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Research Article

Nutritional profile of nigiri sushi meal and the usage of citrate synthase activity as freshness parameter

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Abstract

The aim of the study was to access the influence of nigiri sushi meal ingredients ratio on its weight, nutritional profile, physic-chemical characteristics (crude protein, lipid content, ash content, salt content, phosphorus content and thiobarbituric acid assay) and to estimate the usage of citrate synthase activity as freshness index of seafood used for sushi preparation. Nutrition composition of nigiri sushi is highly influence by rice/seafood ratio. Inclusion of processed seafood in sushi can influence significantly weight and consequently nutritional profile of nigiri sushi meal as well. Shrimp cooking resulted in 16.45±3.29% weight loss in whole non-deveined shrimp and 13.03±3.40% in non-deveined shrimp tail. Nigiri sushi meal prepared with salmon and tuna fish can be recognized as good sources of seafood. Nigiri sushi meal can be considered as a low-calorie meal (nigiri salmon: 716.13±24.18 kJ/100g; nigiri tuna: 638.12±10.64 kJ/100g; nigiri shrimp: 672.06 ± 8.72 kJ/100g) but on the contrary it cannot be considered as low salt content meal (nigiri salmon: 0.97±0.04%; nigiri tuna: 0.89±0.10%; nigiri shrimp: 1.06±0.13%). Citrate synthase activity (CSA) increases after each freezing/thawing cycle and at the end (4th cycle) were 5.29±0.67 μmol/mL/min and 6.67±0.63 μmol/mL/min in tuna and salmon samples, respectively. CSA can be recognized as reliable enzymatic kit indicator for fish freshness determination in nigiri sushi meal.

Keywords: Ingredients ratio, Low-calorie meal, High-salt meal, Processed seafood, Citrate synthase activity

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Introduction

Sushi exists in a miscellaneous range of styles, forms, and varieties. Sushi consumption entails a set of questions considering its safety, intrinsic nutritional and energy value, health benefits or health hazards, position and importance of this meal from the viewpoint of public health policy (Feng, 2012).

Globally, sushi is considered as a healthy meal because it has low calories and it contains low amounts of fat and cholesterol. Sushi is rich in high quality protein and it is an excellent source of omega-3 fatty acids because of the seafood ingredients (Cysnerios *et al.*, 2009). Sushi is consumed by a wide range of population and it emphasizes the importance of sushi meal nutritional and other properties (Dordevic *et al*., 2018).

Sushi is often served as raw sliced fish on acidified rice (nigiri) or as longitudinal strips of fish meat rolled with acidified rice and sheets of seaweed “nori” and sliced into 6 or 8 pieces (maki) (Feng, 2012). The mutual ratio of these ingredients in sushi can vary a lot which can have a major impact on its nutritional profile. For this reason, emphasize should be put on sushi ingredients ratio, more precisely which proportion belongs to animal (seafood) and which to plant (rice, seaweed, vegetables, various seeds) component. It is obvious that with the predominance of plant ingredients (especially rice) sushi will contain more saccharides and less animal proteins and healthful fats.

On the other hand, sushi meal is often associated with microbiological and parasitic hazards (Sabater and Sabater, 2000; Kim *et al*., 2008; EFSA, 2010; Leisner *et al*., 2014, Hoel *et al*., 2015; Kulawik *et al*., 2018). Fish intended for raw consumption have to be deep frozen according to the recommended temperature regimes (regulation no 853/2004). It is not easy to distinguish between fresh fish and thawed ones, because their physical and chemical characteristics are very similar. Commercial enzymatic kit (citrate synthase activity) can be used for the detection whether or not a fish has been previously frozen (Simoniova *et al.*, 2013).

Sushi is a food product that is usually prepared and served at the same time; however, pre-packed sushi meal is available in the supermarkets (Mol *et al.*, 2014). In this context, sushi should be eaten immediately or kept properly refrigerated and eaten no later than the “best before” date (Leisner *et al.*, 2014). Keeping the quality of this product became important (Mol *et al.*, 2014) and freshness is the single most important criterion for sushi meal (Feng, 2012). Sensory evaluation and microbial examination or physico-chemical parameters such as pH value, water activity or thiobarbituric acid assay are often used by laboratories for monitoring quality level and freshness of food.

