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## CONTENT



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# Comparative analysis of meat chemical composition of different broiler provenances

*Natasa Glamoclija[1], Marija Starcevic[1], Jelena Ivanovic[1], Jelena Janjic[1], Marija Boskovic[1], Jasna Djordjevic[1], Radmila Markovic[1], Milan Z. Baltic[1]*

*A b s t r a c t: The objective of this study was to determine the effect of breed line and age on carcass chemical composition in broiler chickens. Chemical composition of broiler breast meat and drumstick with thigh were compared within3 lines (Cobb, Ross, and Hubbard). Each line was divided into two groups, aged 42 and 50 days. Chemical composition of meat was determined 48 hours after slaughter and was very variable depending on the breed and age of the broilers. The most significant differences were observed in fat content. Breast meat and meat from drumstick with thigh of younger broilers (42 days old) had significantly lower fat content than older broilers (50 days old). Cobb broilers, 42 days old, had significantly lower percentages of fat ($p<0.01$) in breast meat and meat from drumsticks with thighs than other broiler lines of the same age, while 50 day old Hubbard broilers had lower fat levels in breast meat and meat from drumsticks with thighs than meat from the other two broiler provenances (Cobb or Ross) of the same age. Hubbard broilers (42 days old) had significantly lower ($p<0.01$) levels of water and protein in breast meat and meat from drumsticks with thighs compared to the other broiler lines. Interactions between breed line and age were found for fat percentage in breast meat and meat from drumsticks with thighs. Meat quality of broilers of different provenances can be estimated by determining the chemical composition of breast meat and meat from drumsticks with thighs.*

***Keywords:*** *Cobb, Ross, Hubbard, chemical composition, breast meat, drumstick with thigh.*

## Introduction

Poultry meat production has doubled in the past 40 years in the world, has a trend of constant growth, and its production volume now exceeds beef, but is less than pork. The biggest producers of poultry meat are Asia, North and South America and Europe. The most significant category of poultry meat is that of young chicken (broilers) (*Glamočlija et al*, 2013a).

Broiler meat is a highly valuable food with evident nutritive and biological properties. It contains high biological value proteins with essential amino acids, fat/fatty acids, vitamins and minerals (*Glamoclija et al*, 2015). According to the United States Department of Agriculture (*USDA*, 2006), the average chemical composition of broiler meat is 74.6% water, 12.1% protein, 11.1% fat, 1.2% carbohydrate and 1% minerals.

The quality of broiler meat can be assessed to determine the suitability of meat for processing, storage and sale (*SRPS EN ISO*, 2012). Among others, some of the main attributes which define the quality of broiler meat, and other types of meats as well, are chemical characteristics such as content of water, protein, fat and ash. However, many factors can affect the chemical composition of broiler meat. Some of these factors are age and sex of broilers, diet and fattening, breed line – genetics, breeding processes and muscle type (red and white muscles) (*Ristic et al*., 2008, *Krischek et al*., 2011). Literature data indicate different broiler meats can contain relatively constant amounts of water, protein and ash, while fat content is very variable (*Ristic et al*., 2007).

Improvements in genetics and diet, plus many other factors, have enabled six week old broilers to now reach weights of nearly three kilograms, whereas 50 years ago, achievement of this weight took 16 weeks (*Glamočlija et al*, 2013b). Nowadays, the most common broiler breeding groups are Cobb-Vantress (with the Cobb500, Cobb700, CobbAvian48 and CobbSasso brands), Aviagen (with the Ross, Arbor Acres, Indian River, Rowan Range and Specialty Males brands), and Group Grimaud (with the Hubbard and Grimaud Frere brands) (*Elfick*, 2012). Cobb, Ross and Hubbard broiler provenances are the most common in Serbia (*Glamočlija et al*, 2013b). Other factors – age, sex, housing conditions, and

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post-mortem factors such as processing of carcasses and method of chilling –influence carcass yields (*Bilgili*, 2002, *Bihan-Duval et al*, 1999). Slaughter age and weight of, for example, Cobb500 broilers depend on the requirements of the consumer population, and can vary between countries. The average weight is from 1.70 kg (33 days old, Germany) to 2.92 kg (51 days old, Japan), where the meat yield is above 70 %. (*Anonym*, 2012a). At the most common age for slaughter – 42 days, Cobb 500 have an average weight before slaughter of 2.73 kg, Ross 308 – 2.65 kg and Hubbard Classic – 2.59 kg (*Anonym*, 2012a, b, c, ).

## Materials and Methods

The study was conducted on 36 commercial market broilers (100 Cobb 500, 100 Ross 308 and 100 Hubbard Classic) which were divided into six groups. Each line was divided into two groups of broilers, which were 42 and 50 days old, respectively. Broilers were between 2000 and 2600 g live weight. The broilers were fed according to a breeder feeding program based on recommended nutrient levels. Broilers were slaughtered in a registered slaughterhouse.

Determination of chemical composition (water, protein, fat and ash) was carried out on breast meat and meat from drumsticks with thighs from each group. The chemical composition was determined using standard methods (*SRPS ISO,* 1998; *SRPS ISO,* 1992; *SRPS ISO,* 1992; *SRPS ISO,* 1999).

Statistical analysis of the results was conducted using the software GraphPad Prism Version 5.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Mean values were calculated and the groups were compared with one-way ANOVA with Tukey's multiple comparison statistical test. Two-way ANOVA with Bonferroni post hoc test was performed to test the effect of broilers line (Cobb, Ross and Hubbard) and age (42 and 50 days) as main effects, and their interaction on the chemical composition values. Values of $p<0.05$, $p<0.01$ and $p<0.001$ were considered significant. The results are expressed as mean ± SD.

## Results and Discussion

Results of the chemical composition of meat (breast and drumstick with thigh) of three different broiler lines are shown in Tables 1 and 2.

The chemical composition of broiler meat reported in the literature is very variable and depends on many factors. According to *Van Heerden et al*. (2002), breast meat contained on average 74.01% water, 23.29% protein, 2.91% fat, and 1.11% ash (Ross 308 and Cobb broilers), while meat from drumsticks with thighs contained 72.47% water, 19.16% protein, 8.91% fat, and 1.0% ash. *Wattanachant et al*. (2004) found that breast meat (CP707 broilers) contained an average of 74.87% water, 20.59% protein, 0.68% fat and 1.10% ash and meat from drumsticks with thighs was 77.22% water, 19.08% protein, 0.81% fat and 1.06% ash. According to *Lonergan et al*. (2003), breast meat (commercial broilers) contained on average 73.42% water, 24.02% protein and 1.08% fat. *Baltic et al*, 2015 found that commercial broiler breast meat contained 78.10±0.13% water, 16.33±0.51% protein, 1.92±0.20% fat and 3.65±0.07% ash. Djordjevic (2005) found that breast meat of broiler hybrid Hybro G contained on average 73.81% water, 24.17% protein, 0.94% fat and 1.22% ash, while meat from drumsticks with thighs contained 72.35% water, 17.50% protein, 9.24% fat and 1.05% ash. According to *Ristic* (2007), the average chemical composition of broiler breast meat

**Table 1.** The effect of breed line and age on chemical composition of breast meat (*Pectoralis major*) of broiler chickens (n=6)

| Parameter (%) | 42 days old | | | 50 days old | | | L | A | L × A |
|---|---|---|---|---|---|---|---|---|---|
| | Cobb (n=6) | Ross (n=6) | Hubbard (n=6) | Cobb (n=6) | Ross (n=6) | Hubbard (n=6) | P value | | |
| Water | 72.99±0.34 | 73.23±0.14 | 73.12±0.16 | $73.50^{a}$±0.45 | $73.01^{a}$±0.17 | 73.32±0.34 | ns | ns | * |
| Protein | $25.44^{a,b}$±0.31 | $25.01^{a}$ ±0.13 | $24.94^{b}$±0.18 | $24.57^{a}$±0.42 | $24.96^{a}$±0.13 | 24.87±0.40 | ns | ** | ** |
| Fat | $0.54^{A,B}$±0.07 | $0.76^{A,a}$±0.04 | $0.91^{B,a}$±0.10 | 0.90±0.07 | $1.01^{C}$±0.14 | $0.79^{C}$±0.13 | *** | *** | *** |
| Ash | 1.03±0.02 | 1.02±0.03 | 1.03±0.03 | 1.04±0.03 | 1.03±0.03 | 1.02±0.04 | ns | ns | ns |

**Legend:** Means within a row with a common superscript letter differ significantly: $^{a,b}$ – ($p<0.05$) and $^{A, B, C}$ – ($p<0.01$); ns – not significant ($p>0.05$); * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$; L – line factor; A – age factor; L × A – Interaction between line and age factor

**Table 2.** The effect of breed line and age on chemical composition of meat from drumsticks with thighs of broiler chickens (n=6)

| Parameter (%) | 42 days old | | | 50 days old | | | L | A | L × A |
|---|---|---|---|---|---|---|---|---|---|
| | Cobb (n=6) | Ross (n=6) | Hubbard (n=6) | Cobb (n=6) | Ross (n=6) | Hubbard (n=6) | P value | | |
| Water | 74.32A±0.69 | 74.44B ±0.21 | 73.28$^{A,B}$±0.64 | 74.49$^{±}$0.74 | 73.29$^{±}$0.50 | 74.26±0.26 | * | * | ns |
| Protein | 19.85$^{A±0}$.14 | 19.78$^{B}$±0.27 | 19.09$^{A,B}$±0.37 | 19.34$^{±}$0.31 | 19.07$^{C}$±0.28 | 19.72C±0.38 | ns | ns | *** |
| Fat | 4.46$^{B±0}$.28 | 4.65$^{C±0}$.22 | 6.77$^{B,C}$±0.37 | 5.15A±0.63 | 6.62$^{A,D}$±0.62 | 5.01$^{D}$±0.22 | *** | * | *** |
| Ash | 1.04±0.04 | 1.03±0.03 | 1.03±0.05 | 1.03±0.03 | 1.03±0.03 | 1.02±0.02 | ns | ns | ns |

**Legend:** Means within a row with a common superscript letter differ significantly: $^{A, B, C, D}$ – ($p<0.01$); ns – not significant ($p>0.05$); * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$; L – line factor; A – age factor; L × A – Interaction between line and age factor

(Ross and Cobb hybrids) was 74.9±0.7% water, 23.6±0.7% protein, 0.6±0.38% fat and 1.2±0.1% ash, and of meat from drumsticks with thighs was 75.4±1.1% water, 19.6±0.9% protein, 3.88±1.33% of fat and 1.1±0.1% ash. *Castellini et al*. (2002) found the breast meat of 56 day old Ross broilers contained 75.54% water, 22.39% protein, 1.46% fat and 0.61% ash.

In all cases, breast meat was different to meat from drumstick with thigh, especially with respect to the fat content. Meat from drumstick with thigh has a higher fat content than breast meat. However, the protein content was consistently higher in breast meat compared to meat from drumstick with thigh. Additionally, variation was observed in chemical composition (water, protein and fat) in breast meat from different broiler provenances; this was also the case with meat from drumsticks with thighs.

Results of chemical analysis of breast and drumsticks with thigh meat from the examined groups of broilers in this study were in agreement with the results of other authors. Our results for breast meat and meat from drumsticks with thighs from three different broiler lines and two different ages, showed significant differences in the fat content. Finally, fat content certainly depends on both broiler line and age, but also depends on interactions of these factors. Cobb broilers at 42 days old contained a significantly lower percentage of fat ($p<0.01$) in both breast meat and in meat from drumsticks with thighs than Cobb broilers at 50 days old. Fat content was the highest in 42 day old Hubbard broilers. Meat from drumsticks with thighs derived from Hubbard broilers (42 days old) had significantly lower ($p<0.01$) amounts of water and protein compared to other broiler provenances. In contrast, older Hubbard broilers (50 days) had lower percentages of fat in breast meat and meat from drumsticks with thighs than the other two broiler provenances (Cobb and Ross) of the same age.

## Conclusion

Beside other factors, genetics, age, diet and breeding have a significant impact on broiler carcass quality parameters, such as chemical composition of breast meat and meat from drumsticks with thighs. Fat content was the most variable meat quality indicator measured, and depended on broiler line and age and both of these factors together. Cobb broilers, 42 days old, had the lowest percentage of fat in breast meat and meat from drumsticks with thighs, compared to meat from the other broiler lines of the same age. Hubbard broilers, 50 days old, had the lowest percentage of fat in breast meat and meat from drumsticks with thighs than the other two broiler provenances (Cobb and Ross) of the same age. Younger broilers (42 days old) always had lower fat levels in breast meat and meat from drumsticks with thighs than older broilers (50 days old).

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## References

**Anonym, (2012a).** www.cobbvantress.com/Products/Product-Profile/Cobb_50

**Anonym, (2012b).** http://en.aviagen.com/

**Anonym, (2012c).** http://www.hubbardbreeders.com/products.php?id=7

**Baltic T., Baltic Z. M., Misic D., Ivanovic J., Janjic J., Boskovic M. & Dokmanovic, M. (2015).** Influence of marination on *Salmonella* spp. growth in broiler breast fillets, *Acta Veterinaria, 65, 3*, 417–428.

**Bihan-Duval E., Milet N. & Remignon, H. (1999).** Broiler meat quality: Effect of selection for increased carcass quality and estimates of genetic parameters. *Poultry Science, 78*, 822–826.

**Bilgili S. F. (2002).** Poultry meat processing and marketing – what does the future hold? *Poultry International, 10, 41*, 12–22.

**Castellini C., Mugnai C. & Dal Bosco, A. (2002).** Effect of organic production system on broiler carcass and meat quality. *Meat Science, 60,* (3), 219–225.

**Djordjevic M. (2005).** *Uticaj supstitucije ribljeg brašna dehidrovanim brašnom larvi domaće muve (Musca domestica L.) na proizvodne rezultate i kvalitet mesa brojlera, doktorska disertacija*, Fakultet veterinarske medicine, Univerzitet u Beogradu.

**Elfick D. (2012).** A brief history of broiler selection: How chicken became a global food phenomenon in 50 years. *Aviagen International*, 1–7.

**Glamoclija N., Drljacic A., Mirilovic M., Markovic R., Ivanovic J., Loncina J., Baltic, Z. M. (2013a).** Analysis of poultry meat production volume in Serbia from 1984. to 2009. *Veterinarski glasnik, 67*, 3–4, 269–278.

**Glamoclija N., Dokmanovic M., Ivanovic J., Markovic R., Loncina J., Boskovic M. & Baltic, Z. M. (2013b).** *The effect of different broiler provenances on carcass meatiness, International* 57th Meat Industry Conference, Belgrade, June 10th–12th, 2013, 169–172.

**Glamoclija N., Starcevic M., Janjic J., Ivanovic J., Boskovic M., Djordjevic J., Markovic R. & Baltic, M. Z. (2015).** The effect of breed line and age on measurements of pH-value as meat quality parameter in breast muscles (m. Pectoralis Major) of broiler chickens. *Procedia Food Science, 5*, 89–92.

**Krischek C., Janisch S., Gunther R. & Wicke, M. (2011).** Nutrient composition in broiler and turkey breast meat in relation to age, gender and genetic line of the animals. *Journal of Food Safety and Food Quality, 3* (62), 73–104.

**Lonergan, S. M., Deeb N., Fedler C. A. & Lamont, S. J. (2003).** Breast meat quality and composition in unique chicken populations, *Poultry Science 82,* (12) 1990–1994.

**Ristic M., Freudenreich P., Damme, K. (2008).** Hemijski sastav živinskog mesa – poređenje brojlera, kokoši, ćuraka, pataka i gusaka. *Tehnologija mesa 49,* (3–4), 94–99.

**Ristic M., P. Freudenreich, Renate Werner, Gabriele Schüssler, Ute Köstner & S. Ehrhardt. (2007).** Hemijski sastav mesa brojlera u zavisnosti od porekla i godine proizvodnje. *Tehnologija mesa 48,* (5–6), 203–207.

**SRPS EN ISO 5492:2012.** Sensory analysis – Institute for Standardization of Serbia.

**SRPS EN ISO 936/1999.** Meat and meat products – Determination of total ash (reference method). Institute for Standardization of Serbia**.**

**SRPS EN ISO 1442:1998.** Meat and meat products – determination of moisture content (reference method). Institute for Standardization of Serbia.

**SRPS EN ISO 937/1992.** Meat and meat products determination of nitrogen content (reference method). Institute for Standardization of Serbia**.**

**SRPS EN ISO 1443/1992.** Meat and meat products – determination of total fat content (reference method). Institute for Standardization of Serbia

**USDA. (2006).** National Nutrient Database for Standard Reference, release 19. http//www.nalusde.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl.

**Van Heerden S. M., Schonfeldt H. C., Smith M. F. ^ Jansen van Rensburg D. M. (2002).** Nutrient content of South African chickens, *Journal of Food Composition and Analysis* (15), 47–64.

**Wattanachant S., Benjakul S. & Ledward D.A. (2004).** Composition, color and texture of Thai indigenous and broiler chicken muscles, *Poultry Science, 83*, (1), 123–128.

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# Effect of modified atmosphere on sensory, chemical and physico-chemical properties of Serbian traditional smoked meat products

*Zoran Petrovic[1], Ivana Brankovic-Lazic[1], Mladen Raseta[1], Dejana Trbovic[1], Nenad Parunovic[1], Aleksandra Nikolic[2], Boris Mrdovic[1]*

*A b s t r a c t: The products included in this study were beef prosciutto and pork prosciutto, common traditional Serbian dry-cured and smoked meat products. The products were packaged with a gas mixture containing 30% vol carbon dioxide and 70 % vol nitrogen (excluded oxygen in packs was below the limit of detection) and stored between 4°C and 7°C. Packaging units were formed from bottom foil based on APET (amorphous polyester) with a sealing layer of polyethylene and top foil composite film containing oriented polyester and a peelable polyethylene-sealant with a barrier layer. A total of 120 packages were examined (60 packages/product) during the storage period of 180 days. The measured concentration of oxygen in packages during the whole storage period was less than 0.1%. A decline in the carbon dioxide concentration at the end of the storage period was registered in packages of pork prosciutto (to 10% vol). Based on the results obtained, both of the products maintained acceptable sensory and chemical qualities during the storage period, meaning they were suitable for consumption.*

***Keywords:*** *traditional food, MAP packaging, sensoric quality.*

## Introduction

The definition of traditional food products may not necessarily reflect opinions of consumers. According to research conducted by *Vanhonacker et al*. (2008), European consumers include well-known food and food they have eaten already or which their ancestors consumed among traditional products.

Despite technological progress and the obligation to comply with modern food safety regulations, production and/or processing must still be consistent with the methods that were originally used, and the food products obtained must preserve the intrinsic features (physical, chemical, and microbiological) (*Grujic et al.*, 2012). Meat production systems using traditional recipes use modern industrial equipment and adapt some processing steps in order to ensure quality and specific characteristics of traditional product.

In Serbia, traditional smoked meat products constitute a diverse group of food products. Originating from distinct Serbian geographic regions, they bear characteristic sensorial properties typical in high-quality meat products.

These products were made of the most valuable parts of beef and pork carcasses (round muscles, loin muscles and tenderloin) (*Tomic et al.,* 2008). The majority of traditionally processed meat products in Serbia are made on the basis of personal knowledge and rarely upon counselling with an expert technologist. Packaging and marketing are almost completely neglected. Modern trends in the processed meat market show that consumers tend to satisfy their needs for food with flavours which combine the tradition and culture of the area they come from, without concern for the price.

Developments in packaging materials and technologies have made the application of modified atmosphere packaging (MAP) feasible on a larger scale to meat and meat products (*Brody*, 1989). For most food products, preservation is a vital function of packaging which ensures that the product is sold fresh (*Helström and Saghir*, 2006). By using MAP with one or more gases, atmospheric conditions can be designed to maximise product life and develop desired product characteristics (*Viana et al.,* 2005). The main purposes of MAP of meat products are to ensure the microbiological shelf life and the sensory

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quality of the product, including the colour, door and palatability.

The aim of the present work was to study the effect of modified atmosphere (30% $CO_2$ /70% $N_2$) on the chemical, physico-chemical and sensory quality of two traditional products, beef prosciutto and pork prosciutto, stored for 180 days between 4°C and 7°C, after slicing and packaging in retail packs.

## Materials and Methods

### *Preparation of meat products*

The products included in this study were: beef prosciutto – traditionally cured, smoked and dried beef sirloin and pork prosciutto – traditionally cured, smoked and dried pork sirloin. Basically, both of the products were made in the common, traditional way, widely established in Serbia. The products were manufactured by and taken directly from respective industrial meat processing facilities who have been adapting their meat processing and packaging operations, combining traditional (curing and smoking) and modern (slicing and packaging) steps.

### *Packaging machine and materials*

For analysis in this study, packaging units containing very thin slices of beef or pork prosciutto were formed using an industrial packaging machine MULTIVAC R 230 (Wolfertschwenden, Germany). Net weight of packaged products was 80g. Individual packs contained 100 ml of gas (30% $CO_2$:70% $N_2$) per 100g of product packaged. Packaging units were formed from bottom foil (declared thickness 300 microns) based on APET (amorphous polyester) with a sealing layer of polyethylene and top foil composite film (declared thickness 80 microns) containing oriented polyester and a peelable polyethylene sealant with a barrier layer. Manufacturer-declared oxygen-permeability measurements (23°C/35% r.F; DIN 53380) of bottom foil and top covering foil were: ≤14 bar $cm^3$ $m^{-2}$ $day^{-1}$ and ≤4.2 bar $cm^3$ $m^{-2}$ $day^{-1}$, respectively. Declared water-vapour permeability (23°C/85% r.F; DIN 53122) of bottom and top foils were: ≤2.3 g $m^{-2}$ $day^{-1}$ and ≤1.5 g $m^{-2}$ $day^{-1}$, respectively.

### *Storage and sampling of packaged products*

A total of 120 packages were examined (60 packages/product). Packages were stored between 4°C and 7°C. Sensory and chemical evaluation of packaged products was performed on the second day of packaging and after 2, 35, 60, 90, 105, 135, 150, 165 and 180 days of storage.

### *Gas analyses in package headspace*

The concentration of gases ($O_2$ and $CO_2$) in the headspace atmosphere during storage was measured using a WITT Oxybaby V® analyser (WITT, Gasetechnik GmbH & Co. KG, Witten, Germany).

### *Sensory analyses*

Sensory evaluation was performed by a panel of five experienced members using a point system of descriptive analytical tests according to *SRPS* (2013). Surface colour of slices, taste, odour and consistency were evaluated using a point system with a scale of 1 to 5, where optimal single characteristics were scored as 5 (1 – unacceptable; 2 – ultimate acceptable limit; 3 – acceptable; 4 – very acceptable; 5 – exceptionally acceptable).

### *Chemical analyses*

Lipid oxidation was measured in duplicate from each of the six packs for each treatment in each trial during the storage period. Peroxide value (PV) was determined by standard method (*SRPS,* 2011), and thiobarbituric acid reactive substances (TBARS) according to *Tarladgis et al.* (1964) and *Holland* (1971). The pH of meat was measured by laboratory pH-meter (EUTECH Instruments, Landsmeer, Holland), according to standard method (*SRPS,* 2004), and the water activity ($a_w$) was measured with a hygrometer (GBX Scientific Instruments, Dublin, Ireland) according to standard method (*ISO,* 2004).

### *Statistics*

Statistical analysis was performed using the MINITAB INC. software package, version 16.0. Before choosing the appropriate statistical analysis, individual distribution identification was conducted to identify the native distribution (Normal, Lognormal, Weibull, Exponential), as the first step. Box-Cox and Johnson transformation of raw data were used to follow a normal distribution. The One Way ANOVA and the post-hoc HSD Tukey's test were used to examine statistical differences of chemical and physico-chemical parameters during storage periods. The differences were considered statistically significant when $p \leq 0.05$. Mean values and standard deviations were used to illustrate both a measure of central tendency and variability of the data.

## Results and Discussion

Decreases in $CO_2$ concentration in the packaged beef prosciutto were from the initial 27.0% to 19.0% at the end of the storage period. Pork prosciutto packages showed $CO_2$ decreases from initial 30.0% to 9.3% during the study period. The average percentage of $CO_2$ in gas from the beef prosciutto and pork prosciutto packages during the study period was 21.63% and 17.33%, respectively. The larger decline in the concentration of $CO_2$ in MAP packaged pork prosciutto (Figure 2) can be attributed to a slightly higher moisture content in comparison to beef prosciutto (Figure 1), which allowed $CO_2$ to dissolve into the meat's liquid phase (*Quintavalla and Vicini*, 2002). The absorption of $CO_2$ is highly dependent on the moisture and fat content of the product, initial $CO_2$ concentration in the gas-phase and the gas/product ratio (*Rubio et al.*, 2007)

Chemical and physico-chemical parameters (TBARS, PV, pH and $a_w$) of beef prosciutto and pork prosciutto packaged in MAP are presented in Table 1.

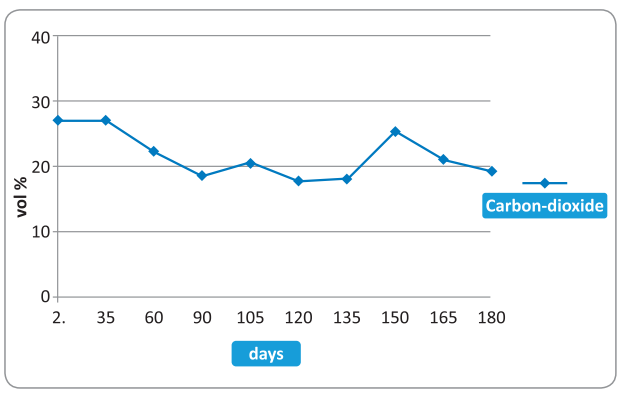


**Figure 1.** Changes in $CO_2$ concentration in the MAP packaging of beef prosciutto during 180 days of storage

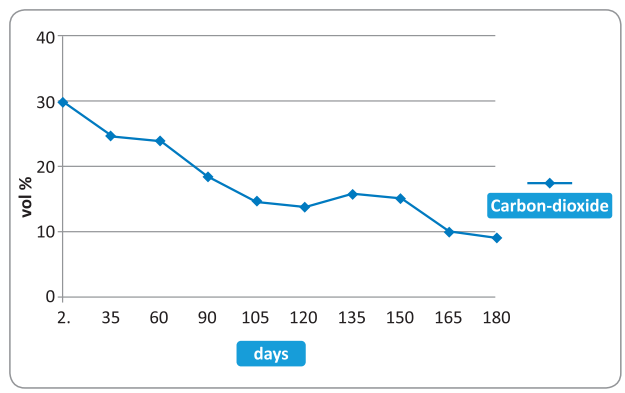


**Figure 2.** Changes in $CO_2$ concentration in the MAP packaging of pork prosciutto during 180 days of storage

**Table 1.** Chemical and physico-chemical parameters of two traditional meat products packaged in MAP during 180 days of storage

| | Beef prosciutto | | | | Pork prosciutto | | | |
|---|---|---|---|---|---|---|---|---|
| Days | TBARS[1] (mg MAL kg$^{-1}$) | PV[2] (mmol kg$^{-1}$) | pH | $a_w$[3] | TBARS (mg MAL kg$^{-1}$) | PV (mmolkg$^{-1}$) | pH | $a_w$ |
| 1 | ≤ LOQ[4] | 0.22±0.02$^{a}$ | 5.79±0.02$^{a}$ | 0.913±0.006$^{a}$ | ≤ LOQ | ≤ LOQ | 5.32±0.02$^{a}$ | 0.874±0.002$^{a}$ |
| 35 | ≤ LOQ | 0.42±0.01$^{b}$ | 6.03±0.01$^{a}$ | 0.904±0.008$^{a}$ | 0.02±0.01$^{a}$ | 0.08±0.01$^{a}$ | 5.61±0.02$^{a}$ | 0.907±0.002$^{a}$ |
| 60 | 0.02±0.01$^{a}$ | 0.68±0.04$^{c}$ | 5.81±0.02$^{a}$ | 0.900±0.006$^{a}$ | 0.05±0.01$^{b}$ | 0.30±0.02$^{b}$ | 5.46±0.02$^{a}$ | 0.874±0.001$^{a}$ |
| 90 | 0.09±0.02$^{b}$ | 0.90±0.02$^{d}$ | 6.00±0.02$^{a}$ | 0.910±0.006$^{a}$ | 0.06±0.01$^{c}$ | 0.52±0.02$^{c}$ | 5.39±0.01$^{a}$ | 0.881±0.002$^{a}$ |
| 105 | 0.13±0.01$^{c}$ | 1.05±0.02$^{e}$ | 5.82±0.04$^{a}$ | 0.869±0.006$^{b}$ | 0.09±0.01$^{d}$ | 0.68±0.02$^{d}$ | 5.25±0.04$^{a}$ | 0.868±0.004$^{b}$ |
| 120 | 0.15±0.02$^{d}$ | 1.30±0.01$^{f}$ | 5.75±0.02$^{a}$ | 0.874±0.008$^{b}$ | 0.11±0.02$^{e}$ | 0.93±0.04$^{e}$ | 5.21±0.04$^{a}$ | 0.871±0.006$^{b}$ |
| 135 | 0.15±0.02$^{d}$ | 1.58±0.02$^{g}$ | 5.73±0.02$^{a}$ | 0.866±0.010$^{b}$ | 0.13±0.02$^{f}$ | 1.20±0.04$^{f}$ | 5.19±0.02$^{a}$ | 0.869±0.008$^{b}$ |
| 150 | 0.15±0.01$^{d}$ | 1.68±0.01$^{g}$ | 5.73±0.02$^{a}$ | 0.865±0.006$^{b}$ | 0.15±0.02$^{g}$ | 1.75±0.06$^{g}$ | 5.16±0.02$^{a}$ | 0.866±0.006$^{b}$ |
| 165 | 0.19±0.01$^{e}$ | 1.80±0.01$^{h}$ | 5.62±0.04$^{a}$ | 0.866±0.006$^{b}$ | 0.19±0.04$^{h}$ | 1.78±0.04$^{g}$ | 5.40±0.04$^{a}$ | 0.863±0.005$^{b}$ |
| 180 | 0.20±0.01$^{e}$ | 1.92±0.02$^{i}$ | 5.64±0.04$^{a}$ | 0.879±0.006$^{b}$ | 0.20±0.01$^{h}$ | 1.86±0.06$^{g}$ | 5.32±0.01$^{a}$ | 0.897±0.006$^{b}$ |

[1] TBARS – thiobarbituric acid reactive substances;
[2] PV – peroxide value;
[3] $a_w$ – water activiey;
[4] LOQ – limit of quantification of the method applied;
$^{a, b, c, d, e, f, g, h}$ within column values – mean±SD (standard deviation) – that do not share a letter are significantly different ($p \leq 0.05$).

The average pH and $a_w$ values obtained for beef prosciutto in MAP were 5.79, and 0.885, respectively. These results are similar to results obtained by *Garriga et al.* (2004) (pH 5.81; $a_w$ 0.890), during 120 days at 4°C. *Rubio et al.* (2007) found that pH value was 5.85, and $a_w$ value was 0.900 in a typical Spanish dry-cured beef product. There were no statistically significant differences in pH values during the storage period for the two products ($p>0.05$). Significant differences regarding TBARS and PV mean values were registered within the first half of the storage period (between 1, 35, 60 and 90 days) and also in relation to periods within 105-150, 165 and 180 days ($p\leq0.05$) for both of the products. TBARS and PV values differed significantly on all days within the first half of storage period for both of the products ($p\leq0.05$) (Table 1).

Statistically significant differences in measured $a_w$ values were registered ($p\leq0.05$) between results obtained in the first half of the storage period and on all other days until the end of the study (105,120,135,150,165 and 180 days). The $a_w$ values in the two products did not differ significantly within the first half of storage period ($p>0.05$). The water activity of meat products is that part of the water that is available for biochemical reactions and microbial growth. Keeping the $a_w$ value below 0.910 is a key factor in prolonging shelf life of meat products (*Karolyi*, 2004).

The average pH value (5.33) of pork prosciutto in MAP during the 180 day storage period was lower in comparison to Spanish dry-cured ham (pH 5.84; $a_w$ 0.880) reported by *Bover-Cid et al*. (2011) and also by *De Alba et al.* (2012) (pH 5.80; $a_w$ 0.900). The $a_w$ measured in pork prosciutto in the current study was 0.877 on average and this value is similar to those mentioned above. The lower pH the product has, the more effective microbial inactivation is reached. On the other hand, $a_w$ of the product plays an important quality trait due to its synergetic/antagonist role *(Hyperbaric*, 2014).

Determination of PV is one of the most important and commonly implemented quality control measurements for assessment of food quality and safety. The PV indicates the concentration of peroxides and hydroperoxides that are produced during the early stages of lipid oxidation (primary oxidation products) (*Ivanovic et al*., 2015). The PV of beef prosciutto gradually increased in the packs and ranged from 0.22 mmol $kg^{-1}$ (day 1) to 1.92 mmol $kg^{-1}$ (day 180). In pork prosciutto, the PV also gradually increased during the period of storage, and ranged from below the LOQ (limit of quantification of the method) (day 1) to 1.86 mmol $kg^{-1}$ (day 180) (Table 1). These PVs indicate slight formation of primary oxidation products in both of the products. However, results for the content of malonaldehyde (TBARS) indicated that there were no signs of secondary lipid oxidation (Table 1). TBARS values increased slightly during the whole study and they ranged from below the LOQ (day 1) to 0.20 mg $kg^{-1}$ at the end of storage period for both of the products. Values above about 0.5 are considered critical since they indicate a level of lipid oxidation of products which would produce a rancid odour and taste which can be detected by consumers (*Wood et al.*, 2008). The oxidation of unsaturated fats in high-fat products also results in unappetizing taste and smell. *Rhee and Ziprin* (1987) showed that lipid oxidation correlated with total pigment and myoglobin content of raw muscle.

In cooked meat products (e.g. cured ham), low residual levels of oxygen promote pigment denaturation which imposes a dull greyness to the meat surface (*Møller et al.*, 2000). Registered changes in surface colour of the two products examined in this

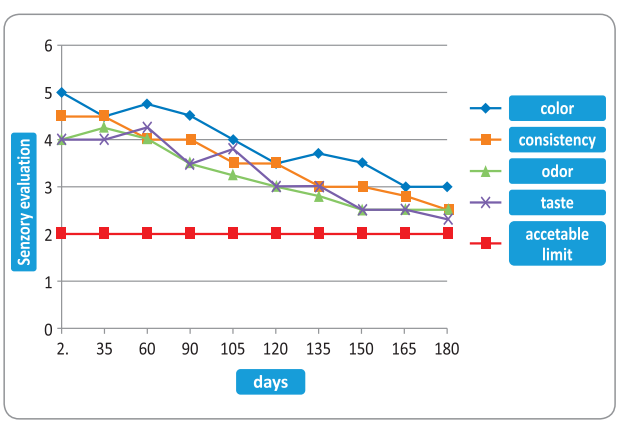


**Figure 3.** Sensory evaluation of beef prosciutto in MAP packaging during 180 days of storage

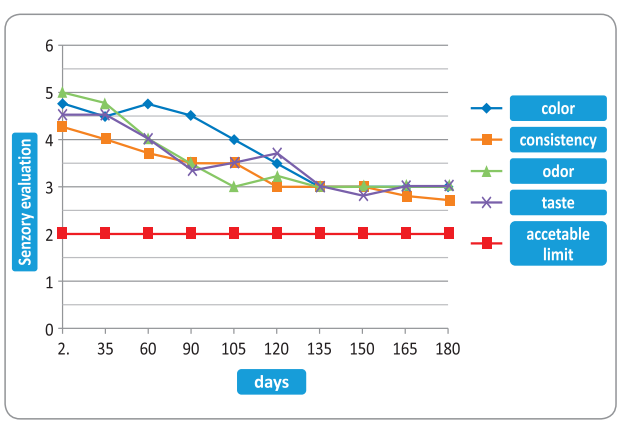


**Figure 4.** Sensory evaluation of pork prosciutto in MAP packaging during 180 days of storage

study were not particularly large, but they were detected towards the end of the storage period (Figures 3 and 4). Very low oxygen atmospheres minimise lipid oxidation and aerobic microorganism growth. Lipid oxidation in both products occurred at a slower comparative rate than discoloration and should not be the major determinant of shelf-life (*Kennedy et al.*, 2004).

During sensory evaluation, the ultimately acceptable level 2 was reached after 180 days storage for both of the products. The generally common limit of acceptability (3 in the 1 to 5 point scale), was reached after 135 days for beef prosciutto (Figure 3) and after 150 days for pork prosciutto (Figure 4).

## Conclusions

Based on the obtained results and discussion, it can be concluded that beef and pork prosciutto packaged in this material for MAP, with 30% $CO_2$ and 70% $N_2$, can be stored between 4°C and 7°C for up to 180 days. At the end of that storage period, both the meat products were still of acceptable sensory quality.

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## References

**Alba, D. M., Bravo, D. & Medina, M. (2012).** High pressure treatments on the inactivation of *Salmonella Enteritidis* and the physicochemical, rheological and color characteristics of sliced vacuum-packaged dry-cured ham. *Meat Science*, *91* (2), 173–8.

**Bover-Cid, S., Belletti, N., Garriga, M. & Aymerich, T. (2011).** Model for *Listeria monocytogenes* inactivation on dry-cured ham by high hydrostatic pressure processing. *Food Microbiology*, *28* (4), 804–809.

**Brody, A. L. (1989).** Modified atmosphere/vacuum packaging of meats. In Brody A. L. (Ed.), *Controlled/modified atmosphere/vacuum packaging of foods*, (pp. 17−38). Trumbull, CT, USA: Food and Nutrition Press.

**Garriga, M., Grebol, N., Aymerich, M. T., Monfort, J. M & Hugas, M. (2004)**. Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, *5* (4), 451–457.

**Grujic, R., Antonic, B. & Vukic, M. (2012)**. Traditional meat products in function of a market offer. *Proceedings of Third International Scientific Symposium, Agrosym*, pp. 472−477.

**Hellström, D., & Saghir, M. (2006).** Packaging and logistics interactions in retail supply chain. *Packaging Technology and Science*, 20 (3), 197–216. http://dx.doi.org/10.1002/pts.754.

**Holland, C. D. (1971)**. Determination of malonaldehydes as an index of rancidity in nut meats. *Journal of Association of Official Analytical Chemists*, *54* (5), 1024−1026.

**Hyperbaric. (2014)**. *Dry, Cured and Fermented Meat Products* (1-10, page 5).
http://www.hiperbaric.com/media/uploads/productos/documentos/WP_cured_and_fermented_meat_-_Hiperbaric_2014.pdf, retrieved May 15th, 2016.

**ISO. (2004).** ISO 21807. Microbiology of food and animal feeding stuffs − Determination of water activity.

**Ivanovic, J., Saicic, S., Milanovic, S. M., Petrovic, N. & Zizovic, I. (2015)**. High efficiency of supercritical rosemary extract in long term oxidative stabilization of pork liver pate. *Tehnologija Mesa*, *56* (1), 41−49.

**Karolyi, D. (2004).** Activity of water as a key factor of prolonged shelf life of meat. *Meso*, *6*, 9−13.

**Kennedy, C., Buckley, D. J. & Kerry, J. P. (2004)**. Meat display life of sheep meats retail packaged under atmospheres of various volumes and compositions, *Meat Science*, 68, 649–658.

**Møller, J. K., Jensen, J. S., Olsen, M. B., Skibsted, L. H. & Bertelsen, G. (2000).** Effect of residual oxygen on colour stability during chill storage of sliced, pasteurised ham packaged in modified atmosphere. *Meat Science*, *54* (4), 399−405.

**Quintavalla, S. & Vicini, L. (2002).** Antimicrobial food packaging in meat industry. *Meat Science*, *62*, 373–380.

**Rhee, K. S. & Ziprin, Y. A. (1987).** Lipid oxidation in retail beef, pork and chicken muscles as affected by concentration of heme pigments and nonheme iron and microsomal enzymic lipid peroxidation activity. *Journal of Food Biochemistry*, *11*, 1−15.

**Rubio, B., Martınez, B., Gonzalez-Fernandez, C., Dolores, Garcıa-Cachana & M. D. (2007)**. Effect of high pressure preservation on the quality of dry cured beef "Cecina de Leon." *Innovative Food Science and Emerging Technologies*, *8* (1), 102–110.

**SRPS. (2011). EN ISO 3960.** Animal and vegetable fats and oils – Determination of peroxide value - Iodometric (visual) endpoint determination.

**SRPS. (2003)**. **ISO 2917.** Meat and meat products - Measurement of pH – Reference method.

**SRPS. (2013)**. **ISO 6658.** Sensory analysis: Methodology – General guidance

**Tarladgis, B. C., Pearson, A. M & Dugan, L. R. (1964)**. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II Formation of the TBA malonaldehyde complex without acid heat treatment. *Journal of the Science of Food and Agriculture*, *15* (9), 602−607.

**Tomic, N., Tomasevic, I., Radovanovic, R. & Rajkovic, A. (2008).** "Uzice beef prshuta": Influence of different salting processes on sensory properties. *Journal of Muscle Foods*, *19*, 237–246.

**Vanhonacker, F., Almli, V. L., Hersleth, M. & Verbeke, W. (2008).** Profiling European Traditional Food Consumers. *British Food Journal*, *112*, 871−886.

