

## **EFFECT OF PRETREATMENT WITH NATURAL ANTIOXIDANTS ON THE COLOUR SURFACE PROPERTIES OF CHILLED-STORED SALMON DISCS**

D. BALEV<sup>1</sup>, G. IVANOV<sup>2\*</sup>, H. NIKOLOV<sup>2</sup> and St. DRAGOEV<sup>1</sup>

<sup>1</sup>*University of Food Technologies, Department of Meat and Fish Technology,  
BG - 4002 Plovdiv, Bulgaria*

<sup>2</sup>*University of Food Technologies, Department of Food Preservation and Refrigeration Technology,  
BG - 4002 Plovdiv, Bulgaria*

### **Abstract**

BALEV, D., G. IVANOV, H. NIKOLOV and St. DRAGOEV, 2009. Effect of pretreatment with natural antioxidants on the colour surface properties of chilled-stored salmon discs. *Bulg. J. Agric. Sci.*, 15: 379-385

The CIE Lab color properties of chilled-stored ( $1\pm 1^{\circ}\text{C}$ ) salmon (*Salmo Salar*) discs were investigated depending on the pretreatment with solution containing either  $0.05\text{ g.l}^{-1}$  or  $0.1\text{ g.l}^{-1}$  of dihydroquercetin isolated from Siberian larch (*Larix sibirica Ledeb*). The superficial treatment with  $0.1\text{ g.l}^{-1}$  dihydroquercetin solution was more effective in stabilizing cross-section appearance colour, as indicated by smaller decrease of C and H values representing the color purity and tonality, respectively. It also contributed to an increase of the colour brightness ( $L^*$ ) with 2.3% and of the  $a^*/b^*$  ratio with 38 %.

*Key words:* salmon; colour stability; CIE Lab color properties; natural antioxidant

### **Introduction**

The oxidative changes of fish deteriorate its sensory attributes and quality. The oxidative rancidity of fish lipids is caused by the activity of tissue enzymes and the oxygen radical species. The accumulated secondary products are responsible for developing of yellow or dark color of the superficial subcutaneous layer and subsequently in depth of muscle layers (Decker and Xu, 1998). The type of fish handling influences initiation of the lipid hydrolysis and oxidation. Fishes of identical type show different oxidative stability when are chilled whole, filleted, cut into discs and with

or without skin (Undeland et al., 1999). For inhibition of the lipid oxidation in chilled fish it is necessary to limit or avoid the oxygen admission (Decker and Xu, 1998). For avoiding the oxygen admission several approaches are suggested: vacuum packaging (Osogul et al., 2004); wrap with low density polyethylene wrapping folio, covered with butylhydroxitoluene (BHT) (Torres-Arreola et al., 2007); superficial treatment with grape phenol extracts (Pazos et al., 2005) or immersing into erythrostate solutions (Santos and Regenstein, 1990).

The possibilities for inhibition of chilled fish lipid and pigment oxidations using antioxidant treatments

\*E-mail: [ivanovgalin@yahoo.com](mailto:ivanovgalin@yahoo.com)

are discussed during the last few years. Banerjee (2006) suggests inhibition of the muscle lipoxigenase by treatment of fish with polyphenols from green tea or synthetic antioxidants, such as butylhydroxyanisole (BHA), BHT, esculetin, caffeic acid, ascorbic acid and ethylenediaminetetraacetic acid (EDTA). Becker et al. (2007) demonstrated that the rutin is effective antioxidant only towards liposomes, where showed pronounced synergism with quercetin, but not for whole muscle. Studies on the cytotoxicity of different flavonoids proved, that in contrast to other compounds the rutin and dihydroquercetin (DHQ) do not show cytotoxic properties (Matsuo et al., 2005).

The objective of the present study is to determine the effect of pre-treatment with the natural antioxidant dihydroquercetin (DHQ) on the instrumentally measured color characteristics of salmon (*Salmo salar*) discs during their storage at  $1\pm 1^{\circ}\text{C}$ .

## Materials and Methods

### *Fish and handling procedures*

Atlantic salmon (*Salmo salar*) farmed by "Hallward Leroy" AS (Efta, Norway) was used in this study. The fish was delivered by plain from Grieg Seafood Rogaland avd. Bokn (Tollaskholmen, Norway) with the intermediation of Metro Bulgaria SA (Sofia, Bulgaria). The fish was bought after the sixth day from the death (*post mortem*). The fish was size 6/7 with excellent quality.