The objectives of the present study were firstly to compare weights and ratios of nigiri sushi (prepared with three types of seafood: salmon, tuna, shrimp) components, secondly to analyse selected physico-chemical freshness parameters, and thirdly to examine whether or not a fish used for sushi meal has been previously frozen.

Materials and methods

The study was carried out by using samples of nigiri sushi prepared with salmon, tuna and shrimp. The number of 108 pieces of nigiri sushi were analysed in total. The research was conducted in 2015 at the Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno and it was financed by institutional research fond.

Weight parameters

Weight parameters (accurate to 0.00 g) were obtained by weighing on the equipment PionnerTH (Ohaus corp., China). The samples of non-deveined thawed shrimps (*Litopenaeus vannamei*) (whole bodies and tails separately) were weighted before cooking in water bath (GFL 1012, Turnov, BDL s.r.o., Czech Republic) and after cooking (10 minutes at 90ºC).

Physico-chemical examination

The dry matter/moisture content (M/MC), crude protein (CP), lipid content (L), ash content (A) and salt content (NaCl) were determined according to Czech national standards (ČSN ISO 1442:1997, ČSN ISO 937:1978, ČSN ISO 1443:1973, ČSN ISO 936:1978 and ČSN ISO 1841-1:1999), respectively. The amount of total phosphorus was determined gravimetrically (Standard operating procedure of Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno**.** Saccharide (S) content [S=100–(MC–CP–L-A)] and energetical value [(EV): EV=(CP+S)×17+ L×37)] were determined mathematically. The following physico-chemical parameters of freshness were investigated: water activity (a_w) (using the LabMaster-aw, Novasina Ltd., Switzerland), pH value (using InoLab pH 730 digital pH-metre, WTW GmbH, Germany) and TBA (thiobarbituric acid assay in mg/kg) (distillation method).

Citrate synthase activity

Citrate synthase activity (CSA) was measured in all samples of nigiri sushi (salmon, tuna, and shrimp). CSA measuring were also conducted on separate fresh fillet samples without skin (retail Ocean 48, Brno, Czech Republic) and after each freezing/thawing cycle (4 freezing/thawing cycles). Four muscle samples (approximately 150 g of *Thunnus albacores* and *Salmo salar*) originated from different fillets were analyzed separately. After analyzing the fresh meat exudates, samples were vacuum-packed (Vacuum: approx. 12.5

kg) using the device VAC-STAR S 223 chamber packaging machine (VAC-STAR AG, Switzerland). Samples were frozen at -40°C in quick freezing unit F.R.C. BF 031AF (Friulinox, Italy) to muscle core temperature of -18°C and they were stored at freezer temperature of -20± 2°C for one week. Frozen samples were thawed in a refrigerator (+2±2°C) for 10 hours and analysed immediately. Citrate Synthase Assay Kit (Technical Bulletin no. CS0720, Sigma-Aldrich, St. Louis, USA) was used to measure the activity of citrate synthase enzyme, using the GENESYS™ 6 spectrophotometr (Thermo Electron Corporation, USA) at 412 nm. The final activity was calculated according to the below formula:

$$U \text{ (in } \mu\text{mol/mL/min)} = (\Delta A_{412})/\text{min} \times V(\text{mL}) \times \text{dil} / \varepsilon^{mM} \times L(\text{cm}) \times V_{enz} (\text{mL})$$

where ΔA_{412}/min is difference between the endogenic and the overall activity of citrate synthase at 0 and 1 minute, V is total volume of sample (1 mL), dil is the dilution factor of the original sample (10^{-1}), ε^{mM} is the extinction coefficient of 5-thio-2-nitrobenzoic acid at 412 nm (13,6 mM^{-1}/cm), L is length (1 cm) for 1 mL a cuvette and V_{enz} is sample volume (10 µL = 0,01 mL).

Statistical analysis was performed using statistical software SPSS Version 20 for windows (SPSS IBM Corporation, Armonk, USA).

