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INFLUENCE OF GARLIC EXTRACT AND PACKAGING METHODS ON THE QUALITY PARAMETERS OF FISH FILLETS STORED AT LOW TEMPERATURES

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Abstract

Fish meat has very high water content, a very low content of connective tissue but has high digestibility. Raw fish is a very perishable food because it has all substances necessary for the development of microorganisms. Fish meat lipids are also very sensitive because they oxidize very quickly during storage. The aim of our research was to examine the influence of garlic extract and packaging methods on the total volatile basic nitrogen (TVB-N), thiobarbituric acid content and pH value in fish fillets during storage at low temperatures. 48 fillets of rainbow trout (Oncorhynchus mykiss) were studied. The fish fillets were randomly assigned to four batches of 12 samples each (C-control and experimental batches E1, E2 and E3) and marinated in a solution with garlic extract in proportion of 0‰ (C), 3‰ (E1), 6‰ (E2) and 9‰ for 12 hours. For each batch, six fillets were individually vacuum packed and six fillets were individually packed in plastic boxes. The samples were stored in the refrigerator at a temperature of 0...+4°C for six days. The studied parameters were determined after marinating, on the 1st, 3rd and 6th day of storage.The raw data obtained was processed by biostatistical methods using the One-Way Anova test, including the Tukey HSD test of the SPPS Statistics application. In the storage period, the amount of TBA increased progressively at each stage of the examination (30 min., 1, 3 and 6 days, respectively), and the amounts in the control samples were significantly higher ($p \leq 0.05$) than those in the E1, E2 and E3 samples. Also, the lowest values after 6 days of refrigeration were recorded in the marinated samples and vaccum packed. The lower values recorded in marinated samples were influnced by the antioxidant effect of the garlic extract used. Regarding the TBA amount in fish and fish products, the admissibility limit recommended for the consumer is 2.0 mg MDA/kg, and in our case the values have been much lower.

Key words: *fish filet, garlic extract, marinating, refrigeration, thiobarbituric acid (TBA).*

INTRODUCTION

Fish meat is an important food source with high biological value, high in protein, minerals, vitamins, unsaturated fatty acids, high digestibility, low cholesterol and superior organoleptic quality (Duan et al., 2010). From a nutritional point of view, fish is one of the most important foods for humans because it has an energetic and plastic role (Francesco et al., 2011).

Fish and fish products are highly perishable, as they have a high content of water and free amino acids and a lower content of connective tissue compared to other types of meat, leading to faster spoilage (Jinadasa, 2014).

Refrigeration and freezing have been used extensively to preserve the sensory and nutritional properties of fish. However, fish meat has a short storage time (Giannini et al., 2007), because it has a high content of unsaturated fatty acids and an important presence of pro-oxidant molecules that lead to rancidity and loss of nutritional quality (Richards and Hultin, 2002).

Lipids contain large amounts of unsaturated fatty acids. Therefore, lipids oxidation is an important problem, which leads to a decrease in meat quality during storage (Secci & Parisi, 2016) and loss of nutritional value (Maqsood et al., 2012). Also, several studies shown the effect of protein oxidation during storage and changes in meat quality (Bertram et al., 2007; Lund et al., 2007; Milijašević et al., 2017). Moreover, the pH value can change the rate of lipid oxidation during storage. Thus, at high pH the TBA value were lower compared to lower pH which accelered lipid oxidation (Balev et al., 2011; Mozuraityte et al., 2016; Wei et al., 2017).

Vacuum packaging is important for the quality of the fish as it extends the shelf life of the fish and to minimize undesirable effects, various antioxidants are used (Taheri et al., 2012; Medina et al., 2009). Also, Navaro-Segura et al. (2020) showed that vacuum-packaging of fish with oregano essential oil vapours had positively effect of shelf life. This technology was applied to sea bream fresh fillets that were kept for 28 days at a temperature of +4°C and the shelf life for refrigerated fillets was established in at least 28 days.

In order to extend the storage time, much attention is directed to antioxidant substances to prevent lipid oxidation. Currently, research activity is focused on replacing synthetic substances with natural antioxidants, due to the possible adverse side effects of synthetic antioxidants and the beneficial effects of natural antioxidants (Sarkardei & Howel, 2008).