**Viana, E. S, Gomide, L. A. M. & Vanetti, M. C. D. (2005).** Effect of modified atmospheres on microbiological, color and sensory properties of refrigerated pork. *Meat Science*, *71*, 696–705.

**Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., & Whittington, F. M. (2008)**. Meat fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*, 343–358.

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# Liver patè: process hygiene, quality parameters and thermal process

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*A b s t r a c t: The aim of this paper was to assess the manufacturing process of liver patè (150 g) packed in cans designed for the domestic market. The investigation included hygiene of the manufacturing process, product quality and control of heat treatment. Process hygiene was assessed through determining microbial numbers in canned stuffing before heat treatment, immediately after filling and during the wait time for the canning process (up to 150 minutes). Sulphate reducing Clostridia were not detected, Escherichia coli numbers ranged from 3,08 up to 3,25log CFU/g$^{-1}$, Enterobacteriaceae from 2,96 up to 3,08 log CFU/g$^{-1}$ and total aerobic colony count from 5,75 up to 6,36 log CFU/g$^{-1}$. Average chemical and physico-chemical product paramaters were determined: total protein content (9,66±0,61%), fat content (22,32±1,05%), sodium chloride content (1,49±0,04%), $a_w$ (0,96±0,005), pH (6,44±0,11) and nitrite content as $NaNO_2$ (7,44±1,97 mg/kg$^{-1}$). Determined chemical and physico-chemical parameters were in accordance with the requirements of domestic legislation. Heat tr eatment lasted 1 hour and 30 minutes. Effective sterilization time was 55 minutes, at autoclave medium temperature 114°C and a pressure of 3.2 bar. Lethality of the heat treatment was controlled by thermocouple probes placed in thermal centre of cans in six point checks and determining $F_o$ values which ranged from 7,24–8,58. This thermal regime, supported by can hermeticity, was sufficient to ensure the safety of the canned liver patè.*

***Keywords:*** *Liver patè, process hygiene, quality, heat treatment.*

## Introduction

Liver patè is a meat product derived from meat, fat, liver (at least 10%), other offal, connective tissue, blood, blood preparations, bouillon, soup, onion and additives, and protein preparations may also be included. Mostly, these patè are placed on the Serbian market as sterilized canned products as well as pasteurized liver patè in casing (*Anonymous*, 2015). Pork liver patè is a very popular and cheap cooked meat product manufactured and consumed all around the world (*Ivanovic et al.*, 2015). Due to high amounts of fat and non-haeme iron as well as the manufacturing process itself, liver patè is highly sucseptibile to lipid oxidation (*Lorenzo and Pateiro*, 2013).

The shelf life of canned meat products, when hermeticity is preseverved, in the first place depends on the degree of microbial destruction during heat treatment. Cans are considered commercially sterile. Comercial sterilization implies successful destruction of vegetative forms of bacteria (*Enterobacteriaceae*, staphylococci, micrococci) and especially spores of *Clostridium botulinum* and *Cl. perfringens,* as well as the absence of toxins and tissue enzymes, but with a tolerance for non-pathogenic microbiota that are not capable of causing failure of cans (*Anonymous*, 2005). The desirable taste of liver patè is significantly influenced by tissue composition (porcine, bovine, poultry meat, fat and liver) and spices and additives used. Also the intensity of heat treatment, which contributes to the appearance of a bitter taste, is correlated with the sterilization temperature. Because of that and especially in order to preserve the biological value of the product, the heat treatment should be carried out at temperatures ≤120°C, but with the imperative that the product must stay safe during the defined shelf life. The type of package (plastic film, cans) causes no difference in the taste of products which are subjected to an equal thermal treatments (*Anomymous*, 2011; *Uhler*, 2016).

According to epidemiological data and risk analysis, the major causes of foodborne diseases are microorganisms and toxins of microorganisms.

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Therefore, with the new approach to food safety it is necessary to set up adequate microbiological criteria for foods (*Buncic and Katic*, 2011). Principles for the development of risk assessment of microbiological hazards have been developed by the Codex Alimentarius (CAC) and EU Scientific Committee for Food (*Anonymous*, 2011a). This takes into account the principles of CAC and opinions, the EU Commission laid down the microbiological criteria for food in 2005 (*Anonymous*, 2005a).

The aim of this study studywas to evaluate the manufacturing process hygiene, safety and quality parameters of commercially sterilized liver patè packed in 150 g amounts in a three-part hard tin can intended for the local market. The process hygiene of liver patè production was determined by systematic monitoring of microorganisms present in patè stuffing at different waiting times before heat treatment. Product safety was monitored by determining the $F_o$ value during the heat treatment.

## Materials and Methods

Examinations were carried o ut in a meat processing plant while a total of six test series were performed. The tests consisted of three parts. The first part was related to the determination of process hygiene, the second to the examination of quality parameters, and the third to the heat treatment.

During the first stage, samples were taken after the closure of the stuffing (150 grams) into cans (three parts hard tin, manufacturer Silgan Holdings Inc., Skydra, Greece) before the heat treatment. The filled cans were sampled and investigated four times:

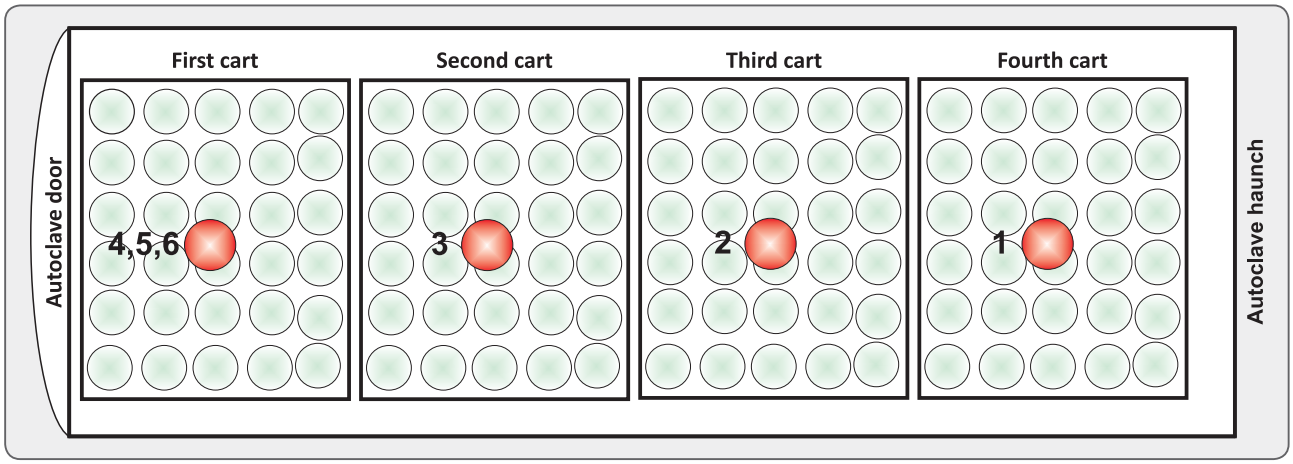
1. Immediately after the filling
2. After 90 minutes of waiting before the heat treatment
3. After 120 minutes of waiting before the heat treatment
4. After 150 minutes of waiting before the heat treatment

**Table 1.** List of reference methods used for enumeration of microbiota testing in the liver patè

| Microbiota | Reference method |
|---|---|
| Sulphate-reducing clostridia | ISO (2011) 15213:2011 – Horizontal method for the enumeration of sulfitereduction bacteria that grow under anaerobic conditions |
| *Escherichia coli* | ISO (2008) 16649–2: 2008 – Horizontal method for the enumeration of β-glucuronidase positive *Escherichia coli* Part 2: Colony count technique at 44°C with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide; |
| *Enterobacteriaceae* | ISO (2009) 21528–2: 2009 – Horizontal method for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method; |
| Total aerobic colony count | ISO (2008b) 4833: 2008 – Horizontal method for the enumeration of microorganisms-count technique at 30°C |

**Table 2.** List of reference methods used for quality parameters testing in the samples

| Quality parameter | Reference method |
|---|---|
| Total protein content | ISO (2008a) 16634:2008. Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content |
| *Sodium chloride content* | ISO (2004). 1738:2004. Meat and meat products – Determination of salt content |
| *$a_w$ value* | ISO (2004b). 21807:2004. Microbiology of food and animal feeding stuff-Determination of water activity |
| *Fat content* | ISO (1998). 1444:1998. Meat and meat products – Determination of free fat content |
| *pH value* | ISO (2004a). 2917:2004. Meat and meat products – Measurement of pH – Reference meth*od* |
| Nitrite content | ISO. (1999). 2918:1999. Meat and meat products – Determination of nitrite content |




**Legend:** Probe placed into the the thermal centre of the product.

**Figure 1.** Schematic diagram of probes positions in thermal centre of the liver patè cans in carts, in autoclave during heat treatment, viewed from above

Each sample consisted of five filled and closed units (cans). Five units were labeled and transported under cold chain conditions (+4°C) for up to two hours to the laboratory. Immediately upon arrival at the laboratory, the samples were examined for numbers of of micro-organisms: sulphite reducing *Clostridia*, *E. coli*, *Enterobacteriaceae* and total aerobic colony counts, in order to determine the process hygiene conditions. Micro-organisms were determined using reference methods, as presented in table 1.

The quality parameters of liver patè were determined in parallel by means of physico-chemical (pH value, $a_w$), and chemical tests (protein, fat, sodium chloride and nitrite contents).

Quality parameters in liver patè were determined using reference methods, as presented in table 2.

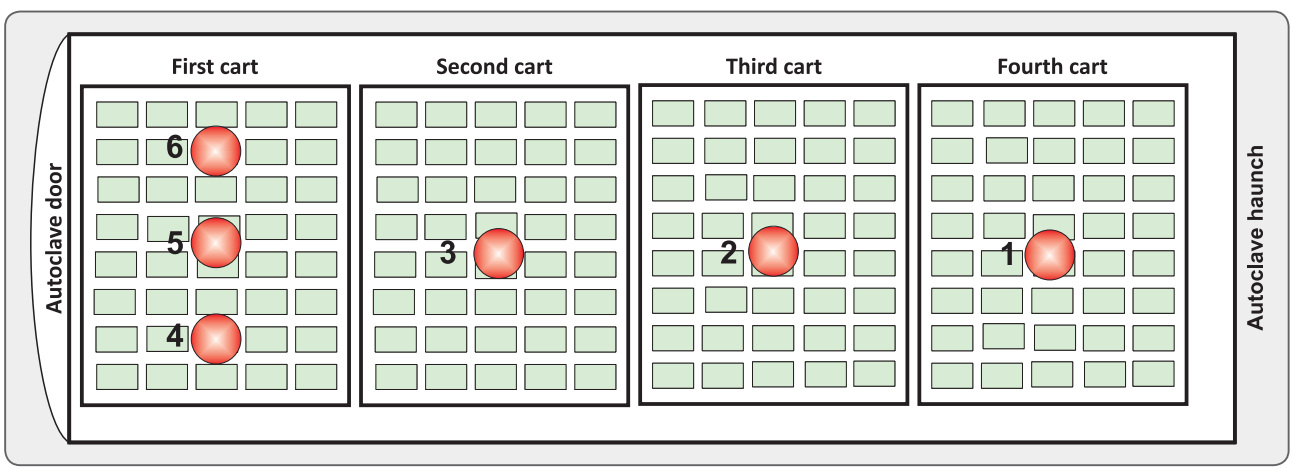
Heat treatment was validated for the same production batch. Validation was performed in a horizontal autoclave with overpressure that can receive four carts ("Sterilflow, type 1341, EA, manufactured 2013" – Roanne France), with thermocouple "ELLAB", model CTF 84with six compensating cables). The temperature and $F_o$ values were recorded and printed every five minutes. A total of six probes were placed in four carts in the middle section, in the thermal centre of filled cans (Figures 1 and 2). Heat treatment was performed in accordance to the following formula:

$$To = 15' + \frac{55'}{114°C/3.2bar} + 20'$$

15' – was the heating time to the required autoclave temperature

55' – was the effective sterilization time under autoclave medium (114°C and pressure 3,2 bar)

20' – was the cooling time




**Legend:** Probe placed into the the thermal centre of the product.

**Figure 2**. Schematic diagram of probes positions in thermal centre of the liver patè cans in carts, in autoclave during heat treatment, viewed from viewed from the side

Statistical analysis was performed by using commercial statistical software (MS Office 2010, Excel 2010)

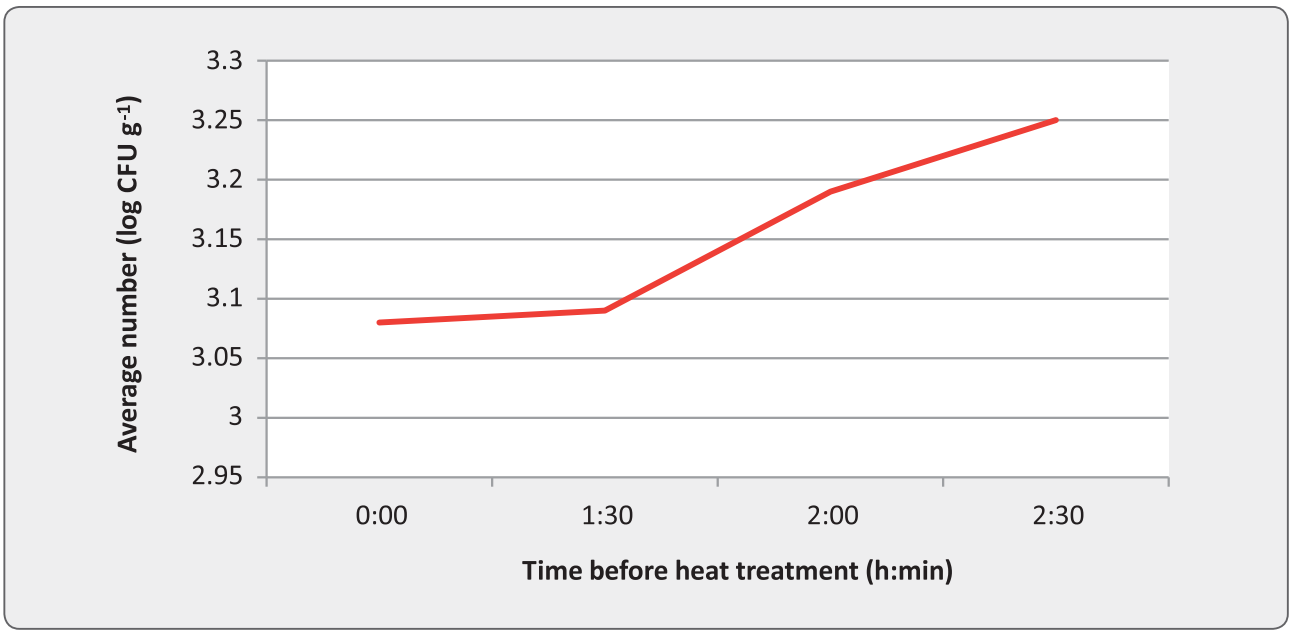
## Results

Microbiological investigation showed that sulphite reducing *Clostridia* were not detected in any sample of canned stuffing before heat treatment.

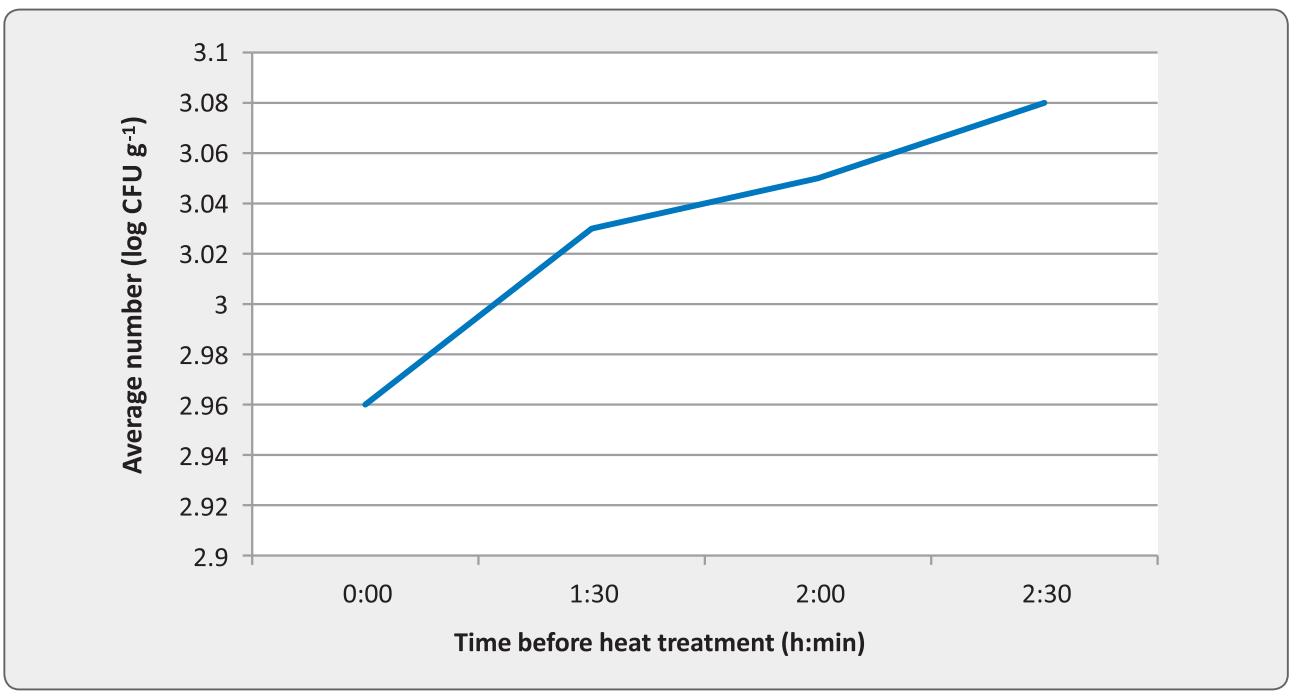
The presence of other tested microbiota is presented in Figures 3–5.

During the waiting time, in hermetically sealed cans with stuffing, an increase of the number of tested microorganism was observed.

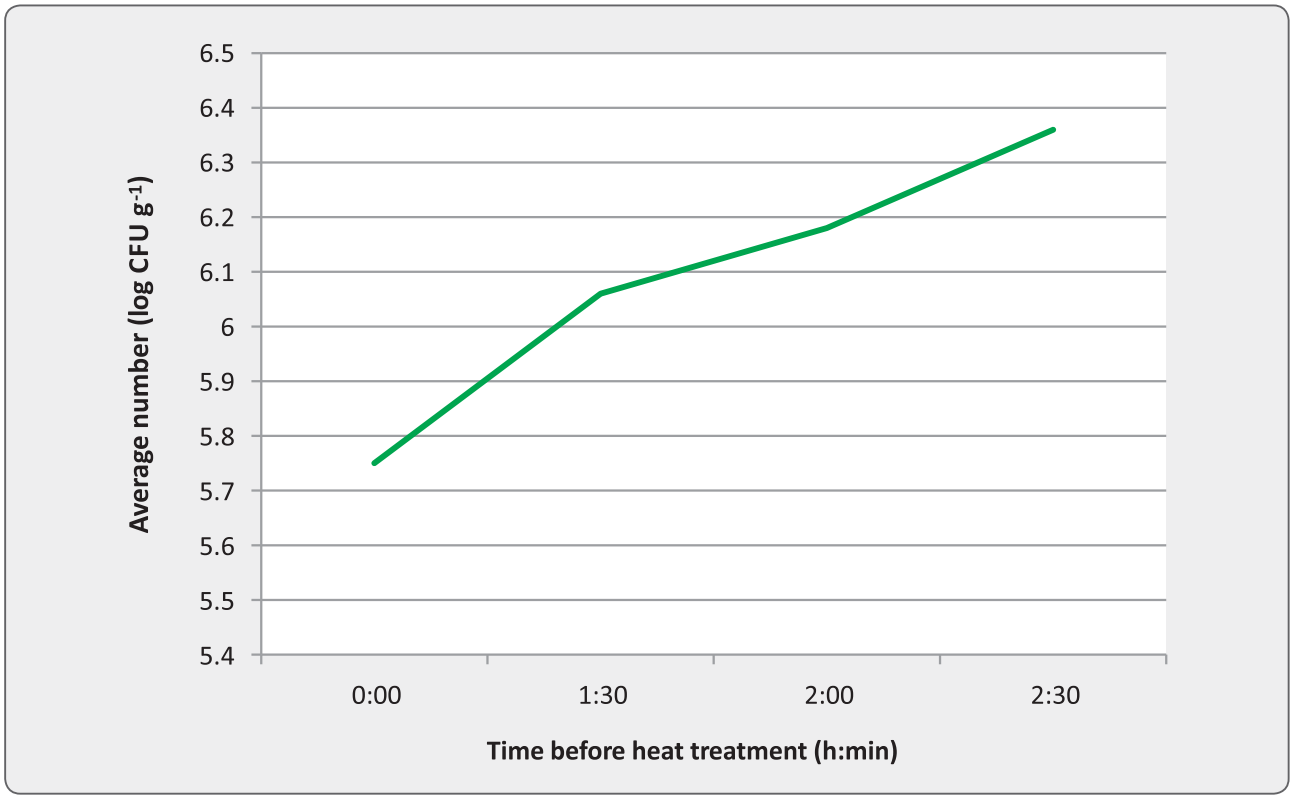
The number of *E. coli* increased from 3,08±0,36 log CFU $g^{-1}$, (immediately after sealing) to 3,25±0,24 log CFU $g^{-1}$, (after 2 hours and 30 minutes, before heat treatment).




**Graph 3.** Numbers of *Escherichia coli* in canned liver patè stuffing before heat treatment




**Graph 4.** Numbers of *Enterobacteriaceae* in canned liver patè stuffing before heat treatment




**Graph 5**. Numbers of total aerobic colony count in canned liver patè stuffing before heat treatment

The number of *Enterobacteriaceae* increased from 2,96±0,26 log CFU $g^{-1}$, (immediately after sealing) to 3,08±0,16 log CFU $g^{-1}$, (after 2 hours and 30 minutes, before heat treatment)

The total aerobic colony count increased from 5,75±0,39 log CFU $g^{-1}$, (immediately after sealing) to 6,36±0,33 log CFU $g^{-1}$, (after 2 hours and 30 minutes, before heat treatment)

Before heat treatment, quality parameters were determined in six repetitions, through determination of chemical and physico-chemical properties. Results are presented in table 3.

The chemical and physico-chemical parameters determined were in accordance with domestic legislation (*Anonymous*, 2015).

Heat treatment was monitored during regular production (Figures 6 and 7).

**Table 3.** Physical and psysico-chemical parameters of liver patè

| Parameters | X±SD | Min | Max | Iv |
|---|---|---|---|---|
| Total protein content (%) | 9,66±0,61 | 8,57 | 10,13 | 1,56 |
| Fat content (%) | 22,32±1,05 | 20,97 | 23,5 | 2,53 |
| Sodium chloride content (%) | 1,49±0,04 | 1,45 | 1,56 | 0,11 |
| $a_w$ | 0,96±0,005 | 0,954 | 0,972 | 0,018 |
| pH | 6,44±0,11 | 6,32 | 6,56 | 0,24 |
| Nitrite as $NaNO_2$ content (mg $kg^{-1}$) | 7,44±1,97 | 5,42 | 9,36 | 3,96 |

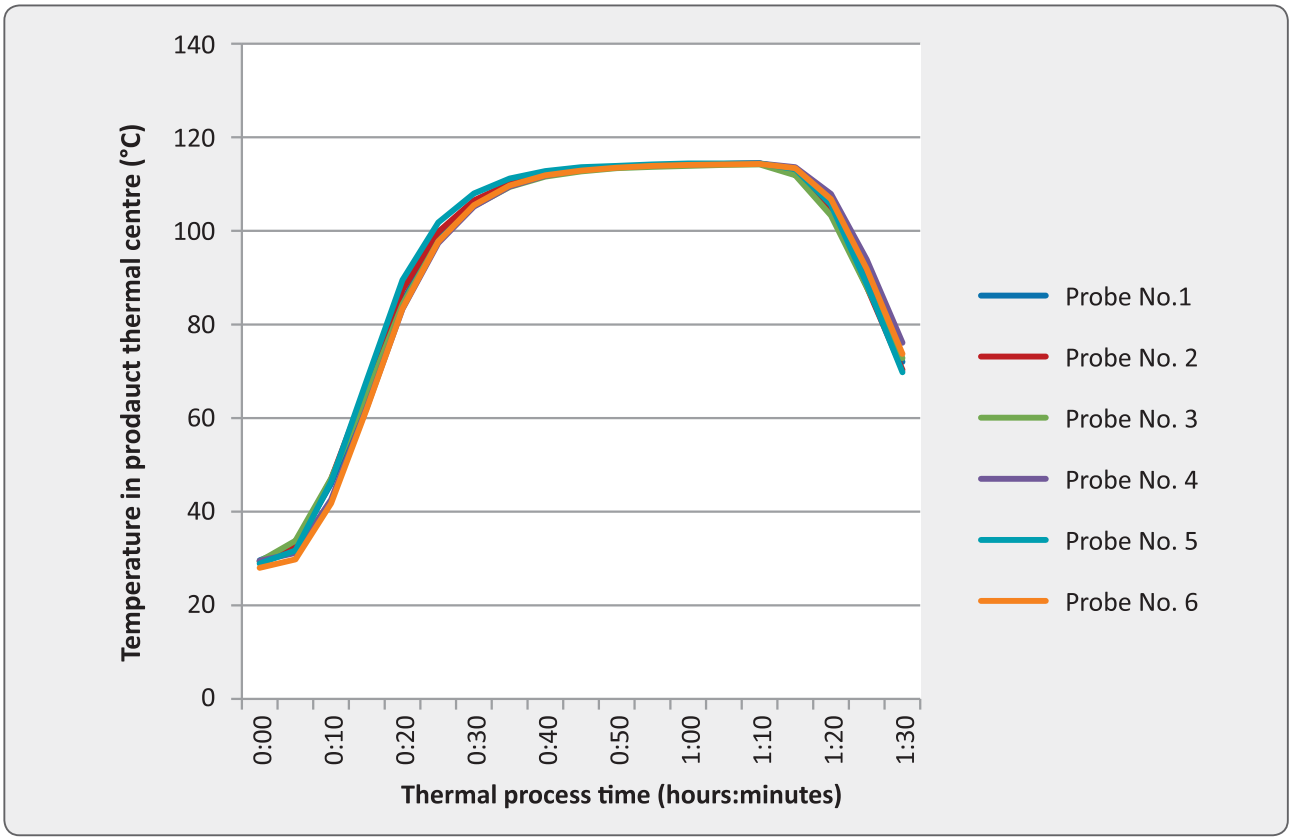



**Figure 6.** Temperature change measured in thermal centre during heat treatment of liver patè (150 g, in three part tinplate)

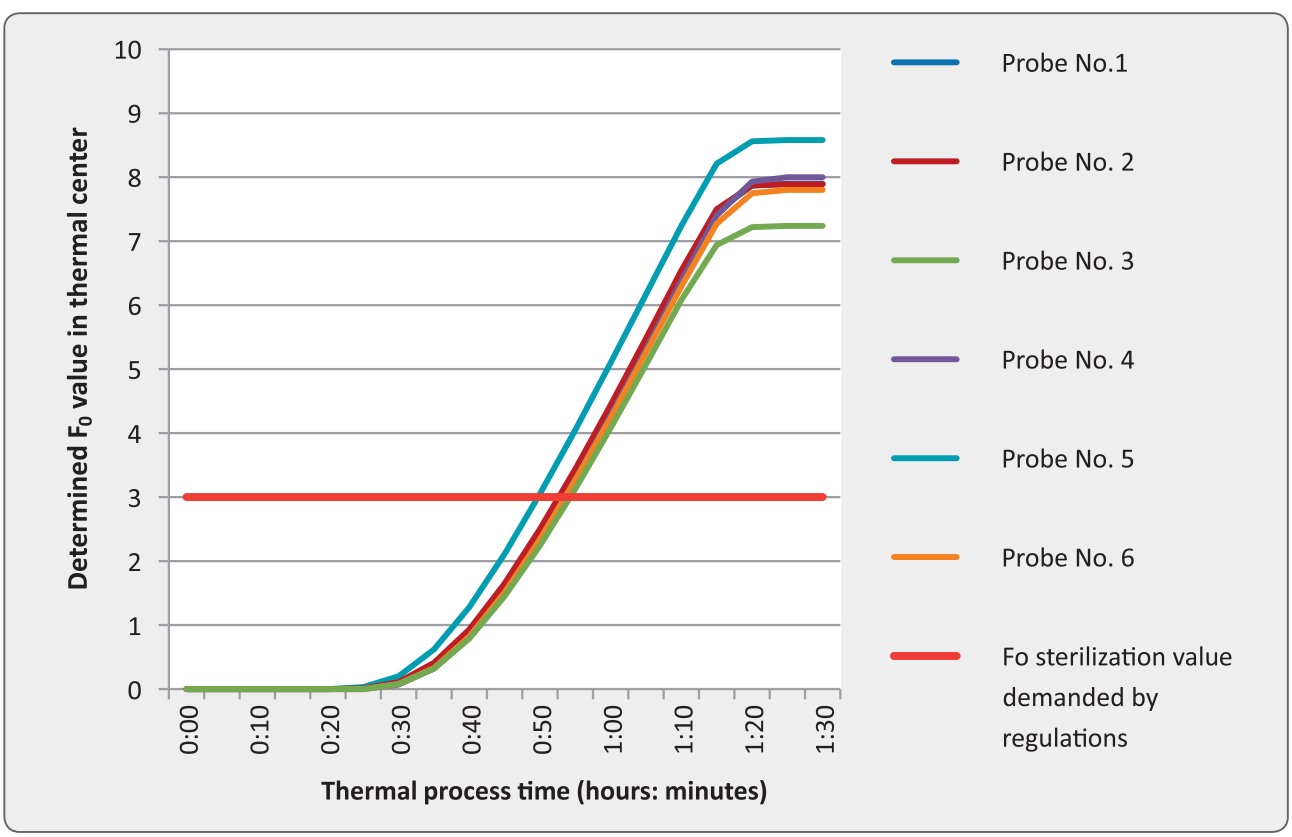



**Figure 7.** $F_o$ value change during heat treatment of liver patè (150 g, in three part tinplate)

## Discussion

### *E. coli* in canned stuffing before heat treatment

*E. coli* is considered as an indicator of fecal contamination. Its presence in raw material generally indicates improper hygiene and/or improper handling of raw material. *E. coli* O157:H7 (causative agent of hemolytic uraemic colitis and thrombotic thrombocytopaenic purpura syndrome) is especially significant for human health. Since the early 1980s, *E. coli* O157 has emerged as one of the most significant pathogens of public health relevance not because of the incidence of the illness, which is much lower than that of other foodborne pathogens such as *Campylobacter* or *Salmonella*, but becouse of the severity of symptoms, the low infections dose and potential sequelae (*Nastasijevic et al.*, 2014). The EU Scientific Council of Veterinary Public Health issued an opinion on verotoxin-positive *E. coli* (VTEC) in food (*Anonymous*, 2003). The Council concluded that it is unlikely that the application of microbiological standards for VTEC O157 to the end product has led to a significant reduction of the related risks for consumers. However, microbiological guidelines aimed at reducing faecal contamination along the food chain can contribute to reducing public health risks, including VTEC (*Anonymous*, 2005a). Therefore, it is difficult to provide clear recommendations on the admissibility of the presence of *E. coli* in raw material which is being directed to the intensive heat treatment at temperatures of over 100°C. However a statement in Serbian regulation covering general and specific food hygiene requirements in any stage of production, processing and transport pertaining to the processing of meat preparations, (Annex 2, 2.1.8.) relevant and requires counts of 2,7–3,7 log CFU $g^{-1}$, immediately before heat treatment (*Anonymous*, 2010). Unsatisfactory microbial counts require measures to improve production hygiene and improvements in selection and/or origin of raw materials. Although these statements are present in the scientific literature (*Peran et al.,* 2015*)* there are also different opinions because the infectious dose of E. coli O157 is not known. In some cases of foodborne disease, only a few cells, perhaps lower than 2 log CFU, may have been ingested (*Tilden et al.,* 1996*).* Therefore, the prevention of foodborne *E. coli* O157 infections requires not only growth suppression in foods, but also elimination of the pathogen from foods (*Buncic et al.,* 2004).

In the current study *E. coli* numbers ranged from 3,08 to 3,25 log CFU $g^{-1}$ (depending on the waiting time of canned stuffing before heat treatment), which was acceptable from the aspect of process hygiene, because all production batches were sent to commercial sterilization. Additionally the *E. coli* level was in accordance with the recommended limit (*Anonymous*, 2010). Good hygiene and manufacturing principles must be applied in all production stages, in order to maintain *E. coli* (if present at low levels) (waiting time of canned stuffing intended for heat treatment must be as short as the production process allows). At the same time, the infectious dose for vulnerable groups (elderly, children, pregnant women) is often lower than 2 log CFU $g^{-1}$ (*Tilden et al.*, 1996), particularly in the case of the most pathogenic serovars. Therefore delays between preparation of stuffing, filling and heat treatment process must be avoided or reduced to minimum. Any deviation is unacceptable, and such product must be removed from production and adequately destroyed (*Wiliam and Doyle*, 2009).

### *Enterobacteriaceae* in canned stuffing before heat treatment

The family *Enterobacteriaceae* includes a large group of biochemically and genetically-related bacteria, the presence/absence of which in feed/food generally indicates the level of hygiene. Their presence in heat-treated foods indicates an inadequate thermal regime or contamination after the completion of heat treatment. The European Food Safety Authority (EFSA) issued an opinion on the microbiological risks, which stated that the presence of *Enterobacteriaceae* can be used as an indicator of risk in the finished food (*Anonymous*, 2004). Moreover EFSA recommended monitoring and testing for *Enterobacteriaceae* in the production setting, and in finished product intended for special vulnerable groups (*Anonymous,* 2005). However, besides pathogenic species, the family *Enterobacteriaceae* also includes saprophytic species, which often occur in environments where food is produced without posing any health hazard. Therefore, the family *Enterobacteriaceae* can be used for routine monitoring and, if present, can trigger testing for specific pathogens. The presence of *Enterobacteriaceae* in raw materials intended for heat treatment is taken as an indication of the general hygienic manufacturing process and manufacturing plant hygiene (*Anonymous*, 2011a).

In the current study the average *Enterobacteriaceae* count in canned stuffing intended for heat treatment ranged from 2,96 to 3,08 lof CFU $g^{-1}$. This may be the result of poor selection of raw materials or errors during manipulation of raw material during the preparation of the product.

## Total aerobic colony count of canned stuffing before heat treatment

Aerobic colony count on the surface of fresh meat directly influences its shelf life. Spoilage of fresh meat occurs when the aerobic colony count reaches 6 log CFU $cm^{-2}$ (*Anonymous*, 2016), followed by strange smell, discolorations and texture changes if total aerobic colony counts reach 8 log CFU $cm^{-2}$. Because of that, the principles of Good Hygiene (GHP) and Good Manufacturing practice (GMP) are of particular importance. In the Serbian legislation on general and specific food hygiene requirements at any stage of production, processing and transport (*Anonymous*, 2010) of minced meat (which has increased surface contact with microorganisms) in Annex 2 (hygiene parameters of the production process), paragraph 2.1.6. cited the limit for aerobic colony count as being 5,7 to 6,7 log CFU $g^{-1}$, whereby counts ≤ 5,7 log CFU $g^{-1}$ are satisfactory, counts between 5,7–6,7 log CFU $g^{-1}$ are acceptable, but counts > 6,7 log CFU $g^{-1}$ are unacceptable. Unsatisfactory results trigger improvements to production hygiene and improvements in selection and/or origin of raw materials.

In the current study the average total aerobic colony countin canned stuffing intended for heat treatment ranged from 5,75 to 6,36 log CFU $g^{-1}$ and so were satisfactory (*Anonymous*, 2010). Special attention must be devoted to the waiting time of canned stuffing prior to heat treatment. Excessive waiting time, over two hours, led to increases of total aerobic colony count.

## Physical and physico-chemical parameters of liver patè

Physical and physico-chemical parameters of liver patè can influence the optimal regime of thermal processing, canning and sustainability at recommended storage temperature.

The $a_w$ of 0,96±0,005 was not an obstacle to microbial growth and development. Generally, common spoilage bacteria are inhibited at approximately $a_w$ 0,97 and some pathogens (e.g. *Clostridia* spp.) at 0,94. In order to ensure human health protection by lowering food $a_w$, this should be lowered to at least 0,62 (*Karolyi*, 2004) to 0,75 (*Vukovic,* 2012).

Nitrite, measured as $NaNO_2$, 7,44±1,97 mg $kg^{-1}$ was relatively low. Similar levels were observed in Germany – 11 mg $kg^{-1}$ (*Sabine et al*., 2011), while the highest observed nitrite levels in that study were around 50 mg $kg^{-1}$, which is significantly higher than in our study (highest nitrite level: 9,36 mg $kg^{-1}$). Nitrite is applied as a preservative through curing salt, a homogenous mixture of table salt and nitrite (0,5–0,6%). In the EU the use of nitrite and nitrate in meat products is regulated (*Anonymous*, 2008). The amount of residual nitrite allowed in this type of product (patè) in Serbia is up to 100 mg $kg^{-1}$ (*Anonymous*, 2013). Nitrites are able to prevent formation of toxins in products containing spores of *Cl. botulinum* types A and B, via an inhibitory effect on spore germination. The amount of nitrites decrease during the thermal treatment of meat products because of reaction with proteins and other components in the meat stuffing. The presence of nitrite residues ensure sustainability of the product during its shelf life. Sodium chloride plays a very important role in the production of meat products and provides a favorable effect on the texture, smell, taste and sustainability. The perception of saltiness of sodium chloride arises from a combination of sodium and chlorine ions (*Miller and Bartosuk*, 1991) and this combination gives a clean salty taste in the mouth receptor corpuscles (*Lilic et al.,* 2014).

The pH of the liver patè was 6,44±0,11 on average. This pH would not on its own effectively prevent the growth of microorganisms in the stuffing. In a product such as liver patè, low pH would negatively affect the sensory properties of the products, so the other factors are needed to achieve microbial stability.

The fat content of the liver patè was 22,32±1,05% on average. Fat content can be of great importance to the dynamics of heat treatment, as fat can act as an insulator and prevent effective heat penetration to the can thermal centre, so the heat treatment of a product containing more fat should last longer than the same product containing less fat.

Total protein content is prescribed as a quality parameter in domestic law (*Anonymous*, 2015), were liver sausage and patè must contain at least 9% meat proteins or total proteins. The average total protein content in the liver patè was 9,66±0,61 so this product met local requirements.

During storage, over longer periods of time , can stuffing is susceptible to oxidative changes. Longer storage leads to increases in peroxide and thiobarbituric acid content, due to the decomposition of fat. To decrease the intensity of fat oxidation processes antioxidants can be justifiably added to stuffing, during production. Natural antioxidant, such as that based on rosemary, would provide added value to commercial liver patè due to both its natural origin and potential bioactive properties (*Ivanovic et al.*, 2015).

## Heat treatment

Commercial sterilization is a heat treatment of cans, at temperatures exceeding 100 °C, wherein, a lethality of at least $F_0$=3 must be achieved in the thermal centre of the product (*Anonymous,* 2015). $F_o$ value describes heat treatment lethality. It is used for reliable control of sterilization and expresses the lethal effect of the reference temperature (121,1°C) for one minute (*Teodorovic et al*., 2015; *Vukovic*, 1996, 2006). According to FAO recommendations (*Anonymous*, 2016a) based on microbiological risk assessment, sterilization of canned meat products should achieve $F_o$ values of 4–5,5, while the temperature should be in the range 117–130°C, depending on the characteristics of the products. If these thermal requirements are met, 1–4 year shelf life is achieved by storage temperature ≤ 25°C.

In the current study, the heat treatment of liver patè (150 g, three piece tinplate can) lasted 1 hour and 30 minutes (15 minutes warming up, 55 minutes effective sterilization and 20 minutes cooling). In the thermal centre of the cans, the achieved Fo values ranged from 7,24–8,58, using a six point check (with six probes) which ensured the safety of the product. During the sterilization process, workflow variations were not observed, which indicated that the heat treatment process occurred uniformly in all areas inside the autoclave.

Sterilization should eliminate vegetative bacteria, spores of clostridia, especially thermoresistant spores of mesophilic *Cl. botulinum* types A and B, while spores of some thermophilic bacteria, such as *Bacillus stearothermophilus*, could survive sterilization, with lethality of $F_o$ 4–5,5. Because of that, proper storage conditions (temperature ≤25°C,dark and dry place) should be provided in order to ensure stability of the product without microbial growth. However, the general commercial intention is to implement mild thermal processing in order to preserve nutritional value and sensory characteristics (*Anonymous*, 2016a).

In order to ensure product safety food business operators must have HACCP-based self-control plans. The EU regulation on hygiene of foodstuffs states that food and staff who come into contact with food must be controlled and must adhere to all food safety instructions (*Anonymous*, 2004). Regular hygiene control must be performed by taking swabs from surfaces that come into contact with food. The elapsed time from filling stuffing cans to the beginning of the thermal treatment should not be longer than two hours as is recommended in FSIS documents.