Results

Ingredients ratio and nutrition profile of nigiri sushi meal

Nigiri sushi ingredients weights, energy value and phosphorus content are shown in Table 1.

Sushi chefs are trying to make sushi pieces as standardized as possible, but as shown in Table 1, it is possible to see that there are differences among weights of rice, seafood and even wasabi between nigiri sushi prepared with salmon, tuna and shrimp. It was found statistically significant difference (p<0,05) between rice weights of nigiri salmon and nigiri tuna. The major portion of seafood was in nigiri tuna (37.48±3.79%), while the lightest weights were measured in nigiri shrimp (18.99±2.79%).

Protein content in nigiri sushi samples was the highest in nigiri tuna (104.14±16.77 g kg^{-1}) and the lowest in nigiri shrimp (56.09±17.36 g kg^{-1}). There was also found statistically significant difference ($p<0.05$) between these three types of nigiri sushi in protein contents. There was found that protein content vary between different types of nigiri sushi and thus samples of nigiri tuna had significantly higher protein content both in rice and seafood part (rice: 33.80±5.84 g kg^{-1}; tuna: 250.34±10.47 g kg^{-1}) in comparison with nigiri salmon (rice: 29.23±3.91; salmon: 221.55±16.64) and nigiri shrimp (rice: 25.85±0.76 g kg^{-1}; shrimp: 221.00±13.42 g kg^{-1}).

Table 1: Weight, nutritional composition, caloric value and phosphorus content of nigiri sushi made by salmon (*Salmo salar,* Atlantic salmon), tuna (*Thunnus albacores,* Bigeye tuna) and shrimp (*Penaeus vannamei,* Pacific white shrimp).

	units	**nigiri salmon n = 36 mean ± s.d.**	**nigiri tuna n = 36 mean ± s.d.**	**nigiri shrimp n = 36 mean ± s.d.**
ingredients		**weight / percentage**		
one piece sushi	g	38.08 ± 2.58^{b}	38.44 ± 2.68^{b}	31.27 ± 4.47^{a}
rice	g	25.52 ± 1.93^{a}	23.95 ± 2.39^{b}	24.69 ± 1.36
	%	67.00 ± 4.07	62.25 ± 3.79	79.72 ± 6.19
seafood	g	12.52 ± 2.05^{b}	14.39 ± 1.57^{a}	5.89 ± 0.85^{c}
	%	32.82 ± 4.10	37.48 ± 3.79	18.99 ± 2.79
wasabi	g	0.07 ± 0.05^{a}	0.10 ± 0.04^{b}	0.10 ± 0.05^{b}
	%	0.18 ± 0.13	0.27 ± 0.10	0.31 ± 0.16
parameters		**nutritional composition / energetical value**		
dry matter / moisture	g/kg	413.5 ± 7.2^{a}	388.4 ± 6.3^{b}	410.0 ± 4.9^{c}
		586.5 ± 7.2^{a}	611.6 ± 6.3^{b}	590.0 ± 4.9^{c}
proteins	g/kg	84.20 ± 10.20^{b}	104.14 ± 16.77^{a}	56.09 ± 17.36^{c}
proteins one piece sushi	g	3.21 ± 0.22	4.00 ± 0.28	1.75 ± 0.25
proteins rice	g/kg	29.23 ± 3.91^{b}	33.80 ± 5.84^{a}	25.85 ± 0.76^{b}
proteins seafood	g/kg	221.55 ± 16.64^{b}	250.34 ± 10.47^{a}	221.00 ± 13.42^{b}
fat	g/kg	17.7 ± 7.4^{a}	0.4 ± 0.3^{b}	0.3 ± 0.3^{b}
fat one piece sushi	g	0.65 ± 0.04	0.02 ± 0.00	0.01 ± 0.00
ash	g/kg	13.1 ± 0.8^{b}	13 ± 0.4^{b}	14.96 ± 1.00^{a}
saccharides	g/kg	298.49 ± 11.86^{b}	270.45 ± 19.61^{a}	338.65 ± 16.82^{c}
saccharides one piece sushi	g	11.37 ± 0.77	10.40 ± 0.72	10.59 ± 1.51
energy value	$kJ·100g^{-1}$	716.13 ± 24.18^{c}	638.12 ± 10.64^{a}	672.06 ± 8.72^{b}
energy value one piece sushi	kJ	272.67 ± 18.45	245.25 ± 17.08	210.13 ± 30.06
energy value 8 pieces / meal size	kJ	2181.76 ± 147.60	1962.34 ± 136.67	1681.20 ± 240.48
phosphor content	$mg·100g^{-1}$	85.01 ± 11.88^{b}	112.86 ± 17.80^{a}	51.04 ± 9.37^{c}

