

Analysis of Chemical and Sensory Parameters in Different Kinds of Escolar (*Lepidocybium flavobrunneum*) Products

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Abstract

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The chemical composition (nutritional and freshness parameters) and sensory characteristics were evaluated of escolar (*Lepidocybium flavobrunneum*) defrosted raw fillets and steaks that were marinated differently (pepper, onion, chilly, herbs, knusper, and hot) and packaged in a manner suitable for microwave heating (Darfresh® Simple Steps®). The study was carried out by using 8 skinless fillets of *Lepidocybium flavobrunneum*. The selected 18 parameters, i.e. dry matter/moisture, crude protein, net protein, collagen, net muscle protein, lipid, ash, saccharide, energy value, pH, water activity, salt content, total volatile basic nitrogen, trimethylamine, free fatty acids, peroxide value, and thiobarbituric acid assay were evaluated. The results indicate that the sample of Chilly marinated steaks was evaluated as the best one (90.29 ± 9.82), however, the samples of Herbs (89.6 ± 13.89) and Knusper (88.64 ± 15.59) marinated steaks also received more than 85 points for the overall impression. Control sample received the smallest number of points (71.7 ± 26.54) for the overall impression.

Keywords: Escolar fish; nutritional value; sensory acceptance; freshness; marination; packaging

In the Czech Republic, fillets (steaks) of marine fish *Lepidocybium flavobrunneum*, which belongs to the family Gempylidae, are sold as deep-frozen or a thawed vacuum-packed product (Council Regulation (EC) 854/2004). They are sold as butterfish or Escolar fish.

The meat of this fish is considered a delicacy due to its flavour, although its consumption may cause serious foodborne illnesses, the reasons being the presence of chemical hazards (biogenic amines/histamine, wax esters) and health risks (scombroid poisoning, oily diarrhea) in its meat (KAN *et al.* 2000; FELDMAN *et al.* 2005; AKHTAR *et al.* 2009; LING *et al.* 2009). The levels of free histidine, a precursor of histamine, in cooked fish samples can range from 8.0 to 11.0 mg/g (KAN *et al.* 2000) and the content of histamine from 2000 to 3800 mg/kg (FELDMAN *et al.* 2005). Headache, palpitation and urticaria are the main symptoms of scombroid poisoning. The lipid fraction of *Lepidocybium flavobrunneum* is

principally composed of wax esters (esters of fatty acid with long chain fatty alcohols), as well as phospholipids, triglycerides, hydrocarbons, and sterols (ZARZA *et al.* 1993). Lipid content fluctuates from 21% to 25% (KAN *et al.* 2000) and comprises more than 90% of wax esters that are equally distributed throughout the fillet (KARL & REHBEIN 2004). Wax esters (human digestive system is not capable to digest them) may cause diarrhea and other acute gastro-intestinal symptoms (cramps, headache, and vomiting) (ZARZA *et al.* 1993). The ingestion of *Lepidocybium flavobrunneum* with high concentrations of wax esters in the flesh can cause an oily diarrhea illness (watery, orange in colour) that is self-limited and rarely life threatening. Illness is known as “Escholarrhea” or “Kerriorrhea” (AKHTAR *et al.* 2009). The recommended portion size of Escolar fish for adults is 170 g although some studies showed that only much bigger portions caused gastrointestinal

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problems (MEDELLITIN 2014). According to European Food Safety Authority, it is not possible to establish an intake level of Escolar fish due to different sensitivity between consumers (EFSA 2004).

More recent works describe the morphological characteristics of *Lepidocybium flavobrunneum* (KELLER & KERSTETTER 2014; LANDGREN *et al.* 2014) as the occurrence, distribution, and genetic variation at the DNA level (QUIGLEY *et al.* 2005; BRENDTRO *et al.* 2008; KERSTETTER *et al.* 2008) but the studies that analysed *Lepidocybium flavobrunneum* as an important fish food from the consumer perspective (way of marketing, sensory properties, chemical composition, freshness parameters) are very rare (KARL & REHBEIN 2004; HWANG 2012).

Fish consumption is health beneficial not only due to the content of omega-3 fatty acids but also because of the contents other ingredients in fish meat such as proteins which have anti-inflammatory properties and can act as prevention against obesity (PILON *et al.* 2011). Anti-inflammatory properties of fish meat are also explained by synergistic effects of omega-3 fatty acids and the unique amino acid composition (RUDKOWSKA *et al.* 2010).