### *Natural antioxidant dihydroquercetin isolate „Flavit”<sup>®</sup>*

The natural antioxidant dihydroquercetin isolate „Flavit”<sup>®</sup> (DHQ) was extracted from Siberian larch (*Larix sibirica Ledeb*). The product was manufactured by Flavit Ltd (Pushchino, Russia) in cooperation with the Biological Instrument-making Institute of the Russian Academy of Sciences. DHQ appears like white or pale yellow crystal powder, without odor, with melting point  $220\text{--}222^{\circ}\text{C}$ . It has good solubility in acetone, methanol, ethanol, ethylacetate and very

poor solubility in water. The natural antioxidant dihydroquercetin isolate „Flavit”<sup>®</sup> contains 96 % dihydroquercetin, 3 % dihydrokaempferol and approximately 1% naringenin.

### *Experiment design*

The fish was cut on 1.5-2.0 cm discs by saw, model Bizerba, type FK 32 (Bizerba GmbH, Balingen, Germany). The discs were divided into nine equal portions, each of them including peaces from different fishes and different parts of fish's body. The experiments were carried out with three samples: first - *control samples*, packed without antioxidant treatment; second - *experimental samples 1*, packed after superficial treatment with a 0.005 % solution of DHQ and third - *experimental samples 2*, packed after superficial treatment with a 0.01 % solution of DHQ. Four portions representing *control samples* were hermetically packaged in multilayer barrier polyethylene/polyamide folio bags Oberfolien, type 406 INC80/500 (Sudpack Verpackungen GmbH & Co. KG, Ochsenhausen, Germany) immediately after dosage. The vacuum-making and the thermal conglomeration was made with vacuum-packaging machine Multivac, Model A300/15 (Multivac Sepp Haggemuller GmbH & Co. KG, Wolfertschwenden, Germany). Three portions representing *experimental samples 1* were spray treated by solution containing 0.5 g DHQ.l<sup>-1</sup> 5 % ethanol water solution before packaging, and the remaining three portions (*experimental samples 2*) representing were spray treated by solution containing 1.0 g DHQ.l<sup>-1</sup> 5 % ethanol water solution. The salmon discs were drained away for 15 min at  $0\pm 1^{\circ}\text{C}$  and packed. After packaging the samples were quickly chilled at a temperature of  $-18^{\circ}\text{C}$  until the temperature in the samples' center reached  $2^{\circ}\text{C}$ , measured by an electronic thermometer EBI-2T-F (Ebro Electronic GmbH, Ingolstadt, Germany). They were then stored for 12 d at  $1\pm 1^{\circ}\text{C}$ . The color surface properties changes were measured on 1, 4, 7 and 12 d of storage (e.g. 6, 9, 12 and 17 d *post mortem*).

### ***Instrumentally determination of color characteristics of fish discs cross-section appearance***

The color of salmon plays crucial role when the consumer is making decision for purchasing. The color characteristics of Atlantic salmon (*Salmo salar*) were determined instrumentally according to the CIE Lab system. The chromameter Konica Minolta, model CR 410 (Konica Minolta Sensing, Inc., Tokyo, Japan) was used and the brightness of the color ( $L^*$ ), red ( $a^*$ ) and yellow ( $b^*$ ) colour components, the hue of the colour tone (H) and the colour saturation (C) were measured.

### ***Data analysis***

Data were analyzed using the Microsoft Excel program, Version 5.0 (SPSS Inc., Chicago, IL, USA). All determinations were carried out in triplicate and data were subject to analysis of variance (ANOVA). ANOVA was carried out with the General Linear Models (GLM) with a significant level of  $P \leq 0.05$  (Draper and Smith, 1998). The Fischer's test with a significant difference set at  $P \leq 0.05$  was used to compare sample means (Kenward, 1987). In addition, the correlation coefficient between colour properties

of the samples was calculated (Draper and Smith, 1998).