The ability to produce microbially safe commercially sterilized canned liver patè depends on low initial microbial contamination of raw materials, adequate application of Good Hygiene (GHP) and Good Manufacturing Practice (GMP), adequately conducted technological processes, as well as adequate heat treatment.

## Conclusions

The safety of canned liver patè is based on proper hermetic sealing and consecutively applied stages of intensive heat treatment. The commercial sterilization process of liver patè studied in cans with 150 g net weight, which lasted 1 hour and 30 minutes, with an effective sterilization time of 55 minutes, autoclave medium temperature 114°C and a pressure of 3,2 bar bar resulted in $F_o$ values ranging from 7,24–8,58. This values are adequate and suitable for the canned liver patè produced.

However the efficiency of heat treatment of canned liver patè also depends on the microbiological profile of the stuffing, and the waiting time prior to heat treatment.

The microbiological profile of the liver patè stuffing before heat processing (*E. coli* from 3,08 to 3,25 log CFU $g^{-1}$; *Enterobacteriaceae* from 2,96 to 3,08 log CFU $g^{-1}$; total aerobic colony count from 5,75 to 6,36 log CFU $g^{-1}$) indicates a need to improve general hygiene conditions during the liver patè production process.

In general the main recommendations for the treatment of canned meat products are: 1) proper selection of raw materials with suitable of hygienic standards; 2) treatment of raw material in accordance with food safety procedures; 3) a high level of hygiene built in to the product design and manufacture; 4) manufacturing, including adequate heat treatment, in accordance with food safety objectives; 5) the preservation of can hermeticity after closure and 6) proper can storage.

Physico-chemical parameters ($a_w$ 0,96±0,04; pH 6,44±0,11) and chemical composition (sodium chloride 1,49±0,04, nitrite content 7,44±1,97 mg $kg^{-1}$) of liver patè are not sufficient to prevent microbial growth on their own, so these antimicrobial parameters have to be combined with some other hurdles including the heat treatment.

The quality of the liver patè was ensured by total protein content being on a average 9,66±0,61% which is in accordance with regulations.

## References


**Anonymous. (2003).** Opinion of the scientific committee on veterinary measures relating to public health on Verotoxigenic E. coli (VTEC) in foodstuffs. European Commission Health and Consumer Protection Directorate-General, 1–64.

**Anonymous. (2004).** Regulation on hygiene of foodstuffs (EU) No. 852/2004. *Official Journal of the European Union*, L 338/1–25.

**Anonymous. (2005).** FSIS Directive, 7530.2 10/20/05, Verification activities in canning operations that choose to follow the canning regulations (http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7530_2.pdf)

**Anonymous. (2005a).** Regulation on microbiological criteria for foodstuffs (EU) No. 2073/2005

**Anonymous. (2008).** Regulation on food additives (EU) No. 1333/2008. *Official Journal of European Union*, L 354/16.

**Anonymous. (2010).** Rules on general and specific food hygiene requirements at any stage of production, processing and transport. *Official Gazette of the Republic of Serbia*, 72/2010.

**Anonymous. (2011).** Code of hygienic practice for low and acidified low acid canned foods, Codex Alimentarius, CAC/RCP 23–1979; Rev. 1989, 1993; Ed. Corrections 2011.

**Anonymous. (2011a.)** Guide for microbiological criteria for foods, Ministry of Agriculture, Trade, Forestry and Water Management, Belgrade, June, first edtion (http://www.vet.minpolj.gov.rs/veterinarsko_javno_zdravstvo/instrukcije_i_vodici/Vodic%20za%20mikrobioloske%20kriterijume%20za%20hranu.pdf).

**Anonymous. (2013).** Rules on food additives. Part D 8.22 – Heat treated meat products, rules of food additives. *Official Gazette of the Republic of Serbia*, 63/2013.

**Anonymous. (2015).** Rules on the quality of ground meat, semi-finished meat and meat products. *Official Gazette of the Republic of Serbia*, 94/2015).

**Anonymous. (2016).** FAO: Effect of bacterial growth on the shelf-life of meat and meat products – General hygiene principles for handling meat (http://www.fao.org/docrep/004/t0279e/t0279e03.htm).

**Anonymous. (2016a).** FAO: Canning and Sterilization, (http://www.fao.org/docrep/010/ai407e/ai407e22.htm)

**Buncic, O. & Katic, V. (2011).** Food safety and microbiological criteria. Tehnologija Mesa, 52 (1), 47–51.

**Buncic, S., Avery, S. M. & De Zutter, L. (2004).** Epidemiology of *Escherichia coli* O157 in cattle from farm to fork. In International EU-RAIN Conference – Food Pathogen epidemiology: microbes, maladies and methods, Padua, Italy, 2–3. December 2004. Proceedings, 67–84.

**Ivanovic, J., Saicic, S., Milanovic-Stevanovic, M., Petrovic, N., Zizovic, I. & Petrovic, S. (2015).** High efficiency of supercritical rosemary extract in long term oxidative stabilization of pork liver patè. *Tehnologija Mesa*, *56* (1), 41–49.

**ISO (1998)** 1444:1998. Meat and meat products – Determination of free fat content.

**ISO (1999)** 2918:1999. Meat and meat products – Determination of nitrite content.

**ISO (2004)** 1738:2004. Meat and meat products – Determination of salt content.

**ISO (2004a)** 2917:2004. Meat and meat products – Measurement of pH – Reference method.

**ISO (2004b)** 21807:2004. Microbiology of food and animal feeding stuff – Determination of water activity.

**ISO (2008)** 16649–2:2008. Horizontal method for the enumeration of β-glucuronidase positive Escherichia coli Part 2: Colony count technique at 44°C with 5-bromo-4-chloro-3-indolyl-β -D-glucuronide.

**ISO. (2008a)** 16634:2008. Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content.

**ISO (2008b)** 4833: 2008. Horizontal method for the enumeration of microorganisms-count technique at 30°C.

**ISO (2009)** 21528–2:2009. Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count method.

**ISO (2011)** 15213:2011. Horizontal method for the enumeration of sulfitereduction bacteria that grow under anaerobic conditions.

**Karolyi, D., (2004).** Activity of water as a key factor of prolonged shelf-life of meat, *Meso*, *6*, 9–13.

**Lilic, S., Milanovic-Stevanovic, M., Karan, D., Lukic, M., Petronijevic, R., Velebit, B. & Lakicevic, B. (2014).** Examination of sensory perceived saltiness of chloride salt's aqueous solutions with different pH values. *Tehnologija Mesa*, *55* (1), 81–87.

**Lorenzo, J. M. & Pateiro, M., (2013).** Influence of fat content on physico-chemical and oxidative stability of foal liver patè. *Meat Science*, *95*, 330–335.

**Miller, I. J. & Bartoshuk, L.M. (1991).** Taste perception, taste bud distortion, and spatial relationship. In T.V. Gretchell, R.L. Dotv, L.M. Bartoshuk&J.B.Snow (Eds.), Smell and taste in healt disease, 205–233, New York, Reven Press.

**Nastasijevic, I., Mitrovic, R. & Jankovic, V. (2014)**. STEC in the beef chain-One Health Approach. *Tehnologija mesa*, *55* (2), 93–116.

**Teodorovic, V., Karabasil, N., Dimitrijevic, M. & Vasilev, D. (2015).** Meat Hygiene and Technology. First edition. Belgrade, Serbia: Faculty of Veterinary Medicine, Belgrade, First Edition, 351.

**Tilden, J., Young, W., McNamara, A. M., Custer, C., Boesel, B., Lambert-Fair, M. A., Majkowski, J., Vugia, D., Werner, S. B., Hollingsworth, J. & Morris, J. G. (1996).** A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American Journal of Public Health*, *86*, 1142–1145.

**Peran, R., Balabarca, V., Etoundi, J.M., Odame-Darkwat, J., Oppong-Otoo, J., Hinson, D. & Wouafo, M. (2015).** Establishment of good hygiene practice-based microbiological criteria in food industries: Guidelines using an example for meat preparations. *Food Control*, *58*, 7–11.

**Sabine, A.**, **Wolfgang, J., Schwagele, F., Schwind, K.H. & Wagner, H. (2011).** Chemical safety in meat industry. *Tehnologija Mesa, 52* (1), 80–96.

**Wiliam, S. & Doyle, M. (2009).** Food Microbiology and Food Safety. In Compendium of the Microbiological Spoilage of Foods and beverages, 190–202.

**Uhler, P. (2016).** FSIS, Thermally Processed, Commercially Sterile Products http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97–013N/PUhler_Canning.pdfI (assessed 02.06.2016.)

**Vukovic, I. (1996).** F value – the determination and control of the process of thermal treatment of canned meat. *Tehnologija Mesa*, *37* (3–4), 177–180.

**Vukovic, I. (2006).** Meat technology fundamentals. Third edition. Belgrade, Serbia: Faculty of Veterinary medicine.

**Vukovic, I. (2012)**. Meat technology fundamentals, Fourth edition. Belgrade, Serbia: Faculty of Veterinary medicine.

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# Effect of sodium chloride reduction in dry fermented sausages on sensory quality parameters and instrumentally measured colour

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*A b s t r a c t : Modern trends in human nutrition require decreasing the sodium chloride content in food due to several negative health impacts of excessive sodium intake from food. The goal of this study was to investigate the possibility of reducing the amount of sodium chloride used in the production of dry fermented sausages by using different salt mixtures to partially replace the sodium chloride. Control sausages were produced only with sodium chloride (3%), while sausages from other groups were produced by partially replacing sodium chloride with other salts in different amounts. In group 1 and 2 sausages, sodium chloride was partially replaced by potassium chloride and in group 3 and 4 sausages, sodium chloride was partially replaced by ammonium chloride.*

*Moderate reductions of sodium chloride in the dry fermented sausages by partial replacement with potassium chloride ( group 1) and with ammonium chloride (group 4) led to a slight reduction in saltiness, although this was still at an acceptable level. The overall acceptability of sausages from these groups was lower in relation to sausages from the control group, but despite that, their smell, colour and taste were at an acceptable level.*

*The most highly expressed bitterness was determined in group 2 and 3 sausages, and these were significantly more bitter than sausages from other groups. The use of different salt mixtures did not affect redness (a*) or yellowness (b*) in sausages, but led to greater expressed lightness in the sodium-adjusted sausages in comparison to sausages from the control group.*

***Keywords:*** *sodium chloride reduction, dry fermented sausages, sensory evaluation, colour.*

## Introduction

Modern trends in human nutrition require the reduction of sodium chloride in food due to several negative health impacts of excessive sodium intake from food. Increased intake of sodium is one of the major causes of hypertension, which is the greatest risk factor for development of cardiovascular diseases. Excessive dietary sodium intake could be a cause of essential hypertension and also can lead to direct risk of heart attack (*Perry and Beevers*, 1992), hypertrophy of the left heart chamber (*Schmieder and Messerli*, 2000), sodium retention in extracellular fluid (*MacGregor and de Wardener*, 1997), greater possibility of infection by *Helicobacter pylori* and risk of gastric cancer (*Tsugane et al.,* 2004), increase of urinary excretion of calcium and risk of forming of kidney calculi (*Cappuccio et al.*, 2000), risk of reduced bone density (*Devine et al.*, 1995), exacerbations of asthmatic seizures (*Mickleborough et al.*, 2005) and increase of HOMA (homeostasis model assessment) insulin resistance in patients with essential hypertension (*Kuroda et al.,* 1999).

Sodium chloride (salt) content can be reduced in meat products in different ways but most common is partial replacement of sodium chloride with potassium chloride (*Terell,* 1983; *Guàrdia et al.,* 2006). According to some data (*Ruusunen and Puolanne*, 2005), the lowest sodium chloride content in dry fermented sausages is 2.5%, particularly in salamis. Sausages with lower salt content are not firm enough and cannot be easily sliced; thus, such low-salt sausages lack one of the main characteristics of dry fermented sausages. Besides potassium chloride, other chloride salts, mainly salts of magnesium and calcium and ascorbates can be used as replacers (Ruusunen and Puolanne, 2005). The main problem in this case is the occurrence of a bitter taste, because only sodium chloride has a clearly salty taste.

The aim of this study was to examine the effects of reducing sodium chloride in dry fermented sausages by replacing it with potassium chloride

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or ammonium chloride. To that end, sensory quality parameters and instrumental measured colour were determined in the sausages.

## Material and Methods

*Sausage production*

Five groups of sausages were produced. Pork for production of control group sausages was cured with nitrite curing salt only, while sausages from other groups were cured with various salt mixtures, according to Table 1. Meat and fat were minced to a granulation of 6 mm, mixed with salt or salt mixtures, and after that was filled into pig small intestine, diameter 22-24 mm. Smoking, fermentation and drying lasted for 21 days in the smoking house.

*Sensory evaluation*

Surface colour, cut colour, intensity of saltiness and bitterness and overall acceptability were assessed by a sensory panel. Numeric-descriptive scales with 5 points were used. For evaluation of colour and overall acceptability, 5 points was the best attribute, while 1 point was the worst attribute. For evaluation of intensity of saltiness and bitterness, 5 points equated to product with the most highly expressed attribute (the most salty or the bitterest), while 1 point was the product with the least expressed attribute. Sensory evaluation was carried out by 10 trained assessors under the same conditions.

*Instrumental colour determination*

Colour of sausages was evaluated using colorimeter (Minolta Chroma Meter RC-400). The CIE system colour profile of lightness (L*), redness (a*) and yellowness (b*) was measured by a reflectance colorimeter using illuminant source D65, 8-mm aperture and 10° observation angle (*CIE*, 1976). The colorimeter was calibrated throughout the study using a standard white ceramic tile. Colour was measured on three cut surfaces of sausage and on each surface, three measurements were taken.

*Determination of sodium content*

Aliquots of approximately 0.3 g of sausage were transferred into Teflon vessels and 5mL nitric acid (p.a. Sigma) and 1.5mL hydrogen peroxide (30%, p.a., Merck) were added. The microwave digestion program consisted of three steps as follows: 5 min from room temperature to 180°C, 10 min hold 180°C, 20 min vent. After cooling at room temperature, the digested solutions were quantitatively transferred into disposable flasks and diluted to 100ml with deionized water (Elga).

The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS). Measurements were performed using an iCap Q (Thermo Scientific, Bremen, Germany), equipped with a collision cell and operating in kinetic energy discrimination (KED) mode. The isotope $^{23}Na$ was measured.

Torch position, ion optics and detector settings were adjusted daily using tuning solution (Thermo Scientific Tune B), in order to optimise measurements and minimise possible interferences. For qualitative analysis, a five-point calibration curve (including zero) was constructed for each isotope in the concentration range of 0.1 – 2.0 mg $L^{-1}$. An additional line of the peristaltic pump was used for online introduction of multi-element internal standard ($^{6}Li$, $^{45}Sc$ – 10 ng $mL^{-1}$; $^{71}Ga$, $^{89}Y$, $^{209}Bi$ – 2 ng $mL^{-1}$)

**Table 1.** Composition of sausages, g

| Group | Raw material | Sodium chloride | Potassium chloride | Ammonium chloride | Sodium nitrite |
|---|---|---|---|---|---|
| **Control** | Pork shoulder, 2400<br>Fat, 600 | 90.00 | – | – | 0.4500 |
| **1** | Pork shoulder, 2400<br>Fat, 600 | 60.00 | 30.00 | – | 0.4500 |
| **2** | Pork shoulder, 2400<br>Fat, 600 | 45.00 | 45.00 | – | 0.4500 |
| **3** | Pork shoulder, 2400<br>Fat, 600 | 45.00 | – | 30.00 | 0.3750 |
| **4** | Pork shoulder, 2400<br>Fat, 600 | 60.00 | – | 7.50 | 0.3375 |

covering a wide mass range. Concentrations of each measured isotope were corrected for response factors of both higher and lower mass internal standard by interpolation.

The quality of the analytical process was controlled by analysis of the standard reference material (NIST SRM 1577c). Measured concentrations were within the range of the certified values for all isotopes.

*Statistical evaluation*

The results are presented as mean±SD. Between averages statistical differences were significant at the levels P≤0.05 and P≤0.01 by Student's t-test. Significant differences in the tables are expressed as different superscript letters.

## Results and discussion

Results of sensory evaluation are shown in Table 2. Sausages from all groups had high scores for surface and cut colour and there were no significant differences between the different groups of sausages (P≥0.05). The saltiest sausage was the control sausage (4.22±0.32), while sausages from other groups, although moderately salty, were significantly less salty than control sausages (P≤0.05). The most highly expressed bitterness was in group 2 and 3 sausages (3.94±1.07 and 3.56±0.86, respectively), and these sausages were significantly more bitter (P≤0.01) than the more moderate group 1 and 4 sausages (2.06±0.90 and 2.89±0.34, respectively). Control sausages were evaluated as the least bitter, which was expected, because only sodium chloride had been added to these products.

The best sensory scores for overall acceptability were obtained by control sausages (4.67±0.47), and this was significantly better than sausages from other groups (P≤0.01). Group 1 and 4 sausages were evaluated similarly (P≥0.05; 3.56±1.26 and 3.83±0.58, respectively), while group 2 and 3 sausages achieved the lowest evaluations (2.89±0.74 and 2.78±0.92, respectively). Despite worse scores for overall acceptability, sausages from groups 2 and 3 were still had acceptable colour, smell and taste. The only differences noted were a slightly bitter taste and less saltiness.

*Gou et al.* (1996) used potassium chloride, potassium lactate and glycine as sodium chloride replacers in fermented sausages and concluded that replacing 40% or more of the sodium chloride with these compounds or their mixtures lead to undesirable and irreversible changes in sensory quality of product. Also, product texture problems occured when 30% of the sodium chloride was replaced with potassium lactate or 50% of the sodium chloride with glycine. The same authors (1996) cited a fall in overall sensory acceptability when 30% of the sodium chloride was replaced with potassium lactate, 20% with glycine or 40% with potassium chloride.

*Askar et al.* (1993) did not find statistically important differences in odour or taste acceptability when the replacers, potassium lactate and potassium chloride, in total amounts of 50% of the sodium chloride, were used.

*Ibañez et al.* (1997) did not find differences in overall acceptability between dry fermented sausages produced with 3% sodium chloride (common amount) and sausages produced with 1.5% sodium chloride and 1% potassium chloride, whereby the sodium content was decreased by one half.

The results of the instrumental determination of cut surface colour of sausages are presented in Table 3. In this study, only the lightness of control sausages (32.57±1.43) was significantly

**Table 2.** Sensory evaluation of sausages

| Group | Surface colour | Cut colour | Saltiness | Bitterness | Overall acceptability |
|---|---|---|---|---|---|
| **Control** | 4.94±0.16 | 4.72±0.42 | 4.22±0.32$^{a}$ | 1.39±0.46$^{x}$ | 4.67±0.47$^{x}$ |
| **1** | 4.94±0.16 | 4.72±0.42 | 3.78±0.79$^{b}$ | 2.06±0.90$^{y}$ | 3.56±1.26$^{y}$ |
| **2** | 4.94±0.16 | 4.72±0.42 | 3.61±0.66$^{b}$ | 3.94±1.07$^{z}$ | 2.89±0.74$^{z}$ |
| **3** | 4.94±0.16 | 4.72±0.42 | 3.56±0.86$^{b}$ | 3.56±0.86$^{z}$ | 2.78±0.92$^{z}$ |
| **4** | 4.83±0.33 | 4.72±0.42 | 3.61±0.94$^{b}$ | 2.89±0.34$^{q}$ | 3.83±0.58$^{y}$ |

$^{a,b}$ Numbers within one column with different superscript letters are significantly different (P≤0.05)

$^{x, y, z, q}$ Numbers within one column with different superscript letters are significantly different (P≤0.01)

**Table 3.** Results of the instrumental determination of cut surface colour of sausages, CIE Lab system

| Group | L* – lightness | a* – redness | b* – yellowness |
|---|---|---|---|
| **Control** | 32.57±1.43[a] | 14.61±2.80 | 5.35±1.44 |
| **1** | 34.80±1.54[b] | 15.72±1.98 | 6.91±1.04 |
| **2** | 36.11±2.20[b] | 15.48±1.71 | 6.44±1.01 |
| **3** | 36.14±2.50[b] | 14.78±1.94 | 6.40±1.52 |
| **4** | 35.25±1.53[b] | 17.23±1.00 | 7.04±0.90 |

a,b Numbers within one column with different superscript letters are significantly different (P≤0.05)

lower (P≤0.05) compared to the lightness of group 1 sausages (34.80±1.54), group 2 sausages (36.11±2.20), group 3 sausages (36.14±2.50) and group 4 sausages (35.25±1.53), while there were no differences between lightness values of sausages from these four groups (P≥0.05). No significant differences (P≥0.05) were determined between redness or yellowness for all examined groups of sausages. The total colour difference (ΔE) between: control and group 1 sausages was 2.94; control and group 2 sausages was 3.80; control and group 3 sausages was 3.72 and; control and group 4 sausages was 4.11.

*Gimeno et al.* (1998) reduced the salt content in Chorizo sausage, using a mixture of 1% sodium chloride, 0.55% potassium chloride, 0.23% magnesium chloride and 0.46% calcium chloride, with the aim of replacing some of the 2.6% sodium chloride that is common for this sausage. They determined that sensory acceptability was reduced due to reduced saltiness intensity as well as decreased red colour intensity because of the reduction in the amounts of nitrosohaeme pigments. Instrumentally measured colour (CIE L*a*b*) showed there were no important difference in the colour between control and experimental group of sausages.

The sodium levels in the sausages are presented in Table 4. As would be expected, the highest sodium content was determined in control sausages (16084.15±1156.50) due to usage of only sodium chloride and this level was statistically higher than the sodium content determined in sausages from other groups (P≤0.01). Sodium levels were similar in sausages from group 1 and 4 sausages (14620.78±475.22 and 14197.06±11.73, respectively), and these were significantly higher than the sodium content determined in group 2 and 3 sausages (9847.71±847.30 and 10706.42±459.37, respectively). Moderate sodium reductions were seen in group 1 sausages of 9.09%, in group 2 of 38.77%, in group 3 of 33.43% and in group 4 of 11.73% in relation to control sausages.

**Table 4.** Sodium content in sausages

| Group | Sodium, mg/kg |
|---|---|
| **Control** | 16084.15±1156.50[x] |
| **1** | 14620.78±475.22[y] |
| **2** | 9847.71±847.30[z] |
| **3** | 10706.42±459.37[z] |
| **4** | 14197.06±11.73[y] |

x,y,z Numbers within one column with different superscript letters are significantly different (P≤0.01)

## Conclusion

Moderate reductions of sodium chloride in the production of dry fermented sausages by partial replacement of sodium chloride with potassium chloride (group 1) and with ammonium chloride (group 4) led to slightly reduced saltiness, although this was still at an acceptable level. Also, sausages from these two groups were acceptable and their overall acceptability was evaluated favourably, despite statistical differences from the overall acceptability of sausages from the control group.

The most highly expressed bitterness was determined in group 2 and 3 sausages, and these sausages

were significantly more bitter than the moderately bitter group 1 and 4 sausages, as well as the control sausages.

The use of different salt replacer mixtures did not affect redness (a*) or yellowness (b*) in sausages from all groups, but did lead to greater expressed lightness in all sausage groups compared to sausages from the control group.

Moderate sodium reductions were seen in group 1 sausages of 9.09%, in group 2 of 38.77%, in group 3 of 33.43% and in group 4 of 11.73% in relation to control sausages.

## References


**Askar, A., El-Samahz S. K., Shehata, H. A. & Tawfi, k M. (1993).** Pasterma and beef bouillon. The effect of substituting KCl and K/lactate for sodium chloride. *Fleischwirtschaft, 73*, 289–292.

**Cappuccio, F. P., Kalaitzidis, R., Duneclift, S. & Eastwood, J. B. (2000).** Unravelling the links between calcium excretion, salt intake, hypertension, kidney stones and bone metabolism. *Journal of Nephrology, 13*, 169–177.

**CIE: Colorimetry: Official Recommendations of the International Commission on Illumination, (1976)**. Paris: *Comisión Internationale de l'Èclairage* [International Commission on Illumination] (CIE No. 15 (E-1.3.1)

**Devine, A., Criddle, R. A., Dick, I. M., Kerr, D. A. & Prince, R. L. (1995)**. A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. *American Journal of Clinical Nutrition, 62*, 740–745.

**Gimeno, O., Astiasarán, I. & Bello, J. (1998).** A mixture of potassium, magnesium, and calcium chlorides as a partial replacement of sodium chloride in dry fermented sausages. *Journal of Agricultural and Food Chemistry, 46*, 4372–4375.

**Gou P., Guerrero L., Gelabert J. & Arnau J. (1996).** Potassium chloride, potassium lactate and glycine as sodium chloride substitutes in fermented sausages and in dry cured pork loin. *Meat Science, 42*, 1, 37–48.

**Guàrdia, M. D., Guerrero, L., Gelabert J., Gou P. & Arnau, J. (2006).** Consumer attitude towards sodium reduction in meat products and acceptability of fermented sausages with reduced sodium content. *Meat Science 73*, 484–490.

**Ibañez, C., Quintanilla, L., Astiasarán, I. & Bello J. (1997).** Dry fermented sausages elaborated with *Lactobacillus plantarum – Staphylococcus carnosus*. Part II. Effect of partial replacement of NaCl with KCl on the proteolytic and insolubilization processes. *Meat Science, 46*, 277–284.

**Kuroda, S., Uzu T., Fujii, T., Nishimura, M., Nakamura, S., Inenaga, T. & Kimura, G. (1999)**. Role of insulin resistance in the genesis of sodium sensitivity in essential hypertension. *Journal of Human Hypertension, 13*, 257–262.

**MacGregor, G. A. & de Wardener, H. E. (1997)**. Idiopathic edema. In: *Schrier, R.W., Gottschalk CW, eds. Diseases of the Kidney*. Boston, MA: Little Brown and Company, 2343–2352.

**Mickleborough, T. D., Lindley, M. R. & Ray S. (2005)**. Dietary salt, airway inflammation, and diffusion capacity in exercise-induced asthma. *Medicine and Science Sports Exercise*, 37, 904–914.

**Perry, I. J. & Beevers D. G. (1992)**. Salt intake and stroke: a possible direct effect. *Journal of Human Hypertension, 6*, 23–5.

**Ruusunen, M. & Puolanne E. (2005).** Reducing sodium intake from meat products. *Meat Science, 70, 3*, 531–541.

**Schmieder, R. E. & Messerli, F. H. (2000)**. Hypertension and the heart. *Journal of Human Hypertension, 14*, 597–604.

**Terell, R. N. (1983)**. Reducing the sodium content of processed meats. Food Technology, 37, 7, 66–71.

**Tsugane, S., Sasazuki, S., Kobayashi, M. & Sasaki S. (2004)**. Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. *British Journal of Cancer, 90*, 128–134.


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*Review paper*

# Bacterial hazards in fish meat: The aetiologic agents of foodborne diseases

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*A b s t r a c t: Nowadays, consumers, fish industry professionals and scientists are increasingly directing their attention towards safety requirements associated with the consumption of fish due to the presence of bacterial hazards. There is no doubt that good knowledge and management of bacterial hazards associated with the consumption of fish are of great economic and medical importance. This study describes the bacterial pathogens that most often cause fishborne outbreaks of disease (Vibrio, Escherichia coli, Staphylococcus aureus, Salmonella, Aeromonas, Clostridium, Campylobacter, Listeria), as well as some individual disease outbreaks. In addition, the measures that can be implemented to reduce the risk of bacterial hazards related to consumption of fish are reviewed. The most important risk factor regarding bacterial zoonoses where fish was a vector was consumption of raw or undercooked processed fish, including cold smoked fish, but recontamination is also an important risk factor.*

**Key words:** *fish, microbiological safety, monitoring, zoonotic diseases, heat treatment.*

## Introduction

The fish trade is bringing increasing economic benefits at the global level, but given the facts that natural resources are limited and that further increases in the amount of wild fish harvested is unsustainable, aquaculture is becoming one of the fastest growing industries in the production of food of animal origin. The rapid growth of aquaculture production is undoubtedly the result of increased demand for fish. One of the reasons is the well-known fact that fish meat is, to a much lesser extent, the cause of zoonotic diseases compared to the meat of other farm animals (*Baltic et al.,* 2009). In addition, processed fish products contain much fewer additives than meat products from other species (*Okanovic et al.,* 2013ab). It is well known that fish meat is a valuable source of nutrients that are present in optimal amounts for human requirements (*Cirkovic et al.,* 2011; 2012; *Ljubojevic et al.,* 2013abc) and the fact that this is a very high-quality food is the main reason for increased consumption of fish meat worldwide. Despite the global increase in the consumption of fish meat, average annual fish consumption per capita in Serbia is significantly lower than the global and European average – only 7 kg (*Janjic et al.,* 2015). Increased fish consumption and changed consumer habits will likely result from continuously promoting this highly valuable food.

Scientific research related to aquaculture is of increasing importance and objectives of this research are improved production (*Spriric et al.,* 2009; *Trbovic et al.,* 2009; *Ljubojevic et al.,* 2013d), as well as the preservation of sensory properties of fish meat (*Babic et al.,* 2009, 2014, 2015). A large number of authors from different countries have studied the chemical composition of fish meat, as well as its nutritive value (*Vranic et al.,* 2010; 2011; *Zsuzsánna et al.,* 2011; *Trbovic et al.,* 2013; *Ljubojevic et al.,* 2014; 2015; *Pavlicevic et al.,* 2014). Studies have also included the safety of fish meat, such as the content of chemical pollutants (heavy metals, persistent organic pollutants) in the fish (*Djinovic et al.,* 2010; *Trbovic et al.,* 2011; *Jankovic et al.,* 2012; *Djinovic-Stojanovic et al.,* 2013; *Djordjevic et al.,* 2013), as well as the microbiological quality of the fish meat (*Davies et al.,* 2001; *Cabrera-García et al.,* 2004; *Milijasevic et al.,* 2012). Today, the attention of consumers as well as fish industry professionals and scientists is increasingly directed towards the safety requirements associated with the consumption of fish meat, due to the presence of bacterial hazards. Taking these factors into account, there is a need to emphasise the bacterial pathogens that most commonly cause illness after consumption of fish meat.

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In addition, the measures that can be implemented to minimise the risk to human health of bacteriological hazards in fish meat are described in the present study. The main objectives of this study were to describe the most important bacterial hazards in fish meat, to point out the most important measures to ensure the safety of fish meat and to raise public awareness when it comes to the safety of fish meat.

## Fish as the cause of foodborne diseases

The main causes of foodborne illness according to the available epidemiological data and risk analyses are microorganisms and microbial toxins (*Buncic and Katic*, 2011). Diseases caused by fish contaminated with microorganisms usually occur after consumption of inadequately heat-treated fish or fish products, including cold-smoked fish, which can be contaminated both during and after production. Outbreaks of foodborne illness, including food poisoning, associated with the consumption of fish and fish products have been recorded in almost all European countries (*EFSA,* 2014), as well as countries around the world. This indicates that the microbiological safety of fish meat on the market is a very important parameter in terms of public health, and there is great public interest because consumption of these products can lead to serious health problems. *Huss et al.* (2000a) found that about 12% of disease outbreaks caused by fish in the United States (US) were of bacterial aetiology (*Clostridium botulinum*, *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Vibrio*, *Bacillus cereus*). *Shigella*, *Vibrio*, *Aeromonas*, *E. coli* and *Campylobacter* are pathogenic bacteria that can be transmitted to humans (*Djordjevic et al.,* 2012; *Raissy et al.,* 2014). *Listeria*, *Salmonella*, *Staphylococcus aureus* and *C. botulinum* type E are potential hazards in cold smoked fish (*Gram,* 2001; *Gram and Dalgaard,* 2002; *Dondero et al.,* 2004). Pathogens that are naturally found in water (*C. botulinum* type E, *Vibrio*, *Aeromonas*) or generally in the environment (*C. botulinum* types A and B, *Listeria monocytogenes*) can normally be found in both live fish and fish immediately after slaughter (*Huss et al.,* 2000a). However, if they are in insufficient numbers to cause illness, they do not pose a risk to consumers. Thus, the International Commission on Microbiological Specifications for Foods (ICMSF) found that food containing less than 100 *L. monocytogenes* $g^{-1}$ is not a risk to the health of people who are not particularly vulnerable (*Vaz-Velho et al.,* 2001). A serious health concern exists when a very low infectious dose is capable of inducing disease, as is the case for *Salmonella, Shigella, E. coli* O157:H7, or in cases where there is potential for growth and the production of toxins (such as toxin production by *C. botulinum* type E) in raw or processed fish (*Huss et al.,* 2000a). It is known that fish meat is very susceptible to microbial deterioration/spoilage, mostly due to *Shewanella putrefaciens*, *Pseudomonas*, *Vibrio* and *Aeromonas* (*Milijasevic et al.,* 2010).

The health-hygienic properties of farmed fish meat are influenced by the entire production chain, from the environmental conditions in the pond, the quality of food used to feed fish, handling during harvesting, slaughter, evisceration and further processing to storage, transport and sale (*Orban et al.,* 2008; *Milijasevic et al.,* 2012), which forces producers along the chain to fulfil requirements associated with quality, and above all, consumer safety. It should be noted that modern principles that apply to food safety, including Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Points (HACCP) are required to improve the safety of fish meat for consumers. The main objective of HACCP is the control of biological, chemical and physical harmful agents that could be hazardous to human health (*Joffraud et al.,* 2001, 2006; *Djordjevic et al.,* 2006).

It is very often difficult to determine the exact number of people who suffer from a bacterial disease occurring as a result of fish meat consumption, since in most cases, especially when it comes to diseases of the gastrointestinal tract, they are not reported because symptoms usually do not last long in healthy individuals. In addition to exposure to environmental factors, internal factors, such as the physiological state of an individual, and especially their immunosuppression and stress status, contribute significantly to the development of infectious diseases. Temperature, water activity and pH are among the most important factors affecting the survival and growth of bacteria in fish meat and fish meat products, and this has led to these factors being used in different processes and heat treatments in order to ensure longer shelf life of fish meat, as well as to prevent the occurrence of foodborne illness in humans. Compulsory hygiene requirements for staff handling food are found in EU legislation (*European Council*, 2004). Microbiological criteria, including sampling plans and methods to be applied in the analyses are provided when there is a need to protect public health. Microbiological criteria for fish and fish meat products require quantification of *E. coli* and pathogenic *V. parahaemolyticus* and are implemented during production. At the end of the production cycle, monitoring measures are quantification of *S. aureus* and detection of

*Salmonella*, and their presence indicates recontamination of the finished product (*European Council*, 1991).

The microbiological quality of fish on the market is of concern for public health due to the incidence of foodborne diseases associated with the consumption of fish and fish products in European countries (*EFSA*, 2014). The European Union (EU) has established monitoring systems which include notification and management for biological hazards in foods including fish meat. The systems are supported by EU legislation and coordinated by the European Commission (EC), the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (*Ribó et al.*, 2009). Collection of relevant data on biological hazards is crucial and these data contribute to risk assessments conducted by EFSA. Taking into account the opinion of the Scientific Committee on Veterinary Measures relating to Public Health, the EU Commission adopted microbiological criteria for foods (*European Commission*, 2005). Harmonisation of legislation in the field of food safety in Serbia with the EU includes the harmonisation of legislation on microbiological criteria for foods. In 2010, the Ministry of Agriculture, Forestry and Water Management adopted a new food hygiene regulation (*Serbia*, 2010) which is compliant with EU regulation (*European Commission*, 2005) and in accordance with Serbian food safety law (*Serbia*, 2009). Undoubtedly, though, there is still a need to establish permanent monitoring, improve the management of microbiological hazards, and utilise all other elements in risk analysis to make the Serbian food safety system function according to the principles of risk analysis.

## The most important bacterial hazards isolated from fish

### *Vibrio*

Consumption of raw or inadequately cooked fish infected with *Vibrio* can cause gastroenteritis in humans (*Raissy et al.,* 2012). *Vibrio parahaemolyticus*, in most cases, produces acute gastroenteritis which usually passes unreported and has a very short duration, but in some cases hospitalisation is necessary, and very rarely, it leads to septicaemia. *V. parahaemolyticus* is frequently isolated from fish and other aquatic organisms throughout the year in tropical areas and during the summer in areas with moderate or cold climates (*Cabrera-García et al.,* 2004). Some traditional Asian dishes such as fish-balls, fried mackerel, tuna and sardines have been associated with infections caused by *V. parahaemolyticus*. These meals can include both raw and undercooked fish and fish products, or heat-treated products which are subject to recontamination (*Baffone et al.,* 2005). *V. parahaemolyticus* was first established as a foodborne pathogen in Japan in the 1950s (*Fujino et al.,* 1974). In the suburbs of Osaka in Japan in October 1950, 272 patients developed gastritis and 20 people died as a result of the consumption of semi-dry small sardines known as shirasu. The occurrence of diarrhoea caused by *V. parahaemolyticus* was recorded in Japan and Taiwan after consumption of inadequately cooked fish and raw local dishes such as sushi and sashimi (*Vuddhakul et al.,* 2000). Disease caused by *V. parahaemolyticus* occurs most often in Japan, Taiwan and other Asian coastal regions, although cases of outbreaks of disease have been observed in many countries around the world. However, cases of disease caused by *V. parahaemolyticus* in Europe occur sporadically. During twenty years, only two cases of *V. parahaemolyticus* gastroenteritis were recorded in Denmark (*Joseph et al.,* 1982). *Smolikova et al.* (2001) isolated *V. parahaemolyticus* and *Vibrio alginolyticus* from patients during an outbreak of acute enteric diseases in Russia in 1997 and the *V. parahaemolyticus* isolates from humans produced thermostable exotoxin haemolysin. They proved that the alimentary toxico-infection was caused by *V. parahaemolyticus* O3:K6. Acute enteric disease was described in Russia in 1999 (*Boĭko,* 1999), wherein the aetiological agents were *V. fluvialis* (30.3%), *V. parahaemolyticus* (27.3%), *V. costicola* (21.2% ) and *Photobacterium damselae* (21.2%) and according to the results of that study, vibriosis inducers, with the exception of *V. costicola*, were more associated with contamination of water than with contamination of fish. In the US, the occurrence of gastroenteritis in 14 people was recorded as a result of *V. parahaemolyticus* during the 1970s, and sporadic cases were recorded during the 80s and 90s, whereby 59% of the cases manifested as gastroenteritis, 34% as wound infections, 5% as septicaemia, and 2% as other symptoms, with most of these disease incidences occurring during the hot summer months and being associated with marine fish, and especially with molluscs (*Daniels et al.,* 2000). Normally, rehydration is sufficient for recovery after food poisoning caused by *V. parahaemolyticus*, and the use of antibiotics should be reserved for serious cases of illness that last a long time. *V. alginolyticus* has been described in fish and shellfish in Europe (*Di Pinto et al.,* 2005).

*Vibrio cholerae*

Cholera is a highly contagious disease caused by infection of the small intestine with *Vibrio cholerae* O1 or O139 and is characterised by severe acute diarrhoea, vomiting and consequent dehydration, while death may occur due to severe and untreated infections (*Colwell,* 1996). *V. cholerae* is mainly transmitted through water, although fish and fish products that have been in contact with contaminated water or faeces of infected persons can also be a source of infection (*Rabbani and Greenough,* 1999). *Kam et al.* (1995) recorded 12 outbreaks of cholera caused by *V. cholerae* biotype El Tor inaba, which occurred in Hong Kong for a period of three weeks in June and July of 1994. Only adults of both sexes were affected. Epidemiological research connected all cases with the consumption of seafood, including mussels, crabs and shrimp, and microbiological test results showed that contaminated sea water in the holding tanks used to keep the animals was most likely the source of the infection. Extensive control measures were implemented that involved a ban on the use of contaminated water in the tanks, rigorous microbiological control of used sea water, control of storage and more. These measures were enhanced through an active campaign and health education on food safety and personal hygiene and abruptly ended the epidemic.

*Escherichia coli*

The presence of *Enterobacteriaceae* in food and water is a common cause of diarrhoea and dysentery, especially among children, and *E. coli* is a classic example of a bacterium that leads to the onset of the disease. *E. coli* is the most common enterobacteria that causes gastrointestinal illness, and it is known that these diseases are the most common causes of mortality and morbidity in developing countries. It is important to note that in addition to other coliform bacteria, the presence of *E. coli* indicates poor hygienic conditions during processing of fish, because these microorganisms are not found on freshly harvested fish (*Milijasevic et al.,* 2012). Contamination of fish or fish products with pathogenic strains of *E. coli* most likely occurs during handling or processing of fish. Fish and fish products are often the vector of infection, especially if they are sold at local fish markets that do not meet the elementary hygienic conditions (*Vieira et al.,* 2001). Verocytotoxic *E. coli* (VTEC) are a group of *E. coli* which are characterised by the ability to produce verocytotoxins (synonym Shiga-like toxin). Most reported cases of human infection with VTEC are sporadic cases. The symptoms associated with VTEC infection in humans range from mild to bloody diarrhoea, which is usually accompanied by stomach cramps, commonly without the appearance of fever. VTEC can lead to haemolytic uremic syndrome (HUS), characterised by acute renal failure, anaemia and decrease in the number of platelets. HUS occurs in approximately 10% of patients infected with VTEC O157:H7 and this syndrome is the leading cause of acute kidney failure in children. Fish and fish products were the vector in 9.2% of foodborne outbreaks of VTEC associated disease in the EU in 2012 (*EFSA,* 2014). In Vietnam, *E. coli* was isolated from raw fish (*Dao and Yen,* 2006). *Mitsuda et al.* (1998) described disease that was manifested by diarrhoea, and caused by ingestion of food contaminated with enterotoxigenic *E. coli* (ETEC) in Japan. The outbreak was recorded in four Japanese primary schools in 1996 and spread to more than 800 people, while the disease itself was associated with tuna pâté. Examination of faeces from symptomatic patients revealed the presence of ETEC O25:NM that produced a heat stable toxin. *Vieira et al.* (2001) isolated 18 strains of ETEC in 3 of 24 samples of raw fish taken from the market in Brazil, 13 of which produced heat-labile enterotoxin. *Ayula et al.* (1994) also isolated (from 37.7% of samples) 317 *E. coli* isolates from fish in Brazil. *Asai et al.* (1999) described the occurrence of diseases caused by salted salmon roe most likely contaminated during the manufacturing process with enterohaemorrhagic *E. coli* O157:H7 in Japan in 1998. *Pierard et al.* (1999) recorded infection with VTEC as a result of fish consumption in Belgium.