Parameters values a, b, c are indicators for statistical significance at $p<0.05$; different letters indicate statistically significant difference between different types of nigiri sushi.

Fat content was noticeably ($p<0.05$) highest in nigiri salmon samples (17.7±7.4 g/kg), same as saccharide content (298.49±11.86 g/kg) and energy value (716.13±24.18). Phosphorus content ranged from 51.04±9.37 mg/100g (nigiri shrimp) to 112.86±17.80 mg/100 g (nigiri tuna). Moisture content was higher in nigiri tuna (611.6±6.3 g/kg) and nigiri shrimp (590.0±4.9 g/kg) in comparison with nigiri salmon (586.5±7.2 g/kg). Ash content in nigiri shrimp (14.96±1.00 g kg^{-1}) was significantly ($p<0.05$) higher than in nigiri salmon (13.1±0.8 g kg^{-1}) and nigiri tuna (13±0.4 g kg^{-1}) samples.

Freshness and physicochemical characteristics of nigiri sushi meal

Water activity, pH, salt (NaCl) and TBA contents of nigiri sushi meal are shown in Table 2 and figure 1.

Table 2: Water activity (aw), pH value, salt (NaCl) content and thiobarbituric acid assay (TBA) of nigiri sushi prepared with salmon *Salmo salar*, Atlantic salmon), tuna (*Thunnus albacores*, Bigeye tuna) and shrimp (*Penaeus vannamei*, Pacific white shrimp).

	units	nigiri salmon n = 36 mean ± s.d.	nigiri tuna n = 36 mean ± s.d.	nigiri shrimp n = 36 mean ± s.d.
aw		0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.01
pH whole sushi		5.08 ± 0.14^{Bb}	5.25 ± 0.07^{Ba}	4.94 ± 0.13^{Bc}
pH rice		4.21 ± 0.08^{A}	4.31 ± 0.10^{A}	4.21 ± 0.10^{A}
pH seafood		6.12 ± 0.09^{C}	6.19 ± 0.21^{Ca}	5.98 ± 0.19^{Cb}
salt whole sushi	%	0.97 ± 0.04^{Bb}	0.89 ± 0.10^{Ba}	1.06 ± 0.13^{Bc}
salt rice	%	1.19 ± 0.19^{A}	1.32 ± 0.15^{A}	1.27 ± 0.15^{A}
salt seafood	%	$0.71 \pm 0,12^{C}$	0.92 ± 0.23^{B}	0.84 ± 0.16^{C}
TBA	mg/kg	1.43 ± 0.42^{b}	3.08 ± 1.04^{a}	1.40 ± 0.54^{b}

Parameters values a, b, c are indicators for statistical significance at P<0.05 between rows, while A, B, C are indicators for statistical significance at *p*<0.05 between columns; different letters indicate statistically significant difference.