The consumption of fish once or two times per week is very beneficial for human health as proven by many research studies (SVEINSDOTTIR *et al.* 2009). *Lepidocybium flavobrunneum* fish consumption can be increased by an attractive method of putting spicy marinades on the steaks surfaces and packaging them in multi-purpose packaging e.g. Darfresh packaging which is suitable for microwave heating (ANONYMOUS 2014).

The aim of this study was to evaluate chemical composition (nutritional, freshness and other selected physico-chemical parameters) and sensory characteristics of Escolar fish steaks that had been differently marinated (6 kinds of spicy marinades) and packaged in a manner suitable for microwave heating (Darfresh® Simple Steps®).

MATERIALS AND METHODS

The study was carried out by using 8 skinless fillets of Escolar fish (*Lepidocybium flavobrunneum*), which were purchased on the market as vacuum packed thawed products (Seafood AG, Prague, Czech Republic). The country of fish origin was Vietnam/Indonesia, the fishing area Pacific/Indian Ocean FAO 71/57 (month/year of fish harvest/freeze: 03/2013;

month/year of thawing: 10/2013; freezing time before thawing in the Czech Republic: 7 months; defrosting conditions: thawing in air at max. +12°C for 12–16 h, temperature after thawing: max. 2°C). The shelf-life period provided by the distributor was nine days from the date of defrosting/packaging in the Czech Republic when stored at $2 \pm 2^\circ\text{C}$. Further processing: (cutting), marinating, Darfresh packaging, storage at $2 \pm 2^\circ\text{C}$. Sensory and laboratory analyses were conducted in the Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, Veterinary and Pharmaceutical University in Brno (DMHT FVHE VFU Brno).

Chemical examination. For the evaluation of chemical composition (dry matter/moisture contents, crude protein, net protein, collagen, net muscle protein, lipid, ash) 3 cm cuts were used from the middle parts of 6 fillets pieces (average weight of fillets 2.66 kg).

The dry matter/moisture content (M/MC) was determined gravimetrically according to the Czech national standard (ČSN ISO 1442:1997) by drying the sample with sand up to the constant weight at $103 \pm 2^\circ\text{C}$ (Binder FD53, Tuttlingen, Germany). The crude protein (CP) content (ČSN ISO 937:1978) was determined as the amount of organically bound nitrogen (recalculating coefficient $f_1 = 6.25$) using the analyser Kjeltec 2300 (FOSS Tecator, Höganäs, Sweden). The net protein (NP) content was determined as the amount of organically bound nitrogen by Kjeldahl method (conversion factor $f_1 = 6.25$) after precipitation with hot tannin solution using the analyser Kjeltec 2300 (FOSS Tecator, Höganäs, Sweden). The content of collagen (C) was computed from the content of hydroxyproline amino acid (conversion factor $f_2 = 8$). Hydroxyproline was determined quantitatively by photometric measurement of the absorbance at 550 nm on a GENESYS™ 6 spectrophotometer (Thermo Electron Corporation, Beverly, USA). Net muscle protein (NMP) content was determined mathematically ($\text{NMP} = \text{NP} - \text{C}$). The lipid (L) content was determined quantitatively (ČSN ISO 1443:1973) by extraction with solvents using Soxtec 2055 after acid hydrolysis of the samples using SoxCap 2047 (both FOSS Tecator, Höganäs, Sweden). The ash (A) content was determined gravimetrically (ČSN ISO 936:1978) by burning weighed samples in a muffle furnace (Elektro LM 212.11, Germany) at 550°C until the disappearance of black carbon particles.

Saccharide (S) content: $S = 100 - (\text{MC} - \text{CP} - \text{L} - \text{A})$ and energetical value (EV): $\text{EV} = (\text{CP} + \text{S}) \times 17 + \text{L} \times 37$ were determined mathematically. The salt (NaCl)

content was determined by precipitation titration with silver nitrate – Volhard's method (ČSN ISO 1841-1:1999).