In the food industry, some plants are generally used to improve the sensory characteristics of foods (Burt, 2004). Moreover, these plants have substances with antimicrobial and antioxidant activity (Baydar et al., 2004; Frank et al., 2014). According to Griffiths et al. (2002), garlic species are revered as possessing antibacterial and antimicrobial activities and contain potent antioxidants, sulfur, and numerous other phenolic compounds that have attracted great interest in the food industry. These beneficial properties seem to be closely related to the high content of sulfur compounds and flavonoids, due to their activity as antioxidants and anti-carcinogens, their effects on lipid metabolism and the cardiovascular system, their antibiotic effects (Griffiths et al., 2002).

In this work, the antioxidant effect of garlic extract and vacuum packaging on trout fillets during storage at +4°C was studied by determining the physicochemical parameters.

MATERIALS AND METHODS

Twenty-four specimens of trout (Oncorhynchus mykiss) with an average weight of 725.25 ± 16.15 g were purchased during the winter from a local market (Timișoara, Romania), transported in polyethylene bags containing cold water to the Food Processing Technology Laboratory, Faculty of Animal Resources Bioengineering. Then each fish was gutted, skinned, filleted and washed with tap water in the laboratory. The fillets were then randomly divided into four batches of 12 fillets for each treatment. Fish fillets were randomly assigned to one of the four treatments shown below:C - Control batch, garlic extract (0‰); E1 - Experimental batch, garlic extract 3‰; E2 - Experimental batch, garlic extract 6‰; E3 - Experimental batch, garlic extract 9‰.

Preparation of garlic extract: Thirtieth fresh garlic bulbs average sized were obtained from market in Timisoara. They were peeled, washed, chopped and oven dried at a temperature of 40°C until constant weight was reached. 800 mL of methanol was used to soak 100 g of the oven dried garlic for 24 hours. The methanol extracts was decanted and placed in evaporator to concentrate the extract up to 80 mL.

The marinating solutions were obtained from 0 mL, 3 mL, 6 mL and 9 mL of garlic extract and 3.5 g of seea salt added to 1 litter of water. The marinating solutions were stored at a temperature of +4°C before immersed the fish fillets.

For each tratament, 12 fish fillets were immersed in the marinating solution together. The samples were marinated in sealed closed plastic bags and kept in a refrigerator for 12 h at a temperature of +4°C.

After 12 h, the fillets were removed from the solutions and drained on sanitized stainless steel grids for 5 min at room temperature. The fillets were individually wrapped and stored in a refrigerator at 4°C. Twenty four fillets were vacuum packed (six fillets for each batch) and twenty four fillets were packed in plastic boxes (six fillets for each batch).

For the physico-chemical examination, samples were taken from the raw fillets (30 min.) and from the marinated fillets after: 30 min, 1, 3 and 6 days. TVB-N was determined according to the method of Antonacopoulos & Vyncke (1989). For total TVB-N, fish muscle (10 g) was homogenized with 6% perchloric acid (90 mL) for 1 min, filtered through a filter paper, alkalized by NaOH (20%) and distilled. The distillate was titrated with a HCl solution 0.01 N. The TVB-N is an indicator of protein degradation in fish muscle and TVB-N level was expressed as mg N/100 g fish muscle.

Thiobarbituric acid reactive substances (TBARS) were determined according to the method of Buege and Aust, (1978). Thus, 0.5 g

of fish fillet was homogenized in 10 ml of the mixture containing TBA (0.375 g/100 mL), TCA (15 g/100 mL) and HCl (0.25 mol/L). The mixture was heated in the boiling water for 10 min, followed by cooling with tap water. The mixture was centrifuged at 3600 x g for 20 min and the absorbance was measured at 532 nm. The TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

pH was measured directly in the fish muscle with an FC 232D glass electrode connected to a Hanna HI99163 portable meat pH meter. The pH was measured at three different places on each fillet. The electrode was also cleaned after each sample and calibrated frequently.

Statistical analysis: The raw data obtained from measurements were processed using methods of biostatistics with Microsoft Excel spreadsheet application (Brudiu, 2010). Significant differences were defined at $P \leq 0.05$. To test the statistical significance of differences between mean values of the characters studied, an analysis of variance with Anova test, included Tukey HSD test from SPSS Statistics, was used.

RESULTS AND DISCUSSIONS

Table 1 showed changes in TVB-N from marinated fish fillets during the whole period of refrigerated storage at a temperature of +4°C.

The total volatile basic nitrogen (TVB-N) is an important indicator of meat resulting from protein and amine degradation. According to Fatih &mental samples (E1, E2 and E3) than in the control samples for which a faster growth was recorded during the six days of storage.