*Staphylococcus aureus*

*S. aureus* is a pathogen of public health concern in fish and fish products (*Vieira et al.,* 2001). *Ayula et al.* (1994) concluded that much more care should be taken during harvesting and post-harvest handling of fish and seafood in order to reduce contamination with *S. aureus*. In research carried out by *Ayula et al.* (1994) on fish, 20% of 175 samples examined carried *S. aureus*, including 60% of the samples of shellfish meat. However, only 9 of 109 strains of *S. aureus* produced enterotoxins, including enterotoxin A (4), D (1) and AB (4). During growth in foods, many strains of *S. aureus* produce enterotoxins, which can cause staphylococcal food poisoning. Seven different enterotoxins have been identified: A, B, C1, C2, C3, D and E. Enterotoxin A is the most common enterotoxin involved in cases of *S. aureus* food poisoning (*Bergdoll,* 1990). In cases of food poisoning with *S.* aureus*,* vomiting and diarrhoea typically occur 2 to 6 hours after intake of food containing one or more enterotoxins (*Ayula et*

*al.,* 1994). *Vieira et al.* (2001) detected, in 3 of 10 samples of fresh fish, higher counts of *S. aureus* than allowed according to Brazilian legislation. *Eklund et al.* (2004) studied *S. aureus* during the process of drying and smoking salmon in Alaska. During the process, the *S. aureus* counts increased to more than 105 CFU $g^{-1}$, after 2 to 3 days. Subsequent laboratory tests determined that there was rapid wrinkling of the skin on the smoked fish when there was strong air circulation in the smoking room, resulting in the bacteria being tied to or pulled into the creases, and thus, they were able to grow in spite of smoke deposition. The elimination of drying as a preceding process and reduction of the air flow during smoking resulted in deposition of smoke before creases formed on the skin and allowed the product to more quickly reach the stage where the salt and water activity inhibited the growth of *S. aureus*. This modification was then applied during fish drying and *S. aureus* was not isolated from the final product.

*Salmonella*

Salmonellosis in humans is usually characterised by acute occurrence of high temperature, fever, abdominal pain, nausea and vomiting after the incubation period, which lasts 12-36 hours (*Round and Mazmanian*, 2009). Symptoms are usually mild and in such cases the disease lasts only for several days, but the illness can be more serious in some patients, particularly when dehydration occurs, and it can lead to a lethal outcome. However, the mortality rate is usually very low, below 1% in patients who are diagnosed with *Salmonella* (*Day et al.,* 2011). *Friesema et al.* (2012) recorded a large number of cases of infection with *S.* Thompson in 2012, where the vector was smoked salmon, and some cases developed systemic infections such as septicaemia. Salmonellosis is also associated with long-term and sometimes chronic consequences, such as reactive arthritis. In the view of *Das et al.* (2010), since no clinical symptoms caused by *Salmonella* appear in fish, they are probably just passive carriers of *Salmonella*. However, the importance of fish in human salmonellosis is that they excrete *Salmonella* into the environment. *Fenlon* (1983) observed that aquatic birds carry *Salmonella* strains which can be found in the environment and, consequently, *Salmonella* can be isolated from pond-farmed fish (*Lotfy et al.,* 2011). In the Czech Republic and Latvia, *Salmonella* was not isolated in fish from streams, or farmed fish (*Hudecova et al.,* 2010; *Terentjeva et al.,* 2015). In contrast, in Germany, salmonellosis caused by *S.* Blockley where the vector consumed was smoked eel carrying *Salmonella*, originating from fish farm ponds in Italy as reported by *Fell et al.* (2000) who further stated that the smoking process does not eliminate bacterial contamination of raw fish. *Ling et al.* (2002) described illness caused by multi-drug resistant *S.* Typhimurium DT104L, after consumption of dried anchovies in Singapore. In Kenya and Malaysia, the presence of *Salmonella* in fish meat was associated with poor hygiene and unsanitary handling of fish during harvesting, processing and sale (*David et al.,* 2009; *Budiati et al.,* 2013).

*Aeromonas*

Foodborne gastroenteritis caused by *Aeromonas* (*Feldhusen,* 2000) is described in people of all ages and is particularly prevalent among vulnerable groups such as very young children and older immunocompromised people. *Aeromonas hydrophila* has been isolated from freshwater fish, crustaceans and molluscs (*Tsai and Chen* 1996; *Karabasil et al.,* 1999*; Djordjevic et al.,* 2012). *Karabasil et al.* (2002) studied motile *Aeromonas* in fish and other seafood products obtained from Belgrade retail markets in Serbia. They isolated nine motile *Aeromonas* from 78 fish/seafood products, three *A. sobria* and six *A. hydrophila*. Eight isolates were from freshwater fish and one from marine fish. Enteropathogenic strains are generally *Aeromonas veronii* sobria and *A. hydrophila*. *Aeromonas* can produce different exotoxins (*Karabasil et al.,* 2002) some of which are enterotoxins.

*Clostridium*

*Khatib et al.* (1994) reported that *Clostridium perfringens* was the causative agent of food poisoning after consumption of tuna salad. *Weber et al.* (1993) described a case of botulism after consumption of fish salads, and the causative agent was *C. botulinum* type B. *Telzak et al.* (1990) reported eight cases of type E botulism where the epidemiological survey found that all eight patients consumed kapchunka, fish that are not eviscerated, but are salted and air dried whole. There was no record that the fish had been prepared incorrectly, but nonetheless, the small amount of salt in the abdominal cavity and internal organs allowed the multiplication of bacteria and toxin production.

*Campylobacter*

The average incubation time for *Campylobacter* in humans is from two to five days. Mild or serious symptoms can appear in patients, and common clinical symptoms include watery and

sometimes bloody diarrhoea, abdominal pain, fever, headache and nausea, while infections are usually self-limiting and only last for few days. In rare cases, extra intestinal infection or post-infection complications can occur, such as reactive arthritis and neurological disorders. It is important to note that *Campylobacter jejuni* is the most common predecessor of Guillain-Barré syndrome, a form of paralysis that can lead to respiratory disorders and severe neurological disorders and even death. *Campylobacter* is commonly found in various types of food, including meat, raw milk and dairy products, fish and fish products, a variety of seafood and fresh vegetables (*Kumar et al.,* 2001). The presence of *Campylobacter* has been recorded in fish and fish products from around the world (*Feldhusen,* 2000; *Raissy et al.,* 2014).

*Listeria*

Listeriosis occurs rarely in humans, but it is a very serious and severe zoonotic disease with high morbidity, a large number of hospitalisations, and a very high mortality rate in susceptible groups. Foods which are suitable for the growth of *Listeria* include thermally unprocessed food, food that is stored for a long time, food that is produced in unsanitary production facilities, and ready-to-eat prepared meals. Listeriosis is characterised by mild but also more severe symptoms such as meningitis, encephalitis, septicaemia, which usually occur in susceptible groups. The occurrence of *L. monocytogenes* in smoked salmon is very common (*Vaz-Velho et al.,* 2001; *Rotariu et al.,* 2014), and therefore is an issue of public health concern since this fish product is usually consumed without further heat treatment. *Kuzmanovic et al.* (2011) examined fish, fish products and seafood from the Serbian market, including chilled fresh fish, frozen foods (fish and seafood – cuttlefish, squid, octopus, clams, crabs and shrimp), breaded products, smoked fish, salted fish, heat-treated fish and canned fish for *Listeria*. *Listeria* was detected in 58 samples (12.34%) of fish, fish products and seafood. Among the isolates, nine (15.52%) were *L. monocytogenes* (1.92% of the fish harboured this species). Other *Listeria* species found were: *L. innocua* (8.51%), *L. welshimeri* (1.28%), *L. welshimeri/innocua* (0.21%), *L. grayi* (0.21%) and *L. seeligeri* (0.21%). The presence of other species of *Listeria* in fish and fish products indicates failures of GHP during production (*Round and Mazmanian*, 2009). The prevalence of *L. monocytogenes* recorded in freshwater farmed and wild fish harvested in Denmark and Finland was 8.6% and 14.6%, respectively (*Vogel et al.,* 2001 ; *Miettinen and Wirtanen,* 2005). During 2012, 17 EU member states reported information on the presence of *L. monocytogenes* in fish prepared for consumption and fish products (*EFSA,* 2014). Smoked fish was the product that was most commonly monitored, and most of the tests were carried out within the production plant. During 2012, the presence of *L. monocytogenes* in fish prepared for consumption was found in 12 out of 16 qualitative studies. In total, *L. monocytogenes* was found in 12.0% of the total surveyed 10,831 units, although the lack of representativeness must be noted when interpreting the overall results, since most of the samples were from Poland. Additionally, *L. monocytogenes* was recorded in 9 of 16 quantitative studies of fish stored for consumption in 2012, wherein a total of 6,141 units were tested, and in six studies, counts >100 cfu $g^{-1}$ were obtained. *L. monocytogenes* counts of over 100 cfu $g^{-1}$ were recorded in 1.4% of the fish samples tested by the counting method in 2012 (compared to 0.5% in 2011). However, this increase can be attributed to the large number of samples from Poland included in the study. Listeriosis associated with vacuum-packed, raw, thinly-sliced cold-smoked fish was described in at least eight people during 11 months in Sweden (*Tham et al.,* 2000). Cold-smoked, raw and thinly-sliced trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) have been the focus of attention in recent years as potential sources of infection with *L. monocytogenes*. Studies have shown that up to 10% of vacuum-packed products in retail contain *L. monocytogenes* (*Norton et al.,* 2001). In the Czech Republic, two deaths from *Listeria* infection resulted from consumption of herring without heat treatment.

## The importance of heat treatment and the cold chain

Heat treatment during the cooking process eliminates the risk of pathogens such as *Salmonella, Shigella* and *E. coli* (plus enteric viruses) which contaminate fish prior to harvesting and can pose a risk, since in some cases, a very low infectious dose is sufficient to produce disease (*Huss et al.,* 2000a). Therefore, the risk of these pathogens causing disease in humans is primarily related to the consumption of raw fish. *Gould* (1999) found that the combination of heat treatment at 90°C for a period of 10 minutes led to a 6 log reduction in numbers of spores of psychrotrophic *C. botulinum*, so this heat treatment is undoubtedly advantageous. Heat treatment

at 70°C for 2 min ensures destruction of *L. monocytogenes* in fish (*Huss et al.,* 2000b). Moderate heat treatment is acceptable for products with a short storage time, or where any potential growth of pathogens can be prevented.

Cold-stored fish products can allow the survival or growth the survival of psychrotrophic pathogens (*Huss et al.,* 2000a). Reducing bacterial proliferation is achieved by rapid cooling of fish immediately after harvest, thus somewhat reducing the risk to human health. A range of pathogenic bacteria can be isolated from fresh fish, especially *L. monocytogenes, C. botulinum* types A and B, *C. perfringens*, *Bacillus*, and also bacteria that are transmitted/carried by humans or animals (*Salmonella, Shigella, E. coli, S. aureus*) and rarely *C. botulinum* type E and non-proteolytic types B and F, pathogenic *Vibrio* species, *A. hydrophila,* and *Plesiomonas shigelloides*.

## The impact of fish packaging on the growth of microorganisms

The biggest concerns when packing fish under anaerobic conditions are *C. botulinum* type E and non-proteolytic type B and *L. monocytogenes* (*Baltic et al.,* 2009). *Milijasevic et al.* (2010) examined chemical, physico-chemical and microbiological changes in carp steaks packaged in a modified atmosphere during fifteen days of storage. They found that the rate of the chemical and microbiological breakdown of fish meat can be affected by the way the product is packaged (*Milijasevic et al.*, 2010). Modified atmosphere packaging has a synergistic effect on bacterial growth compared with vacuum-packaging, as the 40 to 100% $CO_2$ in the headspace gas inhibits microbial growth, primarily *Pseudomonas, Vibrio* and *Aeromonas*. This gas diffuses into the fish tissue, dissolves in the aqueous phase and creates carbonic acid, which slows down the oxidation process. Nitrogen can be used to replace oxygen in the package, as it slows down the formation of rancidity and inhibits the growth of aerobic microorganisms. Oxygen is also used in the modified atmosphere packaging technology for fish, as it inhibits the growth of *C. botulinum* type E, which is often found in fish (Özogul and Özogul, 2006; *Radetic et al.,* 2007). Modified atmosphere packaging significantly reduces the total number of *Enterobacteriaceae* on fish (*Milijasevic et al.,* 2010). *Babic et al.* (2009) found that the shelf-life of chilled fresh fish can be extended in vacuum or modified atmosphere packaging.

## Measures to reduce biological hazards in fish meat

The most important risk factor when it comes to bacterial zoonoses, as is the case for parasitic zoonoses (*Novakov et al.,* 2012; *Cirkovic et al.,* 2013), is the consumption of raw or inadequately heat processed fish meat. In addition, recontamination after heat processing is a significant risk factor. It is worth noting that the number of cases of disease caused by consumption of fish as a source and/or vector of pathogenic bacteria is very low when compared with the number of cases that are caused by the consumption of poultry meat, pork, beef and meat obtained from other farm animals (*Newell et al.,* 2010). Reduction of the initial microbial contamination is one of the main strategies in most countries aiming to reduce the risk associated with fish meat, but if conditions are suitable for pathogen growth, the initial level of contamination is of relatively little significance (*Beuchat*, 2006). With this in mind, more attention must be paid to the role of temperature, water activity, pH and other extrinsic factors which can significantly affect the microbiological safety of fish meat, as well as the interaction between these parameters to reduce the occurrence and/or numbers of pathogenic bacteria to a minimum. Preventing pre-harvest contamination with pathogens that are naturally found in the aquatic environment is either very difficult or in many cases, impossible, while hazards associated with contamination or recontamination during the processing of fish can be controlled by applying GMP, GHP and suitable HACCP programs (*Panisello and Quantick*, 2001). In addition, there are methods to prevent the growth of biological hazards during transportation and storage of fish meat and fish meat products (*Sivertsvik et al.,* 2002). Hazard analysis and qualitative assessment of the risk of microbiological contamination of fish meat are very important and a connection between these measures and the frequency of disease outbreaks caused by fish meat that is microbiologically contaminated has been observed (*Huss et al.,* 2000a). Continuous medical education is certainly a key factor in fighting zoonotic infections, and experience in many countries has shown that successful implementation of control measures requires broad cooperation between medical and veterinary bodies, including government departments at all levels, and in addition, the cooperation of and constant communication with the public is necessary. However, avoiding consumption of raw or inadequately cooked fish is still the best measure to prevent fishborne disease caused by bacterial pathogens.

## Conclusions

The following conclusions and recommendations can be made:

- The microbiological quality and safety of fish available on the market is very important from the standpoint of public health.
- Good knowledge and management of microbiological hazards associated with the consumption of fish meat is of great economic and health importance.
- Fish can be contaminated with bacterial pathogens pre- or post-harvest, depending on the pathogen.
- The most important bacterial hazards isolated from fish which cause foodborne outbreaks include *Vibrio*, *E. coli*, *S. aureus*, *Salmonella*, *Aeromonas*, *Clostridium*, *Campylobacter*, *Listeria*.
- The most important risk factor for bacterial foodborne disease where fish is the vector is the consumption of raw or inadequately heat processed fish.
- Fish and fish products are at risk of recontamination during processing, and so this aspect of fish and fish product hygiene must be strictly controlled.
- Continuous monitoring, including appropriate microbiological testing of fish meat and fish meat products in order to determine the presence of zoonotic bacteria, is necessary.

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## References

**Asai, Y., Murase, T., Osawa, R., Okitsu, T., Suzuki, R., Sata, S., Yamai, S., Terajima, J., Izumiya, H., Tamura, K. & Watanabe, H. (1999).** Isolation of Shiga toxin-producing *Escherichia coli* O157: H7 from processed salmon roe associated with the outbreaks in Japan, 1998, and a molecular typing of the isolates by pulsed-field gel electrophoresis. Kansenshogaku zasshi. *The Journal of the Japanese Association for Infectious Diseases*, *73* (1), 20−24.

**Ayula, A. M., Machado, R. A. & Scussel, V. M. (1994).** Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. *International Journal of Food Microbiology*, *24*, 171–178.

**Babic, J., Milijasevic, M., Baltic, Z. M., Spiric, A., Lilic, S., Jovanovic, J. & Djordjevic, M. (2009).** Uticaj različitih smeša gasova na očuvanje senzornih svojstava odrezaka šarana (*Cyprinus carpio*). *Tehnologija Mesa*, *50* (5–6), 328–334.

**Babic, J., Dimitrijevic, M., Milijasevic, M., Djordjevic, V., Petronijevic, R., Grbic, S. & Spiric, A. (2014).** Uticaj pakovanja u modifikovanoj atmosferi i vakuumu na odabrane hemijske parametre svežine kalifornijske pastrmke (*Oncorhynchus mykiss*) i odrezaka šarana (*Cyprinus carpio*). *Hemijska Industrija*, *68*, 1.

**Babic, J., Milijasevic, M. & Dimitrijevic, M. (2015).** Uticaj pakovanja u modifi kovanoj atmosferi i vakuumu na očuvanje senzornih svojstava odrezaka šarana (*Cyprinus carpio*). *Tehnologija Mesa*, *56* (1), 58–66.

**Baffone, W., Casaroli, A., Campana, R., Citterio, B., Vittoria ,E., Pierfelici, L. & Donelli, G. (2005).** 'In vivo' studies on the pathophysiological mechanism of *Vibrio parahaemolyticus* TDH+—induced secretion. *Microbial Pathogenesis*, *38* (2), 133−137.

**Baltic, Z. M., Kilibarda, N. & Dimitrijevic, M. (2009).** Činioci od značaja za održivost ribe i odabranih proizvoda od ribe u prometu. *Tehnologija Mesa*, *50* (1−2), 166−176.

**Bergdoll, M. S. (1990)**. Analytical methods for *Staphylococcus aureus*. *International Journal of Food Microbiology*, *10*, 91−100.

**Beuchat, L. R. (2006).** Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *British Food Journal*, *108* (1), 38−53.

**Boĭko, A. V. (1999).** The etiological structure of acute intestinal infections caused by noncholera vibrios in the Volga delta. *Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii*, *1*, 15−17. In Russian, English Abstract.

**Budiati, T., Rusul, G., Wan-Abdullah, W. N., Arip, Y. M., Ahmad, R. & Thong, K. L. (2013).** Prevalence, antibiotic resistance and plasmid profiling of *Salmonella* in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture*, 372−375.

**Buncic, O. & Katic, V. (2011).** Food safety and microbiological criteria. *Tehnologija Mesa*, *52* (1), 47−51.

**Cabrera-García, M. E., Vázquez-Salinas, C. & Quiñones--Ramírez, E. I. (2004).** Serologic and molecular characterization of *Vibrio parahaemolyticus* strains isolated from seawater and fish products of the Gulf of Mexico. *Applied and Environmental Microbiology*, *70* (11), 6401−6406.

**Colwell, R. R. (1996).** Global climate and infectious disease: the cholera paradigm. *Science*, *274* (5295), 2025−2031.

**European Council. (1991).** Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Official Journal of the European Union L 268, 24/09/1991 P. 0015–0034.

**European Commission. (2005).** Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union. L 338/1.

**Cirkovic, M., Trbovic, D., Ljubojevic, D. & Djordjevic, V. (2011).** Meat quality of fish farmed in polyculture in carp ponds in Republic of Serbia. *Tehnologija Mesa*, *52* (1), 106–121.

**Cirkovic, M., Ljubojevic, D., Djordjevic, V., Novakov, N., Petronijevic, R., Matekalo-Sverak, V. & Trbovic, D. (2012).** The breed effect on productivity and meat nutrient composition of fish. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *18* (5), 775−780.

**Cirkovic, M., Novakov, N., Petrovic, J., Ljubojevic, D., Apic, J., Babic, J. & Teodorovic, V. (2013).** Finding of parasitic nematodes of fishes present in the market. *Archives of Veterinary Medicine*, *6* (2), 3−13.

**Daniels, N. A., MacKinnon, L., Bishop, R., Altekruse, S., Ray, B., Hammond, R. M., Thompson, S., Wilson, S., Bean, N. H., Griffin, P. M. & Slutsker, L. (2000).** *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *Journal of Infectious Diseases 181* (5), 1661−1666.

**Dao, H. T. A. & Yen P. T. (2006).** Study of *Salmonella*, *Campylobacter*, and *Escherichia coli* contamination in raw food available in factories, schools, and hospital canteens in Hanoi, Vietnam. *Annals of the New York Academy of Sciences*, *1081* (1), 262−265.

**Das, K. P., Chatterjee, S. & Chattopadhyay, J. (2010).** Occurrence of chaos and its possible control in a predator-prey model with density dependent disease-induced mortality on predator population. *Journal of Biological Systems*, *18* (2), 399−435.

**David, O. M, Wandili, S., Kakai, R. & Waindi, E. N. (2009).** Isolation of *Salmonella* and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *Journal of Infection in Developing Countries*, *3*, 99−104.

**Davies, A. R., Capell, C., Jehanno, D., Nychas, G. J. & Kirby, R. M. (2001).** Incidence of foodborne pathogens on European fish. *Food Control*, *12* (2), 67−71.

**Day, J. B., Sharma, D., Siddique, N., Hao, Y. Y. D., Strain, E. A., Blodgett, R. J. & Al-Khaldi S. F. (2011).** Survival of *Salmonella* Typhi and *Shigella dysenteriae* in dehydrated infant formula. *Journal of Food Science, 76* (6), M324-M328.

**Di Pinto, A., Ciccarese, G., Tantillo, G., Catalano, D. & Forte, V. T. (2005).** A collagenase-targeted multiplex PCR assay for identification of *Vibrio alginolyticus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. *Journal of Food Protection*, *68* (1), 150−153.

**Dondero, M., Cisternas, F., Carvajal, L. & Simpson, R. (2004).** Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food Chemistry*, *87* (4), 543−550.

**Djinovic, J., Trbovic, D., Vranic, D., Jankovic, S., Spiric, D., Radicevic, T. & Spiric, A. (2010).** Stanje ekosistema, kvalitet i bezbednost mesa šarana (Cyprinus carpio) iz akvakulture u toku uzgoja. *Tehnologija Mesa, 51* (2), 124–132.

**Djinovic-Stojanovic, J., Spasic, J., Popovic, A., Vranic, D., Nikcevic, M., Hegedis, A. & Nikolic, D. (2013).** Distribucija organohlornih pesticida i polihlorovanih bifenila u dve vrste riba iz Dunava. *Tehnologija Mesa, 54* (1), 69–78.

**Đorđević, V., Baltić, M. Ž., Kilibarda, N., Mitrović, R. & Karabasil, N. (2006).** Evaluation of trout freshness using torrymeter. *Tehnologija Mesa*, *47* (1−2), 45−52.

**Djordjevic, V., Baltic, M., Karabasil, N., Cirković, M., Jankovic, V., Mitrovic, R. & Đuric, J. (2012).** Biohemijske karakteristike roda *Aeromonas* izolovanih iz kalifornijske pastrmke (*Oncorhynchus mykiss*.) *Tehnologija Mesa*, *53* (1), 20–25.

**Djordjevic, V, Ćirković, M. & Ljubojević, D. (2013).** Contamination of the ecosystem with organic pollutants and its impact on the protection of less valuable fish species in Sava and Danube Rivers. International 57th Meat industry conference meat and meat products – perspectives of sustainable production; Proceedings. Belgrade, Serbia 10–12 June 2013., 92−104.

**EFSA (2014).** (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. *EFSA Journal 12* (2), 3547. 312.

**Eklund, M. W., Peterson M. E., Poysky F. T., Paranjpye R. N. & Pelroy, G. A. (2004).** Control of bacterial pathogens during processing of cold-smoked and dried salmon strips. *Journal of Food Protection*, *67* (2), 347−351.

**Fell, G., Hamouda, O., Lindner, R., Rehmet, S., Liesegang, A., Prager, R., Gericke, B. & Petersen, L. (2000).** An outbreak of *Salmonella* Blockley infections following smoked eel consumption in Germany. *Epidemiology and Infection*, *125* (1), 9−12.

**Fenlon, D. R. (1983).** A comparison of *Salmonella* serotypes found in the faeces of gulls feeding at sewage works with serotypes in sewage. *Journal of Hygiene*, *91*, 47−52.

**Friesema, I. H., de Jong, A. E., Fitz, James, I. A., Heck, M. E., van den Kerkhof, J. H., Notermans, D. W., van Pelt & W., Hofhuis, A. (2012).** Outbreak of *Salmonella* Thompson in the Netherlands since July 2012. *Eurosurveillance*, 17, 43. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20303.

**Fujino, T., Sakazaki, R. & Tamura, K. (1974).** Designation of the type strain of *Vibrio parahaemolyticus* and description of 200 strains of the species. *International Journal of Systematic Bacteriology*, *24* (4), 447−449.

**Gould, G. W. (1999).** Sous vide foods: conclusions of an ECFF Botulinum working party. *Food Control*, *10*, 47−51.

**Gram, L. (2001).** Potential Hazards in Cold-Smoked Fish: *Listeria monocytogenes*. *Journal of Food Science*, *66* (s7), S1072−S1081.

**Gram, L. & Dalgaard, P. (2002).** Fish spoilage bacteria–problems and solutions. *Current Opinion in Biotechnology*, *13* (3), 262−266.

**Hudecova, K., Buchtova, H. & Steinhauserova, I. (2010).** The effects on modified atmosphere packaging on the microbiological properties of fresh common carp (*Cyprinus carpio*). *Acta Veterinaria Brno*, *79,* 93−100.

**Huss, H. H., Reilly, A. & Embarek, P. K. B. (2000a).** Prevention and control of hazards in seafood. *Food Control*, *11* (2), 149−156.

**Huss, H. H., Jørgensen, L. V. & Vogel, B. F. (2000).** Control options for *Listeria monocytogenes* in seafoods. *International Journal of Food Microbiology*, *62* (3), 267−274.

**Jankovic, S., Antonijević, B., Ćurčić, M., Radičević, T., Stefanović, S., Nikolić, D. & Ćupić, V. (2012).** Assessment of mercury intake associated with fish consumption in Serbia. *Tehnologija Mesa*, *53* (1), 56–61.

**Janjić, J., Ivanovic, J., Popovic M., Dokmanovic M., Boskovic M., Glamoclija N., Sarcevic, D. & Baltic, Z. M. (2015).** Kvalitet paniranih proizvoda od ribe na nasem tržištu. *Tehnologija Mesa*, *56* (1), 67−74.

**Joffraud, J. J., Cardinal, M., Cornet, J., Chasles, J. S., Leon, S., Gigout, F. & Leroi, F. (2006).** Effect of bacterial interactions on the spoilage of cold-smoked salmon. International *Journal of Food Microbiology*, *122*, 51−61.

**Joffraud, J. J., Leroi, F., Roy, C. & Berdague, J. L. 2001.** Characterization of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *International Journal of Food Microbiology*, *66*, 175−184.

**Joseph, S. W., Colwell, R. R. & Kaper, J. B. 1982.** *Vibrio parahaemolyticus* and related halophilic vibrios. *CRC Critical Reviews in Microbiology*, *10* (1), 77−124.

**Kam, K. M., Leung, T. H., Ho, Y. Y. P., Ho, N. K. Y. & Saw, T. P. (1995).** Outbreak of *Vibrio cholerae* O1 in Hong Kong related to contaminated fish tank water. *Public Health*, *109* (5), 389−395.

**Karabasil, N., Baltić, M. Ž., Teodorović, V. & Aleksić, J. (1999).** Prevalenca identifikacija pokretnih *Aeromonas* vrsta u plodovima voda. *Tehnologija Mesa*, *40* (6), 294−296.

**Karabasil, N., Asanin, R., Baltic, M. Z., Teodorovic, V. & Dimitrijevic, M. (2002).** Isolation of motile *Aeromonas* spp. from fish and their cytotoxic effect on Vero cell cultures. *Acta Veterinaria*, *52, 1*, 3−10.

**Khatib, R., Naber, M., Shellum, N., Ashton, L., Knowles, K., Giardina, V. & Wilson, F. M. (1994).** A common source outbreak of gastroenteritis in a teaching hospital. *Infection Control*, *15* (08), 534−535.

**Kumar, A., Agarwal, R. K., Bhilegaonkar, K. N., Shome, B. R. & Bachhil, V. N. (2001).** Occurrence of *Campylobacter jejuni* in vegetables. *International Journal of Food Microbiology*, *67* (1), 153−155.

**Kuzmanović, J., Ašanin, R., Baltić, M., Mišić, D., Dimitrijević, M., Stojanović, M., Ašanin, N. & Kovačević, I. (2011).** Presence of *Listeria* spp. in fish samples, fish products and sea products. *Acta Veterinaria*, *61* (2−3), 193−203.

**Ling, M. L., Goh, K. T., Wang, G. C. Y., Neo, K. S. & Chua, T. (2002).** An outbreak of multidrug-resistant *Salmonella enterica* subsp. *enterica* serotype Typhimurium, DT104L linked to dried anchovy in Singapore. *Epidemiology and Infection*, *128* (01), 1−5.

**Lotfy, N. M., Hassanein, M. A., Kh, F., El-Taweel, G. E. & Bassem, S. M. (2011).** Detection of *Salmonella* Spp in aquatic insects, fish and water by MPN-PCR. *World Journal of Fish and Marine Sciences*, *3*, 58−66.

**Ljubojevic, D., Bjelic-Cabrilo, O., Novakov, N., Cirković, M., Davidov, I., Jovanovic, M. & Aleksic, N. (2012).** Eustrongylides sp. in freshwater fish species as a potential hazard for humans. In Proceedings of the International Conference Biological Food Safety and Quality. Belgrade (Serbia).

**Ljubojevic, D., Cirkovic, M., Djordjevic, V., Puvaca, N., Trbovic, D., Vukadinov, J. & Plavsa, N. (2013a).** Fat quality of marketable fresh water fish species in the Republic of Serbia. Czech *Journal of Food Sciences*, *31* (5), 445−450.

**Ljubojevic, D., Cirkovic, M., Novakov, N., Jovanovic, R., Jankovic, S., Djordjevic, V. & Masic, Z. (2013b).** Productivity and meat nutrient in fish: The diet effect. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 19* (1), 43−49.

**Ljubojevic, D., Trbovic, D., Lujic, J., Bjelic-Cabrilo, O., Kostic, D., Novakov, N. & Cirkovic, M. (2013c).** Fatty acid composition of fishes from inland waters. *Bulgarian Journal of Agricultural Science*, *19* (1), 62−71.

**Ljubojevic, D., Cirkovic, M., Djordjevic, V., Trbovic, D., Vranic, D., Novakov, N. & Masic, Z. (2013d).** Hemijski sastav, sadržaj holesterola i sastav masnih kiselina šarana (*Cyprinus carpio*) iz slobodnog izlova, poluintenzivnog i kaveznog sistema gajenja. *Tehnologija Mesa*, *54* (1), 48–56.

**Ljubojevic, D., Cirkovic, M., Novakov, N., Puvaca, N., Aleksic, N., Lujic, J. & Jovanovic, R. (2014).** Comparison of meat quality of tench, *Tinca tinca*, reared in extensive and semi-intensive culture systems. *Journal of Applied Ichthyology*, *30*, 50−57.

**Ljubojevic, D., Radosavljevic, V., Puvaca, N., Balos, M. Z., Djordjevic, V., Jovanovic, R., & Cirkovic, M. (2015).** Interactive effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio L.*). *Journal of Food Composition and Analysis*, *37*, 44−50.

**Miettinen, H. & Wirtanen, G. (2005).** Prevalence and location of *Listeria monocytogenes* in farmed rainbow trout. *International Journal of Food Microbiology*, *104* (2), 135−143.

**Milijasevic, M., Babic, J., Baltic, Z. M., Spiric, A., Velebit, B., Borovic, B. & Spiric, D. (2010).** Uticaj različitih smeša gasova na promene nekih mikrobioloških i hemijskih parametara u odrescima šarana (*Cyprinus carpio*) upakovanih u modifikovanu atmosferu. *Tehnologija Mesa*, *51* (1), 66–70.

**Milijasevic, M., Babic, J., Baltic, Z. M., Djordjevic, V., Spiric, D., Jankovic, S. & Spiric, A. (2012).** Parametri higijenske ispravnosti četiri vrste riba koje su najzastupljenije na tržištu Srbije. *Tehnologija Mesa*, *53* (2), 127–133.

**Mitsuda, T., Muto, T., Yamada, M., Kobayashi, N., Toba, M., Aihara, Y., Ito, A. & Yokota, S. (1998).** Epidemiological Study of a Food-Borne Outbreak of Enterotoxigenic *Escherichia coli* O25:NM by Pulsed-Field Gel Electrophoresis and Randomly Amplified Polymorphic DNA Analysis. *Journal of Clinical Microbiology*, *36* (3), 652−656.

**Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F. & van der Giessen, J. (2010).** Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, *139*, S3-S15.

**Norton, D. M., Scarlett, J. M., Horton, K., Sue, D., Thimothe, J., Boor, K. J. & Wiedmann, M. (2001).** Characterization and pathogenic potential of *Listeria monocytogenes* isolates from the smoked fish industry. *Applied and Environmental Microbiology*, *67* (2), 646−653.

**Okanovic, Dj., Cirkovic, M., Novakov, N., Ljubojevic, D., Karan, D., Matekalo-Sverak, V. & Masic, Z. (2013a).** Sensory and chemical characteristics of sausages produced of cyprinid meat. *Food and Feed Research*, *40*, 53−58.

**Okanovic, Dj., Ljubojevic, D., Cirkovic, M., Djordjevic, V., Karan, D., Vranic, D. & Novakov, N. (2013b).** Characteristics of sausages produced of carp meat. *Savremena Poljoprivreda*, *62* (1−2), 83−90.

**Orban, E., Nevigato, T., Di Lena, G., Masci, M., Casini, I., Gambelli, L. & Caproni, R. (2008).** New trends in the seafood market. Sutchi catfish (*Pangasius hypophthalmus*) fillets from Vietnam: Nutritional quality and safety aspects. *Food Chemistry*, *110*, 383–389.

**Özogul, F. & Özogul, Y. (2006).** Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. *Food Chemistry*, *99*, 574–578.

**Panisello, P. J. & Quantick, P. C. (2001).** Technical barriers to hazard analysis critical control point (HACCP). *Food Control*, *12* (3), 165−173.

**Pavlicevic, N., Baltic, M., Dimitrijevic, M., Karabasil, N., Djordjevic, V., Markovic, R. & Grbic, S. (2014).** Polinezasicene masne kiseline u mesu ribe i njihov znacaj za zdravlje ljudi. *Tehnologija Mesa*, *55* (1), 1−7.

**Pierard, D., Crowcroft, N., De Bock, S., Potters, D., Crabbe, G., Van Loock, F. & Lauwers, S. (1999).** A case-control study of sporadic infection with O157 and non-O157 verocytotoxin-producing *Escherichia coli*. *Epidemiology and infection*, *122* (03), 359−365.

**Serbia. (2010).** Regulation on general and specific food hygiene requirements at any stage of production, processing and transport. Pravilnik o opštim i posebnim uslovima higijene hrane u bilo kojoj faziproizvodnje, prerade i prometa. *Official Gazette of the Republic of Serbia*, 72.

**Rabbani, G. H. & Greenough III, W. B. (1999).** Food as a vehicle of transmission of cholera. *Journal of Diarrhoeal Diseases Research*, 1−9.

**Radetic, P., Milijasevic, M., Jovanovic, J. & Velebit, B. (2007).** Pakovanje svežeg mesa u modifikovanoj atmosferi – trend koji traje. *Tehnologija Mesa*, *48* (1–2), 99–109.

**Raissy, M., Moumeni, M., Ansari, M. & Rahimi, E. (2012)**. Occurrence of *Vibrio* spp. in lobster and crab from the Persian Gulf. *Journal of Food Safety*, *32* (2), 198−203.

**Raissy, M., Khamesipour, F., Rahimi, E. & Khodadoostan, A. (2014).** Occurrence of *Vibrio* spp., *Aeromonas hydrophila*, *Escherichia coli* and *Campylobacter* spp. in crayfish (*Astacus leptodactylus*) from Iran. *Iranian Journal of Fisheries Sciences*, *13* (4), 944−954.

**European Council. (2004).** Regulation (EC) no 852/2004 of the European parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs.

**Ribó, O., Candiani, D. & Serratosa, J. (2009).** Role of the European Food Safety Authority (EFSA) in providing scientific advice on the welfare of food producing animals. *Italian Journal of Animal Science*, *8* (1s), 9−17.

**Rotariu, O., Thomas, D. J. I., Goodburn, K. E., Hutchison, M. L., Strachan, N. J. (2014).** Smoked salmon industry practices and their association with *Listeria monocytogenes*. *Food Control*, *35* (1), 284−292.

**Round, J. L. & Mazmanian, S. K. (2009).** The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, 9, 5, 313−323.

**Sivertsvik, M., Jeksrud, W. K. & Rosnes, J. T. (2002).** A review of modified atmosphere packaging of fish and fishery products–significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, *37* (2), 107−127.

**Smolikova, L. M., Lomov, I., Khomenko, T. V., Murnachev, G. P., Kudriakova, T. A., Fetsaĭlova, O. P., Sanamiants, E. M., Makedonova, L. D., Kachkina, G. V. & Golenishcheva, E. N. (2000).** Studies on halophilic vibrios causing a food poisoning outbreak in the city of Vladivostok. *Zhurnal Mikrobiologii, Epidemiologii, i Immunobiologii*, *6,* 3−7.

**Spiric, A., Trbovic, D., Vranic, D., Djinovic, J., Petronijevic, R., Milijasevic, M., Jankovic, S., & Radicevic, T. (2009).** Uticaj masnih kiselina u hrani na sastav masnih kiselina i količinu holesterola kod kalifornijske pastrmke (*Oncorhynchus mykiss*). *Tehnologija Mesa*, *50* (3–4), 179–188.

**Telzak, E. E., Bell, E. P., Kautter, D. A., Crowell, L., Budnick, L. D., Morse, D. L. & Schultz, S. (1990).** An international outbreak of type E botulism due to uneviscerated fish. Journal of *Infectious Diseases*, *161*, 340–342.

**Terentjeva, M., Eizenberga, I., Novoslavskij, A., Strazdina, V., Valcina, O., Osmjana, J., & Berzins, A. (2015).** Bacterial microflora of freshwater fish orginated from Usmas lake in Latvia. *Journal of Microbiology, Biotechnology and Food Sciences*, *4*, 74−77.

**Tham, W., Ericsson, H., Loncarevic, S., Unnerstad, H. & Danielsson-Tham, M. L. (2000)**. Lessons from an outbreak of listeriosis related to vacuum-packed gravid and coldsmoked fish. *International Journal of Food Microbiology*, *62*, 173–175.

**Trbovic, D., Vranic, D., Djinovic, J., Borovic, B., Spiric, D., Babic J. & Spiric, A. (2009).** Masnokiselinski sastav i sadržaj holesterola u mišićnom tkivu jednogodišnjeg šarana (*Cyprinus carpio*) u fazi uzgoja. *Tehnologija Mesa*, *50* (5–6), 276–286.

**Trbovic, D., Jankovic, S., Cirkovic, M., Nikolic, D., Matekalo-Sverak, V., Djordjevic, V., & Spiric, A. (2011).** Bezbednost i kvalitet mesa nekih slatkovodnih riba u Srbiji. *Tehnologija Mesa*, *52* (2), 276–282.

**Trbovic, D., Markovic, Z., Petronijevic, R., Milijasevic, M., Spiric, D., Vranic, D. & Spiric, A. (2013).** Promene hemijskog i masnokiselinskog sastava mesa šarana u toku poluintenzivnog uzgoja. *Tehnologija Mesa*, *54* (1), 39–47.

**Tsai, G. J. & Chen, T. H. (1996).** Incidence and toxigenicity of *Aeromonas hydrophila* in seafood. *International Journal of Food Microbiology*, *31* (1), 121−131.

**Vaz-Velho, M., Duarte, G., McLauchlin, J. & Gibbs, P. (2001).** Characterization of *Listeria monocytogenes* isolated from production lines of fresh and cold-smoked fish. *Journal of Applied Microbiology*, *91* (3), 556−562.