The following chemical parameters of freshness were investigated at the beginning of the research and after 9 days of storage at $2 \pm 2^\circ\text{C}$: water activity (a_w), pH value, TVBN (total volatile basic nitrogen in mg/100 g), TMA-N (trimethylamine in mg/100 g), FFA (free fatty acids in % total lipid as oleic acid), PV (peroxide value in meq active oxygen/kg), and TBA (thiobarbituric acid assay in mg/kg). The pH values were measured using the InoLab pH 730 digital pH-metre (WTW GmbH, Weilheim in Oberbayern, Germany). The total volatile basic nitrogen (TVBN) was determined by direct distillation followed by titration on a Kjeltac 2300 (FOSS Analytical AB, Höganäs, Sweden) according to regulation (EC) 2074/2005, Chapter III. Trimethylamine (TMA-N) was determined using the same method as for TVBN determination after formaldehyde addition to the samples to release primary and secondary amines. Free fatty acids (FFA) and peroxide value (PV meq active oxygen/kg) were determined after fat extraction with diethyl ether. FFA were determined in accordance with ČSN ISO 660:1997. Peroxide value were determined by a modified method according to ČSN ISO 3960:2010. The thiobarbituric acid assay (TBA) value was determined by the distillation method and the oxidation products were quantified as malondialdehyde equivalents (MDA mg malondialdehyde/kg).

Marination (6 pieces of seasoning mixture). For marinating and subsequent sensory evaluation whole fillets were used (average fillet weight: 3.37 kg), which were cross-portioned in cranio-caudal direction into steaks (a total of 14 pieces with a thickness of about 2.5 cm). The first two steaks (just beneath the head) of each fillet (total of 4) were used as controls. The remaining 12 fillet steaks were used for the marination in seasoning mixes (pepper – 1039248-002 Pfeffer Willy, onion – 1000712 Magic Zwiebel, chilly – 38330 Chilli Willi, herbs – 1037397-003 Kräuter Willy, hot – 1090803 Magic Hot, and knusper – 1093692-001 Knusper Willy). The producer of the spicy marinades is RAPS GmbH & Co. KG (Kulmbach, Germany), which guarantees quality, food safety, and standardisation of the products (certified under DIN EN ISO 9001).

Two anatomically identical steaks from each fillet (total of 4 sections) were coated with the same type of marinade. Each steak was individually wrapped in Darfresh® Simple Steps® packaging and stored at $2 \pm 2^\circ\text{C}$ for 24 hours. Trays (MWBK500 Tray Black 140 ×

230 × 16 mm, 0514 S45) for packaging and barrier films were used (Darfresh Top Webs), both materials being suitable for microwave heating (Sealed Air s.r.o., Prague, Czech Republic). Darfresh® Simple Steps® packaging was performed on the device VS26 Food Packaging Systems (Sealed Air, Food Care/Cryovac®, Root, Switzerland).

Sensory evaluation. The steak samples were evaluated raw and immediately after the microwave heating. The heat treatment of the steaks (marinated and control sample/not marinated) was performed in Darfresh packaging in the microwave GM Electronic MTDG-25X (Pantin Ltd., Jenec near Prague, Czech Republic) for 3 min (power 1450 W, 900 W power). The temperature in spicy marinades was $90\text{--}92^\circ\text{C}$ after the microwave heating for 3 minutes. Sensory evaluation was carried out in the laboratory environment equipped in the way that meets the requirements of standard ISO 8589:2008, and included a panel of trained persons. The evaluation was performed by the employees or students of the postgraduate study program at DMHT FVHE VFU Brno, Czech Republic. The protocol used for the sensory evaluation was with unstructured graphical scales of 100 mm length, with one edge of the scale representing fully satisfactory status of the parameter and the second edge completely unsatisfactory state of the parameter. The evaluators assessed the following parameters: raw fillets – general appearance, cooked fillets – smell, fish smell, flavour, fish flavour, intensity, spiciness, texture, juiciness, palatability, the overall impression.

Statistical significance at $P < 0.05$; $P < 0.01$ was evaluated using *t*-test and one-way ANOVA analysis of variance, and post hoc Tukey test for finding differences between the groups. SPSS 20 statistical software (IBM Corporation, Armonk, USA) was used.

