters superscripts indicate significant ($p<0.05$) differences between treatments within the same storage period for each sensory attribute. Different small letters superscripts indicate significant ($p<0.05$) differences between same treatments within the different storage period for each sensory attributes.

reached by the samples processed at 65 ºC after 21 days (Table 5). A similar effect was also observed by other researchers in odor of sous-vide salmon by González-Fandos et al. (2005). Odor score decreased steadily from 2.23 at the beginning to a final 1.18. However, in meat and meat products, Schafheitle (1990) and Nyati (2000) found no significant ($p<0.05$) differences during storage.

The decrease in score of the sensory characteristic in the sous-vide samples could be related to bacterial and chemical changes during processing. Since trout has a high fat content it is very vulnerable to oxidation and rancid taste development. However, using vacuum packaging most of the oxygen in the surrounding atmosphere is removed and rancidity delayed. As oxidation is delayed, sous-vide technology is more adequate in maintaining the quality of fat fish than other technologies such as freezing. Since trout is vulnerable to heat treatment, since there is a protein precipitation during heat treatment at about 70 ºC (Bergslien, 1996). A white protein layer on the fish treatment can also cause a hard and a dry texture of the fish products (González-Fandos et al., 2005).

4. Conclusion

Based on these results, sous-vide samples had an acceptable sensory quality after 21 days of storage at 4 ºC, thus highlighting the importance of heat treatment. It can be concluded that treatment of 85 ºC was the most effective for extending the shelf life of fish. The heat treatment has great importance in microbiological and sensory characteristics to ensure the quality and safety of sous-vide products, together with the storage time.

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