**Vieira, R. H. S. D. F., Rodrigues, D. D. P., Gonçalves, F. A., Menezes, F. G. R. D., Aragão, J. S. & Sousa, O. V. (2001).** Microbicidal effect of medicinal plant extracts (*Psidium guajava Linn*. and *Carica papaya Linn*.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *43* (3), 145−148.

**Vogel, B. F., Huss, H. H., Ojeniji, B., Ahrens, P. & Gram, L. (2001).** Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. *Applied and Environmental Microbiology*, *67* (6), 2586−2595.

**Vranic, D., Trbovic, D., Djinovic, J., Mazic, Z., Spiric, D., Milicevic, D. & Spiric, D. (2010).** Nutritivna vrednost kalifornijske pastrmke (*Oncorhynchus mykiss*) i šarana (*Cyprinus carpio*) iz akvakulture. *Tehnologija Mesa*, *52* (2), 159–168.

**Vranic, D., Djinovic-Stojanovic, J. & Spiric, A. (2011).** Rainbow trout (*Oncorhynchus Mykiss*) from aquaculture–meat quality and importance in the diet. *Tehnologija Mesa*, *52* (1), 122–133.

**Vuddhakul, V., Chowdhury, A., Laohaprertthisan, V., Pungrasamee, P., Patararungrong, N., Thianmontri, P., Ishibashi, M., Matsumoto, C. & Nishibuchi, M. (2000).** Isolation of a pandemic O3: K6 clone of a *Vibrio parahaemolyticus* strain from environmental and clinical sources in Thailand. *Applied and Environmental Microbiology*, *66* (6), 2685−2689.

**Weber, J. T., Hibbs, R. G., Darwish, A., Mishu, B., Corwin, A. L., Rakha, M., Hatheway, C. L., Elsharkawy, S., Elrahim, S.A., Alhamd, M. F. S., Sarn, J. E., Blake, P. A. & Tauxe, R.V. (1993).** A massive outbreak of type E botulism associated with traditional salted fish in Cairo. *Journal of Infectious Diseases*, *167*, 451–454.

**Serbia. (2009).** Law on food safety. Službeni glasnik RS broj 41 od 2009. *Official Gazette of the Republic of Serbia*, 41.

**Zsuzsánna, S., Zsuzsanna, G. P., István, C. & Zsigmond, J. (2011).** Fish meat quality and safety *Tehnologija Mesa*, *52* (1), 97–105.

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# Health aspects of dry-cured ham

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*A b s t r a c t: Different factors (pig breed, animal production practices) are responsible for nutritional characteristics of pork and dry-cured hams thereof, and their potential effects on human health. Traditional production of dry-cured ham is very popular all over Europe (Vrsacka ham, Iberian ham, Serrano ham, Corsican ham, Parma ham, Modena ham, Nazionale ham, San Daniele ham etc.). Dry-cured ham is an important source of biologically valuable proteins, iron, B-complex vitamins, and phosphorus. Although the potential role of meat products, including such traditionally produced hams, in the healthy human diet have not been completely clarified, many studies are starting to draw a picture of their impact on human health. The object of this review was to provide an analysis of the nutritional composition, including some micronutrients and vitamins, of traditionally produced dry-cured ham and the role these meat products could play in a healthy diet.*

***Keywords:*** *dry-cured ham, health, nutritional composition, micronutrient supply.*

## Introduction

Pork is recognized as a food with essential nutritional properties because it is an important source of proteins, minerals and fats (*Baltic et al.*, 2014; *Boskovic et al.*, 2015; *Jiménez-Colmenero et al.*, 2001; *Jiménez-Colmenero et al.*, 2010; *Kauffman*, 2001; *Lucarini et al*., 2013; *Reig et al.*, 2013). However, pork also contributes to the intake of fat, saturated fatty acids, cholesterol, and other substances that, in inappropriate amounts, can have negative physiological effects on human health (*Toldrá and Reig*, 2011).

Red meat (100 g) contains about 20–24 g of protein in the raw state or 27–35 g of heat-treated protein (*Wyness et al.*, 2011). Meat contains eight essential amino acids and histidine, an essential amino acid for children (*Higgs*, 2000; *Wyness et al.*, 2011). Essential amino acids play a role in muscle tissue regeneration after injuries or surgery (*Boskovic et al.*, 2015; *Higgs*, 2000). Some healthy components of meat and their target markers are presented in Table 1.

The fat content in meat varies widely depending on various factors including animal feeding system, meat cut, cooking conditions etc. The fatty acid composition of pork has an important effect on the diet/health relationship for pork consumers. Among pork lipids, less than 50% constitutes saturated fatty acids (SFAs) (*Jiménez-Colmenero et al.*, 2001).

Dietary fatty acids in pig feed are incorporated unchanged into pig adipose tissue (*Jakobsen*, 1999; *Toldrá et al.*, 1996). The extent of incorporation can vary depending on the specific fatty acid and the type of feed. Different types of dietary oils and their effects on the proportions of fatty acids have been studied (*Reig et al.*, 2013). The use of linseed oil in pig feed increased the n-3 fatty acid content. It significantly increased the linolenic acid (C18:3n-3), slightly increased the eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) acids, and decreased the linoleic acid (C18:2n-6) in pork (*Jiménez-Colmenero at al.*, 2006; *Reig et al.*, 2013). Other dietary oils such as soy, peanut, corn, and sunflower oil increased the content of linoleic acid (C18:2n-6). The addition of fish oils increased the content of EPA and DHA and reduced the n-6/n-3 ratio to almost 2 (*Irie and Sakimoto*, 1992; *Jakobsen*, 1999; *Reig et al.*, 2013). Also, some studies investigated incorporation of omega-3 fatty acids in meat because of their ability to reduce the level of low density lipoproteins (LDL), cholesterol and blood triacylglycerols (*Harris*, 2007; *Markovic et al.*, 2015).

When pig feed is rich in saturated fats, the levels of palmitic (C16:0), palmitoleic (C16:1),

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**Table 1.** Healthful components of meat products (adapted from *Olmedilla-Alonso et al.*, 2013)

| Target markers | Compounds associated with beneficial effect |
|---|---|
| *Cardiovascular* | |
| Lipids | MUFA, PUFA, CLA, dietary fibre, vitamin C, vitamin E, bioactive peptides, L-carnitine |
| Blood pressure | bioactive peptides |
| Obesity | CLA, dietary fibre |
| Other | Folic acid, vitamin B6, vitamin B12, lycopene |
| *Cancer* | Selenium, CLA, folic acids |
| *Bone diseases* | Calcium, magnesium |
| *Anemia* | Iron |
| *Growth* | Iodine |

**Legend:** MUFA – monounsaturated fatty acids; PUFA – Polyunsaturated fatty acid; CLA – Conjugated linoleic acid

stearic (C18:0) and oleic (C18:1) acids in pork are significantly higher and the PUFA/SFA ratio is lower (*Leszczynski et al.*, 1992; *Morgan et al.*, 1992; *Reig et al.*, 2013). Feed rich in linoleic acid, an n-6 fatty acid which is most commonly found in soy, corn, maize, sunflower and barley, resulted in pork with significantly increased concentrations of this fatty acid (*Larick et al.*, 1992; *Toldrá et al.*, 2004). The content of conjugated linoleic acid (CLA) can also vary depending on the feed type. CLA has potential benefits for human health (anticarcinogenic, antidiabetic and antiatherogenic effects) (*Lauridsen et al.*, 2005; *Schmid et al.*, 2006; *Reig et al.*, 2013). Studies with CLA added to pig feed had the goal of increasing the CLA content in pork. *Ivanovic et al*. (2015) added the recommended 2.0% CLA to pig feed and reported 3.56±0.71% CLA (c9t11CLA+t10c12CLA) in the muscle tissue, while the control pork from pigs without added dietary CLA did not contain detectable CLA. Similar results were reported with 1% CLA added to pig feed which resulted in CLA levels in muscle tissue of 5.5 mg 100 $g^{-1}$ (*Eggert et al.*, 2001).

As a major source of high quality proteins, meat and meat products are one of the most highly investigated sources for isolation of bioactive peptides in recent years (*Baltic et al.*, 2014; *Ryan et al.*, 2011). Bioactive peptides have a range of potential positive effects on human health, such as antioxidative, antimicrobial, antihypertensive, antithrombotic, cytomodulatory, immunomodulatory, anticancer, hypocholesterolemic and anti-obesity effects, which mainly depend on their structure. Considering the activities of bioactive peptides and their wide-spectrum benefits to human health, it is clear that these peptides could be suitable candidates to be used for health promotion and disease risk reduction (*Baltic et al.*, 2014). In addition to myosin and actin being used for peptide generation, other proteins originating from thick and thin filaments and from connective tissue like fibrillar collagen can be used (*Udenigwe and Ashton*, 2013).

The iron content in pork is quite constant as is the content of the trace elements selenium, magnesium and zinc (*Reig et al.*, 2013). Also, pork contains folate, vitamin B12 and vitamin A, all cancer protecting factors (*Bieasalski*, 2005). Pork contains about 1.8 mg iron, 2.6 mg zinc per 100 g, and meat can provide up to 50% of the Recommended Dietary Allowances (RDA) for iron, zinc, selenium, vitamins B12, B1, B2, B6 and 100% of vitamin A (*Bieasalski*, 2005).

## Ham components and health implications

Dry-cured ham is a typical meat product in the Mediterranean area, but also in many other countries, including the United States and Japan (*Bermúdez et al.*, 2012; *Jiménez-Colmenero et al.*, 2010; *Lucarini et al.*, 2013; *Marusic et al.*, 2013; *Toldra and Reig*, 2011). The differing manufacturing features applied in dry-cured ham production result in chemical composition differences (*Lucarini et al.*, 2013). However, many factors affect the physico-chemical and nutritional aspects and also sensory properties of ham, including the rearing system,

**Table 2.** Chemical composition of traditional dry-cured hams from Italy, Serbia and Montenegro (adapted from *Lucarini et al.*, 2013)

| Traditional ham | Moisture | Ash | Proteins | Lipids | Energy Kcal portion$^{-1}$, 50 g |
|---|---|---|---|---|---|
| | g 100 g$^{-1}$ (%) | | | | |
| *Italy* | | | | | |
| Modena | 45.6±3.0 | 5.8±0.6 | 25.6±1.6 | 22.9±3.5 | 154 |
| Nazionale | 50.5±3.2 | 6.6±1.4 | 27.8±2.5 | 13.7±5.1 | 117 |
| Parma | 50.3±2.0 | 5.5±0.4 | 25.9±1.5 | 18.3±2.8 | 134 |
| San Daniele | 50.2±2.1 | 5.3±0.5 | 25.7±1.4 | 18.6±2.8 | 135 |
| *Serbia* | | | | | |
| Vrsacka* | 51.65 | 4.97 | 32.82 | 10 | 99 |
| *Montenegro* | | | | | |
| Dry-cured ham from Martex company | 49.61 | 4.71 | 31.02 | 14.61 | 128 |

* Chemical analyses of Vrsacka ham and dry-cured ham (Martex) were according to ISO standard methods (moisture - ISO 1442:1998; ash- ISO 936:1998, proteins - ISO 937:1978, lipids- ISO 1444:1996

animal age, pig genotype, as well as processing conditions (*Andrés et al.*, 2004).

Table 2 shows moisture, ash, protein, lipid and energy content of the most popular dry-cured hams from Italy, Serbia and Montenegro. Dry-cured ham is a good source of proteins (25.6–32.82 g 100 g$^{-1}$) and lipids (9.56–22.9 g 100 g$^{-1}$). The content of free amino acids in dry-cured ham rises during processing as a result of proteolysis (*Alfaia et al*., 2004; *Jiménez- Colmenero et al.*, 2010; *Toldra et al.*, 2000). Some of the amino acids in dry-cured ham, like taurine, glutamine, tryptophan, leucine and valine, can have benefits for human health (*Ventanas*, 2006).

**Figure 1** Vrsacka ham (*Baltic et al*., 2015a)

Vrsacka ham contains about 33% protein, which is higher than the protein content of dry-cured ham from Italy (Table 2). The fat content of Vrsacka ham is lower than that of Italian hams. This Serbian ham has a designated protected geographical indication (PGI) (http://www.zis.gov.rs) (Figure 1).

Nevertheless, the main factor that determines ham price is the fattening diet for the animals (*Narváez-Rivas et al.*, 2011). Furthermore, there is an increased awareness about the need for more "healthy" fats in the human diet that has focused research on the nutritional characterisation of fat from different meat products.

There are studies which show that dietary fat content plays a significant role in prevention of some chronic disorders (*Jiménez-Colmenero et al.*, 2010). The World Health Organization (*WHO*, 2003) recommended an optimal intake of total unsaturated fatty acids (between 15–30% of total diet energy), for SFA to be no more than 10% of dietary energy, and polyunsaturated fatty acid (PUFA) to be between 6–10% of dietary energy. Many studies provide results about fat and fatty acid profiles in dry-cured ham (*Gandemer*, 2009; *Lo Fiego et al.*, 2005; *Santos et al.*, 2008; *Ventanas et al.*, 2007). The total *trans* fatty acid content in the human diet should be less than 1% (*World Health Organization*, 2003). The results of different studies suggest that dry-cured ham is a healthy food and can be consumed as a regular diet component (*Fernández et al.*, 2007; *Jiménez-Colmenero et al.*, 2009).

**Table 3**. Fatty acid profile (% of total fatty acids) of the different types of dry-cured ham (commercial feeding system) (adapted from *Jiménez-Colmenero et al.*, 2010)

| Ham | SFA | MUFA | PUFA | P/S* | n-6/n-3 |
|---|---|---|---|---|---|
| Iberian (Spain) | 35.15 | 51.39 | 13.44 | 0.38 | 31.2 |
| Serrano (Spain) | 32.70 | 52.7 | 10.2 | 0.31 | 16.2 |
| Bayonne (France) | 36.4 | 52.9 | 10.7 | 0.29 | 14.1 |
| Corsican (France) | 34.9 | 55.4 | 9.7 | 0.28 | 8.7 |
| Parma (Italia) | 35.99 | 54.04 | 8.59 | 0.23 | 39.9 |
| San Daniele (Italia) | 38.5 | 51.9 | 9.6 | 0.25 | – |
| Jinhua (China) | 37.10 | 46.63 | 14.24 | 0.38 | – |

* P/S – PUFA/SFA ratio

On average, the fatty acids in dry-cured ham comprise 35–40% SFA, 45–50% MUFA and 10–15% PUFA (Table 3). Many nutritionists currently tend to focus more on the PUFA/SFA ratio and the n-6/n-3 PUFA ratio than on individual levels of fatty acids. The PUFA/SFA ratio in dry-cured ham ranges from 0.23 to 0.38 (Table 3). Note that a high amount of PUFA is not necessarily healthy, especially if the n-6/n-3 ratio is not balanced. *Simopoulos* (2002) presented an argument that the n-6/n-3 ratio should not exceed 4, while the *British Nutrition Foundation* (1992) stated that the n-6/n-3 ratio should be <6. When the n-6/n-3 ratio is very high, it can promote cancer, autoimmune and inflammatory diseases (*Simopoulos*, 2002). Genetic and feeding strategies have proven to be effective in supporting production of dry-cured ham with good PUFA/SFA and n-6/n-3 ratios, and therefore, with desirable fat characteristics (*Bermúdez et al.*, 2012).

Dry-cured ham is a good source of iron, zinc, potassium, magnesium and selenium (Table 4), which is particularly important for the nutrition of pregnant women and children (*Benoist*, 2001). It contains iron levels of 1.8–3.3 mg 100 $g^{-1}$ (Table 4). Dry-cured ham contains zinc at levels of 2.2–3.0 mg 100 $g^{-1}$ (Table 4), an essential element in nutrition, as it is involved in the activity of more than 200 enzymes (*Neumann et al.*, 2002). Magnesium is important, especially in the prevention of cardiovascular diseases and osteoporosis (*Fleet and Cashman*, 2003). The magnesium content in dry-cured ham depends on the pig diet and the type of salts added, such as magnesium aspartate, magnesium aspartate hydrochloride or magnesium fumarate (*D'Souza et al.*, 1999). Selenium is an important trace element in the human diet, because of its role in antioxidative processes in the human body (*Higgs*, 2000). Many studies have shown the influence of selenium added to feed on pork quality (*Baltic et al.*, 2015b; *Gjerlaug-Enger et al.*, 2015; *Naik et al.*, 2015). Pork can be enriched with selenium through dietary supplementation of pig feed with sodium selenite or selenium-rich yeast.

In addition, dry-cured ham contains a high level of the B-complex vitamins, whereby the nutritional profile of this vitamin group depends on the raw meat vitamin content and manufacturing procedures (*Lucarini et al.*, 2013). Vitamin B6 is one of the major vitamins from this group in pork (*Ball*, 1994). *Higgs* (2000) presented data showing that animal origin foods are the only dietary source of vitamin B12. Also, dry-cured ham is a source of tocopherol (vitamin E, important in prevention of cardiovascular diseases). Vitamin E is a very effective antioxidant because it is accumulated in tissues and subcellular structures, including membranes. Pig muscle can be enriched with vitamins E and A through their supplementation in pig feed. Depending on the concentration (usually about 100–200 mg $kg^{-1}$ of feed) and supplementation duration (several weeks prior to slaughter) the content of these vitamins in the muscles may be proportionally increased (to the value of almost 13 mg $kg^{-1}$ of dry muscle) (*Isabel et al.*, 2003; *Mercier et al.*, 1998). Vitamin E tends to deposit in the muscles of the thoracic limb and neck (*O'Sullivan et al.*, 1997).

**Table 4.** Content of trace elements and vitamins (mg 100 $g^{-1}$ and µg 100 $g^{-1}$) in traditionally-produced dry-cured ham (adapted from *Jiménez-Colmenero et al.*, 2010*; Lucarini et al.*, 2013)

| Parameter | Italian ham | Serbian ham (Vrsacka) | All types of traditional ham (included Spanish ham), per 100 g | Montenegro ham |
|---|---|---|---|---|
| Ca (mg) | / | / | 12–35 | / |
| Fe (mg) | 0.92–1.05 | 1.8 | 1.8–3.3 | 2.2 |
| Zn (mg) | 2.08–2.72 | 2.2 | 2.2–3.0 | 4.3 |
| Mg (mg) | / | / | 17–18 | / |
| K (mg) | / | / | 156–160 | / |
| Cu (mg ) | / | 0.03 | / | 0.03 |
| Mn (mg) | / | 0.01 | / | 0.01 |
| P (mg) | / | | 157–180 | |
| Se (µg) | 11–17 | 14 | 29 | 17 |
| Na (mg) | / | / | 1100–1800 | / |
| Thiamine (B1) (mg) | 0.58–0.90 | / | 0.57–0.84 | / |
| Riboflavin (B2) (mg) | 0.19–0.22 | / | 0.20–0.25 | / |
| Niacin (B3) | 5.13–5.90 | / | 4.5–11.8 | / |
| Vitamin B6 (mg) | 1.0–1.13 | / | 0.22–0.42 | / |
| Folic acid (µg) (B9) | / | / | /−13.49 | / |
| Vitamin B12 (µg) | 0.33–0.67 | / | /−15.68 | / |
| Vitamin E (mg) | 0.11–0.24 | / | 0.08–1.5 | / |

**Legend:** Fe, Cu, Mn, Zn analyses of Vrsacka ham and dry-cured ham (Martex) were according to ISO 6869:2008; Se analysis according to ISO 16159:2012

Dry-cured ham contains several endogenous antioxidants (ubiquinone, ascorbic acid, uric acid, spermine, carnosine, anserine) (*Decker et al.*, 2000). Ubiquinone (Coenzyme Q10) has an effect on gene expression (*Mattila et al.*, 2000), ameliorating endothelial function (*Belardinelli et al.*, 2006), and it is considered as a bioactive compound (*Marusic et al.*, 2013). Many meat compounds have been identified as antioxidant regulating substances.

Carnosine and anserine are know as antioxidants in meat and they are absorbed into the blood plasma intact (*Marusic et al.*, 2013). These dipeptide antioxidants are involved in xenobiotic metabolism and protection against free radicals and oxygen toxicity (*Winters et al.*, 1995). Dipeptides help oxidation control through prevention of lipid oxidation (*Decker and Crum*, 1993). Also, they reduce rancid tastes and improve colour stability in finished hams (*Jiménez-Colmenero et al.*, 2010). Research into the effects of these substances in dry-cured ham and their potential benefits for human health is currently in progress.

The major source of free L-carnitine (an amino acid derivative) for humans is meat and meat products. L-carnitine plays a role in energy production processes (*Marusic et al.*, 2013), helps the human body to absorb calcium and protects skeletal muscle (*Demarquoy et al.*, 2004). Creatine (another amino acid derivative) is a key substance in muscle, particularly involved in the transfer of high energy phosphate to ADP in muscle cells (*Wyss and Kaddurah-Daouk*, 2000).

## Feeding and management plan for pig production with potential health implications

There are numerous factors in pig breeding and management that affect the content of many important substances in pork, which should be taken into consideration (*Reig et al.*, 2013). Genetic strategies and pig selection are based on morphological parameters of pure Iberian breed or white sows. Duroc origin pork contains more intramuscular fat than pork from European white breeds (*Carrion et al.*, 2004). Also, in recent years, there has been growing interest of producers in the genotyping of pigs used for dry-cured ham production. Genetic strategies can improve the fatty acid profile of dry-cured ham (and other pork products) (*Lai et al.*, 2006).

The chemical composition of pork, and especially the fat content, is a polygenetically determined characteristic (*Carrion et al.*, 2004). However, the chemical composition of pork is also related to the pig population genotype, nutrition and feeding management (*Garnier et al.*, 2003). Numerous studies indicate that loin and ham from Iberian pigs contain significantly higher amounts of intramuscular fat that those cuts in Landrace pigs (*Ventanas et al.*, 2007). Also, the dietary fatty acid composition provided by the pig feed is an extremely important contributor to the fatty acid profile of the resultant pork. *Jiménez-Colmenero et al*. (2001) showed that increased amounts of unsaturated fatty acids in meat increased the possibility of oxidation. All strategies based on pig production make it possible to increase concentrations of beneficial and reduce concentrations of detrimental components in pork, in order to improve human health.

Reduced fat, cholesterol and sodium content, plus reduced calories are among the most common targets for reformulation of pork products with the aim of positively influencing human health (*Jiménez-Colmenero et al.*, 2001). Fat reduction can be achieved by combining pre-selected raw pork meat. The cholesterol content can be reduced by replacing fat with other vegetable materials (peanut, canola, sunflower, olive), without cholesterol. Finally, the salt content can be reduced by using lactates and collagen hydrolysates as flavour enhancers.

## Conclusion

Dry-cured ham has potential to be a component of a healthy human diet, as it contains important essential amino acids and micronutrients (trace elements and minerals). During the dry-cured ham production process, the amino acid content in the product actually increases due to proteolysis. Therefore, these meat products should be recommended in the human diet, especially for groups with special dietary requirements. (pregnant women and growing children). However, dry-cured ham cannot be recommended for hypertensive consumers, because of its high salt content.

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## References

**Alfaia, C. M., Castro, M. F., Reis, V. A., Prates, J. M., De Almeida, I. T., Correia, A. D. & Dias, M. A. (2004).** Changes in the profile of free amino acids and biogenic amines during the extended short ripening of Portuguese dry-cured ham. *Food Science and Technology International*, *10* (5), 297−304.

**Andrés, A. I., Cava, R., Ventanas, J., Thovar, V. & Ruiz, J. (2004).** Sensory characteristics of Iberian ham: Influence of salt content and processing conditions. *Meat Science*, *68*, 45–51.

**Ball, G. F. M. (1994).** Chemical and biological nature of the water soluble vitamins. In *Water soluble vitamin assays in human nutrition*. London: Chapman and Hall.

**Baltic, Z. M., Boskovic, M., Ivanovic, J., Janjic, J., Dokmanovic, M., Markovic R. & Baltic, T. (2014).** Bioactive peptides from meat and their influence on human health. *Tehnologija Mesa*, *55* (1), 8−21.

**Baltic, Z. M., Dokmanovic, M., Djordjevic, J., Markovic, R., Ivanovic, J., Rajkovic, Z., Djordjevic, V. & Boskovic, M. (2015a)** Vrsacka ham. *Technical Solution*-06-00-80/2013–16/1-29. http://www.mpn.gov.rs/nauka/najava-konkursa/

**Baltic, Z. M, Dokmanovic-Starcevic, M., Basic, M., Zenunovic, A., Ivanovic, J., Markovic, R., Janjic, J. & Mahmutovic, H. (2015b).** Effects of selenium yeast level in diet on carcass and meat quality, tissue selenium distribution and glutathione peroxidase activity in ducks. *Animal Feed Science and Technology*, *210*, 225–233.

**Biesalski, H. K. (2005).** Meat as a component of a healthy diet–are there any risks or benefits if meat is avoided in the diet? *Meat Science*, *70* (3), 509−524.

**Benoist, B. (2001).** Deficiency anemia: Re-examining the nature and magnitude of the public health problem. Introduction. *Journal of Nutrition*, *131* (2S-II), 564.

**Bermúdez, R., Franco, I., Franco, D., Carballo, J. & Lorenzo J. M., (2012).** Influence of inclusion of chestnut in the finishing diet on fatty acid profile of dry-cured ham from Celta pig breed. *Meat Science*, 92 (4), 394−399.

**Boskovic, M., Baltic, Z. M., Ivanovic, J., Djuric, J., Dokmanovic, M., Markovic, R., Sarcevic, D., Baltic, T. (2015).** The impact of pork meat and lard on human health. *Tehnologija Mesa*, *56* (1), 8−15.

**British Nutrition Foundation (1992).** Unsaturated fatty acids. Nutritional and physiological significance. *The Report of British Nutrition Foundation's Task Force*. London: Chapman and Hall.

**Carrion, D., Sosnicki, A. A., Klont, R. E. & Diestre, A. (2004).** Impacto de la genética porcina sobre la calidad del jamón curado. In: J. Ventanas (Coordinator), *Avances en la Ciencia, Tecnología y Comercialización delJamón* (pp. 31–41). Cáceres, Spain: Conjamón 2003.

**D'Souza, D. N., Warner, R. D., Dunshea, F. R. & Leury, B. J. (1999).** Comparison of different dietary magnesium supplements on pork quality. *Meat Science*, *51*, 221–225.

**Decker, E. A., Crum, A. D. (1993).** Antioxidant activity of carnosine in cooked ground pork. *Meat Science*, *34*, 245–253.

**Decker, E. A., Livisay, S. A. & Zhou, S. (2000).** Mechanisms of endogenous skeletal muscle antioxidants: Chemical and physical aspects. *Antioxidants In Muscle Foods*, 25−60.

**Demarquoy, J., Georges, B., Rigault, C., Royer, M. C., Clairet, A., Soty, M. & Le Borgne, F. (2004).** Radioisotopic determination of L-carnitine content in foods commonly eaten in Western countries. *Food Chemistry*, *86* (1), 137−142.

**Eggert, J. M., Belury, M. A., Kempa-Steczko, A., Mills, S. E. & Schinckel, A. P. (2001).** Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. *Journal of Animal Science*, *79* (11), 2866−2872.

**Fernández, M., Ordóñez, J. A., Cambero, I., Santos, C., Pin, C. & de la Hoz, L. (2007).** Fatty acids compositions of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry*, *101*, 107–112.

**Fleet, J. C. & Cashman, K. D. (2003)**. Magnesio. In B. A. Bowman & R. M. Russell (Eds.), *Conocimientos Actuales Sobre Nutrición*. Publicación Cientifico y Técnica No. 592 (8th ed., pp. 318–329). Washington, DC: ILSI y Organización Panamericana de la Salud.

**Gandemer, G. (2009)**. Dry cured ham quality as related to lipid quality of raw material and lipid changes during processing: A review. *Grasas y Aceites*, *60*, 297–307.

**Garnier, J. P., Klont, R. & Plastow, G. (2003).** The potential impact of current animal research on the meat industry and consumer attitude towards meat. *Meat Science*, *63*, 79–88.

**Gjerlaug-Enger, E., Haug, A., Gaarder, M., Ljøkjel, K., Stenseth, R. S., Sigfridson, K. & Berg, P. (2015).** Pig feeds rich in rapeseed products and organic selenium increased omega-3 fatty acids and selenium in pork meat and backfat. *Food Science and Nutrition*, *3* (2), 120−128.

**Harris, W. S. (2007).** International recommendations for consumption of long chain omega-3 fatty acids. *Journal of Cardiovascular Medicine*, *8*, 50–52.

**Higgs, J. D. (2000).** The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science and Technology*, *11*, 85−95.

**Irie, M. & Sakimoto, M. (1992).** Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *Journal of Animal Science*, *70* (2), 470−477.

**Isabel, B., López-Bote, C. J., de la Hoz, L., Timón, M., Garcia C. & Ruiz, J. (2003).** Effects of feeding elevated concentrations of monosaturated fatty acids and vitamin E to swine on characteristics of dry cured hams. *Meat Science*, *64*, 475–482.

**Ivanovic, J., Pantic, S., Dokmanovic, M., Glamoclija, N., Markovic, R., Janjic, J. & Baltic, M. Z. (2015).** Effect of Conjugated Linoleic Acids in Pig Nutrition on Quality of Meat. *Procedia Food Science*, *5*, 105−108.

**Jakobsen, K. (1999).** Dietary modifications of animal fats: status and future perspectives. *European Journal of Lipid Science and Technology*, *101*, 475–483.

**Jiménez-Colmenero, F., Carballo, J. & Cofrades, S. (2001).** Healthier meat and meat products: their role as functional foods. *Meat Science*, *59* (1), 5−13.

**Jiménez-Colmenero, F., Ventanas, J. & Toldrá, F. (2009).** El jamón curado en una nutrición saludable. *Proceeding Of 5th World Congress Of Dry-Cured Ham*. Aracena, Spain.

**Jiménez-Colmenero, F., Reig, M. & Toldrá, F. (2006).** 11 New Approaches. *Advanced Technologies for Meat Processing*, 275.

**Jiménez-Colmenero, F., Ventanas, J. & Toldrá, F. (2010).** Nutritional composition of dry-cured ham and its role in a healthy diet. *Meat Science*, *84* (4), 585−593.

**Kauffman, R. G. (2001).** Meat composition. In Y. H. Hui, W. K. Nip, R. W. Rogers, & O. A. Young (Eds.), *Meat Science and Applications* (pp. 1–19). New York: Marcel Dekker, Inc.

**Lai, L., Kang, J. X., Li, R., Wang, J., Witt, W. T., Yong, H. Y. & Samuel, M. (2006).** Generation of cloned transgenic pigs rich in omega-3 fatty acids. *Nature Biotechnology*, *24* (4), 435−436.

**Larick, D. K., Turner, B. E., Schoenherr ,W. D., Coffey, M. T. & Pilkington, D. H. (1992).** Volatile compound contents and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, *70*, 1397–1403.

**Lauridsen, C., Mu, H. & Henckel, P. (2005).** Influence of dietary conjugated linoleic acid (CLA) and age at slaughtering on performance, slaughter- and meat quality, lipoproteins, and tissue deposition of CLA in barrows. *Meat Science*, *69*, 393–399.

**Leszczynski, D. E., Pikul, J., Easter, R. A., McKeith, F. K., McLaren, D. G. & Novakofski, J. (1992).** Characterization of lipid in loin and bacon from finishing pigs fed full-fat soybeans or tallow. *Journal of Animal Science*, *70*, 2175–2181.

**Lo Fiego, D. P., Macchioni, P., Santoro, P., Pastorelli, G. & Corino C. (2005)**. Effect of dietary conjugated linoleic acid (CLA) supplementation on CLA isomers content and fatty acid composition of dry-cured Parma ham. *Meat Science*, *70*, 285–291.

**Lucarini, M., Saccani, G., D'Evoli, L., Tufi, S., Aguzzi, A., Gabrielli, P. & Lombardi-Boccia, G. (2013).** Micronutrients in Italian ham: A survey of traditional products. *Food Chemistry*, *140* (4), 837−842.

**Markovic, R., Todorovic, M., Pantic, S., Baltic, M., Ivanovic, J., Jakic-Dimic D., Sefer D., Petrujkic, B. & Radulovic, S. (2015).** Effect of the diet on improvement of the fatty acid composition of pig meat. *First International Symposium of Veterinary Medicine*, Vrdnik, Serbia ISVM215, 148−156.

**Marusic, N., Concepción, Aristoy, M. & Toldrá, F. (2013).** Nutritional pork meat compounds as affected by ham dry-curing. *Meat Science*, *93* (1), 53−60.

**Mattila, P., Lehtonen, M. & Kumpulainen, J. (2000).** Comparison of in-line connected diode array and electrochemical detectors in the high-performance liquid chromatographic analysis of coenzymes Q9 and Q10 in food materials. *Journal of Agricultural and Food Chemistry*, *48* (4), 1229−1233.

**Mercier, Y., Gatellier, P., Viau, M., Remignon, H. & Renerre, M. (1998).** Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Science*, *48*, 301–318.

**Morgan, C. A., Noble, R. C., Cocchi, M. & McCartney, R. (1992).** Manipulation of the fatty acid composition of pig meat lipids by dietary means. *Journal of the Science of Food and Agriculture*, 58, 357–368.

**Naik, S. K., Tiwari, S. P., Sahu, T. & Doneria, R. (2015)**. Effect of dietary supplementation of organic selenium and vitamin E on growth performance, carcass characteristics and meat quality of broiler chickens. *The Indian Journal of Animal Sciences*, *85*, 9.

**Narváez-Rivas, M., Gallardo, E., Ríos, J. J. & León-Camacho, M. (2011).** A new high-performance liquid chromatographic method with evaporative light scattering detector for the analysis of phospholipids. Application to Iberian pig subcutaneous fat. *Journal of Chromatography A*, *1218* (22), 3453−3458.

**Neumann, C., Harris, D. M. & Rogers, L. M. (2002).** Contribution of animal source foods in improving and function in children in the developing world. *Nutrition Research*, *22*, 193–220.

**Olmedilla-Alonso, B., Jiménez-Colmenero, F. & Sánchez--Muniz, F. J. (2013).** Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, *95* (4), 919−930.

**O'Sullivan, M. G., Kerry, J. P., Buckley, D. J., Lynch, P. B. & Morrisey P. A. (1997).** The distribution of dietary vitamin E in the muscles of the porcine carcass. *Meat Science*, *45*, 297–305.

**Reig, M. & Toldrá, F. (1998).** Protein nutritional quality of muscle foods. *Recent Research Developments in Agricultural and Food Chemistry*, *2*, 71−78.

**Reig, M., Concepción, Aristoy, M. & Toldrá, F. (2013).** Variability in the contents of pork meat nutrients and how it may affect food composition databases. *Food Chemistry*, *140* (3), 478−482.

**Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F. & Stanton, C. (2011).** Bioactive peptides from muscle sources: meat and fish. *Nutrients*, *3* (9), 765−791.

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**Santos, C., Hoz, L., Cambero, M. I., Cabeza, M. C. & Ordoñez, J. A. (2008).** Enrichment of dry-cured ham with a-linolenic acid and a-tocopherol by use of linseed oil and α-tocopheryl acetate. *Meat Science*, *80*, 668–674.

**Schmid, A., Collomb, M., Sieber, R. & Bee, G. (2006).** Conjugated linoleic acid in meat and meat products: a review. *Meat Science*, *73*, 29–41.

**Simopoulos, A. P. (2002).** The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy*, *56* (8), 365−379.

**Toldrá, F. & Reig, M. (2011).** Innovations for healthier processed meats. *Trends in Food Science and Technology*, *22*, 517–522.

**Toldrá, F., Aristoy, M. C. & Flores, M. (2000).** Contribution of muscle aminopeptidases to flavor development in dry-cured ham. *Food Research International*, *33* (3), 181−185.

**Toldrá, F., Flores, M., Aristoy, M. C., Virgili, R. & Parolari, G. (1996).** Pattern of muscle proteolytic and lipolytic enzymes from light and heavy pigs. *Journal of the Science of Food and Agriculture*, *71*, 124–128.

**Toldrá, F., Rubio, M. A., Navarro, J. L. & Cabrerizo, L. (2004).** Quality aspects of pork and its nutritional impact. In F. Shahidi, A. M. Spanier, C-T. Ho & T. Braggins (Eds.). *Quality Of Fresh And Processed Foods. Advances In Experimental Medicine And Biology* (Vol. 542, pp. 25–32). New York: Kluwer Academic/Plenum Publications.

**Udenigwe, C. C. & Ashton, H. (2013).** Meat proteome as source of functional biopeptides. *Food Research International*, *54*, 1021–1032.

**Ventanas, J. (2006).** El jamón ibérico. Mundi-Prensa Libros.

**Ventanas, S., Ruiz, J., García, C. & Ventanas, J. (2007).** Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, *77* (3), 324−330.

**WHO, (2003)**. Diet, nutrition and the prevention of chronic diseases. *WHO Technical Report Series 916.*

**Winters, R. A., Zukowski, J., Ercal, N., Matthews, R. H. & Spitz, D. R. (1995).** Analysis of glutathione, glutathione disulfide, cysteine, homocysteine, and other biological thiols by high-performance liquid chromatography following derivatization by n-(1-pyrenyl) maleimide. *Analytical Biochemistry*, 227 (1), 14−21.

**Wyness, L., Weichselbaum, E., O'Connor, A., Williams, E. B., Benelam, B., Riley, H. & Stanner, S. (2011).** Red meat in the diet: An update. *British Nutrition Foundation Nutrition Bulletin*, *36*, 34–77.

**Wyss, M. & Kaddurah-Daouk, R. 2000.** Creatine and creatinine metabolism. *Physiological Reviews*, *80* (3), 1107−1213.

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# UPLC-MS/MS determination of histamine levels in canned fish collected from Belgrade retail markets

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*A b s t r a c t: The aim of this study was to determine the amount of histamine in canned fish samples collected from Belgrade retail stores using ultra-performance liquid chromatography tandem mass-spectrometry. In addition, the established levels were compared with the maximum levels set by US Food and Drug Administration (FDA) and European Union (EU) in order to assess the risk of this toxic biogenic amine to the city population. Histamine was detected in 54.07% of analyzed canned fish, in concentrations ranging from 5 to 420 mg/kg with a mean level of 60.91 mg/kg. In canned tuna, histamine levels ranged from 6 to 420 mg/kg, while in canned mackerel the concentrations ranged from 5 to 121 mg/kg. Also, the mean histamine level in canned tuna was higher than in canned mackerel (mean values were 60.91 mg/kg and 42.94 mg/kg, respectively). Among the tested canned fish, 20% of samples had higher histamine levels than the maximum level prescribed by the FDA (histamine levels >50 mg/kg), indicating definite decomposition of the fish. Histamine levels lower than 10 mg/kg were found in 51.48% of canned fish, which indicated good-quality fish products. Only 6.67% of examined production lots of canned fish had histamine levels above the regulatory limit according to the EU standard.*

***Keywords:*** *histamine, canned tuna, canned mackerel, UPLC-MS/MS.*

## Introduction

Fish and fishery products are essential for a complete diet, since they contain high amounts of proteins and free amino acids, low amounts of saturated fat and are also rich in omega fatty acids known to provide significant health benefits (*Babic et al.,* 2015; *Belicovska et al.,* 2015). On the other hand, if decomposition processes occur due to bacterial activity, enzymatic breakdown of proteins takes place, resulting in formation of biogenic amines. The enzyme histidine decarboxylase is formed during bacterial growth, and converts histidine to histamine, especially in scombroid and other fish with relatively high free histidine levels (*Zhai et al.,* 2012*).*

Scombroid fish poisoning occurs after consumption of fish or fishery products containing relatively high histamine levels (*Lehane and Olley,* 2000). This poses a significant public health and safety concern, due to the toxicological and physiological effects, as well as being a trade issue, due to potential economic losses (*Lehane and Olley,* 2000*; Sagratini et al.,* 2012). Tuna and mackerel are the most common fish species responsible for scombroid fish poisoning (*McLauchlin et al.,* 2006). Clinical symptoms of the illness are usually mild and vary considerably, with some of the prominent ones being rash, urticaria, nausea, vomiting, diarrhea, flushing, tingling and skin itching (*Taylor, 1986*). Although all biogenic amine intakes can result in clinical symptoms due to their vasoactive effects, histamine is the most commonly found amine and the only one with regulated quantities in certain types of seafood. In relation to this, monitoring of histamine levels has also been globally accepted as one of the key parameters used to determine the safety of fish and fishery products (*Sagratini et al.,* 2012). The European Union (EU) has established an acceptable level of 200 mg/kg of histamine for fish species belonging to *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, *Pomatomidae* and *Scombresocidae* families *(Commission Regulation (EC) No. 1441/2007).* On the other hand, the US Food and Drug Administration (FDA) issued guidelines for fish and fishery products establishing a maximum permissible level for histamine of 50 mg/kg in any sample (*FDA,* 1995). In addition, histamine can be a very useful indicator of good manufacturing practice of canned tuna and canned mackerel fish in oil (*Frattini and Lionetti,* 1998).

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The aim of this study was to determine the amount of histamine in canned fish samples collected from Belgrade (capital of Serbia) retail stores using ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS). In addition, the established levels were compared with the maximum levels set by the FDA and EU in order to assess the risk of this toxic biogenic amine to the city population.