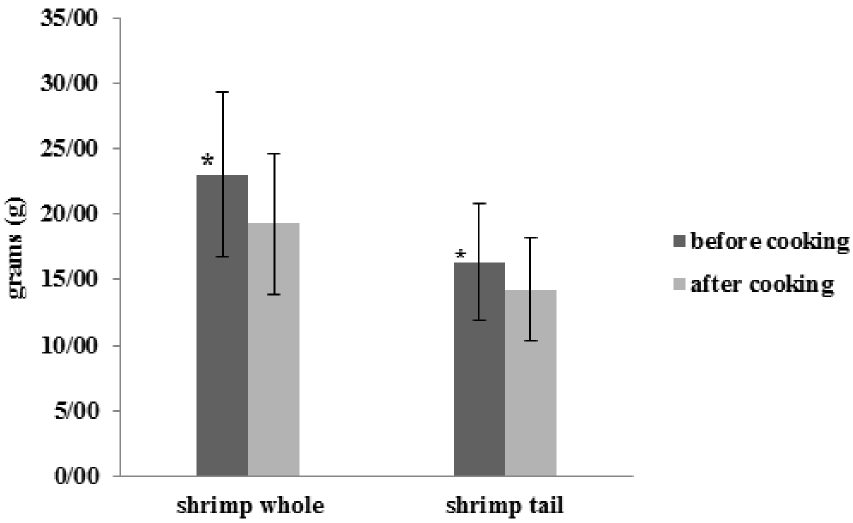

Figure 1: Weights of shrimp (*Penaeus vannamei*) before and after cooking (*t-test P<0.05 (between weights of whole shrimp and shrimp tail, before and after cooking).

The highest acidity among nigiri sushi ingredients in all three types of nigiri sushi was measured in vinegared rice. No significant difference was found between pH of rice among three types of nigiri sushi. pH measured in whole nigiri sushi was statistically significant with the lowest among nigiri shrimp samples (4.94±0.13). The highest (*p*<0.05) pH of seafood was measured in tuna (6.19±0.21) used for nigiri tuna preparation. Same trend as pH was observed for salt content, where the highest significant (*p*<0.05) salt content among used nigiri sushi ingredients was in vinegared rice, ranging from 1.19 ± 0.19% (nigiri salmon) to 1.32±0.15% (nigiri tuna).

The highest (*p*<0.05) level of oxidation measured by TBA was found among nigiri tuna samples (3.08±1.04 mg/kg). Water activity was stable in all three types of examined nigiri sushi samples, amounted to the same value of 0.97±0.01.

Shrimp cooking losses and citrate synthase activity in nigiri sushi/seafood samples

CSA of nigiri sushi samples and seafood samples during freezing/ thawing cycles are shown in Table 3 and figure 2, respectively.

Table 3: Citrate synthase activity (CSA) of nigiri sushi prepared with salmon *Salmo salar,* Atlantic salmon), tuna (*Thunnus albacores,* Bigeye tuna) and shrimp (*Penaeus vannamei,* Pacific white shrimp).

CSA	units	nigiri salmon n = 12 mean ± s.d.	nigiri tuna n = 12 mean ± s.d.	nigiri shrimp n = 12 mean ± s.d.
1st sampling		3.53 ± 0.19[a]	1.54 ± 0.39[Bb]	0.00 ± 0.00
2nd sampling		2.35 ± 0.81[a]	1.12 ± 0.04[Bb]	0.00 ± 0.00
3rd sampling	μmole/mL/min	2.39 ± 0.84[a]	0.15 ± 0.06[Ab]	0.00 ± 0.00
mean of samplings		2.76 ± 0.84[a]	0.94 ± 0.65[b]	0.00 ± 0.00

Parameters values a, b, c are indicators for statistical significance at P<0.05 between rows, while A, B, C are indicators for statistical significance at *p*<0.05 between columns; different letters indicate statistically significant difference.