RESULTS AND DISCUSSION

Deepwater fish *Lepidocybium flavobrunneum*, as the fish of the family Tetraodontidae (Fugu fish), belongs to the controversial types of food. Tasty white tender meat without bones makes this fish a delicacy, on the other hand the consumption of its meat may represent a risk for humans. There is a number of food poisoning incidents in which seafood was involved. In June 1998, a food poisoning incident due to the ingestion of cooked fish occurred in Tokyo. The fish responsible for the poisoning was confirmed to be “Abura-sokomutsu” (KAN *et al.*

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2000). In August 2003, an outbreak of scombroid fish poisoning occurred at a retreat centre in California, USA (FELDMAN *et al.* 2004). Histamin, which belongs to biogenic amines, is the causative agent of scombroid poisoning, a foodborne chemical hazard (HWANG *et al.* 2012). Biogenic amines are formed mainly through the decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with the seafood (TAYLOR & SPECKARD 1983). In the study of HWANG *et al.* (2012), most of histamine-forming isolates belonged to the genus *Enterobacteriaceae*, such as: *Enterobacter* sp., *E. aerogenes*, *E. cloacae*, *E. alvei*, and *Citrobacter freundii*.

Fillets or steaks of *Lepidocybium flavobrunneum* are well accepted by consumers and they are present on the market in the Czech Republic, but health complications occur very rarely due to the information given by Council Regulation (EC) 854/2004. Nutritional composition, freshness, and other selected parameters of the *Lepidocybium flavobrunneum* defrosted steaks are showed in Table 1.

From the nutritional point of view, escolar fish belongs to foods with a higher energy value (1016 ±

68 kJ/100 g), due to the high fat content in muscles (194.07 ± 23.90 g/kg). Fat content decreases, with the exception of the medial part (25.3%), in cranio-caudal direction (steaks closer to head 25.9%, steaks closer to tail 22.9% (KARL & REHBEIN 2004). Indigestible wax esters are the main lipid fraction in muscle tissue of escolar fish (RUIZ-GUTIERREZ *et al.* 1997). The fat fraction of raw fillet of *Lepidocybium flavobrunneum* contained 47–97% (MORI *et al.* 1966; NICHOLS *et al.* 2001), cooked fish 74–88% (KAN *et al.* 2004), and hot smoked pieces of fillets contained more than 90% of wax esters (KARL & REHBEIN 2004). Fatty acid (FA) profile of edible dorsal loin muscle was the following: 2.75% of saturated fatty acids (SFA), 78.7% of monounsaturated FA (MUFA), and 17.72% of polyunsaturated FA (PUFA), of which 7.57% were PUFA_{n-6} and 10.15% were PUFA_{n-3} (SCHWARTZ 1997). In addition, it has to be mentioned that the major part of the lipids are not digestible and hence the consumption of this fish does not lead to an increased omega-3 uptake as fish consumption normally does. In our study, a higher fat content corresponded to lower moisture (621.80 ± 22.80 g/kg) and total pro-

Table 1. Nutritional composition, freshness and other selected parameters of the *Lepidocybium flavobrunneum* defrosted steaks

Parameters	Mean ± SD	
Nutritional composition		
Dry matter/moisture (g/kg)	378.20 ± 22.80/621.80 ± 22.80	
Crude protein (g/kg)	169.78 ± 10.83	
Net protein (g/kg)	148.49 ± 14.74	
Collagen (g/kg)	3.81 ± 1.06	
Net muscle protein (g/kg)	144,68 ± 14,87	
Lipid (g/kg)	194.07 ± 23.90	
Ash (g/kg)	8.21 ± 0.561	
Saccharides (g/kg)	6.15 ± 12,90	
Energy value (kJ/100 g)	1 016 ± 68	
Salt content (NaCl) (%)	0.41 ± 0.08	
Freshness parameters	First day	After 9 days
Free fatty acids (% total lipid as oleic acid)	1.38 ± 0.12 ^a	1.86 ± 0.07 ^b
Peroxide value (meq active oxygen/kg)	0.58 ± 0.09	1.06 ± 0.43
Thiobarbituric acid assay (mg malondialdehyde/kg)	3.22 ± 0.98 ^a	2.50 ± 0.40 ^b
Total volatile basic nitrogen (mg/100 g)	16.52 ± 0.52 ^a	21.16 ± 2.08 ^b
Trimethylamine-N (mg TMA-N/100 g)	12.26 ± 0.58 ^a	18.29 ± 2.63 ^b
pH value	6.30 ± 0.14	6.32 ± 0.15
Water activity a_w	0.98 ± 0.03	0.99 ± 0.00

Parameters values a and b are indicators for statistical significance at $P < 0.05$; values in the same column with different letters are statistically different

teins (169.78 ± 10.83 g/kg) contents. Similar composition for *Lepidocybium flavobrunneum* (water 59.2%, protein 18.0%) was reported in the work by KARL and REHBEIN (2004).