### **Results**

The data from the instrumental determination of the color properties of chilled salmon discs showed that the color brightness ( $L^*$ ) values varied slightly for all samples – between 40.86 and 47.20. At the beginning of the experiment the color brightness ( $L^*$ ) value of control samples was 43.23. At 4 d it decreased by 5.48 %. After that an increase was observed and color brightness ( $L^*$ ) value of 46.10 was reached at the 12 d. Such changes in color brightness ( $L^*$ ) could be explained by the initial oxidation of color pigments in fish muscles tissue. The oxidation process is followed by moisture loss, which concentrates the pigments in the sample. Despite of the higher  $\beta$ -carotene content of salmon muscles, the lipids and muscle pigments oxidize to a great extent during the 12 d of storage. Thus the color brightness ( $L^*$ ) values increase during the refrigeration. A little bit higher color brightness ( $L^*$ ) values were determined for experimental samples 1 (Table 1). For the first 4 days of experiment the color brightness ( $L^*$ ) did not change signifi-

**Table 1**  
**The instrumental readings of fish discs cross-section appearance color properties**

Samples	Color characteristics of the fish discs cross-section appearance					
	Day of storage	Brightness of the color ( $L^*$ )	Red color component ( $a^*$ )	Yellow color component ( $b^*$ )	Hue of the color tone (H)	Color saturation (C)
Control sample	1	43.23± 0.11	26.76± 0.15	24.21± 0.13	33.57± 0.31	44.88± 0.36
	4	40.86± 0.12	22.34± 0.17	22.70± 0.18	30.87± 0.39	41.99± 0.46
	7	42.84± 0.19	22.29± 0.20	25.10± 0.21	31.55± 0.25	41.79± 0.33
	12	46.10± 0.18	19.84± 0.23	18.54± 0.22	25.96± 0.49	41.27± 0.50
Sample 1	1	43.23± 0.11	26.76± 0.15	24.21± 0.13	33.57± 0.31	44.88± 0.36
	4	43.11± 0.14	23.27± 0.13	20.98± 0.18	31.41± 0.49	44.87± 0.49
	7	45.37± 0.13	23.50± 0.21	24.86± 0.21	34.42± 0.37	45.08± 0.47
	12	46.50± 0.16	21.11± 0.19	18.05± 0.20	26.75± 0.44	41.75± 0.35
Sample 2	1	43.23± 0.11	26.76± 0.15	24.21± 0.13	33.57± 0.31	44.88± 0.36
	4	44.45± 0.13	23.32± 0.17	20.52± 0.12	31.90± 0.43	45.42± 0.48
	7	46.51± 0.14	25.17± 0.19	22.13± 0.23	35.75± 0.51	46.83± 0.52
	12	47.20± 0.20	23.75± 0.18	17.43± 0.17	28.10± 0.38	42.19± 0.37

cantly ( $*P > 0.05$ ) and reached values of 43.11. After that up to the 12 d of storage it increased significantly ( $*P < 0.05$ ) by 7.8 % (Table 1). The color brightness ( $L^*$ ) of experimental samples 2 increased constantly during the storage and reached the highest values (Table 1). Significant ( $*P < 0.05$ ) increase of color brightness ( $L^*$ ) values from the initial level of 43.23 (at the first day of experiment) to 47.20 (at the 12 d of experiment) was observed (Table 1). The results obtained showed that the superficial treatment of salmon discs by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol, causes greater increase in the color brightness ( $L^*$ ) at the 12 d of storage compared to the control samples and samples treated by spray containing 0.5 g DHQ.l<sup>-1</sup>.

The color redness ( $a^*$ ) of control samples decreased significantly ( $*P < 0.05$ ) during the 12 d of chilled storage (Table 1). For the first 7 days of storage the colour redness ( $a^*$ ) of experimental samples 1 did not change significantly ( $*P > 0.05$ ), but decreased by 11 % during the last five days (Table 1). The color redness ( $a^*$ ) of experimental samples 2 did not change significantly ( $*P > 0.05$ ) during the chilled storage and gained the highest values (Table 1). Obviously, the superficial treatment of salmon discs by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of

ethanol, contribute to preservation of color redness ( $a^*$ ) of fish muscle tissue by increasing the reflection in higher wave length 600-700 nm and perception of red color ( $a^*$ ) or hue of the colour tone (C). Between this two parameters color redness ( $a^*$ ) and hue of the color tone (H) was found moderate correlation (Table 2).