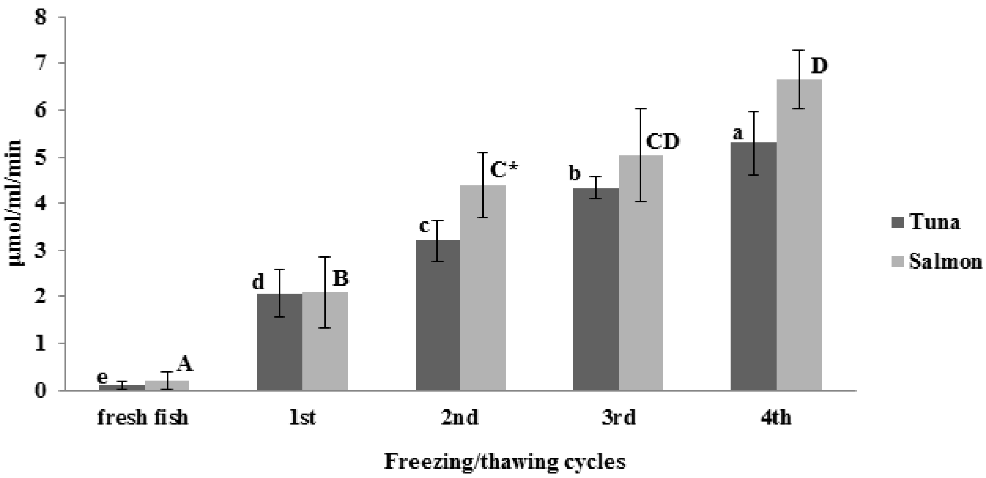

Figure 2: Citrate synthase activity affected by freezing/thawing cycles (Parameters values a. b. c / *A. B. C* are indicators for statistical significance at *p*<0.05; lowercase letters indicate the lower values of the particular parameter. *t-test *p*<0.05 (between tuna and salmon samples).

Exudate of salmon used for nigiri salmon preparation had statistically significant higher CSA content (2.76±0.84 μmol/mL/min) during the all three sampling than tuna used for nigiri tuna (0.94±0.65 μmol/mL/min) preparation.

Freezing/thawing cycles of tuna and salmon samples resulted in constant CSA increase in samples' exudates. At the end of the 4th cycle, CSA was

5.29±0.67 μmol/mL/min and 6.67±0.63 μmol/mL/min in tuna and salmon samples, respectively.

The cooking of shrimps resulted in significant ($p<0.05$) weight losses of 16.45±3.29% (whole shrimp, non-deveined) and 13.03±3.40% (shrimp tail, non-deveined) (Fig. 1).

Discussion

Nutritional profile of nigiri sushi meal

Nigiri-sushi is served in restaurants in pairs and consists of piece of raw fish (seafood) on top of vinegared balled rice (Celeste and Schultz, 2007). It is obvious that nigiri sushi nutritional profile hardly depends on its ingredients ratio. Greater participations of seafood in nigiri sushi means more proteins and more fat (depends on seafood species) but less saccharides. Greater seafood portion in sushi meal can have health beneficial due to facts that seafood represents good source of lean proteins, amino acids, polyunsaturated fatty acids, certain vitamins (especially vitamin D) and iodine (Farrugia *et al.*, 2015). Salmon and shrimps are good sources of polyunsaturated omega-3 fatty acids and 170 g/week of salmon and shrimp can provide 350 mg/day and 35 mg/day of docosahexaenoic acids (DHA), respectively (Oken *et al.*, 2012). Shrimp used for nigiri shrimp preparation was cooked and cooking losses are the reason for the lowest seafood portion in this type of nigiri sushi. Weight losses in our experiment (Fig. 1) are lower in comparison with the results of Carneiro *et al.* (2013), where they observed weight losses of 38.5±0.9% in cooked deveined shrimps, but compared with the results of Erdogdu *et al.*, (2004), where shrimp samples were deveined before cooking, cooking losses (17%) were closer to our results. Weight losses after cooking are influenced by shrimp size and gender due to the fact that female shrimps are bigger in comparison with males (Bono *et al.*, 2012). It is easier for released tissue water to reach surface after protein degradation in smaller shrimps (Erdogdu *et al.*, 2004).

Studies including sushi ingredients' ratio are very rare but results of Adams *et al.* (1990) corresponds with our results, where they found that average salmon slice (in nigiri sushi piece) weighed 11.1 g (range 4.9 g to 25.2 g). Though, among our samples weights of salmon slices did not oscillate in that extant. It also assumed that out of one salmon fish can be sliced 1000 pieces for sushi, if it is taken into consideration that average salmon fish weigh of 2.27 kg.

If it is assumed that average nigiri sushi meal consists of 8 sushi pieces it can be concluded that nigiri salmon and nigiri tuna meal contain more than 100 g of seafood and recommended weekly intake between 150- 300 g (EFSA, 2015). Seafood content in nigiri shrimp meal (8 pieces) is over 40 g, which could be also reasonable source of seafood, considering the fact that daily seafood consumption of 35 g can also lead to decrease of cardiovascular mortality due to bioactive components found in seafood (Hosomi *et al.*, 2012).