TVB-N values during storage for the control samples ranged from 10.29 mg/100 g after first day of storage up to 53.50 mg/100 g after six days of storage while the values from experimental samples had was between 7.96 mg/100 g up to 35.29 mg/100 g in the same days of examination. Thus, in experimental samples E3 the TVB-N values were lower (7.96-22.76 mg/100 g) than in samples E2 (8.34 and 29.04 mg/100 g) and E1 (8.96-35.29 mg/100 g) because it increased more slowly in samples marinated with high amounts of garlic extract.

Also, to samples in vacuum packaging TVB-N values was lowest, for all batches (from 7.96 up to 33.29 mg/100 g) as compared to the fillets packed in plastic boxes which had the highest values (from 14.96 up to 53.30 mg/100 g).

After applying the statistical test, significant differences ($P \leq 0.05$) were identified depending on the concentration of garlic extract, storage time and packaging method.

The values from this study are similary with results obtained by Muhammet & Sevim, (2007) in freshly caught fish (between 5 to 20 mg/100g).

Table 1. Dynamics of total volatile basic nitrogen (TVB-N mg/100 g)

Storage time		C (n=6)	E1 (n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		6.25 ± 0.04^{aA}	6.20 ± 0.05^{aA}	6.20 ± 0.06^{aA}	6.22 ± 0.02^{aA}	0.20912
After marinating	**30 min**	6.49 ± 0.09^{aAx}	6.31 ± 0.06^{bAx}	6.27 ± 0.05^{bAx}	6.24 ± 0.02^{bAx}	0.00001
	1 day	10.29 ± 1.35^{aBy}	8.96 ± 1.41^{abBy}	8.34 ± 0.77^{bBy}	7.96 ± 0.61^{bBy}	0.00763
	3 days	14.13 ± 1.12^{aBz}	10.70 ± 1.27^{bBz}	9.12 ± 0.38^{cBz}	8.49 ± 0.05^{cBz}	0.00001
	6 days	33.29 ± 5.56^{aBw}	19.87 ± 4.14^{bBw}	16.12 ± 1.96^{bcBw}	13.16 ± 1.45^{cBw}	0.00001
Plastic boxes						
Initial values		6.25 ± 0.04^{aA}	6.20 ± 0.05^{aA}	6.20 ± 0.06^{aA}	6.22 ± 0.02^{aA}	0.20800
After marinating	**30 min**	6.50 ± 0.09^{aAx}	6.30 ± 0.05^{bAx}	6.28 ± 0.05^{bAx}	6.24 ± 0.02^{bAx}	0.00000
	1 day	17.80 ± 3.25^{aAy}	16.29 ± 3.07^{aAy}	15.13 ± 2.84^{aAy}	14.96 ± 2.47^{aAy}	0.33233
	3 days	30.30 ± 3.49^{aAz}	20.29 ± 2.63^{bAz}	17.88 ± 1.73^{bcAz}	15.59 ± 2.45^{cAz}	0.00001
	6 days	53.30 ± 4.89^{aAw}	35.29 ± 4.52^{bAw}	29.04 ± 1.94^{bcAw}	22.76 ± 3.74^{cAw}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standard deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

Also, Fan et al. (2009); Gulsun et al. (2009) and Frank et al. (2014) showed a gradual increase in TVB-N values in all samples during storage. According by Zhou et al. (2011) the TVB-N of fish is an indicator of the freshness of the raw material. TVB-N values lower than 25 mg/100 g which are considered as the threshold for a good-quality fish product, while high TVB-N values are unacceptable and are associated with organoleptic changes especially unpleasant smell in the fishmeat (Limbo et al., 2009). On the contrary, according by Fan et al. (2009), the upper limit when a fish can be considered spoiled is between 35 to 40 mg/100 g. Also, in fish and fish products, several authors recommend the level of TVB-N up to 25 mg/100 g for high quality, up to 30 mg/ 100 g for good quality, the limit of acceptability up to 35 mg/100 and above 35 mg /100 g spoiled (EU/CE No. 627/2019, Amegovu et al., 2012).

The results of the present study showed the effectiveness of the garlic extract used in the minimum concentration of 3‰ in the marinating solution of fillets, because antioxidants and antimicrobials limited the increased of TVB-N values in the samples treated and protected by vacuum packaging (Table 1). Also, in samples packed in plastic boxes, at a minimum concentration of 6‰ of garlic extract, lipid oxidation was limited after six days to 29.04 mg/100 g.