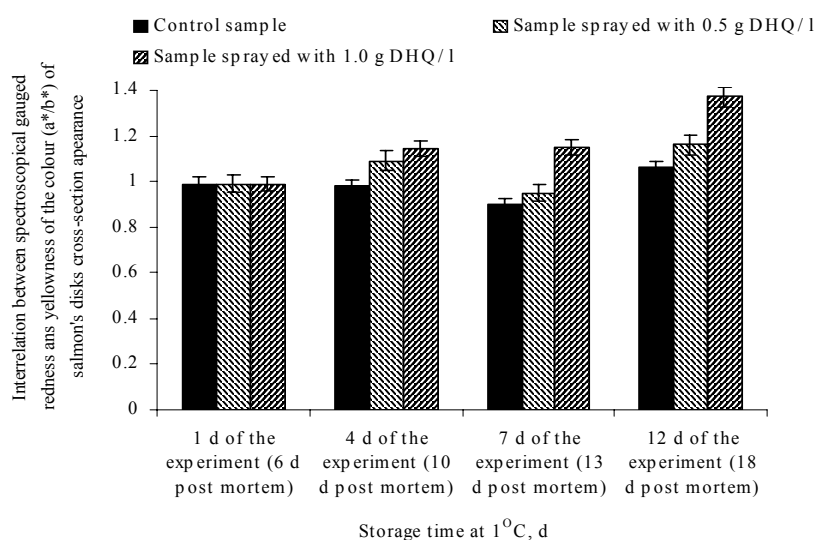
It was established that the colour yellowness ( $b^*$ ) of the control samples varied during the chilled storage. Significant ( $*P < 0.05$ ) decrease and the lowest values of 18.54 for color yellowness ( $b^*$ ) were established at the end of experiment (12 d). Similar tendency was found for the experimental samples 1 and experimental samples 2 (Table 1). The lowest values of color yellowness ( $b^*$ ) for specific storage period were determined for the experimental samples 2 (Table 1). It was found that the superficial treatment of salmon discs by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol contributed to significant ( $*P < 0.05$ ) decreased color yellowness ( $b^*$ ) values of salmon discs cross-section appearance by 6-12 %. The trends in color alterations could be determined by following the changes in red and yellow color components ratio ( $a^*/b^*$ ) (Figure 1). For the control samples  $a^*/b^*$  ratio slightly increased by 0.28 % up to the 4 d, after that decreased significantly ( $*P < 0.05$ ) by 9.51 % to the

**Table 2**

**Determination of statistically significant correlation between examined parameters using correlation coefficient  $|r|$**

Red color component ( $a^*$ )	0.29	0.06	0.69	0.05
Yellow color component ( $b^*$ )		0.50	0.72	0.10
Color brightness ( $L^*$ )			0.15	0.07
Hue of the color tone (H)				0.06
	Yellow color component ( $b^*$ )	Color brightness ( $L^*$ )	Hue of the color tone (H)	Color saturation (C)

Footnote:  $|r| \geq 0.85$  – strongly pronounced correlations;  $0.70 \leq |r| < 0.85$  – moderately strongly pronounced correlations;  $0.60 \leq |r| < 0.69$  → moderately pronounced correlation;  $0.50 \leq |r| < 0.59$  – slightly pronounced correlation



**Fig. 1. Changes of red component/yellow component of the color ( $a^*/b^*$ ) ratio of examined salmon disks**

7 d and increased again to the end of experiment when reached values 9% higher than in the beginning. Similar changes in the  $a^*/b^*$  ratio were found for the experimental samples 1 (Figure 1). Compared to the control samples they reached higher  $a^*/b^*$  values and greater increase (with 16%) at the end of the storage period. The highest values of  $a^*/b^*$  ratio were found for the experimental samples 2 (Figure 1). At the end (12 d) of storage  $a^*/b^*$  increased significantly ( $*P < 0.05$ ) by 38–39% (Figure 1).