The protein fraction is characterised by four parameters: crude protein (169.78 ± 10.83 g/kg) as the amount of organic nitrogen present in the sample (including lower molecular weight protein substances with N-nature), net protein (148.49 ± 14.74 g/kg) represented as sarcoplasmatic content (hemoglobin, myoglobin), myofibrillar (actin, myosin), and stroma (collagen) proteins without low molecular weight substances of N-nature (e.g. peptides, free aminoacids, ammonia, amines, amides), collagen (3.81 ± 1.06 g/kg) as stromatic protein content in muscle, and net muscle proteins (144.68 ± 14.87 g/kg) represented as myofibrillar content without collagen.

The values of pH, a_w , salt content, and the other five parameters which are related to the freshness (shelf-life) of the fillets, matched the state of muscles immediately after thawing (Table 1). The measured value of water activity was high (0.98 ± 0.03), but this is expected for the defrosted fish muscle. The content of NaCl ($0.41 \pm 0.08\%$) was appropriate for raw fish meat. Same parameters were followed by HWANG *et al.* (2012) in 15 pieces of escolar thawed steaks originating from the market in Thailand (12 pieces were prepared of *Lepidocybium flavobrunneum* kind, three of *Ruvettus pretiosus*) who found a lower pH of 5.6 ± 0.3 , and practically the same value for water activity a_w (0.98 ± 0.01) as compared with our results. The salt content was twice as high (0.8 ± 0.1), which could have been caused by the use of salty sea water (in liquid or solid form) for fillets chilling. There were no significant differences between pH and a_w values at the beginning of the research and after 9 days of storage at $2 \pm 2^\circ\text{C}$.

The freshness parameters are important for the evaluation of the course and intensity of proteolytic, lipolytic, and oxidative processes during storage of thawed fillets (BREMNER 2002). The intensity of autolytic and proteolytic processes in proteins was monitored in our experiment by two parameters: total volatile basic nitrogen (16.52 ± 0.52 mg/100 g) and trimethylamine (12.26 ± 0.58 mg/100 g) (Table 1). TMA-N and TVBN contents were statistically significantly higher after 9 days of storage at $2 \pm 2^\circ\text{C}$ (TMA-N: 18.29 ± 2.63 ; TVBN: 21.16 ± 2.08). The values published in the research by HWANG *et al.* (2012) into TVBN parameter (4.8 ± 2.0 mg/100 g) were significantly lower (about 1/4) when compared with our results. The differences in the values may be due to the methodological procedure in which HWANG *et*

al. (2012) used for TVBN determination methodology according to Conway (COBB *et al.* 1973). It is possible that some low molecular compounds of N-nature could not be determined by this method. KARL and REHBEIN (2004) reported for raw thawed fillets of *Lepidocybium flavobrunneum* values more similar to ours: the parameter TVBN 120.1 mg/kg (max. 150.1 mg/kg), for TMA-N 76 mg/kg (max. 129.9 mg/kg).

The quality (freshness) of the lipid fractions was characterised by three parameters: free fatty acids content ($1.38 \pm 0.12\%$ total lipid as oleic acid) expresses the degree of hydrolytic decomposition of fat; peroxide value (0.58 ± 0.09 mekv O_2/kg) evaluates the intensity of the primary oxidation products and thiobarbituric acid (3.22 ± 0.98 mg/kg) which is related to the secondary oxidation processes. The values of FFA, PV, and TBA were very low (Table 1). It can be stated that lipolytic and oxidative processes were markedly inhibited by storage of the fillets after manufacturing (rapid freezing of fresh fillets avoiding the fluctuation in temperature during storage, vacuum packaging eliminating the presence of oxygen, gentle thawing). After 9 days of storage at $+2 \pm 2^\circ\text{C}$, FFA (1.86 ± 0.07) and PV (1.06 ± 0.43) were significantly higher while TBA (2.50 ± 0.40) value was significantly lower in comparison with the results obtained at the beginning of the research. The preservative effects of the storage temperature on lipid hydrolysis and oxidation was evident in the study of AUBOURG (1999) who evaluated the lipid damage detection during the frozen storage of fish. Due to the higher fat content in the steak samples and their physical state (thawed muscle), it is possible to assume that the values of these parameters will increase significantly during the following storage period in the refrigerator. This hypothesis should be experimentally verified due to the fact that the manufacturer guarantees the shelf life of 9 days for fish fillets after thawing. No work on this topic has been published to now.