## Materials and Methods

*Sample collection*

A total of 270 imported canned fish, 135 canned tuna and 135 canned mackerel, were collected from major Belgrade retail stores from 1st of July 2015 to 10th of July 2015. Samples were collected from three areas of the city. Thirty different production lots were analyzed, fifteen of each product type. The samples were collected before their expiration date, all cans were free from any physical damage and were stored at room temperature in their original packaging until analysis. After opening each can, oil was drained off, a piece of the fish muscle was taken and additionally pressed in order to remove excess oil. The analytical method used in this study was a modified method described by *Sagratini et al.* (2012). The method was validated according to the Commission Decision 2002/657/EC. All tests were performed in the Department for Residues of the Institute of Meat Hygiene and Technology, Belgrade.

*Preparation of standards*

Histamine stock solution was prepared by dissolving 100 mg of the analytical standard (Histamine, Sigma-Aldrich, St Louis, MO, USA) in 5% trichloroacetic acid (TCA), in 10 mL volumetric flask. The final concentration was 10 mg/mL. Stock solution was stored in the refrigerator at 4°C. The stock solution was stable for six months.

*Sample extraction*

A total of 1 g of each fish sample was weighed on a technical balance (Ohouse, model "Adventurer", New Jersey, USA), with an accuracy of 0.01 g in a 50 mL polypropylene tube. After addition of 10 mL 5% TCA (Sigma-Aldrich, St. Louis, MO, USA), samples were homogenized for one minute using an Ultra-Turrax S 18N-10 G (IKA-WerkeGmbh & Co., Germany) at 6000 rpm, and centrifuged at 4000 rpm for five minutes in a centrifuge (Thermo Scientific Heraeus™ model "Labofuge 200", Waltham, MA, USA). After centrifugation, supernatant was filtered through nylon syringe filters (pore diameter 0.45 mm) directly into the autosampler vials. Filtered supernatant (10 mL) was injected into the UPLC-MS/MS system.

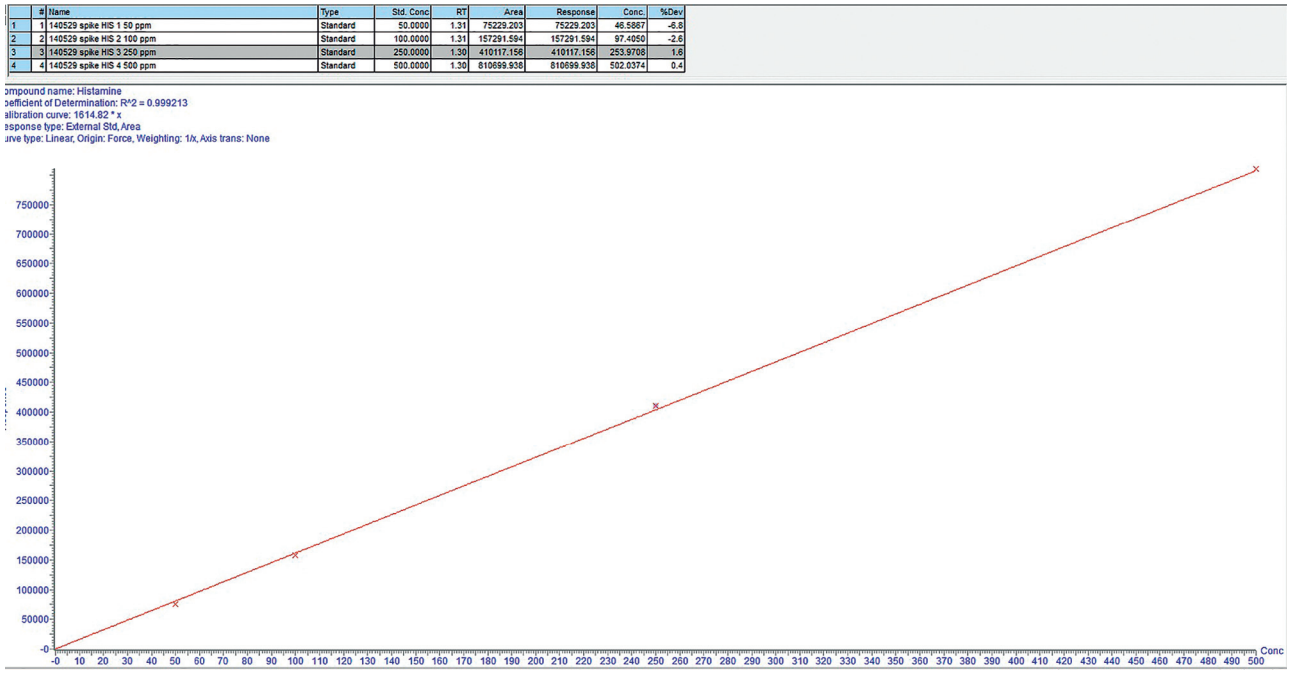
*UPLC-MS/MS*

Analyses were performed on a UPLC–MS/MS instrument consisting of a Waters Acquity UPLC system (Waters, Milford, MA, USA) with quaternary pump, autosampler, column heater and triple quadrupole mass spectrometer (TQD; Waters, Milford, MA, USA). Data acquisition and analysis were performed using MassLynx® software (version 4.1, Waters). Chromatographic separation of histamine was carried-out on Purospher® Star RP-18, reversed-phase column (50 × 2.1 mm, particle size 2 mm; Merck, Darmstadt, Germany) at 35±1°C with an isocratic flow rate of 0.20 mL/min. The mobile phase consisted of 10 mM ammonium acetate and 0.1% of formic acid in water (mobile phase A) and acetonitrile (mobile phase B) in the ratio A:B = 65:35. The temperature in the autosampler was maintained at 20±1°C. Ionization of neutral molecules of histamine to molecular ions was achieved using electrospray system in a positive mode (ESI +). The temperature of the ion source was 120°C, while the temperature of the dessolvatation gas (nitrogen) was 350°C. Capillary voltage and cone voltage were 4 kV and 10 V, respectively. Argon was used as collision gas; collision energy was set to 10 V. The mass spectrometer operated in MRM (multiple reaction monitoring) mode, monitoring the m/z of the molecular ion (112 Da) and two transitional products (95 Da and 64.5 Da). The transition product of 95 Da was used for quantification.

The reported limit of quantification (LoQ) of the method was 5 mg/kg. A five-point calibration curve (including zero) was constructed by injecting a blank sample and four fortified blanks (fish muscle found to contain less than the LoQ of histamine) containing histamine standard corresponding to the final concentrations of 50, 100, 250 and 500 mg/kg. Control samples were analyzed at the beginning and end of every batch of samples. A calibration curve of histamine is displayed in Figure 1.

## Results and Discussion

Histamine was detected in 146/270 (54.07%) of examined canned fish samples (Table 1), while 45.93% (n=124) of examined canned fish samples




| # | Name | Type | Std. Conc | RT | Area | Response | Conc. | %Dev |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 140529 spike HIS 1 50 ppm | Standard | 50.0000 | 1.31 | 75229.203 | 75229.203 | 46.5867 | -6.8 |
| 2 | 2 140529 spike HIS 2 100 ppm | Standard | 100.0000 | 1.31 | 157291.594 | 157291.594 | 97.4050 | -2.6 |
| 3 | 3 140529 spike HIS 3 250 ppm | Standard | 250.0000 | 1.30 | 410117.156 | 410117.156 | 253.9708 | 1.6 |
| 4 | 4 140529 spike HIS 4 500 ppm | Standard | 500.0000 | 1.30 | 810699.938 | 810699.938 | 502.0374 | 0.4 |

**Figure 1.** Calibration curve of histamine

had histamine levels below the LoQ of 5 mg/kg (Figure 2). These results differ slightly from data presented by *Karmi* (2014), who found that the overall frequency distribution rate of histamine in canned fish was 66.7%, whilst 33.3% of samples were free from histamine. However, in the study of *Kalantari et al.* (2015), histamine was detected in 78.9% of examined canned fish, which is a much higher percentage compared to the results obtained in the current study.

Statistical data for histamine concentrations in canned fish obtained from Belgrade retail markets are given in Table 1. Histamine concentrations in canned fish ranged from 5 to 420 (mean: 52.17) mg/kg. These results were higher than the results reported by *Karmi* (2014*)*, but lower than the results obtained by *Zaman et al.* (2009*)*. However, they were in agreement with the data of *Khezri et al.* (2014). Histamine levels in canned tuna, found in the present study, ranged from 6 to 420 mg/kg (Table 1). Histamine concentrations in canned mackerel ranged from 5 to 121 mg/kg (Table 1), which were higher than the results reported by *Tsai et al.* (2005). As opposed to previous studies (*Tsai et al.,* 2005*; Yesudhason et al.,* 2013; *Karmi,* 2014), the mean histamine levels in canned tuna and canned mackerel were 60.91 mg/kg and 42.94 mg/kg, respectively. In addition, canned tuna contained a higher mean histamine level than canned mackerel (60.91 mg/kg and 42.94 mg/kg, respectively), which was in line with the results of *Karmi* (2014*)*.

Five samples of canned tuna taken from Belgrade retail markets contained histamine levels higher than 200 mg/kg (ranged from 212 to 420 mg/kg). All humans are susceptible to histamine and the individual sensitivity, the amount of

**Table 1.** Statistical data of histamine concentrations (mg/kg) in canned fish samples obtained from Belgrade retail markets (n = 270)

| | n > QL[a] | Mean for > QL[b] | SD[c] | SE[d] | Min | Max |
|---|---|---|---|---|---|---|
| **Canned tuna** (n = 135) | 75 | 60.91 | 78.1 | 9.02 | 6 | 420 |
| **Canned mackerel** (n = 135) | 71 | 42.94 | 31.68 | 3.76 | 5 | 121 |
| **Total** (n = 270) | 146 | 52.17 | 60.65 | 5.02 | 5 | 420 |

[a]Number of samples with concentrations above quantification limit. [b]Mean values for samples with concentrations above quantification limit. [c]Standard deviation. [d]Standard error.

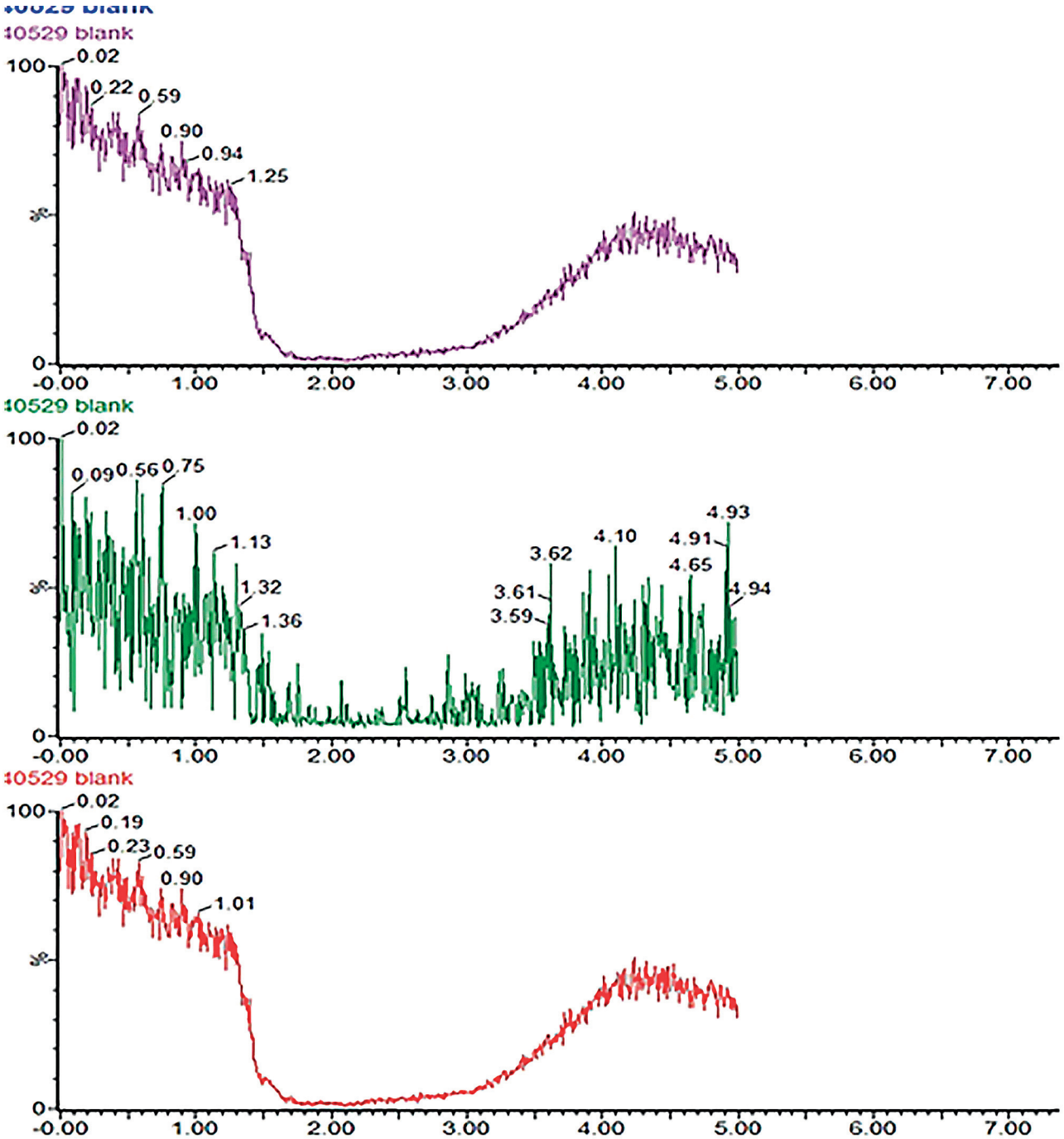



**Figure 2.** MRM chromatograms of histamine in canned fish sample below the LoQ

histamine in food, and the detoxification activity in the human body are the most important factors influencing the toxicological response in consumers (*Visciano et al.,* 2014). The consumption of a portion of 250 g of fish containing 200 mg/kg of histamine was reported to cause the toxicological symptoms of scombroid fish poisoning (*Centers for Disease Control and Prevention (CDC),* 2000*; Food and Agriculture Organization/World Health Organization (FAO/WHO),* 2012). Hence, this indicates that the canned fish with high histamine levels (>200 mg/kg) found in this study posed a health risk to consumers. In contrast, no sample of canned mackerel contained histamine concentrations higher than 200 mg/kg. In January 2014, an outbreak of scombroid fish poisoning occurred in 28 children (aged 2 to 5 years) who consumed canned sardines in a kindergarten in Vojvodina province, northern Serbia (*Petrovic et al.,* 2016). The authors reported that seven of the nine investigated units had

histamine levels above 300 mg/kg, a concentration that leads to classical intoxication symptoms. CDC reported that scombroid fish poisoning contributes 5% of all food-related illness and 37% of all seafood-related illnesses, and cases of histamine poisoning are reported every year (*Yesudhason et al.,* 2013). Scombroid fish poisoning occurs worldwide, while the US, Great Britain and Japan are countries with the highest number of reported incidents (*Lehane and Olley,* 2000). One of the largest reported outbreaks of scombroid fish poisoning in the US occurred in August 2003 in California, where the fish samples contained markedly elevated histamine levels (from 2000 to 3800 mg/kg) (*Feldman et al.,* 2005). The European Food Safety Authority (EFSA) (*EFSA*, 2011) reported that histamine in fish or fishery products were the causative agents of more than 100 outbreaks in Europe between 2005 and 2010.

As one of the most important biogenic amines, histamine was identified by the US FDA as the major chemical hazard of seafood products (*Tao et al.,* 2011). Based on the guidelines issued by the FDA *(FDA*, 2010*)*, good-quality fishery products should contain less than 10 mg/kg of histamine, a level of 30 mg/kg indicates significant deterioration, whereas a level of 50 mg/kg is evidence of definite decomposition, and therefore, fish with this level or greater should be declared unfit for human consumption. Thus, analysis of histamine in fish and fishery products is of interest because of the toxicological risk, but also because histamine levels can be used as chemical indicators of fish spoilage, food quality and degree of freshness of the product (*Muscarella et al.,* 2013).

According to the FDA (*FDA*, 2011)*,* the number of scombrotoxin-forming fish samples necessary to make a judgment about a lot, should not be fewer than 18 samples per lot, unless the lot contains less than 18 fish, in which case a sample should be collected from each fish. In this study, there were fewer than 18 samples per lot, and therefore, each can of fish was calculated as a separate sample. The results of histamine determination in canned fish obtained from Belgrade retail markets according to the FDA criteria are depicted in Table 2. In the present study, histamine levels of less than 10 mg/kg were detected in 50.37% and 52.59% (in total, 51.48%) of examined canned tuna and canned mackerel, respectively, indicating good quality of products. The results of this study are comparable with the report by *Muscarella et al.* (2013), who found that 70% of 216 examined fish samples were good-quality fish products (histamine level<10 mg/kg). Also, in the current study, 30.37% of canned tuna and 26.67% of canned mackerel contained histamine levels between 10 and 50 mg/kg, indicating a lower quality of these products (Table 2).

As can be seen in Table 2, among the tested canned fish, a total of 54 (20.00%) exceeded the FDA regulatory limits for histamine and were unsafe for human consumption. Results from earlier studies (*Karmi et al.,* 2014; *Khezri et al.,* 2014) showed that the use of poor quality fish as raw material for canning and/or defective handling techniques of fish during processing are the main reasons of high percentage of unacceptable canned fish. Histamine levels in 26 of 135 canned tuna (19.26%) ranged from 52 to 420 mg/kg, higher than the tolerance limit of 50 mg/kg accepted by the FDA (Table 2) (Figure 3). In 28 of 135 canned mackerel fish (20.74%), histamine levels were between 50 to 121 mg/kg, which were higher than the tolerance limit of 50 mg/kg accepted by the FDA. This result is consistent with the findings reported by *Muscarella et al.* (2013), who found that 18% of fish samples were unfit for human consumption (histamine levels >50 mg/kg). Comparable results were reported by *Zarei et al.* (2011*)*, who found that 25% of tested samples had histamine levels higher than maximum levels prescribed by the FDA (*FDA*, 1995). In contrast, Yesudhason et al. (2013), *Khezri et al.* (2014) and *Kalantari et al.*

**Table 2.** The results of histamine determination in canned fish samples obtained from Belgrade retail markets according to the US Food and Drug Administration (n = 270)

| | Histamine Levels (mg/kg) | | |
|---|---|---|---|
| | **n (%) < 10** [a] | **10 < n (%) < 50** [b] | **n (%) > 50** [c] |
| **Canned tuna** (n = 135) | 68 (50.37) | 41 (30.37) | 26 (19.26) |
| **Canned mackerel** (n = 135) | 71 (52.59) | 36 (26.67) | 28 (20.74) |
| **Total** (n = 270) | 139 (51.48) | 77 (28.52) | 54 (20.00) |

[a]**Good-quality fish products** – less than 10 mg/kg of histamine. [b]**Significant deterioration of fish products** – a level of histamine higher than 10 mg/kg but lower than 50 mg/kg. [c]**Unfit for human consumption** – a level of histamine higher than 50 mg/kg.

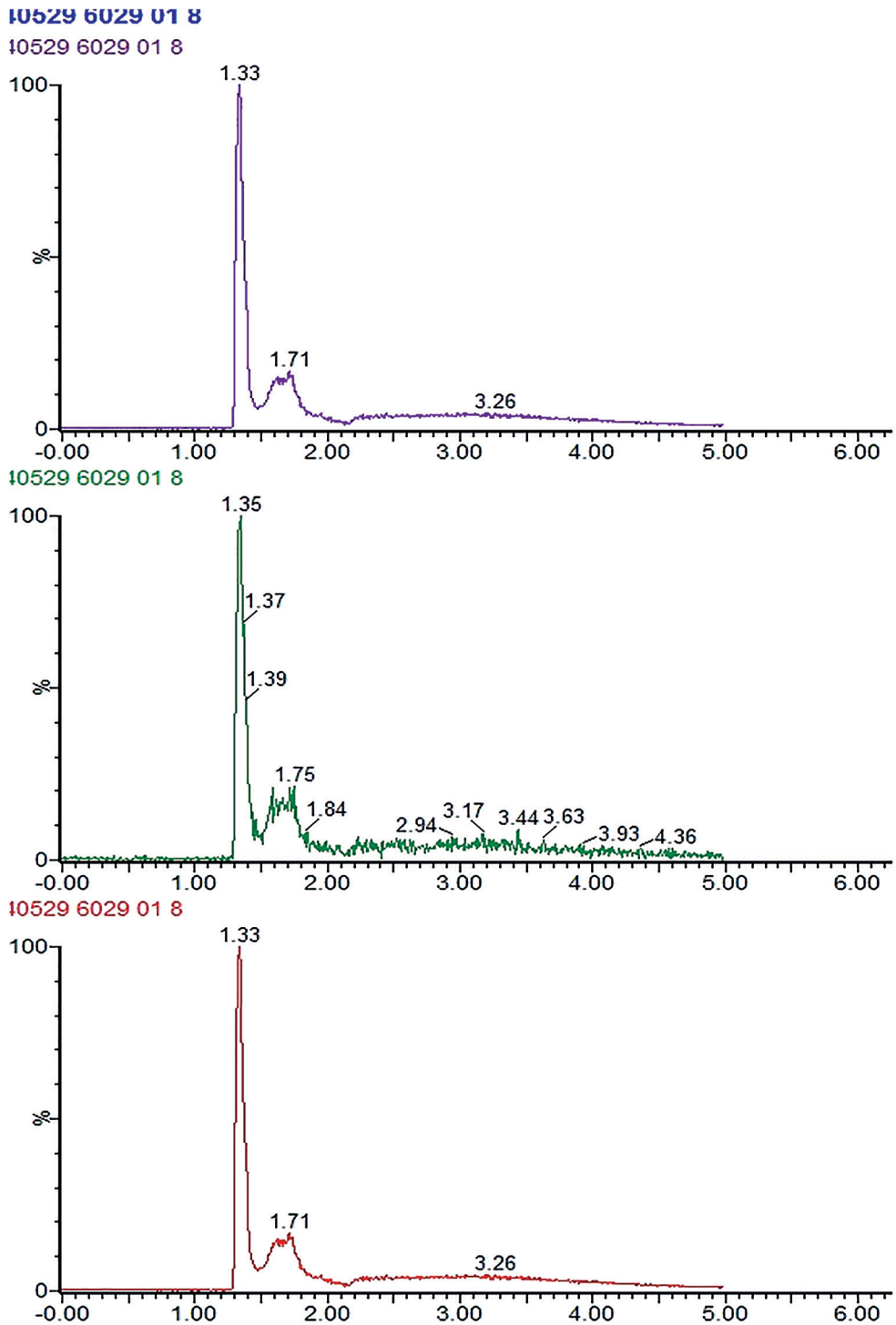



**Figure 3.** MRM chromatograms of histamine in a canned tuna fish sample at a concentration of 90 mg/kg. Chromatograms depict transitional products 112>95 Da, 112>64.5 Da and total ion count (TIC) of the molecular ion of histamine

(2015) found that only 0.79, 6.67% % and 3.70% of samples, respectively, exceeded the FDA regulatory limits for histamine.

The legislation in Serbia (*Official Gazette RS 72/2010*) in respect to histamine level is harmonized with the *EC Regulation 1441/2007*. The examination of one production lot includes testing of nine samples. The permitted level implies that not more than two out of nine samples can have histamine levels of more than 100 mg/kg but less than

200 mg/kg; however, no sample can have a histamine level of ≥ 200 mg/kg, and, finally, the mean histamine level of all nine samples must not exceed 100 mg/kg. Furthermore, the analysis can be performed using HPLC analytical method, or other methods. The results of histamine determination in the 30 lots of canned fish obtained from Belgrade retail markets, analyzed according to the European Commission regulation (*Commission Regulation (EC) No. 1441/2007*) are shown in Table 3. Out of 15 analyzed production lots of canned tuna, only one (6.67%) was above the regulatory limit (Table

**Table 3.** The results of histamine determination in canned tuna and canned mackerel of 30 lots obtained from Belgrade retail markets according to the EU standards

| Lot No. | No. of units between 100 and 200 mg/kg of histamine | No. of units ≥ 200 mg/kg of histamine/ | Mean value of Histamine in 9 units (mg/kg) | Lots exceeding EU tolerance limit (YES/NO) |
|---|---|---|---|---|
| **Canned tuna** | | | | |
| **1.** | 0/9 | 0/9 | 4.5 | |
| **2.** | 0/9 | 0/9 | 19.61 | NO |
| **3.** | 0/9 | 0/9 | 28.33 | NO |
| **4.** | 0/9 | 0/9 | **nd** | NO |
| **5.** | 0/9 | 0/9 | **nd** | NO |
| **6.** | 1/9 | 0/9 | 72 | NO |
| **7.** | 0/9 | 0/9 | 9.5 | NO |
| **8.** | 2/9 | 0/9 | 71 | NO |
| **9.** | 4/9 | 5/9 | 242.1 | **YES** |
| **10.** | 0/9 | 0/9 | 5 | NO |
| **11.** | 0/9 | 0/9 | 13.44 | NO |
| **12.** | 0/9 | 0/9 | 33.94 | NO |
| **13.** | 0/9 | 0/9 | **nd** | NO |
| **14.** | 0/9 | 0/9 | 12.28 | NO |
| **15.** | 0/9 | 0/9 | 5 | NO |
| **Canned mackerel** | | | | |
| **1.** | 0/9 | 0/9 | 8.44 | NO |
| **2.** | 0/9 | 0/9 | 20.56 | NO |
| **3.** | 0/9 | 0/9 | **nd** | NO |
| **4.** | 0/9 | 0/9 | **nd** | NO |
| **5.** | 0/9 | 0/9 | 5.61 | NO |
| **6.** | 0/9 | 0/9 | 9.5 | NO |
| **7.** | 0/9 | 0/9 | **nd** | NO |
| **8.** | 0/9 | 0/9 | 47 | NO |
| **9.** | 0/9 | 0/9 | 4.89 | NO |
| **10.** | 0/9 | 0/9 | 64.67 | NO |
| **11.** | 0/9 | 0/9 | 69.22 | NO |
| **12.** | 0/9 | 0/9 | **nd** | NO |
| **13.** | 3/9 | 0/9 | 96.11 | **YES** |
| **14.** | 0/9 | 0/9 | **nd** | NO |
| **15.** | 0/9 | 0/9 | 18.06 | NO |

**nd** – not detected (<LoQ).

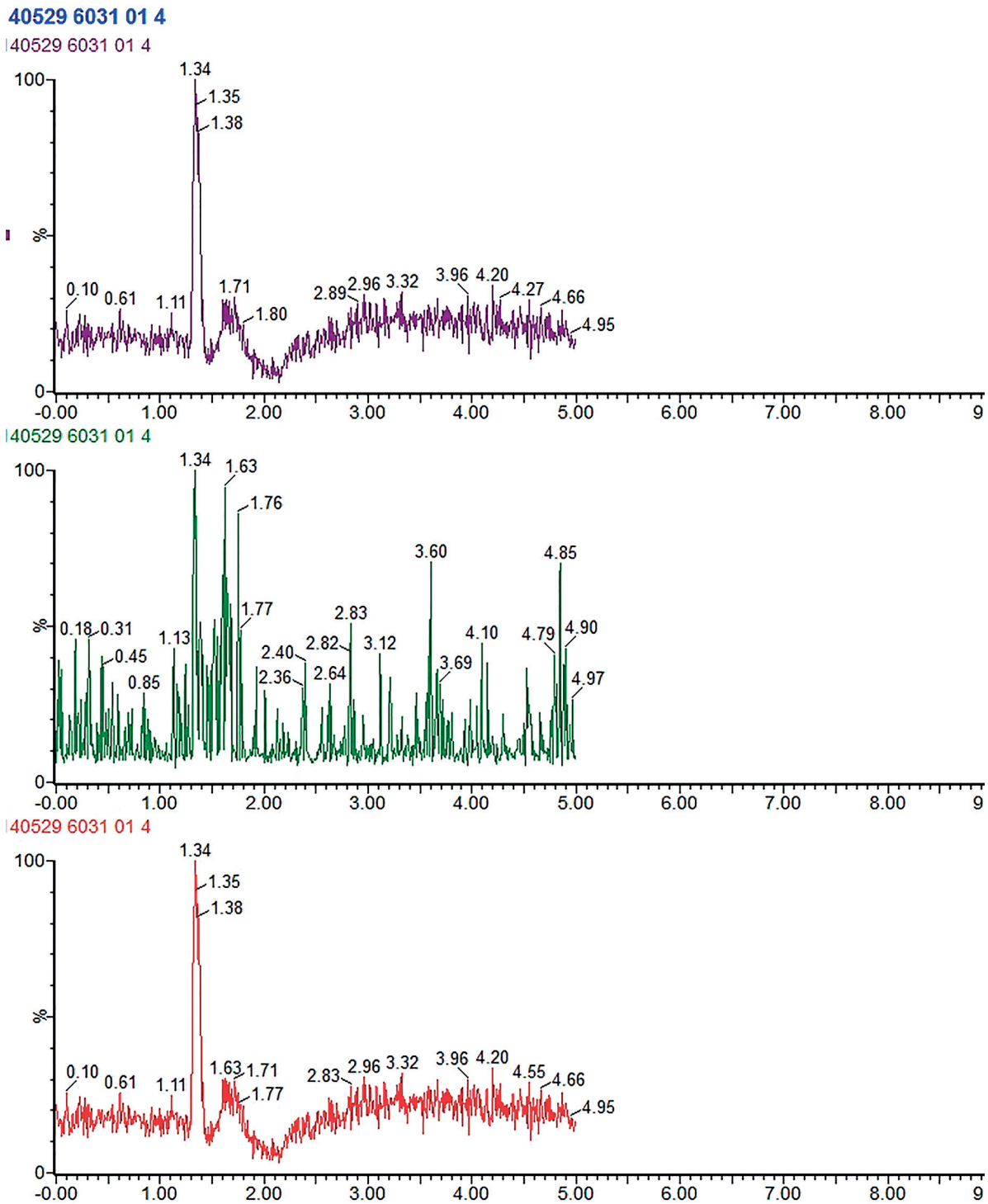



**Figure 4.** MRM chromatograms of histamine in a canned mackerel sample at a concentration of 5 mg/kg. Chromatograms depict transitional products 112>95 Da, 112>64.5 Da and total ion count (TIC) of the molecular ion of histamine

3). In addition, 1 of 15 (6.67%) examined production lots of canned mackerel exceeded the EU regulatory limits for histamine (Table 3) (Figure 4). Similar results were reported in the study of *Babic et al.* (2015), who found that out of 97 analyzed production lots, 3.09% were above the EU regulatory limits for histamine. Likewise, comparable results were reported by *Muscarella et al.* (2013), who found that 5% of tested samples had higher histamine levels than maximum levels prescribed by the EU (*EC Regulation 1441/2007)*. In contrast, *Kalantari et al.* (2015) found that only

0.79% of the total samples exceeded the EU regulatory limits for histamine.

On the whole, 80.00% and 93.33% of the analyzed canned fish samples were in accordance with the FDA and EU regulatory limits for histamine, respectively, indicating that the sampling scheme of US Food and Drug Administration offers more confidence that non-conforming lots will be detected *(Visciano et al.,* 2014). As stated in *EU Regulation 1019/2013*, for the detection of histamine, single samples can be taken at retail level. Despite the fact that the whole lot is not to be deemed unfit for human consumption based on the result of only one sample (unless the result is above 200 mg/kg) (*EU Regulation 1019/2013*), EU regulatory limits for histamine are fourfold higher than FDA tolerance limits (200 mg/kg and 50 mg/kg, respectively). Also, it could be argued that the possibility of preventing scombroid fish poisoning will be much higher if the FDA regulatory limits for histamine are applied, rather than the EU limits. Furthermore, on the basis of the US FDA regulatory limits for histamine, fish or fishery products can be classified according to quality, which is not the case if EU legislation is applied.

## Conclusion

Histamine was detected in 54.07% of analyzed canned fish, in concentrations ranging from 5 to 420 mg/kg with a mean level of 60.91 mg/kg. In canned tuna, histamine levels ranged from 6 to 420 mg/kg, while in canned mackerel, the concentrations ranged from 5 to 121 mg/kg. Also, the mean histamine level in canned tuna was higher than in canned mackerel (mean values were 60.91 mg/kg and 42.94 mg/kg, respectively). After the histamine levels in canned fish were determined, they were compared with the FDA and EU standards. The results showed that among the tested canned fish, 20% of samples had higher histamine levels than maximum levels prescribed by the FDA (*FDA*, 1995) (histamine levels >50 mg/kg), indicating evidence of definite decomposition of product. Histamine concentrations lower than 10 mg/kg were found in 51.48% of canned fish samples, which indicated good-quality fish products. In addition, only 6.67% of examined production lots of canned fish had histamine levels above the regulatory limit according to EC Regulation 1441/2007 (*Commission Regulation (EC) No. 1441/2007*).

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## References

**Babic, J., Milijasevic, M. & Dimitrijevic, M. (2015).** *The impact of packaging in modified atmosphere and vacuum on preservation of sensory properties of carp filets* (*Cyprinus carpio*). Tehnologija mesa, 56, 1, 58–66.

**Babic, J., Petrovic, J., Kartalovic B., Pelic, M., Jaksic S. & Cirkovic, M. (2015).** *The importance of border inspection in control histamine poisoning from canned tuna*, VII International Conference "WATER & FISH", June, 10–12. Faculty of Agriculture, Belgrade, Serbia 165–169.

**Belicovska, K., Belicovska, D., Pejkovski & Uzunoska, Z. (2015).** Chemical composition and pH value of rainbow trout (*Oncorhynchus mykiss*) and river trout (*Salmo trutta fario*) meat grown in fish ponds. *Tehnologija mesa, 56, 1*, 75–80.

**Centers for Disease Control and Prevention, (1998)**. Scombroid fish poisoning – Pennsylvania, *Morbidity and Mortality Weekly Report, 49***,** 2000, 398–400.

**Commission Regulation (EC) No. 1441/2007** of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union, L 322*, 12–29.

**Commission Regulation (EC) No. 657/2002** of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results Official Journal of the European Union, L 221, 8–36

**European Food Safety Authority, (2011)**. Scientific opinion on risk based control of biogenic amine formation in fermented foods, *EFSA Journal, 9*, 10, 2393.

**Feldman, K., Werner, S., Cronan, S., Hernandez, M., Horvath, A., Lea, C., Au, A. M. & Vugia, D. J. (2005).** A large outbreak of scombroid fish poisoning associated with eating escolar fish (*Lepidocybium flavobrunneum*). *Epidemiology and Infection, 133*, 1, 29–33.

**Food and Agriculture Organization/World Health Organization (FAO/WHO), (2012)**. Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products, Joint FAO/WHO expert meeting report, Rome, FAO Headquarters, 1–111.

**Food and Drug Administration, (1995).** Decomposition and histamine-raw frozen tuna and mahi–mahi; canned tuna; and related species; availability of revised compliance policy guide, Federal Registration, 60**,** 1, 39754–39756.

**Food and Drug Administration, (2010)**. Fish and fisheries products hazards and controls guidance. *Scombrotoxin (histamine) formation: a chemical hazard*, Washington, DC: Food and Drug Administration 3rd ed.

**Food and Drug Administration, (2011)**. Fish and Fishery Products Hazards and Controls Guidance Washington, DC: Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, 4th ed.

**Frattini, V. & Lionetti, C. (1998).** Histamine and histidine determination in tuna fish samples using high-performance liquid chromatography derivatization with o-phthalaldehyde and fluorescence detection or UV detection of ''free'' species. *Journal of Chromatography A, 809*, 241–245.

**Kalantari, H., Behfar, A. A., Nazari, Z., Kalatari, M. & Hosseini, H. (2015).** Occurrence of histamine in canned tuna fish produced of two major manufactories in Khuzestan province by HPLC method. *International Journal of Current Research Chemistry and Pharmacy Science, 2, 10*, 9–15.

**Karmi M. (2014).** Determination of histamine and tyramine levels in canned salted fish by using HPLC. *Global Veterinaria 12, 2,* 264–269.

**Khezri, M., Hosseininia, S. A. R., Kamani, M. H. & Safari, O. (2014).** Determination of histamine in canned tuna using ELISA method. *Trends in Life Science 3, 2,* 95–101.

**Lehane, L. & Olley, J. (2000).** Histamine fish poisoning revisited.. *International Journal of Food Microbiology, 58*, 1–37.

**McLauchlin, J., Little, C. L., Grant, K. A. & Mithani, V. (2006).** Scombrotoxic fish poisoning. *Journal of Public Health, 28, 1,* 61–62.

**Muscarella, M., Magro, S. L., Campaniello, M., Armentano, A. & Stacchini, P. (2013).** Survey of histamine levels in fresh fish and fish products collected in Puglia (Italy) by ELISA and HPLC with fluorimetric detection. *Food Control, 31,* 211–217.

**Official Gazette RS, (2010)**. Regulation on general and special conditions of hygiene of food at any stage of production, processing and transport. 72/2010.

**Petrovic, J., Babic, J., Jaksic, S., Kartalovic, B., Ljubojevic, D. & Cirkovic, M. (2016).** Fish Product–Borne Histamine Intoxication Outbreak and Survey of Imported Fish and Fish Products in Serbia. *Journal of Food Protection, 79, 1,* 90–94.

**Regulation 1019/2013/ EU. Commission Regulation EU No 1019/2013**. Amending annex I to regulation EC No2073/2005 as regards histamine in fishery products. Official Journal of the European Union. L.282, 46–47.

**Sagratini, G., Fernández-Franzón, M., De Berardinis, F., Font, G., Vittori, S. & Mañes, J. (2012).** Simultaneous determination of eight underivatised biogenic amines in fish by solid phase extraction and liquid chromatography–tandem mass spectrometry. *Food Chemistry, 132,* 537–543.

**Tao, Z., Sato, M., Han, Y., Tan, Z., Yamaguchi, T. & Nakano, T. (2011).** A simple and rapid method for histamine analysis in fish and fishery products by TLC determination. *Food Control, 22,* 1154–1157.

**Taylor, S. L. (1986).** Histamine food poisoning: toxicology and clinical aspect. *Critical Reviews in Toxicology 17*, 91–117.

**Tsai, Y. H., Lin, C. Y., Chang, S. C., Chen, H. C., Kung, H. F., Wei, C. I. & Hwang, D. F. (2005).** Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology, 22*, 461–467.

**Visciano, P., Schirone, M., Tofalo R. & Suzzi, G. (2014).** Histamine poisoning and control measures in fish and fishery products. *Frontiers in Microbiology, 5*, 1–3.

**Yesudhason, P., Al-Zidjali, M., Al-Zidjali, A., Al-Busaidi, M., Al-Waili, A., Al-Mazrooei, N. & Al-Habsi, S. (2013).** Histamine levels in commercially important fresh and processed fish of Oman with reference to international standards. *Food Chemistry, 140*, 777–783.

**Zaman, M. Z., Abdulamir, A. S., Bakar, F. A., Selamat J. & Bakar, J. (2009)**. Microbiological, physicochemical and high impact of high level of biogenic amines in fish sauce. *American Journal of Applied Science, 6*, 1199–1211.

**Zarei, M., Najafzadeh, H., Enayati, A. & Pashmforoush, M. (2011)**. Biogenic amines content of canned tuna fish marketed in Iran. *America-Eurasian Journal of Toxicological Sciences 3, 3*, 190–193.

**Zhai, H., Yang, X., Li L., Xia, G., Cen, J., Huang H. & Hao, S. (2012).** Biogenic amines in commercial fish and fish products sold in southern China. *Food Control, 25*, 303–308.

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*Review paper*

# Predicting microbial growth: Theory and Application

*Vesna V. Jankovic[1], Ivan Nastasijevic[1], Brankica Lakicevic[1]*

*A b s t r a c t: Predictive microbiology is a recent area within food microbiology, which studies the responses of microorganisms in foods to environmental factors (e.g. temperature, pH, NaCl) through mathematical functions. These functions enable scientists to predict the behavior of pathogens and spoilage microorganisms under different combinations of factors. Predictive microbiology models have immediate practical applications to improve microbial food safety and quality, and are leading to the development of a quantitative understanding of the microbial ecology of foods. Predictive models in foods have developed significantly in the last 20 years due to the emergence of powerful computational resources and sophisticated statistical packages.*

*Modeling microbial responses in food requires the interdisciplinary collaboration of food microbiologists and mathematicians; food technologists and computing scientists; molecular microbiologists and statisticians.*

***Key words:*** *predictive microbiology, microbial ecology of food, food safety and quality.*

## Introduction

Predictive microbiology – or the quantitative microbial ecology of foods – represents a proactive approach to food quality and safety by accumulating information on bacterial responses related to intrinsic and extrinsic factors characterizing the food and its environment and summarizing the responses in databases and mathematical models (*Bjerre*, 2014; *McMeekin et al*., 1997; *McMeekin and Ross*, 2002; *Toldra*, 2009).

Predictive microbiology in foods is a research area within food microbiology intended to provide mathematical models to predict microbial behavior in food environments (*Fakruddin et al.*, 2011). Although the first predictive models date to the beginning of the 20$^{th}$ century, rapid development has occurred in recent decades as a result of computer software advances. In addition to exhaustive knowledge of food microbiology, the predictive microbiology field is based on important mathematical and modeling concepts that should be previously introduced for predictive microbiology beginners (*McMeekin et al.*, 1997).