Proteins consumed out of seafood sources are considered health beneficial and better choice than red meat and processed red meat (Yu *et al.,* 2015), due to excellent amino acid composition and high digestibility (Hosomi *et al.,* 2012). Bioaccessibility of fat and proteins are higher when raw salmon meat is consumed than the grilled one, which can be contemplated as advantage of sushi meal due to participation of raw seafood in this product (Costa *et al.,* 2015). Protein contents in nigiri salmon and nigiri tuna meals can satisfy almost over 50% of daily protein intake requirements for adults (60 g) (Hosomi *et al.,* 2012).

The main component of nigiri sushi is rice which has a medium glycaemic response. On the other way, seafood portion in nigiri sushi, as the valuable source of protein, can lower glycaemic response of rice and consequently makes glycaemic index of the whole nigiri sushi meal more acceptable (George *et al.,* 2014). Sriket *et al.* (2007) found closely related protein content in shrimp (*Penaeus vannamei*) samples (188 g/kg) to ours.

Fat content of nigiri sushi meal is in correlations with rice/seafood content. Fat of rice is almost negligible (Abbas *et al.*, 2011), while the highest fat content of nigiri salmon is explained with the fact that salmon belongs to fish species with higher fat content (Du *et al.,* 2012). Rice used for sushi preparation is always short grain white Japanese or Californian rice. Other varieties of rice are not suitable for sushi preparation due to their size, consistency, taste and smell (Ryuichi *et al.*, 2006).

Sushi meal is characterized by literature data as very-low caloric meal because the whole meal contains less than 1600 kJ (Vaccariell and Hermann, 2010). The calculated energy value in our samples for one meal (if it is considered that one meal consists of 8 nigiri pieces) is slightly over this data, but taking into the account that the average daily requirements is around 10 000 kJ (Walker, 2006) energy value of examined nigiri sushi samples can be still declared as low.

Out of sushi consumers' preferences surveys can be seen that sushi meal is considered tasty, digestible, aromatic, attractive, healthy and also more than 30% of respondents consume it for special occasions (Czarniecka-Skubina *et al.*, 2014; Dordevic and Buchtova, 2014). Compared with sushi meal, the meal consumed during special occasions contains more than 6000 kJ (Angus, 1997).

Phosphorus is the main mineral for bone and teeth mineralization in mammals and birds (Helland *et al.*, 2005). Phosphorus contents in nigiri sushi samples were much lower than the average content estimation in fish meat (200 mg/100 g) (EFSA, 2005) due to rice dominance in nigiri sushi samples. Average portion of 8 pieces of nigiri sushi contains Phosphorus well below the upper intake level (3000 mg/day) of this mineral (EFSA, 2005).

Physico-chemical characterictics and freshness estimation of nigiri sushi meal

Our samples had higher pH values than recommended value for this type of food (pH=4.2), which is thought to be an appropriate for bacterial growth inhibition (Farber and Todd, 2000).

Sushi should be consumed within 12 hours if it is held on the temperatures below 15ºC, and if pH of rice is under 4.8. If the pH of cooked sushi rice is higher than 4.8, prepared sushi should be consumed within 4 hours and after this period should be discarded (Barralet *et al.*, 2004). The rice used for nigiri sushi preparation should have pH less than 4.4, which is assumed as critical control point (Novak *et al.*, 2003). The pH of rice less than 4.6 is advisable for properly acidified sushi rice, and in that way is accomplished the main function of vinegered rice in sushi meal to lower its pH and made barrier for microorganisms (Muscolino *et al.*, 2014). The samples of seafood did not reach to higher pH values (>8), like in the study of Muscolino *et al.* (2014). The pH of fresh fish is around 6.6 (mainly due to the presence of trimethylamine oxide), and fish meat represents good buffer due to its components. During storage period, trimethylamine oxide is reduced to thrimethylamine and pH slightly drops. After disappearance of lactic acid, ammonium is formed and pH starts to rise reaching values over 8, which is characteristic for rotten or putrid fish (Paine and Paine, 1992). Water activity measured in nigiri sushi samples is indicating favourable conditions for Listeria monocytogenes growth, which occurrence is expected due to seafood partition in nigiri sushi meal (Gambarin *et al.*, 2012).