The changes in the hue of the colour tone (H) of studied samples were similar to those in the color yellowness ( $b^*$ ) (Table 1). It was established a moderately strong correlation ( $|r| = 0.72$ ) between these two parameters (Table 2). The hue of the colour tone (H) values of control samples decreased by 8.04% at the 4 d (Table 1). The registered increase at the 7 d was not significant ( $*P < 0.05$ ). At the end of experiment (12 d) an increase by 8.00% compared to the 7 d was observed. The hue of the colour tone (H) of experimental samples 1 take a medium values compared to the control samples and experimental samples 2. After 12 d of storage the hue of the colour tone (H) decreased significantly ( $*P < 0.05$ ) by 20.00%. The highest ( $*P < 0.05$ ) values of hue of the colour tone (H) were determined for experimental samples 2 (Table 1). At the 12 d of experiment hue of the colour tone

(H) decreased significantly ( $*P < 0.05$ ) by 16–17%.

The results obtained showed that the superficial treatment of salmon discs by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol contributes to significant ( $*P < 0.05$ ) increase by 3 to 12% of the hue of the colour tone (H) values and consequently of the differentiation between light and dark colors.

The changes of color saturation (C) followed different trends comparing to the other color characteristics (Table 1). During the 12 d storage of the control samples, color saturation (C) decreased by 8.00–9.00% and reached its minimum of 41.27 at the end of experiment (Table 3). The color saturation (C) of experimental samples 1 did not change significantly ( $*P > 0.05$ ) up to the 12 d of storage (Table 1), and afterwards decreased significantly ( $*P > 0.05$ ) and reached minimum value of 41.75 at the 12 d. Therefore the superficial treatment of salmon discs by 0.005% solution of DHQ preserves the color saturation (C) of fish muscles up to the 7 d of chilled storage. The highest ( $*P < 0.05$ ) values of color saturation (C) were determined for experimental samples 2 (Table 1). The color saturation (C) increased significantly ( $*P < 0.05$ ) by 4.34% during the first seventh days of storage and after that decreased by 10.00% to the minimum levels of 42.19 (Table 1). It was established that the superficial treatment of salmon discs by spray contain-

ing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol did not effect significantly (\*P < 0.05) the color saturation (C) of cross-section appearance.

## Discussion

Better color characteristics were established for the samples superficially treated by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol. The colour of salmon muscles remained stable during the chilled storage.

It has to be noted, that the fish was delivered fresh, chilled in flake ice. Therefore the muscle enzymes, which are responsible for the lipid oxidation, keep their higher activity. The dihydroquercetin shows high inhibition activity towards the lipoxygenase (LD<sub>50</sub>=1mM) (Yamamoto et al., 1984), inhibits the phosphodiesterase of AMP (adeninemonophosphate) and GMP (guaninemonophosphate) (Ferrell et al., 1979; Ruckstuhl and Landry, 1981), effectively inhibits the copper ions catalyzed oxidation of low density lipoproteins (Frankel et al., 1993), as well as the 10 - 500 µM copper, vanadium and cadmium catalyzed lipid peroxidation (Sugihara et al., 1999).

The natively higher b-carotene content of salmon meat also takes part in antioxidant defend system of fish muscles (Zhong et al., 2007) and thus affects the obtained results. The impossibility for entering of the antioxidant solution in the depth of fish muscles also limits its effect. That is also a limitation of the effect of DHQ. Nevertheless the treatment by 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol preserves to the greater extend the color of salmon muscles compared to the control samples and experimental samples 1.

## Conclusions

The obtained results showed that the superficial treatment of salmon discs by spray containing 1 g DHQ.l<sup>-1</sup> 5% water solution of ethanol minimized the color alterations of cross-section appearance at the conditions of experiment. After 12 d of storage at 1±1°C the colour brightness (L\*) increased by 2.3%, the a\*/b\* ratio increased by 38 to 39%, the hue of

the colour tone (H) decreased by 3 to 12% and the colour saturation (C) decreased by 2%. The colour of fish muscles was preserved bright, fresh and orange red. The superficial treatment of salmon discs by spray containing 0.5 g DHQ.l<sup>-1</sup> 5% water solution of ethanol was not so effective and the color of cross-section appearance faded to some extend. The pretreatment with a 0.1 % solution of DHQ was found as a most effective for stabilizing the fish colour.

## Acknowledgements

The authors express their gratitude of the leaderships of the Flavit Ltd (Pushtino, Russia), and the Institute of the Biological Instrument-making attached to Russian Academy of Sciences, which were produced and supplied of the natural antioxidant dihydroquercetin isolate „Flavit”<sup>®</sup> (DHQ), and to their representative company Vita life SA (Sofia, Bulgaria), about the informative and organizing co-operation and financial support given to them, as well as of the team of the fish processing manufactory Nessy 5 Ltd (Plovdiv, Bulgaria) for their technical maintenance and the experiment implementation at industrial conditions.