The different typology of predictive models allows the prediction of growth, inactivation, or the probability of bacterial growth in foods under different environmental conditions and considering additional factors such as the physiological state of cells or interaction with other microorganisms. Nowadays, predictive models have become a necessary tool, allowing rapid responses to specific questions. Predictive models allow the estimation of the shelf-life of foods, define critical points in the production and distribution process and can give insight on how environmental variables affect the behavior of pathogenic or spoilage bacteria. Furthermore, predictive models have been incorporated as helpful elements into the self-control systems such as Hazard Analysis for Critical Control Point (HACCP) programs and food safety risk-based metrics. National and international food safety policies are now based on the development of Quantitative Microbial Risk Assessments studies, which is greatly supported but at the same time is turning into an important tool for improving food safety and quality (*Fakruddin et al*., 2011; *Perez-Rodriguez and Valero*, 2013). Microorganisms of interest are foodborne pathogens such as *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Clostridium botulinum* and spoilage microorganisms such as *Enterococcus* spp., *Pseudomonas* spp. and *Enterobacter* spp. *(Jankovic et al.,* 2013; 2014; 2015; *Lakicevic et al.*, 2014; 2015; *Nastasijevic*, 2011; *Nastasijevic*, 2014; *Nastasijevic et al.*, 2014).

## History

The origin of predictive microbiology, as pointed out by Perez-Rodriguez and Valero (2013) is often linked to the works by *Bigelow et al.* (1920),

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*Bigelow* (1921) and *Esty and Meyer* (1922), in which a log linear model was proposed to describe bacterial death kinetics by heat. Their model found wide application in the food industry, and especially in the canning industry. Indeed, nowadays, these results are still applied by the food industry to reduce *Clostridium botulinum* in "low acid" canned foods. This model simply says that at a given temperature, the relative (or specific) death rate of the bacteria is constant with time. In other words, the percentage of the cell population inactivated in a unit time is constant. This is a simple, logical and understandable model, similar to those commonly used in physical and chemical sciences for processes such as dissipation, diffusion, etc., when the force that causes the decrease of a certain quantity is constant with time (*Baranyi et al.*, 1994; 2004). A step forward was taken by *Scott* (1936), who investigated how the specific death rate depended on the available water, quantified today by the so-called water activity, a dimensionless number between 0 (dry) and 1 (wet). He subsequently studied the effect of the temperature on the specific microbial death rate. Modeling microbial growth was also being done in the field of industrial microbiology (*Monod*, 1949). During the 1960s and 1970s, several efforts were devoted to apply mathematical models to inactivation of pathogens (*Clostridium botulinum* and *Staphylococcus aureus*) and growth of spoilage bacteria (*Nixon*, 1971; *Spencer and Baines*, 1964). Nonetheless, the great development of predictive microbiology started during the 1980s when computers and specific software facilitated the development of more complex and precise models. The term "predictive" microbiology, which is relatively recent, was coined by *Roberts and Jarvis* (1983), establishing the conceptual basis of modern predictive microbiology (*Brul et al.*, 2008). In the first book on the subject, *McMeekin et al.* (1993) defined it as a quantitative science that enables users to evaluate objectively the effect of processing, distribution and storage operations on the microbiological safety and quality of foods. *McMeekin et al.* (1993) suggested, as another possible explanation for the development of predictive microbiology, the marked increase in foodborne diseases during those years together with a major awareness of the limitations of the microbiological methods applied at that time. The scientific discipline of predictive microbiology aims to condense microbiological knowledge and mathematical techniques into mathematical models, capable of describing and predicting microbial growth in various environments, mostly related to food products (*Baranyi and Roberts*, 1994; *Ross and McMeekin*, 1994).

## Development and limitations of predictive models

It is a general goal of food microbiologists to know in advance the behavior of microorganisms in foods under foreseeable conditions. To do so, exhaustive control of physicochemical factors that could influence microbial growth is needed (such as t, pH, $a_w$, salt, etc.), as well as in-depth knowledge about the biological characteristics of the target microorganisms (*Fakruddin et al.*, 2011; *Hajmeer and Cliver*, 2002).

The premise behind the scientific basis of predictive microbiology is that microbial responses in foods are reproducible when considered in the context of several extrinsic and intrinsic environmental factors (*Ross et al*, 2000). This behavior can be translated into diverse mathematical models that estimate microbial growth/inactivation/toxin production/probability of growth etc. This emerging area was redefined recently as modeling microbial responses in foods (*McMeekin et al.*, 2002).

Several authors suggested different classifications of predictive models based on their final purpose, the type of microorganisms to be studied, and their impact on food spoilage or food safety (*Roberts,* 1989; *Ross and McMeekin*, 2003; *van Boeckel*, 2008).

Basically, predictive models are split up into three groups: survival/inactivation models, boundary (growth/no growth) models and growth models. Basing on their development, models can be classified as follows (Figure 1):

1. Primary models: aim to describe the kinetics of a process with as few parameters as possible while being able to accurately define the growth and inactivation phases. They are represented as the increase/decrease in population density against time. Primary models developed in the 90s are still widely used, but are mainly empirical (*Baranyi and Roberts*,1994; *Buchanan et al.*, 1997; *Geeraerd et al*., 2000).
2. Secondary models: describe the effect of environmental conditions (physicochemical and biological factors) on the values of the parameters of a primary model. Most currently-used secondary models can be subdivided into four classes (*Adopted from Van Impe et al.* (2013): (i) square root models (*Ratkowsky et al.,* 1982; *Ratkowsky et al.,* 2003), (ii) cardinal parameter models (*Rosso et al.*, 1995; *Sautour et al.*, 2001), (iii) neural networks (*Geeraerd et al*.,1998; *Panagou et al.*, 2007), and (iv) response surface models (*Baranyi et al.*, 1996; *Geeraerd et al.*, 2004). Secondary

models used for describing the effect of environmental conditions on microbial growth include (*McKellar and Lu*, 2004): Arrhenius-type models, Belehradek type models, models based on the gamma concept, cardinal parameter models, and polynomial models.

3. Tertiary models: based on computer software programs that provide an interface between the underlying mathematics and the user, allowing model inputs to be entered and estimates to be observed through simplified graphical outputs. *Whiting and Buchanan* (1997) called the foregoing integrated software-based model "tertiary models". Tertiary models are the application of the aforementioned models, included in user-friendly software in order that they can be used without additional modeling; moreover, nonmodelers can use these tertiary models (*Fakruddin et al*., 2011). One or more of the primary and secondary models are compiled to provide a prediction, generally coupled with databases gathering the input parameters such as cardinal values, optimal growth rate, and so on required for running the simulation (*McDonald and Sun*, 1999). Usually, various factors can be specified, such as temperature, $a_w$, pH, NaCl concentration, and so on. All of these input parameters used in tertiary models were previously validated in primary and/or secondary models. One of the main uses of such software is in product development, since they let the user examine the effect of formulation changes on the safety of the product without costly pilot plant trials. The software packages that are available include: ComBase (www. combase.cc), Sym'Previus (www.symprevius.net), USDA pathogen (http://www.ars.usda.gov), Food Spoilage and Safety Predictor, (www.fssp.dtu.dk).

Predictive microbiological models are normally developed assuming that microbial responses are consistent (*McMeekin et al*., 2002, 2010b; *McMeekin*, 2007; *Mejlholm et al.*, 2010). While predictive models can provide a cost-effective means to minimize microbiological testing in determining shelf-life, there may be occasions when the model's predictions may not be accurate, due to inconsistent microbial responses and variations in the growth media (*DVFA,* 2014; *EC*, 2005). Finally, models cannot be applied if a validation process is not previously accomplished, which typically consists of confirming the predictions experimentally, using a quantitative method. The validation process is conducted considering biological knowledge of the system and statistical tools. Once models are validated and users are aware of the limitations of the models, they are useful tools to obtain information and make

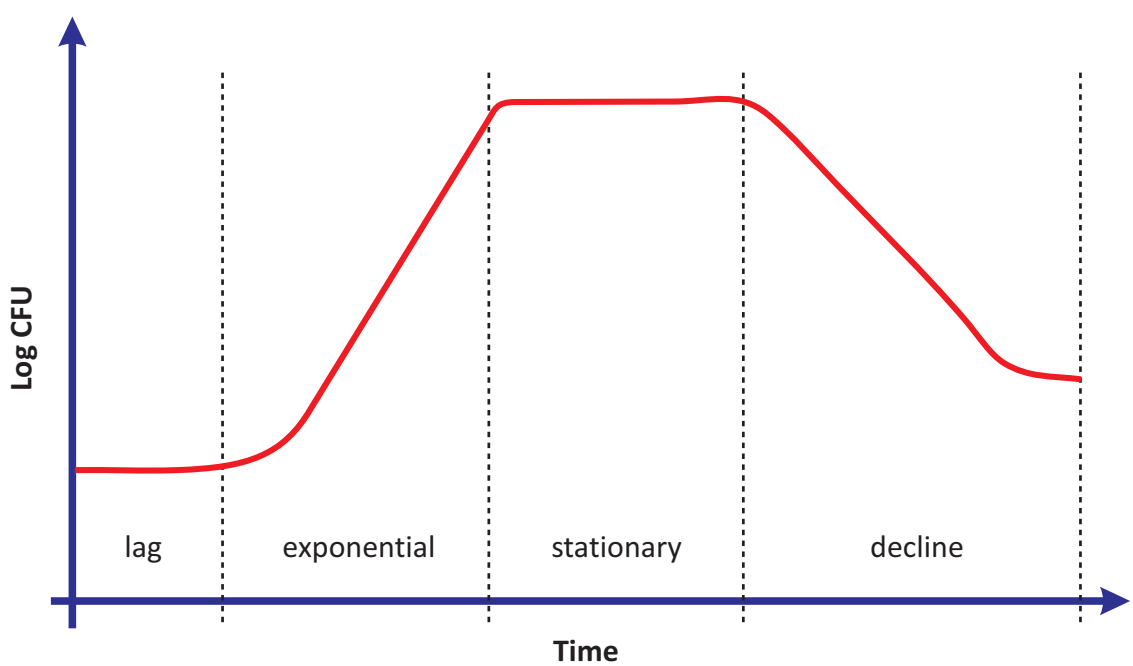


**Figure 1.** Theoretical presentation of the bacterial growth curve with four phases: (i) lag phase, (ii) exponential growth phase, (iii) stationary phase and (iv) decline (*Perez-Rodriguez and Valero*, 2013)

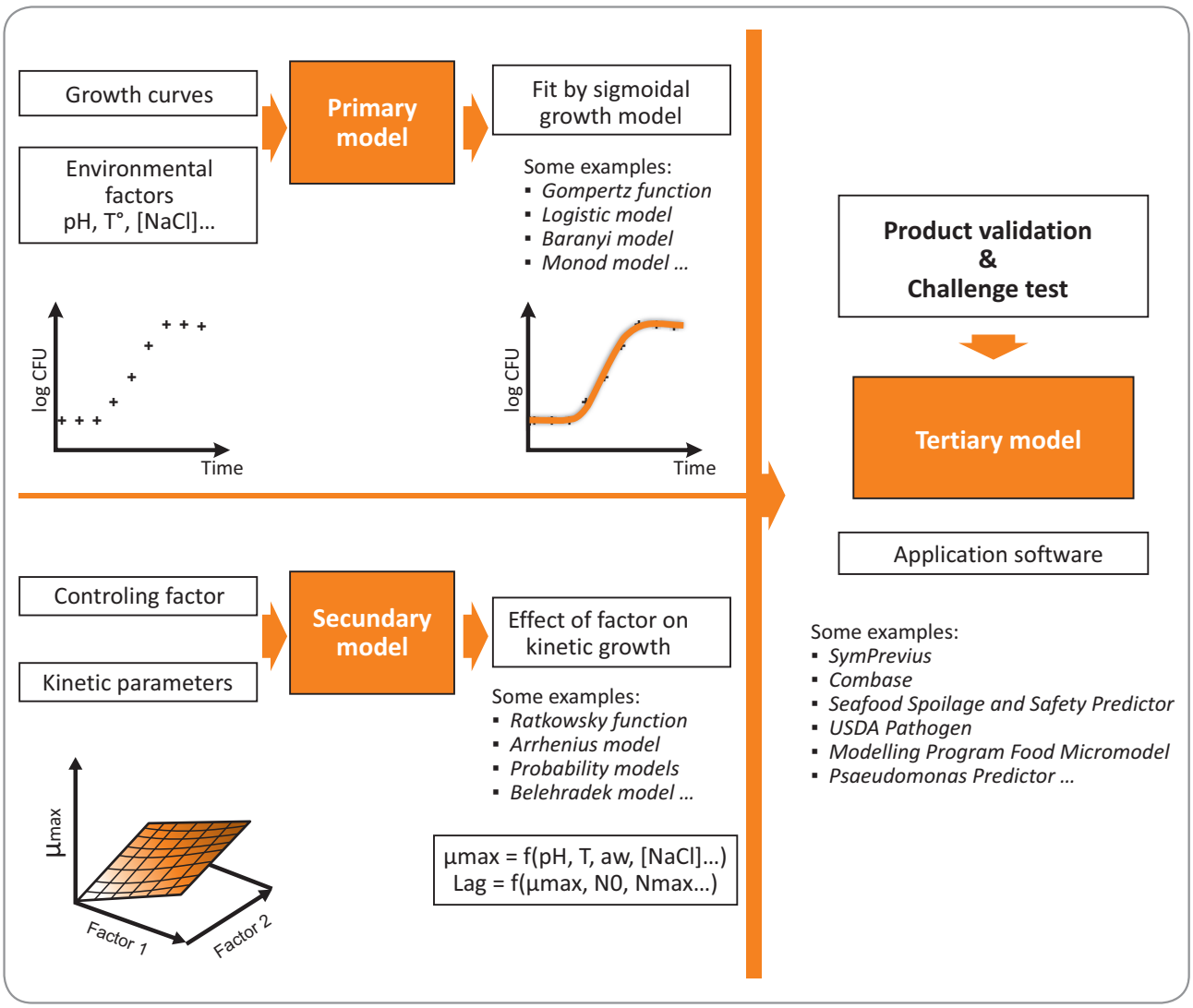


**Figure 2.** Schematic development, classification, and some examples of predictive microbiology models in food products (Adopted from *Fakruddin et al.*, 2011; *McDonald and Sun,* 1999).

decisions for the following situations (*Alzamora et al*., 2000a; *Buchanan and Whiting*, 1997):

1. **Prediction of safety**: Estimate the risk of growth or survival of pathogens during food processing.
2. **Quality control**: Improve systems like HACCP (Hazard Analysis of Critical Control Points) to ensure food safety.
3. **Product development**: Redesign processes and recipes, set priorities in product design and evaluation.
4. **Data analysis and laboratory planning**: The model could save resources, time, and money.
5. **Risk assessment models**: Evaluate the probability that a food could cause foodborne illness

## Microbial modeling and applications of predictive microbiology

### *Microbial modeling*

In all predictive microbiology, a prediction must only be used as a guide to the response of microorganism(s) to a particular set of environmental conditions (pH, $a_w$, t). However, food businesses should never rely solely on any predictive microbiological model to determine the safety of foods and/or processing systems (*Toldra*, 2009). Determining the growth, survival or inactivation of pathogens in food requires (*FDA*, 2015):

1. The determination of the intrinsic and extrinsic properties of the product, taking into account the storage and processing conditions,

the possibilities for contamination and the foreseen shelf-life.

2. Consultation of available scientific literature and research data regarding the survival, growth and inactivation of microorganisms of concern.
3. Where necessary on the basis of these studies food businesses should also conduct additional studies which may include:
4. Laboratory-based microbiological sampling and analysis.
5. Predictive microbiological modeling.
6. Challenge tests to investigate the ability of microorganisms of concern to grow or survive in the food product under reasonably foreseeable conditions of distribution and storage (no challenge testing for Campylobacter spp., Shigella spp. and Yersinia enterocolitica are recommended because other organisms, such as *Salmonella*, have similar routes of contamination and are easier to culture and have less fastidious growth and survival requirements (*FDA*, 2015).
7. Predictive microbiological models are also useful when the shelf-life has been determined, but the product is then subject to a minor process or formulation change (either planned or unplanned through loss of process control). A predictive microbiological model can then be used to initially establish if the change might have any effect on the safety and shelf-life of the product. Table 1 shows predicted pH limits for growth (p=0.5, 0.1, 0.01) of selected pathogens at various $a_w$ and temperature conditions (EFSA, 2012).

**Table 1.** Predicted pH limits for growth (p = 0.5, 0.1, 0.01) of selected pathogens at various $a_w$ and temperature conditions (*EFSA*, 2012)

| Pathogen | $a_w$ | Predicted pH limits at various aw, temperature and p-values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5°C P | | | 10°C P | | | 15°C P | | | 25°C P | | |
| | | 0.5 | 0.1 | 0.01 | 0.5 | 0.1 | 0.01 | 0.5 | 0.1 | 0.01 | 0.5 | 0.1 | 0.01 |
| *Listeria monocytogenes* (Koutsoumanis et al., 2004) | 0.99 | 4.76 | 4.69 | 4.61 | 4.45 | 4.39 | 4.34 | 4.29 | 4.24 | 4.19 | 4.23 | 4.19 | 4.14 |
| | 0.98 | 4.84 | 4.77 | 4.69 | 4.53 | 4.47 | 4.41 | 4.37 | 4.32 | 4.26 | 4.32 | 4.28 | 4.23 |
| | 0.97 | 4.96 | 4.87 | 4.79 | 4.62 | 4.56 | 4.49 | 4.46 | 4.41 | 4.35 | 4.43 | 4.38 | 4.33 |
| | 0.96 | 5.10 | 5.00 | 4.91 | 4.73 | 4.66 | 4.60 | 4.56 | 4.51 | 4.44 | 4.54 | 4.48 | 4.43 |
| | 0.95 | 5.28 | 5.16 | 5.05 | 4.86 | 4.78 | 4.71 | 4.68 | 4.61 | 4.55 | 4.66 | 4.60 | 4.54 |
| *Salmonella* (Koutsoumanis et al., 2004) | 0.99 | – | | | 4.66 | 4.41 | 4.37 | 4.40 | 4.18 | 4.14 | 3.95 | 3.91 | 3.87 |
| | 0.98 | | | | 4.85 | 4.61 | 4.56 | 4.56 | 4.35 | 4.31 | 4.23 | 4.06 | 4.01 |
| | 0.97 | | | | 5.04 | 4.80 | 4.74 | 4.71 | 4.51 | 4.45 | 4.45 | 4.18 | 4.13 |
| | 0.96 | | | | 5.24 | 4.98 | 4.92 | 4.86 | 4.65 | 4.59 | 4.66 | 4.29 | 4.23 |
| | 0.95 | | | | 5.47 | 5.17 | 5.10 | 5.02 | 4.80 | 4.73 | 4.88 | 4.39 | 4.33 |
| *Escherichia coli* O157:H7 (Skandamis et al., 2007) | 0.99 | – | | | 5.31 | 5.10 | 4.89 | 4.46 | 4.33 | 4.20 | 3.94 | 3.83 | 3.72 |
| | 0.98 | | | | 5.16 | 5.02 | 4.88 | 4.63 | 4.51 | 4.38 | 4.03 | 3.92 | 3.80 |
| | 0.97 | | | | 6.20 | 5.95 | 5.69 | 5.28 | 5.09 | 4.89 | 4.38 | 4.22 | 4.05 |
| | 0.96 | | | | – | – | – | – | 6.69 | 6.06 | 5.01 | 4.74 | 4.48 |
| | 0.95 | | | | – | – | – | – | – | – | – | – | 5.80 |
| *Bacillus cereus* (Lanciotti et al., 2001) | 0.99 | – | | | – | | | – | – | – | 4.85 | – | – |
| | 0.98 | | | | | | | | | | 4.92 | | |
| | 0.97 | | | | | | | | | | 5.10 | | |
| | 0.96 | | | | | | | | | | 6.02 | | |
| | 0.95 | | | | | | | | | | – | | |
| *Staphylococcus aureus* (Lanciotti et al., 2001) | 0.99 | – | | | – | | | 5.44 | 5.22 | 5.03 | 4.79 | 4.68 | 4.59 |
| | 0.98 | | | | | | | 5.64 | 5.39 | 5.16 | 4.89 | 4.77 | 4.65 |
| | 0.97 | | | | | | | 5.97 | 5.66 | 5.38 | 5.06 | 4.90 | 4.77 |
| | 0.96 | | | | | | | 6.66 | 6.24 | 5.85 | 5.40 | 5.19 | 5.00 |
| | 0.95 | | | | | | | – | – | – | 7.39 | 6.85 | 6.36 |

**Table 2.** Applications of predictive microbiology (according to *Fakruddin et al.*, 2011)

| Area of Application | Example |
|---|---|
| **Hazard Analysis Critical Control Point (HACCP)** | Preliminary hazard analysis identification and establishment of critical control point(s)<br>Corrective actions<br>Assessment of importance of interaction between variables<br>Risk assessment |
| **Risk assessment** | Estimation of changes in microbial numbers in a production chain<br>Assessment of exposure to a particular pathogen |
| **Microbial shelf life studies** | Prediction of the growth of specific food spoilage microorganisms<br>Prediction of growth of specific foodborne pathogens |
| **Product research and development** | Effect of altering product composition on food safety and spoilage<br>Effect of processing on food safety and spoilage<br>Evaluation of effect of out-of-specification circumstances |
| **Temperature function integration and hygiene regulatory activity** | Consequence of temperature in the cold chain for safety and spoilage |
| **Education** | Education on safety, especially non-technical people |
| **Design of experiments** | Number of samples to be prepared<br>Defining suitable intervals between sampling |

*Applications of predictive microbiology*

Some of the applications of predictive microbiology are listed in Table 2.

*Limitations of predictive microbiology*

Even though predictive microbiology models are widely used when correctly validated, they have several limitations because of the complexity of microbial behavior and food systems.

Mathematical models are simplifications of complex biochemical processes and in some cases, not every important variable or factor that affects the system is included in the model (*Alzamora et al*., 2005; *Buchanan and Whiting*, 1997). Usually, models are not designed for the same conditions in which microorganisms exist in food systems (biofilms, starved and unknown nutrients, among many others), since the majority of the data to generate the predictive models are derived from broth-based experiments. It is known that bacterial pathogens are more resistant in real food products than in broth cultures (*Alzamora et al*., 2005). Most of the models describe changes of microbial behavior for homogeneous populations; nevertheless, competition among microorganisms affects the food environment, and models do not account for this (*Lebert and Lebert*, 2006).

Some models make a good description of linear relationships; but when more than one factor is involved, reparameterization of the model becomes necessary.

It is important the model developer clearly specify directly or through the model what the limits of the model are, i.e., what microorganisms, what factors, what ranges of each factor and what combinations of factors will give valid answers. The presence of additional inhibitory factors in a food, and which have not been included in the model, invalidates the model or requires caution to be used to interpret the predictions. Currently, growth models do not usually include factors such as anion effects from the acidulent used, phosphates, sorbates, and bacteriocins, and humectants other than sodium chloride. No broth models include competition from other microorganisms. Some models developed with foods include the "normal" spoilage microbiota, but how this microbiota changes in species and number with plant or season and the effect upon the modeled microorganism is largely unknown (*Van Impe et al.*, 2013).

Because pathogens grow in most foods, the important question, then, is whether the pathogens will grow to a significant population before the spoilage microbiota causes the food to be rejected by the consumer. There is a need for systematic modeling of representative classes of spoilage microorganisms so tertiary models can then plot comparative growth curves for both pathogenic and spoilage organisms. For some pathogens with very low infective or toxic dose, such as *Listeria*, *Yersinia* and *C. botulinum*, the criteria may be growth-no growth and the spoilage flora has little significance unless they alter the environment by lowering the pH or produce a bacteriocin (*Fakrudin*, 2011).

## Risk analysis and predictive microbiology

Management of foodborne threats is an ongoing challenge due to changes in primary and secondary production, microbial adaption, increases in international trade, changes in consumer demands and behavioral and demographic changes. Risk analysis has been introduced as a means to face these challenges and to evaluate and control microbial risks (*Bjerre*, 2014). Risk analysis includes three components; (i) risk assessment, (ii) risk management and (iii) risk communication (CAC/GL 63- 2007). Risk assessment is the scientific evaluation of known or potential adverse health effects of a food product and comprises: hazard identification, hazard characterization, exposure assessment and risk characterization (*Marvin et al*., 2009). The outcome of the risk characterization is an estimate of the likelihood of adverse health effects in the population due to exposure to the hazard in question (*FAO/WHO*, 1995). In a quantitative microbiological risk assessment, the exposure assessment describes the routes by which the microbiological hazard can be introduced, distributed and altered during the production, distribution and consumption of a given food product (*WHO/FAO*, 2004).

Predictive microbiology is of particular interest in relation to evaluation of alterations in numbers (increase or decrease) of the hazard over time. For quantitative risk analyses, it is often stated that data is lacking and available data often originate from modeling experiments with, for example, unrealistically high initial bacterial numbers. In general, high quality, relevant and timely data is lacking (*Gardner*, 2004; *Ross and Sumner*, 2002; *WHO/FAO*, 2004). In spite of that, as a means to provide information and to fill data gaps, predictive models for growth and inactivation can be helpful and efficient tools. Predictive models, successfully validated in growth environments comparable to the products of concern, can be used to predict the effect of intrinsic and extrinsic factors on the response of the pathogen in question (*Toldra*, 2009). This quantification is important since the effects of both spoilage and pathogenic microorganisms are highly correlated to the number of microbes present in the food product at the point of consumption (*Bjerre*, 2014).

## Future perspectives

Foods are complex feedback systems. Generally, substantial quantities of data have been derived from modeling studies conducted under experimental conditions, but this data is often not immediately relevant to real-life conditions for the pathogen, the food vehicle or the consumer. Mathematical models are able to bridge some gaps but are also an approximation of reality. Evidently, modelers need to be diligent when relating and extrapolating data, and using or interpreting mathematical growth models and their outcomes, particularly when conducting exposure assessment and hazard characterization, as these impact on the validity of a risk characterization. Additionally, estimation of pathogen prevalence and level (number) in food products is key for exposure assessment and indispensable for the generation of reliable risk estimates (*ILSI*, 2010). Thus, risk assessors require an understanding of the biology and ecology of the pathogen(s), and of the properties of food materials they investigate. Often, dose-response models are the element where least information is available. Risk assessments should include an integral evaluation of the quality of data and models that are included and this is often accomplished by including an explicit evaluation concerning uncertainty and variability in the risk characterization outcome.

Besides a move towards stochastic modeling approaches, other subjects are also forecasted to be a part of the future of predictive microbiology. In 2004, Bernaerts and co-workers strongly advocated for the development of more mechanistically-inspired predictive models in order to obtain a better understanding of the underlying mechanisms, but also to develop more robust models (*Bernaerts et al*., 2004). *McMeekin et al*. (2010) suggested focusing on the ecophysiology of foodborne pathogens and to model growth responses from, for example, thermodynamics. The introduction of systems biology into predictive microbiology has been suggested by *Brul et al*. (2008) and *Van Impe et al*. (2013) in order to apply “bottom-up” approaches and to work

at the microscopic level e.g. by developing metabolic network-based modeling approaches. Belief in systems-biology as an integrated part of predictive microbiology has also been expressed by *McMeekin et al*. (2013) in order to induce a shift from empirical predictive microbiology towards mechanistic predictive systems biology models. These new, emerging approaches within predictive microbiology should be considered and, if obtainable, tested when developing new models.

## References


**Alzamora, S. M., López-Malo, A. & Tapia de Daza, M. S. (2000a).** Overview, in: *Minimally processed fruits and vegetables-fundamental aspects and applications*. Alzamora S. M., Tapia M. S., López-Malo A., Eds. Aspen Publishers Inc. Gaithersburg, MD, USA, 1–9.

**Alzamora, S. M., Guerrero, S., Viollaz P. & Welti-Chanes, J. (2005).** Experimental protocols for modeling the response of microbial populations exposed to emerging technologies: some points of concern, in: *Novel food processing technologies*. Barbosa-Cánovas G., Tapia M. S., Cano P. M., Eds. CRC Press. Boca Ratón, FL, USA.

**Baranyi, J. & Roberts, T. A. (1994).** A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology, 23, 3–4*, 277–294.

**Baranyi, J. & Pin, C. (2004).** Modeling the history effect on microbial growth and survival, in: *Modeling microbial responses in food. McKellar R., Lu X., Eds. Contemporary Food Science*. CRC Press. Boca Ratón, FL, USA, 285–301.

**Baranyi, J., Pin, C. & Ross, T. (1999).** Validating and comparing predictive models. *International Journal of Food Microbiology, 48*, 159–166.

**Baranyi, J., Ross, T., McMeekin, T. A. & Roberts, T. A. (1996).** Effects of parameterization on the performance of empirical models used in 'predictive microbiology'. *Food Microbiology, 13*, 83–91.

**Bernaerts, K., Dens, E., Vereecken, K., Geeraerd, A.H., Standaert, A.R., Devlieghere, F., Debevere, J. & Van Impe, J.F. (2004).** Concepts and tools for predictive modeling of microbial dynamics. *Journal of Food Protection, 67*, 2041–2052.

**Bigelow, W. D. (1921)**. The logarithmic nature of thermal death time curves. *Journal of Infection Diseases, 29*, 528–536.

**Bigelow, W. D., Bohart, G. S., Richardson, A.C. & Ball, C.O. (1920).** Heat penetration in processing canned foods. *National Canners Association Bulletin. 16–L.*

**Bjerre, N. (2014).** *Food microbiology: new tools for risk assessments and diary product development*. PhD thesis, Technical University of Denmark, 40–43.

**Brul, S., Mensonides, F. I. C., Hellingwerf, K. J. & Teixeira de Mattos, M. J. (2008)**. Microbial systems biology: new frontiers open to predictive microbiology. *International Journal of Food Microbiology*, 128, 16–21.

**Buchanan, R. L., Whiting, R. C. & Damert, W. C. (1997).** When is simple good enough: a comparison of the Gompertz, Baranyi, and three phase linear models for fitting bacterial growth curves. *Food Microbiology, 14*, 313–326.

**CAC/GL 63-2007 (2007).** Principles and guidelines for the conduct of microbial risk management (MRM). *Codex Alimentarius*, 1–19.

**DVFA (2014).** Danish Veterinary and Food Administration. *Listeria monocytogenes*. http://www.altomkost.dk/Fakta/Foedevarer/maelk_og_ost/forside.htm (accessed 6.4.14).

**EC (2005).** Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union 338*, 1–26.

**EFSA (2012).** Scientific opinion on public health risks represented by certain composite products containing food of animal origin. *EFSA Journal, 10, 5*, 2662.

**Esty, J. R. & Meyer, K. F. (1922).** The heat resistance of the spore of *Bacillus botulinus* and allied anaerobes, XI. *Journal of Infection Disease, 3*, 650–663.

**Perez-Rodriguez, F. & Valero, A. (2013).** Predictive microbiology in Foods, Springer Briefs in Food, *Health and Nutrition, 5*.

**Fakruddin, M., Mohammad, R., Khanjada, S. & Bin Mannan (2011).** Predictive microbiology: modeling microbial responses in food. *Ceylon Journal of Science (Bio. Sci.), 40, 2*, 121–131.

**FAO/WHO (1995).** Application of risk analysis to food standards issues. *Report of the Joint FAO/WHO Expert Consulation, WHO/FNU/FOS.* World Health Organization, Geneva.

**FDA (2015).** Evaluation and definition of potentially hazardous foods – Chapter 6. Microbiological challenge testing. www.fda.gov

**Gardner, I. A. (2004)**. An epidemiologic critique of current microbial risk assessment practices: the importance of prevalence and test accuracy data. *Journal of Food Protection, 67*, 2000–2007.

**Geeraerd, A. H., Herremans, C. H. & Van Impe, J. F. (2000).** Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, 59, 185–209.

**Geeraerd, A. H., Valdramidis, V. P., Devlieghere, F., Bernaert, H., Debevere, J. & Van Impe, J. F. (2004).** Development of a novel approach for secondary modelling in predictive microbiology: incorporation of microbiological knowledge in black box polynomial modelling. *International Journal of Food Microbiology, 91*, 229–244.

**Geeraerd, A., Herremans, C., Cenens, C. & Van Impe, J. (1998).** Application of artificial neural networks as a nonlinear modular modeling technique to describe bacterial growth in chilled food products. *International Journal of Food Microbiology, 44*, 49–68.

**Hajmeer, M. N. & Cliver, D. O. (2002).** Microbiology of food preservation and sanitation, in: *Foodborne diseases*. Cliver D. O., Riemann H., Eds. Academic Press. New York, NY, USA, 330–352.

**ILSI (2010)**. Impact of microbial distributions on food safety. ILSI Europe risk analysis in food microbiology task force, Brussels, Belgium. Report. www.ilsi.org

**Van Impe, J. F., Vercammen, D. & Van Derlinden, E. (2013).** Toward a next generation of predictive models: a systems biology primer. *Food Control, 29*, 336–342.

**Jankovic, V., Lakicevic, B., Dzinic, N., Tasic, T., Ikonic P. & Saric, Lj. (2015)**. Microbiological profile of Petrovská klobása made from warm meat in the traditional way depending on the method of packaging. *Procedia Food Science, 5*, 117–120.

**Jankovic, V., Lakicevic, B., Ikonic, P., Tasic, T., Borovic, B., Veskovic, S. & Saric, Lj. (2014)**. *Enterobacteriaceae* presence during fermentation of traditional dry fermented sausage – petrovská klobása. Proceedings of the II International Congress of Food Technology, Quality and Safety, Novi Sad, Serbia, 202–208.

**Jankovic, V., Petrovic, Lj., Lakicevic, B., Matekalo Sverak, V., Lilic, S. & Mitrovic, R. (2013).** Determination of typical house flora during production process of the Petrovská klobása. *African Journal of Microbiology Research, 7*, 32, 4130–4137.

**Koutsoumanis, K. P., Kendall, P. A. & Sofos, J. N. (2004).** Modeling the boundaries of growth of *Salmonella* Typhimurium in broth as a function of temperature, water activity, and pH. *Journal of Food Protection, 67*, 53–59.

**Lakicevic, B., Nastasijevic, I. & Raseta, M. (2015).** Sources of *Listeria monocytogenes* contamination in retail establishments. *Procedia Food Science 5*, 160–163.

**Lakicevic, B., Velebit, B., Jankovic, V., Spiric, D., Baltic, T., Mitrovic, R. & Babic, J. (2014)**. Taq Man real time PCR detection of *Listeria monocytogenes*: a study of enrichment incubation time affecting sensitivity in experimental dry fermented sausages. *Tehnologija Mesa, 55* (1), 60–65.

**Lanciotti, R., Sinigaglia, M., Gardini, F., Vannini, L. & Guerzoni, M. E. (2001).** Growth/no growth interfaces of *Bacillus cereus, Staphylococcus aureus* and *Salmonella Enteritidis* in model systems based on water activity, pH, temperature and ethanol concentration. Food Microbiology, 18, 659–668.

**Lebert, A. & Lebert, I. (2006).** Quantitative prediction of microbial behaviour during food processing using an integrated modeling approach: a review. *International Journal of Refrigeration, 29*, 968–984.

**Lopez-Malo, A., Alzamora, S. M. & Palou, E. (2005)**. Aspergillus flavus growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. *International Journal of Food Microbiology*, 99, 119–128.

**Marks, B. P. (2008)**. Status of microbial modeling in food process models. Comprehensive Reviews in Food Science and Food Safety, 7, 1, 137–143.

**Marvin, H. J. P., Kleter, G. A., Frewer, L. J., Cope S., Wentholt, M. T. A. & Rowe, G. (2009).** A working procedure for identifying emerging food safety issues at an early stage: implications for European and international risk management practices. *Food Control 20,* 345–356.

**McDonald, K. & Sun ,D. W. (1999).** Predictive food microbiology for the meat industry: a review. *International Journal of Food Microbiology*, 52, 1–27.

**McKellar, R. & Lu, X. (2004).** *Modeling Microbial Responses on Foods*. CRC Press. Boca Ratón, FL, USA.

**McMeekin, T., Brow, J. & Kris, K. (1997)**. Quantitative microbiology: a basis for food safety. Emerging Infectious Diseases, 3, 541–549.

**McMeekin, T. A. (2007).** Predictive microbiology: quantitative science delivering quantifiable benefits to the meat industry and other food industries. *Meat Science, 77*, 17–27.

**McMeekin, T. A., Olley, J. N., Ross, T. & Ratkowsky, D. A. (1993).** Predictive Microbiology. John Wiley & Sons Ltd. Chichester, UK.

**McMeekin, T. A. & Ross, T. (2002)**. Predictive microbiology: providing a knowledge-based framework for change management. *International Journal of Food Microbiology, 78*, 133–153.

**McMeekin, T. A., Tamplin, M. L., Ross T. & Koseki, S. (2010b).** The way forward with predictive microbiology in the dairy industry. *Australian Journal of Dairy Technology, 65*, 91–97.

**McMeekin, T., Olley, J., Ratkowsky, D., Corkrey, R. & Ross, T. (2013).** Predictive microbiology theory and application: is it all about rates? *Food Control, 29*, 290–299.

**Mejlholm, O., Gunvig A., Borggaard C., Blom-Hanssen, J., Mellefont L., Ross T., Leroi F., Else, T. Visser D. & Dalgaard, P. (2010).** Predicting growth rates and growth boundary of *Listeria monocytogenes* – an international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology 141*, 137–150.

**Monod, J. (1949)**. The growth of bacterial cultures. *Annual Review of Microbiology, 3*, 371–394.

**Nastasijevic, I. (2011).** STEC O157 in the beef chain – risk assessment and management. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 6, 061, 1–19 (Animal Science Reviews ISSN 1749-8848, 219–239).

**Nastasijevic, I. (2014).** Verotoxigenic *Escherichia coli* in the meat chain and control strategies. 12th International Conference on Fundamental and Applied Aspects of Physical Chemistry, 4th Workshop: Specific methods for food safety and quality, September 23rd, Belgrade, Serbia, Proceedings, 1–4.

**Nastasijevic, I., Mitrovic, R. & Jankovic, V. (2014)**. STEC in the beef chain – one health approach. *Tehnologija Mesa, 55, 2*, 93–116.

**Nixon, P. A. (1971)**. Report on quality of fish products. Semin. 3, Fish. Ind. Board, Wellington, New Zealand, 34.

**Panagou, E. Z., Tassou, C. C., Saravanos, E. K. A. & Nychas, G. J. E. (2007).** Application of neural networks to simulate the growth profile of lactic acid bacteria in green olive fermentation. *Journal of Food Protection, 70*, 1909–1916.

**Pérez-Rodríguez, F. & Valero, A. (2013).** Predictive Microbiology in Foods. Springer Briefs in Food, Health, and Nutrition. Springer, New York, USA. Volume 5, p. 128. ISBN: 978-1-4614-5519-6.

**Ratkowsky, D. A., Olley, J., McMeekin, T. A. & Ball, A. (1982).** Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology, 149*, 1–5.

**Roberts, T. A. (1989)**. Combinations of antimicrobials and processing methods. *Food Technology, 43*, 156–163.

**Roberts, T. A. & Jarvis, B. (1983).** Predictive modeling of food safety with particular reference to *Clostridium botulinum* in model cured meat systems, in: *Food microbiology: advances and prospects*. Roberts T.A., Skinner F. A., Eds. Academic Press. Orlando, FL, USA, 85–95.

**Ross, T., Dalgaard P. & Tienungoon, S. (2000)**. Predictive modelling of the growth and survival of *Listeria* in fishery products. *International Journal of Food Microbiology 62*, 231–245.

**Ross, T. & McMeekin, T. A. (1994)**. Predictive microbiology. *International Journal of Food Microbiology 23*, 241–264.

**Ross, T. & McMeekin, T. A. (2003).** Modeling microbial growth within food safety risk assessments. Risk Analysis 23, 179–197.

**Ross, T., Ratkowsky, D. A., Mellefont, L. A. & McMeekin, T. A. (2003).** Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli. International Journal of Food Microbiology*, 82, 33–43.

**Ross, T. & Sumner, J. (2002).** A simple, spreadsheet-based, food safety risk assessment tool. *International Journal of Food Microbiology 77*, 39–53.

**Rosso, L., Lobry, J. R., Bajard, S. & Flandrois, J. P. (1995)**. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Applied and Environmental Microbiology, 61*, 610–616.

**Sautour, M., Dantigny, P., Divies, C. & Bensoussan, M. (2001).** A temperature-type model for describing the relationship between fungal growth and water activity. *International Journal of Food Microbiology, 67*, 63–69.

**Scott, W. J. (1936).** The growth of microorganisms. The influence of water content of substrate on rate of growth at –1°C. *Journal of the Council for Scientific and Industrial Research (Australia), 9*, 177–182.

**Skandamis, P. N., Stopforth, J. D., Kendall, P. A., Belk, K. E., Scanga, J. A., Smith, G. C. & Sofos, J. N. (2007).** Modeling the effect of inoculum size and acid adaptation on growth/no growth interface of *Escherichia coli* O157:H7. *International Journal of Food Microbiology, 120*, 237–249.

**Spencer, R. & Baines, C. R. (1964).** The effect of temperature on spoilage of wet white fish. Food Technology, 18, 769–773.