Thiobarbituric acid (TBA) values were used as an index for lipid oxidation in trout fillets during refrigerated storage (6 days). For fish and fish products they will have a TBA value below 2.0 mg malondialdehyde/kg (MDA/kg), if the TBA value is greater than 2.7 mg MDA/kg the fish meat are poor quality is likely to have a rancid smell and taste (Bonnell, 1994; Baron et al., 2007; Limbo et al., 2009). While in other countries such as Egypt for TBA values in fish and fish products, the permissible limit recommended by EOS (2005) is 4.5 mg MDA/kg.

Table 2 shows the TBA values of trout fillets marinated with garlic extract during storage at refrigeration temperatures. Thus, the data in Table 2 showed a progressive increase in TBA (secondary oxidation product) values with the increase in the storage period under refrigeration conditions from 0.002 mg MDA/kg (initial values) up to 0.902 mg MDA/kg (control samples after six days).

The evolutions of TBA values were very slow in the experimental samples that were treated with garlic extract. Also, the present study shows a significant gradual increase in TBA values for control (from 0.003 up to 0.367, at vacuum-packed and from 0.002 up to 0.902, in plastic boxes-packed) and experimental samples (vacuum-packed E1: 0.002-0.208, E2: 0.002-0.130, E3: 0.003-0.061 and plastic boxes-packed E1: 0.003-0.386, E2: 0.003-0.266, E3 0.003-0.139) for both packaging methods (Table 2).

Table 2. Dynamics of thiobarbituric acid (TBA mg MDA/kg)

Storage time		C (n=6)	E1(n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		0.003 ± 0.001^{aAx}	0.002 ± 0.001^{aAx}	0.002 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.20001
After marinating	**30 min**	0.019 ± 0.004^{aAx}	0.016 ± 0.003^{abAx}	0.014 ± 0.003^{abAx}	0.010 ± 0.001^{bAx}	0.00791
	1 day	0.110 ± 0.018^{aBy}	0.085 ± 0.018^{abBy}	0.066 ± 0.019^{bBy}	0.032 ± 0.008^{cBy}	0.00001
	3 days	0.205 ± 0.031^{aBz}	0.141 ± 0.030^{bBz}	0.080 ± 0.014^{cBz}	0.044 ± 0.010^{cBz}	0.00001
	6 days	0.367 ± 0.035^{aBw}	0.208 ± 0.053^{bBw}	0.130 ± 0.038^{cBw}	0.061 ± 0.019^{dBw}	0.00001
Plastic boxes						
Initial values		0.002 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.003 ± 0.001^{aAx}	0.20800
After marinating	**30 min**	0.020 ± 0.003^{aAx}	0.017 ± 0.004^{aBx}	0.013 ± 0.003^{bAx}	0.011 ± 0.003^{bAx}	0.00000
	1 day	0.302 ± 0.023^{aAy}	0.143 ± 0.017^{bAy}	0.113 ± 0.011^{cAy}	0.074 ± 0.014^{dAy}	0.02333
	3 days	0.652 ± 0.051^{aAz}	0.236 ± 0.023^{bAz}	0.183 ± 0.013^{cAz}	0.094 ± 0.010^{dAz}	0.00001
	6 days	0.902 ± 0.046^{aAw}	0.386 ± 0.034^{bAw}	0.266 ± 0.025^{cAw}	0.139 ± 0.027^{dAw}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standard deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

In this study, the initial values for TBA were very low (0.002-0.003) and after marinating were between 0.010 to 0.020 mg MDA/kg. Thus, our results was lower that the values reported by Orban et al. (2011), Frank et al. (2014) and Hassanin and El-Daly (2013).

Moreover, the E3 samples treated with garlic extract 9‰ showed the lowest amount of TBA (from 0.032 mg MDA/kg after the first day to 0.139 mg MDA/kg after six days), while samples E1 (garlic extract 3‰) had higher TBA values (from 0.085 mg MDA/kg after the first day up to 0.386 mg MDA/kg after six days) and in E2 samples (garlic extract 6‰) were intermediate values. During the storage period, the increase in TBA values was higher for the samples packed in plastic boxes compared to the vacuum-packed which had lowest amount of TBA.

Compared to control samples which had 0.110 - 0.302 mg MDA/kg after first day, 0.205-0.652 mg MDA/kg at three days and 0.367-0.902 mg MDA/kg the end of storage period (six days) in experimental samples the values of TBA have showed the antioxidant activity of garlic extract. This claim is supported by statistical differences revealed between experimental and control samples in all stages of examination.