Live fresh water fishes (food intended for the market, most often species *Cyprinus carpio* and *Oncorhynchus mykiss*) are considered to be the freshest food in the Czech Republic, and also high quality fresh (frosted), full or modified (whole gutted, fillets, steaks) fishery products are present on the market. The vacuum packaging method is used for the prolongation of the shelf life, exceptionally packaging in gas modified atmosphere ($30\% \text{CO}_2/70\% \text{N}_2$). The assortment is extended during the year by offering deep frozen fish and their parts.

The advantage of Darfresh® Simple Steps® packaging method, in comparison with vacuum packaging, is the possibility of multi-use packaging material (pre

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made tray + web) suitable for microwave heating (heat treatment), without having to unpack the product prior to heating. This type of packaging is very suitable for seasoned (spicy marinated) intermediate products. Their offer and sale would increase the consumption of fish, which is in the Czech Republic very low (about 5 kg/person/year). In addition, a sophisticated method of packaging must also satisfy consumers' preferences (appearance, aroma, flavour, intensity, spiciness, salinity), which were met in our experiment. More preferred samples in the sensory evaluation of raw and cooked steaks were those kinds of marinades (chilly, herbs, knusper, hot) which were more attractive visually, with more intense colour and taste.

Table 2 shows the results of the sensory evaluation of marinated samples of *Lepidocybium flavobrunneum*.

Best score. Chilly marinade received the best scores for taste, intensity of seasoning, consistency, palatability, and the overall impression, while Herbs marinade achieved the highest scores for fragrance, fish flavour, and juiciness. The best score for the overall appearance was obtained by Hot marinade. **Lowest score.** Control sample had the lowest scores for fragrance, taste, intensity of seasoning, palatability, and the overall impression. This makes sense because Control sample

had not been marinated at all. The lowest scores for fish flavour, consistency, and juiciness were given to Onion marinade, while the lowest score for the overall appearance to Pepper marinade. Knusper marinade was evaluated as the worst for fishy smell. The marinated steaks of escolar fish are shown in Figure 1.

Statistical significance. (1) *Raw steaks.* The overall appearance was significantly ($P < 0.05$) inferior with Pepper sample in comparison with Knusper Hot sample. (2) *Steaks after microwave heating.* Among all the marinated samples, Control sample had the significantly ($P < 0.05$) lowest marks for taste, while Onion sample had significantly ($P < 0.05$) lower marks in comparison with Chilly sample. Control sample also had the significantly ($P < 0.01$) lowest marks for the intensity of seasoning among all other marinated samples, which is in accordance with the fact that Control sample was not marinated at all (Table 2). Pepper and Onion samples were evaluated as worse than control samples only when they were raw (Table 2).

The results of our experiment are a good indication of the acceptance of fish sensory properties. Control sample, which was not marinated at all, is a good indicator about reviewers' perception of *Lepidocybium flavobrunneum*: although it gained the lowest marks,

Table 2. Sensory evaluation of marinated samples of the *Lepidocybium flavobrunneum*