Nigiri sushi meal cannot be declared low salt content food because according to our results salt content was over 2 grams in 8 pieces of nigiri sushi, while adults' daily dietary salt needs is 1.5 g (EFSA, 2005). Higher salt consumption which consequently leads to higher sodium intake leads to higher prevalence of chronic diseases (hypertension, bone diseases, stomach cancer, kidney diseases) (Kim and Lee, 2014).

The freshness of nigiri sushi was characterized by the usage of two parameters: TBA (indicating the level of fish muscle deterioration during storage and the formation of secondary oxidation products such as malondialdehyde) (Su *et al.*, 2014) and citrate synthase activity (representing the amount of mitochondrial enzymes in seafood exudates, released during meat freezing) (Simoniova *et al.*, 2013). Our results are far below 5 mg/kg malondialdehyde limit which is considered as maximum allowed TBA content for fish of good quality (Hu *et al.*, 2008). TBA content in nigiri salmon samples is slightly higher in comparison with results of Papastergiadis *et al.* (2014) where TBA content in fresh salmon was 1.029 mg/kg, while salmon used in the examined nigiri salmon samples was not fresh (proved by CSA determination). If it is assumed that the weight of average nigiri sushi meal is less than 350 g, it could be stated that malondialdehyde content in our samples are much lower than upper intake limit of MDA (30 μg/kg body weight per day) (EFSA, 2012).

Zero values of CSA were measured in boiled shrimp meat (used for nigiri shrimp preparation) because during the heat treatment of shrimps, the thermolabile citrate synthase enzyme was completely inactivated. CSA amounts in salmon and tuna exudates are in accordance with Regulation (EC) No 853/2004 (in the section VIII Fishery products, D. Requirements concerning parasites) requires that raw fishery products or finished products (e.g. sushi), intended to be consumed raw, have to be frozen. However, regulation permits, in substantiated cases, the use of fresh meat or meat that has not been frozen, but has undergone sufficient heat treatment.

The amount of citrate synthase depends on red/white muscles proportion in meat and it was found that transgenic Atlantic salmon contains more aerobic enzymes (such as citrate synthase) in red muscles than in white muscles (Overturf, 2009). Simoniova *et al*. (2013) also found higher CSA in chicken thighs than in chicken breasts after 4 freezing/thawing cycles, which could be explained by the fact that chicken breasts are predominantly composed of white muscles (Barbut, 2002). Tuna and salmon species belong to active fish and they possess more red muscles tissues in comparison with sedentary species (Tyus, 2012).

The results obtained by experiment which included 4 freezing/thawing cycles of tuna and salmon samples are underlying that CSA measurement is a reliable indicator of fish freshness due to constant CSA growth in fish samples after each freezing/thawing cycle. Comparing results obtained by freezing/thawing cycles experiment with measured CSA in fish samples used for nigiri sushi preparation can be observed that salmon used for nigiri sushi preparation were frozen at least one time, while the same observation is not possible to confirm unambiguously for tuna meat used for nigiri tuna preparation. Fresh or not more than one time frozen tuna fillets were used for nigiri tuna preparation.

Sushi meal is starting to be consumed worldwide even in countries where it hasn't been present recently. Sushi restaurants are opening in many continental countries where the seafood consumption is very low per capita, and the consumption of nigiri sushi meal can support higher seafood consumption in those countries. Nutritional profile of nigiri sushi meal is highly depends on rice/seafood ratio. Among sushi types, nigiri sushi contains the highest portion of seafood and in examined samples seafood portions were over 38%. Out of nutritional evaluation of nigiri sushi meal it can be stated that nigiri sushi meal is low calorie and fat meal, while it contains higher amounts of salt and refined grains. Freshness of sushi meal is always questioned due to the inclusion of raw seafood. The enzymatic method, citrate synthase activity kit, showed reliable response to freezing/thawing cycle rounds, emphasizing the usage of this method for determination of seafood freshness and therefore freshness of fish slices used for nigiri sushi preparation.

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