## References

- Banerjee, Sr., 2006. Inhibition of mackerel (*Scomber scombrus*) muscle lipoxygenase by green tea polyphenols. *Food Research International*, **39**: 486 – 491.
- Becker, E. M., G. Ntouma and L. H. Skibsted, 2007. Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organization. *Food Chemistry*, **103**: 1288 – 1296.
- Decker, E. A. and Z. Xu, 1998. Minimizing rancidity in muscle foods. *Food Technol.*, **52** (10): 54 – 59.
- Draper, N. R. and H. Smith, 1998. Applied regression analysis, 3<sup>rd</sup> ed, *John Wiley*, New York, pp. 131-153.
- Ferrell, J. E. J. R., P. D. G. Chang Sing, G. Leow, R. King, J. M. Mansour, T. E. Mansour, 1979. Structure/Activity studies of flavonoids as inhibitors of cyclic AMP phosphodiesterase and relation-

- ship to quantum chemical indices. *Molecular Pharmacology*, **16** (2): 556-568.
- Frankel, E. N., J. Kanner and J. B. German**, 1993. Inhibition of oxidation of human low density lipoprotein substances in red wine. *Lancet*, **341** (3): 454-457.
- Kenward, M. G.**, 1987. A method for comparing profiles of repeated manuscripts. *Appl. Statistics*, **36**: 296-308.
- Matsuo, M., N. Sasaki, K. Saga and T. Kaneko**, 2005. Cytotoxicity of flavonoids toward cultured normal human cells. *Biol. Pharm. Bull.*, **28** (2): 253-259.
- Osogul, F., A. Polat and Y. Osogul**, 2004. The effect of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbial changes of sardines (*Sardina pilchardus*). *Food Chemistry*, **85** (1): 49 – 57.
- Pazos, M., J. M. Gallardo, J. L. Torres and I. Medina**, 2005. Activity of grape phenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chemistry*, **92**: 547 – 557.
- Ruckstuhl, M. and Y. Landry**, 1981. Inhibition of lung cyclic AMP-NADP Cyclic GMP-phosphodiesterases by flavonoids and other chromone-like compounds. *Biochemical Pharmacology*, **30** (7): 697-702.
- Santos, E. E. M. and J. M. Regenstein**, 1990. Effect of vacuum packaging, glazing and erythrobinic acid on the shelf-life of frozen white hake and mackerel. *Journal of Food Science*, **55** (1): 64 - 70, 118.
- Sugihara, N., T. Arakawa, M. Ohnishi and K. Furuno**, 1999. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide - dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linolenic acid. *Free Radicals in Biology and Medicine*, **27** (11-12): 1313-1323.
- Torres-Arreola, W., H. Sato-Valdez, E. Peralta, J. L Cardenas-Lopez, J. M. Ezquerra-Brauer**, 2007. Effect of low-density polyethylene film containing butylated hydroxytoluene on lipid oxidation and protein quality of siera fish (*Scorpaenopsis sierra*) muscle during frozen storage. *Journal of Agriculture and Food Chemistry*, **55** (15): 6140 – 6146, doi: 10.10221/jf070418h S0021-8561(07)00418-9
- Undeland, I., M. Stading and H. Lingnert**, 1999. Influence of skinning on lipid oxidation in different horizontal layers of herring (*Clupea harengus*) during frozen storage. *Journal of the Science of Food and Agriculture*, **78** (3): 441 – 450.
- Yamamoto, S., T. Yoshimoto, M. Furukawa, T. Horie and S. Watanabe-Kohno**, 1984. Arachidonate 5-lipoxygenase and its new inhibitors. *Journal of Allergy and Clinical Immunology*, **84** (2): 349-352.
- Zhong, Y., T. Madhujith, N. Mahfouz and F. Shahidi**, 2007. Compositional characteristics of muscle and visceral oil from steelhead trout and their oxidative stability. *Food Chemistry*, **104** (2): 602-608.

Received April, 20, 2009; accepted for printing September, 2, 2009.