Properties	Experimental samples (mean ± SD)						
	Control	Pepper	Onion	Chilly	Herbs	Knusper	Hot
Salt content*	0.41 ± 0.08	0.65 ± 0.03	0.65 ± 0.04	0.71 ± 0.02	0.68 ± 0.03	0.68 ± 0.63	0.51 ± 0.04
Salt content**		1.57 ± 0.19	0.94 ± 0.14	1.10 ± 0.17	0.57 ± 0.07	1.49 ± 0.08	0.67 ± 0.04
Raw steaks							
Overall appearance	72.4 ± 21.25	56.38 ± 18.96 ^a	71.29 ± 20.02	75.7 ± 18.10	77.13 ± 18.43	79.93 ± 18.08 ^b	86.29 ± 13.77 ^{b***}
Steaks after microwave heating							
Fragrance	63.1 ± 31.97	69.00 ± 23.77	69.71 ± 22.19	78.14 ± 19.30	87.33 ± 11.72	85.71 ± 14.33	82.00 ± 14.12
Fishy smell	74.1 ± 30.25	49.38 ± 33.64	51.21 ± 24.53	58.64 ± 32.80	54.53 ± 31.09	41.43 ± 34.13	53.07 ± 30.83
Taste	28.1 ± 28.69 ^a	66.15 ± 29.16 ^b	57.79 ± 33.41 ^{bc}	87.79 ± 11.52 ^{bd}	74.53 ± 23.15 ^b	78.14 ± 23.69 ^b	77.57 ± 21.45 ^b
Fish flavour	85.7 ± 15.73	83.31 ± 17.78	77.43 ± 19.84	89.79 ± 10.20	91.73 ± 8.45	91.29 ± 10.42	82.43 ± 20.22
Intensity of seasoning	17.8 ± 30.32 ^a	67.00 ± 27.91 ^b	59.79 ± 29.81 ^a	84.07 ± 16.34 ^b	78.67 ± 24.84 ^b	83.5 ± 18.78 ^b	74.71 ± 25.27 ^b
Consistency	83.2 ± 19.50	79.85 ± 14.65	78.64 ± 17.51	88.21 ± 11.76	84.27 ± 15.67	80.43 ± 17.87	85.86 ± 18.71
Juiciness	84.6 ± 16.19	88.62 ± 10.09	80.43 ± 19.55	87.29 ± 13.40	94.33 ± 5.07	83.86 ± 22.54	87.36 ± 12.60
Palatability	69.2 ± 29.11	78.08 ± 19.22	75.93 ± 22.99	90.29 ± 10.13	90.13 ± 11.12	87.86 ± 17.66	82.93 ± 21.55
Overall impression****	71.7 ± 26.54	79.38 ± 18.43	75.71 ± 18.77	90.29 ± 9.82	89.60 ± 13.89	88.64 ± 15.59	83.14 ± 21.32

*NaCl in % in marination day; **NaCl in % in day of sensory evaluation (24 h after marination); ****overall impression represents the mean value of all sensory properties (9 evaluations); ***parameters values a, b, c, d are indicators for statistical significance at $P < 0.05$; values in the same column with different letters are statistically different



Figure 1. Defrosted steaks (Control sample – Kontrola) made from fillets of the *Lepidocybium flavobrunneum* that were marinated differently (1 – Chilly, 2 – Onion, 3 – Hot, 4 – Knusper, 5 – Herbs, 6 – Pepper) and packaged in a manner suitable for microwave heating (Darfresh® Simple Steps®)

the reviewers evaluated it with more than 70 points for the overall impression and more than 80 points for the fish flavour, consistency, and juiciness. In conclusion, it must be noted that the marinated steaks were evaluated positively as a result of 2–3× higher salt content in muscles, which participated along with spices in the enhancement of the flavour (Table 2).

CONCLUSION

The results of the experiment provided new information on the monitored physico-chemical parameters of thawed fillets of the sea fish *Lepidocybium flavobrunneum*, which can be used for further monitoring and evaluation of the quality of this fish under the conditions of their circulation on the market in the Czech Republic. This study has shown that 9 days after packaging, the expiry period of *Lepidocybium flavobrunneum* defrosted fish fillets, specified by the manufacturer, is an appropriate period for this storage conditions. This statement is in accordance with the results obtained in our study, although the values of 4 parameters (free fatty acids FFA, thiobarbituric acid assay TBA, total volatile basic nitrogen TVBN, trimethylamine TMA-N) were significantly increased ($P < 0.05$) after 9 days of storage at $2 \pm 2^\circ\text{C}$. There are not limit values set by Regulations for FFA and TBA but the values of TVBN and TMA-N were lower than those suggested by MOKRANI *et al.* (2012) for fish of good quality. TVBN content after 9 days of storage at $2 \pm 2^\circ\text{C}$ was lower than that allowed by the Regulation (Council Regulation (EC)

854/2004). The results of the sensory evaluation have shown that the consumers prefer steaks marinated in visually more attractive and flavourful marinades (chilly, herbs, knusper, hot). Darfresh® Simple Steps® packaging should increase consumers' interest for this unconventional kind of intermediate fish product which is still missing in our market.

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