Thus, for TBA values after applying the statistical test (ANOVA included Tukey HSD test), significant differences ($P \leq 0.05$) were identified depending on the concentration of garlic extract, storage time and packaging method.

Effectiveness of the garlic extract has also been shown by Harris et al. (2001) and Mariutti et al. (2008) which showed the antioxidant properties of garlic due to its compounds (alliin, allicin, ajoene and allylpropyl) that play an important role in inhibited the lipid oxidation.

Vacuum packaging has the role of protecting the oxidation of lipids in food and especially in fish meat, which is highly perishable (Arashisar et al., 2004; Rashidi et al., 2014). Taheri & Motallebi (2012), in their studies showed that vacuum packaging substantially reduce lipids oxidation in frozen fish and fishe products. Also, Giménez et al. (2002) had studied effect of low oxigen content on trout meat in during storage and showed that lower concentrations of TBA in samples vacuum-packing than in fish stored in simple packaging peroxides in simple packaging resulted in an increase in TBA due to the presence of oxygen. Similar results were published by Manju et al. (2007) in baleen fish, Gandotra et al. (2015) in Labeo Rohita, Jezek & Buchtova (2014) in rainbow trout, Gandotra et al. (2016) in common carp.

Table 3 shows pH values in trout fillets according to the concentration of garlic extract, packaging method and storage time.

Table 3. Dynamics of the pH value

Storage time		C (n=6)	E1(n=6)	E2 (n=6)	E3 (n=6)	P value
Vacuum packing						
Initial values		6.032 ±0.057^{aAx}	6.025±0.110^{aAx}	6.032±0.097^{aAx}	6.023±0.097^{aAx}	0.23001
After marinating	**30 min**	6.173 ±0.029^{aAy}	6.152±0.042^{Ay}	6.132±0.059^{aAy}	6.128±0.023^{aAy}	0.22477
	1 day	6.232 ±0.042^{aBz}	6.198±0.030^{aBz}	6.155±0.023^{bBz}	6.153±0.016^{bBz}	0.00016
	3 days	6.281 ±0.019^{aBw}	6.228±0.024^{aBw}	6.175±0.008^{cBz}	6.162±0.015^{cBw}	0.00001
	6 days	6.316 ±0.019^{aBw}	6.267±0.040^{bBw}	6.184±0.024^{cBz}	6.167±0.014^{cBw}	0.00001
Plastic boxes						
Initial values		6.032 ±0.057^{aAx}	6.038±0.102^{aAx}	6.032±0.097^{aAx}	6.045±0.075^{aAx}	0.24573
After marinating	**30 min**	6.177 ±0.028^{aAy}	6.154±0.036^{aAy}	6.159±0.031^{aAy}	6.127±0.033^{bAy}	0.07436
	1 day	6.308 ±0.038^{aAz}	6.275±0.027^{aAz}	6.270±0.018^{aAz}	6.202±0.042^{aAz}	0.00016
	3 days	6.402 ±0.023^{aAw}	6.324±0.018^{bAz}	6.298±0.047^{bAz}	6.243±0.038^{cAz}	0.00001
	6 days	6.532 ±0.057^{aAw}	6.430±0.037^{bAw}	6.335±0.025^{cAz}	6.286±0.019^{cAz}	0.00001

Means followed by different superscript lowercase letters in the same row and means followed by different superscript uppercase letters in the same column differ significantly to $P \leq 0.05$ by ANOVA test, included Tukey HSD test; Mean±Standar deviation; a, b, c, d-between batches; A, B-between packaging methods; x, y, z, w-between storage time

The pH value of marinated trout fillets increased during storage time, with different dynamics depending of the contraction of garlic extract and the packaging method. Thus, the pH value

ranged between 6.025 and 6.532, with the highest values for the control samples in plastic boxes-packed (from 6.032 up to 6.532) and the lowest values for the vacuum-packed E3 samples (from 6.023 up to 6.167).
Comparaison to raw fillets from day zero, after six days of storage at +4°C the values showed significant increased for all samples. Also, significant differences were recorded between control and experimental samples and between the two packaged methods ($p<0.05$), in all examination stages.

CONCLUSIONS

From the results obtained in this study, it can be concluded that garlic extract had an antioxidant effect on marinated trout fillets. For the physico-chemical indicators studied, the samples marinated with 9‰ garlic extract had the lowest values.
Also, vacuum packaging of samples has been shown to be a suitable way to reduce lipid oxidation in trout fillets and extend shelf life.
The results of this study recommend garlic as a natural herb that could be used to extend the shelf life of fish meat and replace synthetic antioxidant

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