**Toldra, F. (2009)**. *Safety of meat and processed meat*, Marie Laure Delignette-Muller, 535–541.

**Van Boeckel, M. (2008).** Kinetics of microbial growth, in: Kinetic modelling of reactions in foods. CRC Press. Boca Ratón, FL, USA, 12–1; 12–40.

**Van Impe, J. F., Poschet, F., Geeraerd, A. H. & Vereecken, K. M. (2005)**. Towards a novel class of predictive microbial growth models. *International Journal of Food Microbiology, 100*, 97–105.

**Van Impe, J. F., Vercammen, D. & Van Derlinden, E. (2013)**. Toward a next generation of predictive models: a systems biology primer. *Food Control 29*, 336–342.

**Whiting, R. C. & Buchanan, R. L. (1997).** Predictive modelling, in: Food microbiology – fundamentals and frontiers. Doyle M. P., Beuchat L. R., Montville T. J., Eds. ASM Press. Washington, USA, 728–739.

**WHO/FAO (2004).** Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical Report. World Health Organization.

**www.combase.cc**

**www.symprevius.net**

**www.ars.usda.gov**

**www.fssp.dtu.dk**

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# Inhibitory effect of thyme and oregano essential oils and some essential oil components on *Salmonella* Senftenberg *and Salmonella* Give

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*A b s t r a c t: Salmonella is a pathogen of public concern causing health and economic problems worldwide. Salmonella Enteritidis and Salmonella Typhimurium are the serotypes most commonly recognized as causes of human salmonellosis, which is why research is mainly dedicated to prevention or inhibition of these frequently reported serotypes, while less attention is dedicated to the uncommon Salmonella serotypes. Outbreaks of salmonellosis caused by rarer subspecies of Salmonella are increasing, which is why their control is needed. Essential oils derived from plants have gained attention mainly due to their antibacterial properties and potential to be used as a replacement for synthetic additives in the food industry. To the best of our knowledge, there are no literature data about the effect of essential oils on Salmonella Give. Therefore, the aim of this study was to evaluate the effect of thyme and oregano essential oils and thymol, carvacrol, cinnamaldehyde and eugenol on Salmonella Senftenberg and Salmonella Give. Results showed that there were no differences between the susceptibility of the examined Salmonella serovars to these essential oils and active compounds. Oregano essential oil, thymol and carvacrol exhibited greater antibacterial activity, followed by cinnamaldehyde, while the Salmonella serovars examined were most resistant to the effect of eugenol.*

***Keywords:*** *Salmonella, thymol, carvacrol, cinnamaldehyde, eugenol.*

## Introduction

Foodborne diseases are an important cause of morbidity and mortality worldwide (*Van et al.,* 2007). Bacterial pathogens are considered to be the most common agents causing foodborne diseases and among foodborne bacteria, *Salmonella* is the most common cause of illness after *Campylobacter* (*Carrasco et al.,* 2012; *de Silva et al*., 2013). It is estimated that non-typhoidal salmonellosis is the cause of 155,000 deaths annually and of 93.8 million reported cases, 80.3 million are foodborne (*Majowicz et al*., 2010). The major vehicles of this pathogen are eggs, poultry and pork, as well as other types of meat and meat products, but *Salmonella* is often found in low-moisture foods (powdered milk, chocolate, peanut butter, infant formula), vegetables, spices, seafood, milk and milk products (*Carrasco et al*., 2012; *Pires et al*., 2014). *Salmonella* Enteritidis and *Salmonella* Typhimurium followed by *Salmonella* Infantis are the most frequently reported serotypes in human salmonellosis, but other serotypes were also involved in salmonellosis outbreaks (*de Freitas Neto et al*., 2010; *Carrasco et al.*, 2012). The incidence of *Salmonella* infections with more rare serovars is increasing (*David et al*., 2007). *Salmonella* Senftenberg is not one of the serotypes most commonly associated with human infection but is a pathogen of public interest due to its high heat resistance (*Pezzoli et al*., 2008; *Gurman et al*., 2016). *Salmonella* Give was identified as the cause of some cases of illness and minced pork and infant milk formula were detected as sources of infection (*Girardin at al*., 2006; *Berger*, 2015). This serotype was found on beef carcasses, refrigerated and pulp meat (*David et al*., 2007; *Perez-Montaño et al*., 2012). Although infection with this serotype is rare, the hospitalisation rates for patients infected with *S.* Give are higher compared to those infected with *S*. Enteritidis. This possibly indicates that this serotype has a higher virulence compared to other non-typhoidal *Salmonella* spp. (*Girardin et al*., 2006).

Salmonellosis is usually a self-limiting disease and symptoms include fever, chills, nausea, vomiting, abdominal cramping, and diarrhoea (*Chen et*

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*al*., 2013). Infants, young children, the elderly and the immunocompromised are at particular risk for bacteraemia which occurs in 5–10% of infected persons, and may progress to focal infection including meningitis, bone and joint infection (*Chen et al*., 2013; *Crump et al.,* 2015).

Therefore, *Salmonella* infections are a major human public health and economic problem in both developed and developing countries (*EFSA*, 2010) and novel strategies and methods for control of this pathogen are needed.

A number of studies have reported essential oils to be effective antimicrobials with potential application in meat and in the general food industry to increase the safety of these products. Although *Salmonella* is one of the most investigated pathogens, there are little or no data about the effects of essential oils or their components on uncommon *Salmonella* serotypes. Considering the above, the aim of this study was to evaluate the effects of thyme and oregano essential oils, thymol, carvacrol, eugenol and cinnamaldehyde on *Salmonella* Senftenberg and *Salmonella* Give.

## Materials and Methods

### *Essential Oils and Active Compounds*

Thymol, carvacrol, cinnamaldehyde and eugenol were purchased from the manufacturer (Essentico, Kula, Serbia). Oregano and thyme essential oils, extracted by the steam distillation method, were purchased from the manufacturer (Herba doo, Belgrade, Serbia). The major components of thyme (*Thymus vulgaris*) essential oil, determined by GC-MS analysis, were thymol and p-cymene followed by linalool, γ-terpinene and 1,8-clineole and of oregano (*Origanum vulgare*) essential oil were carvacrol followed by *p*-cymene, trans-β-caryophyllene, linalool, γ-terpinene and thymol. Other chemical compounds were in lower concentrations. Essential oils were kept in dark glass bottles at 4°C.

### *Antibacterial Assay*

The antibacterial effects of oregano and thyme essential oils, thymol, carvacrol, eugenol and cinnamaldehyde on *Salmonella enterica subsp. enterica* serovar Senftenberg (6.7:g,m,s :-) and *Salmonella enterica subsp. enteric* serovar Give (3,10:l.v:1.7) were studied. *Salmonella* Senftenberg (Veterinary Institute Subotica, Serbia) was isolated from animal feed and *Salmonella* Give (Veterinary Institute Subotica, Serbia) from poultry meat.

The susceptibility of *Salmonella* isolates to essential oils and active compounds was investigated by the broth microdilution method (*CLSI* 1999; *CLSI* 2009). Broth microdilution method was performed in sterile U-bottom microtitre plates (Spektar, Serbia). The inoculum density was set to 0.5 McFarland (approximately $1–2 \times 10^8$ cfu $mL^{-1}$), diluted 10 times ($1–2 \times 110^7$ cfu $mL^{-1}$) in sterile saline and 5 µL of this suspension was inoculated in 0.1 mL of CAMHB-Cation Adjusted Mueller-Hinton Broth (Becton, Dickinson and Company, Sparks, USA) to reach a final inoculum of 5 x $10^4$ cfu $well^{-1}$. Active substances were diluted in DMSO (Serva, Heidelberg, Germany) and added to CAMHB at levels from 2560 µg $mL^{-1}$ to 1.25 µg $mL^{-1}$ by two-fold dilution in 96-well microtitre plates. After inoculation, plates were incubated at 37°C for 24 hours. Minimal inhibitory concentration (MIC) was determined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in broth dilution susceptibility test (*CLSI*, 2006). From wells without visible growth, 10 µL was subcultivated onto CAMH Agar and incubated at 37°C for 24 hours. Growth of less than five colonies was taken as the minimal bactericidal concentration (MBC) as it represented a kill ratio of over 99.9% (CLSI, 1999). Amikacin (Sigma-Aldrich, USA) in the range of 64–0.03 µg $mL^{-1}$ was used as control.

## Results and Discussion

Results of the antimicrobial activity of thyme and oregano essential oils, thymol, carvacrol, cinnamaldehyde and eugenol on the *Salmonella* serovars studied are presented in Table 1.

The antibacterial effects of the essential oils used in this study were previously reported (*Boskovic et al.*, 2015). As hydrophobic liquids, essential oils interact with the lipid membrane of bacterial cells, causing the collapse of the proton motive force and depletion of the ATP pool, thus changing the membrane permeability and leading to leakage of the inner cell components and eventually to cell death (*Ultee et al.*, 2002; *Burt*, 2004; *Bajpai et al*., 2012). Essential oils also affect potassium ion reflux and cause coagulation of cytoplasm (*Burt*, 2004; *Bakkali et al*, 2008).

Results from a number of studies confirmed that Gram-negative bacteria, including *Salmonella* spp. are more resistant to effects of essential oils than Gram-positive bacteria due to their outer membrane which covers the cytoplasmic membrane and their peptide-glycan layer, which acts as a barrier against

**Table 1.** The minimum inhibitory concentrations of oregano and thyme essential oils and active compounds against *Salmonella* spp.

| | Minimum inhibitory concentration (μg mL$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Essential oils | | Active compounds | | | | Antibiotic |
| | Oregano | Thyme | Thymol | Carvacrol | Cinnamaldehyde | Eugenol | Amikacin |
| S. Senftenberg | 320 | 640 | 320 | 320 | 640 | 1280 | 1 |
| S. Give | 320 | 640 | 320 | 320 | 640 | 1280 | 0.25 |

hydrophobic macromolecules (*Holley and Patel*, 2005; *Hyldgaard et al*., 2012; *Esteban et al*., 2013).

In the current study, results showed that both *Salmonella* serovars were equally sensitive to oregano and thyme essential oil, thymol, carvacrol, cinnamaldehyde and eugenol, showing minimum inhibitory concentrations of 320 μg mL$^{-1}$, 640 μg mL$^{-1}$, 320 μg mL$^{-1}$, 320 μg mL$^{-1}$, 640 μg mL$^{-1}$ and 1280 μg mL$^{-1}$, respectively.

The high antimicrobial activity of thyme and oregano essential oils has been attributed to their phenolic components such as thymol and carvacrol (*Bajpai et al.*, 2012; *Bassolé and Juliani*, 2012). In the present study, oregano essential oil exhibited a stronger antibacterial effect than thyme essential oil, probably as a result of the higher content of phenolic compounds (data not shown). Because essential oils are complex mixtures containing a number of components, the antimicrobial activity cannot be attributed to single compound (*Bajpai et al*., 2012; *Boskovic et al*., 2013). Nevertheless, in the present study, carvacrol and oregano essential oil (which comprised 77.6% carvacrol) exhibited the same antibacterial effect. Other authors also reported the antibacterial effects of thyme and oregano essential oil on *Salmonella* spp. (*Bajpai et al.*, 2012). Still, most of these studies have been conducted on *S*. Typhimurium and *S*. Enteritidis and so there are few literature data about the effect of essential oils on *S*. Senftenberg. *Cherrat et al*. (2014a) reported the antibacterial effect of *Laurusnobilis* and *Myrtuscommunis* essential oils against *S*. Senftenberg. In their study, *Laurusnobilis* showed greater antimicrobial activity but the reported MIC values were much higher than those found for essential oils in the present study. *Menthapulegium*, *Saturejacalamintha* and *Lavandulastoechas* also exhibited an antimicrobial effect on *S*. Senftenberg but in higher concentrations, 4 μL mL$^{-1}$, 14 μL mL$^{-1}$ and 14 μL mL$^{-1}$, respectively (*Cherrat et al.,* 2014b). *Nanasombat and Lohasupthawee* (2005) examined the effect of different essential oils obtained from spices on nine serotypes of *Salmonella* which were potential pathogens and most commonly isolated from fresh and fermented meat, including *S*. Senftenberg. This serotype was the most or equally sensitive to cardamom, coriander, cumin, kaffir lime peel and ginger essential oils (MIC 0.2 μL mL$^{-1}$) less sensitive to mace and nutmeg essential oils (MIC 8.3 μL mL$^{-1}$) and most resistant to garlic (MIC 47.6 μL mL$^{-1}$), kaffir lime leaf and holy basil essential oils (MIC >62.5 μL mL$^{-1}$). The MICs obtained for essential oils in their study were higher than those determined in the present study but it should be noted that they used an inoculum concentration of $10^7$cfu mL$^{-1}$ in contrast to our study where an inoculum concentration of $10^4$ cfu well$^{-1}$ was used. Differences between the effects of essential oils towards bacteria are mainly attributed to its chemical profile (*Burt*, 2004; *Boskovic*, 2013).

Strain biodiversity, among other factors, influences the antimicrobial resistance of *Salmonella* (*Mazzarrino et al*., 2015). Differences were not observed between tested serovars of *Salmonella* in the present study, but as *Boskovic et al*. (2015) reported, the same essential oils were shown to be more effective against *S. Typhimurium* and thyme essential oil was more effective against *S*. Enteritidis. Lu and Wu (2010) did not find differences between the susceptibility of four *Salmonella* serovars (*S. Kentucky, S. Senftenberg, S. Enteritidis* and *S. Typhimurium*) to thyme essential oil, thymol and carvacrol. They obtained higher MIC values for thyme essential oil than those in the present study, and thymol exhibited the strongest antibacterial activity against all four *Salmonella*. Results from the present study showed that thymol and carvacrol exhibited stronger antibacterial activity than cinnamaldehyde and eugenol, with an obtained MIC value of 320 μg mL$^{-1}$ for both *Salmonella* serovars.

A number of studies showed that among constituents of essential oils, carvacrol and thymol exhibited the greatest antibacterial activity which is why these substances are the most investigated and mechanism of their action is well described (*Burt*, 2004). Thymol is structurally analogous to carvacrol, but the locations of the hydroxyl groups are at a different

location on the phenolic ring. Both phenols interact with the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP and potassium ions (*Burt*, 2004; *El Abed et al*., 2014). As mentioned above, a high percentage of phenolic compounds, including eugenol as well as carvacrol and thymol, are considered to be responsible for the antimicrobial activity of essential oils (*Burt*, 2004), but in the present study, eugenol exhibited the lowest antimicrobial activity against both tested *Salmonella* serovars with an obtained MIC value of 1280 μg $mL^{-1}$. Apart from essential oils containing phenols, essential oils containing significant amounts of aldehydes, such as cinnamaldehyde, showed high antibacterial activity (*Bassolé and Juliani*, 2012). The mode of action of cinnamaldehyde, the main component of cinnamon essential oil, is not still fully understood. Cinnamaldehyde, depending on the added concentration, inhibits different enzymes involved in cytokinesis or less important cell functions, acts as an ATPase inhibitor and perturbs cell membranes (*Hyldgaard et al*., 2012; *Shen et al*., 2015). It has been suggested that cinnamaldehyde inhibits cytokinesis (*Hyldgaard et al*., 2012). In the present study, cinnamaldehyde exhibited a moderate antibacterial effect. In contrast, *Zhou et al*. (2007) reported that cinnamaldehyde (MIC 200 mg $L^{-1}$) was more effective against *S*. Typhimurium than thymol and carvacrol (400 mg $L^{-1}$). The MICs reported in the present study for thymol and carvacrol were lower and these results may be caused by different sensitivity between the *Salmonella* serovars studied. Amikacin was used as a positive control. As was expected, amikacin showed a greater antimicrobial effect than the essential oils and active compounds. *S*. Give was more susceptible to amikacin than *S*. Senftenberg. Amikacin has a different mechanism of action compared to essential oils; while essential oils act mainly on the cell membrane, amikacin inhibits translation and amino acid misincorporation, and thus bacterial protein synthesis, by binding to rRNA (*López-Díez et al.*, 2005). Different modes of action could explain differences between susceptibility of *Salmonella* serovars to the antimicrobial substances examined.

## Conclusion

Despite invested efforts to control *Salmonella*, this pathogen is still a public health problem. As many pathogens, *Salmonella* can also be inhibited by essential oils. The results of this study showed that oregano and thyme essential oils and active compounds, in differing concentrations, exhibited antibacterial effects on *Salmonella* Senftenberg and *Salmonella* Give. Oregano essential oil, thymol and carvacrol exhibited the greatest effect on both tested *Salmonella* serovars. Taking into account that there are little or no data about the effect of essential oils on the two examined serovars of *Salmonella*, further research should be undertaken in food substrates to confirm the antimicrobial effects of essential oils and active compounds on these *Salmonella* serovars.

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## References

**Bajpai, V. K., Baek, K. & H., Kang, S. C. (2012).** Control of *Salmonella* in foods by using essential oils: A review. Food Research International, 45, 722–734.

**Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008).** Biological effects of essential oils – a review. *Food and Chemical Toxicology, 46*, 446–475.

**Bassolé, I. H. N. & Juliani, H. R. (2012)**. Essential oils in combination and their antimicrobial properties. *Molecules, 17* (4), 3989–4006.

**Berger, S. (2015).** Salmonellosis: Global Status. Gideon Informatics Inc.

**Boskovic, M., Baltic, M. Z., Ivanovic, J., Djuric, J., Loncina, J., Dokmanovic, M. & Markovic, R. (2013).** Use of essential oils in order to prevent foodborne illnesses caused by pathogens in meat. *Tehnologija mesa, 54* (1), 14–20.

**Boskovic, M., Zdravkovic, N., Ivanovic, J., Janjic, J., Djordjevic, J., Starcevic, M. & Baltic, M. Z. (2015).** Antimicrobial activity of Thyme (*Thymus vulgaris*) and Oregano (*Origanum vulgare*) essential oils against some foodborne microorganisms. *Procedia Food Science, 5*, 18–21.

**Burt, S. (2004).** Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*, 223–253.

**Carrasco, E., Morales-Rueda, A. & García-Gimeno, R. M. (2012).** Cross-contamination and recontamination by *Salmonella* in foods: a review. *Food Research International, 45, 2*, 545–556.

**Chen, H. M., Wang, Y., Su, L. H. & Chiu, C. H. (2013).** Nontyphoid *Salmonella* infection: microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology, 54, 3*, 147–152.

**Cherrat, L., Espina, L., Bakkali, M., García-Gonzalo, D., Pagán, R. & Laglaoui, A. (2014a).** Chemical composition and antioxidant properties of *Laurusnobilis* L. and *Myrtuscommunis* L. essential oils from Morocco and evaluation of their antimicrobial activity acting alone or in combined processes for food preservation. *Journal of the Science of Food and Agriculture, 94, 6*, 1197–1204.

**Cherrat, L., Espina, L., Bakkali, M., Pagán, R. & Laglaoui, A. (2014b).** Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes. *Innovative Food Science and Emerging Technologies, 22*, 221–229.

**Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. & Parry, C. M. (2015).** Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clinical Microbiology Reviews, 28, 4,* 901–937.

**David, C., Trif, R., Tîrziu, E., Irimescu, R. & Gros, R. (2007).** Study of frequency of *Salmonella* strains isolated from meat, meat products and organs. Lucrari Stiintifice-Universitatea de Stiinte Agricole a Banatului Timisoara, *Medicina Veterinara, 40*, 673–677.

**de Freitas Neto, O. C., Penha Filho, R. A. C., Barrow, P. & Berchieri, Junior A. (2010).** Sources of human non-typhoid salmonellosis: a review. *Revista Brasileira de Ciência Avícola, 12,* 1, 1–11.

**de Silva, G. D. D., Abayasekara, C. L. & Dissanayake, D. R. A. (2013).** Freshly eaten leafy vegetables: a source of food borne pathogens? *Ceylon Journal of Science, 42, 2*, 95–99.

**European Food Safety Authority (EFSA) (2010).**The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA Journal, 8, 1*, 1496.

**El Abed, N., Kaabi, B., Smaali, M. I., Chabbouh, M., Habibi, K., Mejri, M., Nejib Marzouki M. & Ben Hadj Ahmed, S. (2014)**. Chemical composition, antioxidant and antimicrobial activities of *Thymus capitata* essential oil with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Evidence-Based Complementary and Alternative Medicine,.

**Esteban, M. D., Aznar, A., Fernández, P. S. & Palop, A. (2013).** Combined effect of nisin, carvacrol and a previous thermal treatment on the growth of *Salmonella* Enteritidis and *Salmonella* Senftenberg. *Food Science and Technology International*.

**Girardin, F., Mezger, N., Hächler, H. & Bovier, P. A. (2006).** *Salmonella* serovar Give: an unusual pathogen causing splenic abscess. *European Journal of Clinical Microbiology and Infectious Diseases, 25, 4*, 272–274.

**Gurman, P. M., Ross, T., Holds, G. L., Jarrett, R. G. & Kiermeier, A. (2016).**Thermal inactivation of *Salmonella* spp. in pork burger patties. *International Journal of Food Microbiology, 219*, 12–21.

**Holley, R. A. & Patel, D. (2005).** Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology, 22, 4*, 273–292.

**Hyldgaard, M., Mygind, T. & Meyer, R. L. (2012).** Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology, 3*, 12.

**López-Díez, E. C., Winder, C. L., Ashton, L., Currie, F. & Goodacre, R. (2005).** Monitoring the mode of action of antibiotics using Raman spectroscopy: investigating subinhibitory effects of amikacin on *Pseudomonas aeruginosa*. *Analytical Chemistry, 77, 9*, 2901–2906.

**Lu, Y. & Wu, C. (2010).**Reduction of *Salmonella* enterica contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *Journal of Food Protection, 73, 12*, 2270–2275.

**Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A. & Hoekstra, R. M. (2010).** The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases, 50, 6,* 882–889.

**Mazzarrino, G., Paparella, A., Chaves-López, C., Faberi, A., Sergi, M., Sigismondi, C., Compagnone, D. & Serio, A. (2015).** *Salmonella* enterica and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils. *Food Control, 50*, 794–803.

**Nanasombat, S. & Lohasupthawee, P. (2005).** Antibacterial activity of crude ethanolic extracts and essential oils of spices against *Salmonella* and other enterobacteria. *Science Technology Journal, 5, 3*, 527–538.

**Perez-Montaño, J. A., Gonzalez-Aguilar, D., Barba, J., Pacheco-Gallardo, C., Campos-Bravo, C. A., Garcia, S. & Cabrera-Diaz, E. (2012).** Frequency and antimicrobial resistance of *Salmonella* serotypes on beef carcasses at small abattoirs in Jalisco State, Mexico. *Journal of Food Protection, 75, 5*, 867–873.

**Pezzoli, L., Elson, R., Little, C. L., Yip, H., Fisher, I., Yishai, R. & Mather, H. (2008).** Packed with *Salmonella*-investigation of an international outbreak of *Salmonella* Senftenberg infection linked to contamination of prepacked basil in 2007. *Foodborne Pathogens and Disease, 5, 5*, 661–668.

**Pires, S. M., Vieira, A. R., Hald, T. & Cole, D. (2014).** Source attribution of human salmonellosis: an overview of methods and estimates. *Foodborne Pathogens and Disease, 11, 9*, 667–676.

**Shen, S., Zhang, T., Yuan, Y., Lin, S., Xu, J. & Ye, H. (2015).** Effects of cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* membrane. *Food Control, 47*, 196–202.

**Ultee, A., Bennik, M. H. J. & Moezelaar, R. (2002).** The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology, 68, 4*, 1561–1568.

**Van, T. T. H., Moutafis, G., Istivan, T., Tran, L. T. & Coloe, P. J. (2007).** Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Applied and Environmental Microbiology, 73, 21*, 6885–6890.

**Zhou, F., Ji B., Zhang, H., Jiang, H.U.I., Yang, Z., Li J., Li, J. & Yan, W. (2007).**The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogen *Salmonella typhimurium*. *Journal of Food Safety, 27, 2*, 124–133.

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# The attitudes and habits of Serbian preschool children in consumption of meat and fish

*Vesna Djordjevic[1], Radivoj Petronijevic[1], Danijela Sarcevic[1], Vesna Jankovic[1], Brankica Lakicevic[1], Branko Velebit[1], Dragana Ljubojevic[2]*

*A b s t r a c t: The goal of this study was to explore attitudes and habits of Serbian preschool children to consumption of meat and fish. Altogether, 60 preschool children from 5 to 7 years old participated in this study. The results showed that 100% of preschool children eat meat and fish. Results showed that 75% of respondents consumed meat once a day. Data analysis of fish consumption frequency showed that participants consumed fish once a week (75%). The most frequently consumed meat was poultry. Preschool children preferred river fish in their diet over sea fish. Also, parental influence in this childhood period is significant.*

***Keywords:*** *preschool children, consumption, fish, meat.*

## Introduction

Healthy eating habits are essential for the normal growth and development of preschool children and to prevent nutrition-related diseases later in life (*Dietz*, 1994). Food habits that develop during childhood are maintained as children entered school (*Singer et al,* 1995), and dietary choices of elementary school-aged children track into adolescent (*Kleder et al*, 1994). Healthy eating habits in childhood are important because they help prevent undernutrition, growth retardation, and acute childhood nutrition problems, such as obesity, coronary heart disease (CHD ), type-2 diabetes, and stroke (*Nicklas and Hayes*, 2008).

Although food habits are not stable and unchanging during a person's lifetime, a basis for healthy food habits can be created in early childhood. Children's food habits can be assumed to be influenced by their parents' food habits and choices (*Nicklas et al*, 2008). Parents can influence their children's food choice by making specific foods available and by acting as models for their behaviour in specific situations. Parental acceptance of meat nutritional recommendations in their own dietary practices may serve to underline attempts to ensure healthful dietary practices of the children (*Brewis and Gartin*, 2006). Therefore, it is conceivable that parental behaviours and child feeding practices interact with genetic predispositions to promote the development of problematic eating behaviours or less nutritious food choices in children. Food preferences play a central role in food choices and consumption, and can be described as a general predisposition for particular food, an expressed degree of liking (*Nicklaus et al,* 2008).

The purpose of this study was to provide information about meat and fish consumption by Serbian preschool children in the Republic of Serbia. Heavy food is integral part of Serbian traditions and culture. For many Serbian families, a meal without meat is a rare exception (*Sarcevic et al*, 2013). On the other hand, consumption of fish is not as frequent as is recommended in most countries (*FAO*, 2008). European public authorities recommended two to three meals that include fish per week. The field of children's food choice and behaviour remains challenging and no children's preferences, purchase behaviour and consumption of food category as well as specific brands within a food category.

## Materials and Methods

The sample frame for this research consisted of preschool children from one public kindergarten in one Belgrade municipality in the Republic of Serbia.

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Convenience sampling was used and a questionnaire was distributed to all preschool children's parents who agreed to participate after they were informed about the goals of this research and to provide information about children's eating habits with respect to consumption of meat and fish. A total of 60 preschool children, aged 5−7, participated in this study.

Parents participated in the investigation instead of their children, and they were asked to fill out a questionnaire designed by authors to collect appropriate data. Descriptive statistics, analysis of variance (ANOVA) and post-hoc Tukey-Kramer HSD test were used for analysis of collected results. Data were arranged and analysed using Microsoft Office Excel software.

## Results and Discussion

In this study, we started the investigation from the hypothesis that preschool children usually consume meals prepared at home, created by their parents. Parents' education and nutritional knowledge might have long-term effects on health outcomes. *Vereecken et al.* (2004) have established that better maternal nutritional knowledge was associated with better diets for children, although their influence increased with child age. Results of our survey presented in Table 1 showed that 100% of respondents ate meat and fish.

**Table 1.** The percentage of responses to the question Do you eat meat/fish?

| Meat | | Fish | |
|---|---|---|---|
| Yes | No | Yes | No |
| 60 | 0 | 60 | 0 |
| 100% | 0 | 100% | 0 |

Parental acceptance of nutritional recommendations for meat in their own dietary practices may serve to underline attempts to ensure healthful dietary practices of the children (*Brewis and Gartin*, 2006). In Figure 1, results showed that there was no significant difference between the answers "I like it very much" and "I like it" and they can be considered as one group of answers to the question "Do you like to eat meat?". Almost 90% of responses to the question "Do you like to eat meat?" were in this group.

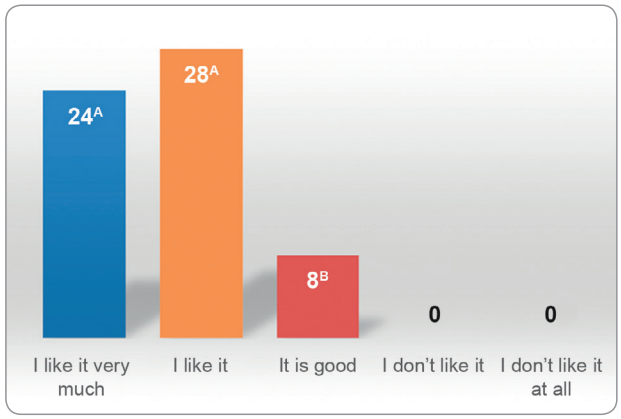



**Figure 1.** Level of preference in consumption of meat by preschool children (different letters indicate statistical significance, P<0.05)

Answers to the question "Do you like to eat fish?" showed that "I like it" was reported by fewer than half of them (Fig 2). Results of ANOVA demonstrated that there was no significant difference between the number of respondents stating "I like it very much" and "It is good". However, no respondents replied "I don't like it" or "I don't like it at all", as was the case for the question "Do you like to eat meat?".

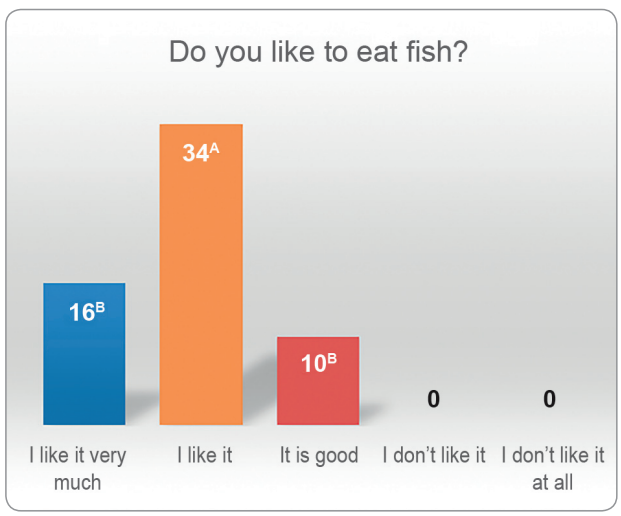



**Figure 2.** Level of preference in consumption of fish by preschool children (different letters indicate statistical significance, P<0.05)

The ratios between the answers "I like it very much" and "I like it" for consumption of meat and fish imply that preschool children prefer to eat meat rather than fish, which is in line with Serbian eating culture and cuisine.

The most common answer to the question "What kind of meat do you usually eat?" (Fig 3) was poultry (98.33%), then pork (51.67%) and then

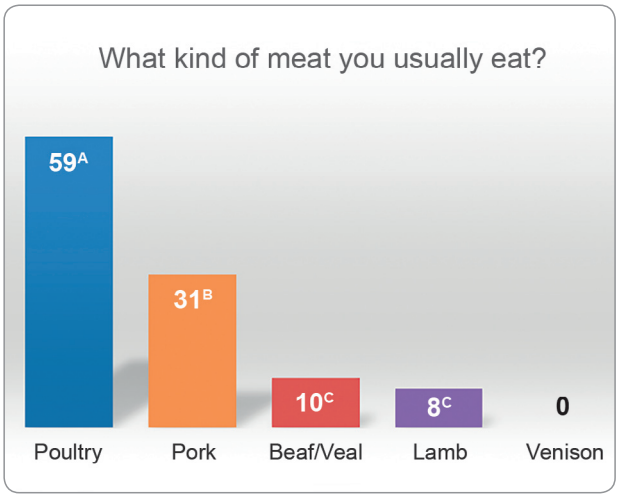



**Figure 3.** Preference for species of meat consumed by preschool children (different letters indicate statistical significance, P<0.05)

followed beef/veal (16.67%) and lamb (13.33%), with no significant difference between these two meat species (P>0.05). Similarly to our investigation, in 2007, the Australian National Children's Nutrition and Physical Activity Survey was conducted to provide data on nutrition and meat consumption of Australian children (*Bowen et al*, 2012). The data indicated that 90% of enrolled children, aged 4–8 years, consumed poultry, pork, beef/veal and lamb, which is very close to the results we obtained. Also, this is in accordance with previous statements regarding the position of meat in Serbian attitudes and eating habits, as a very nutritious food in everyday use (*Sarcevic* et al, 2013).

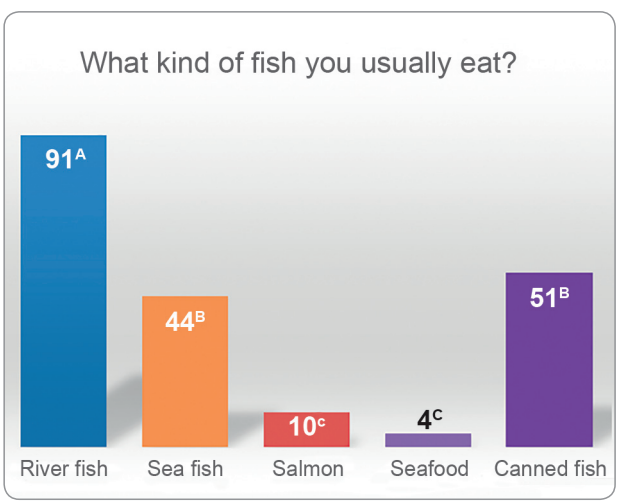



**Figure 4.** Preference for type of fish consumed by preschool children (different letters indicate statistical significance, P<0.05)

Results showed that responses to the question "What kind of fish do you usually eat?" can be sorted into three groups (Fig 4). River fish was the most favoured kind and comprised the first group, sea fish and canned fish comprised the second group, and the third group consisted of salmon and sea food, which were the least preferred types of fish.

Results of statistical analysis shown in Figure 5 showed that there was no significant difference between children's preference for consumption of trout, common carp and catfish. The children enrolled in the study consumed silver carp and pangasius less often than the other three types of river fish.

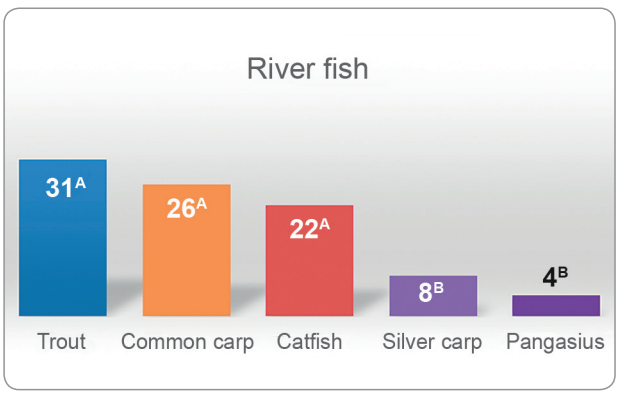



**Figure 5.** River fish consumption by preschool children (different letters indicate statistical significance, P<0.05)

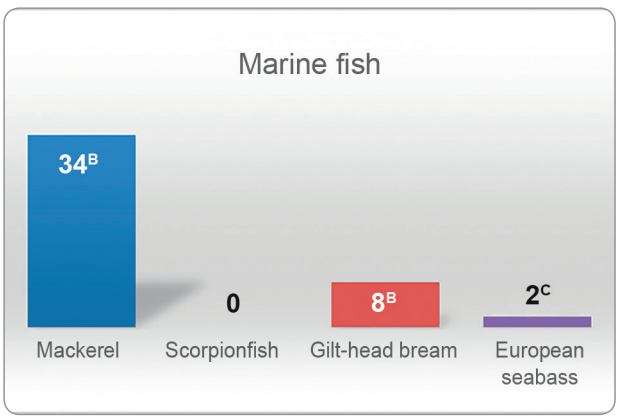



**Figure 6.** Sea fish consumption by preschool children (different letters indicate statistical significance, P<0.05)

Preschool children consumed more mackerel than any other type of marine fish (Fig 6). In Fig 6, no significant differences were observed in consumption frequency of scorpion fish and European sea bass, and these fish seem to be neglected by respondents.

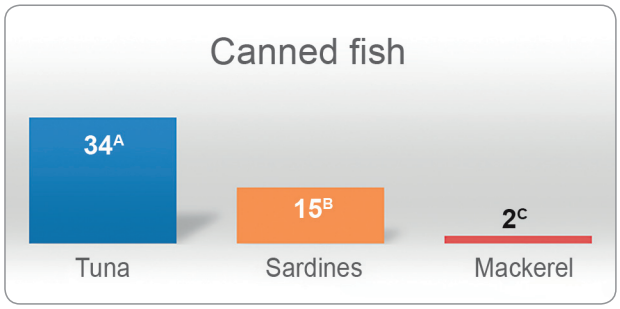



**Figure 7.** Canned fish consumption by preschool children (different letters indicate statistical significance, P<0.05)

Data analysis of the kinds of canned fish consumed showed that, unlike sea fish consumption, mackerel was consumed the least frequently, and tuna was the most favoured canned fish.

Statistical analysis of responses demonstrates that 3/4 of participants consumed meat once a day (Fig 8). There were no observed significant differences between the other answers. Data analysis of fish consumption frequency showed that many participants (75%) consumed fish once a week. No differences were noticed between the other answers.

For both questions regarding place of consumption (Figs 9 and 10), Do you eat meat prepared at…? and Do you eat fish prepared at…?, reported answers in this survey showed that 100% of parents routinely prepared meat and fish meals at home. Children sometimes ate meat or fish outside the home, but these occasions were relatively rare.

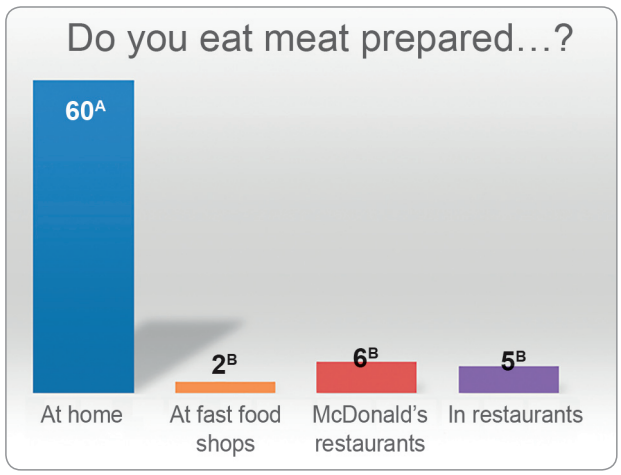
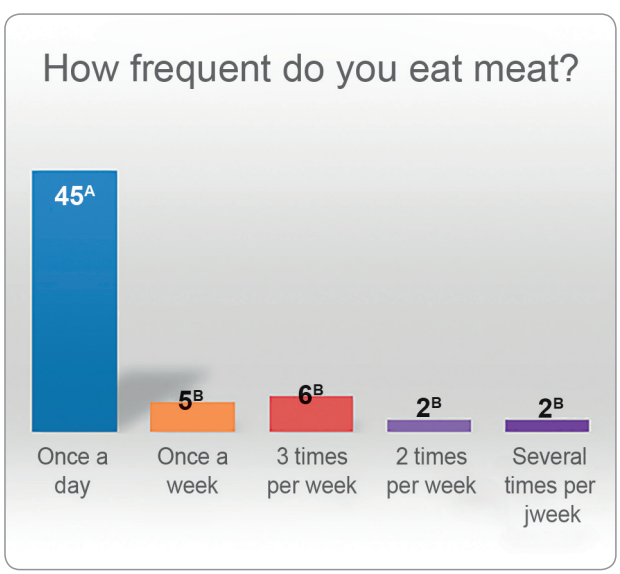



**Figure 9.** Reported place of meat consumption (different letters indicate statistical significance, P<0.05)


**Figure 10.** Reported place of fish consumption (different letters indicate statistical significance, P<0.05)



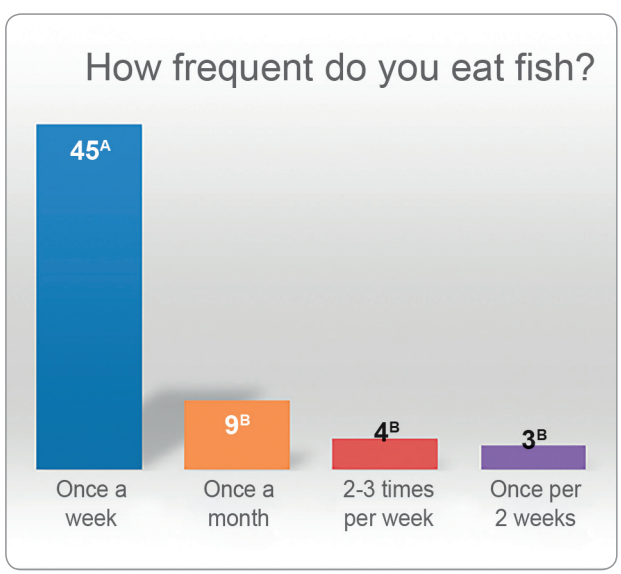




**Figure 8.** Frequency of meat and fish consumption by preschool children

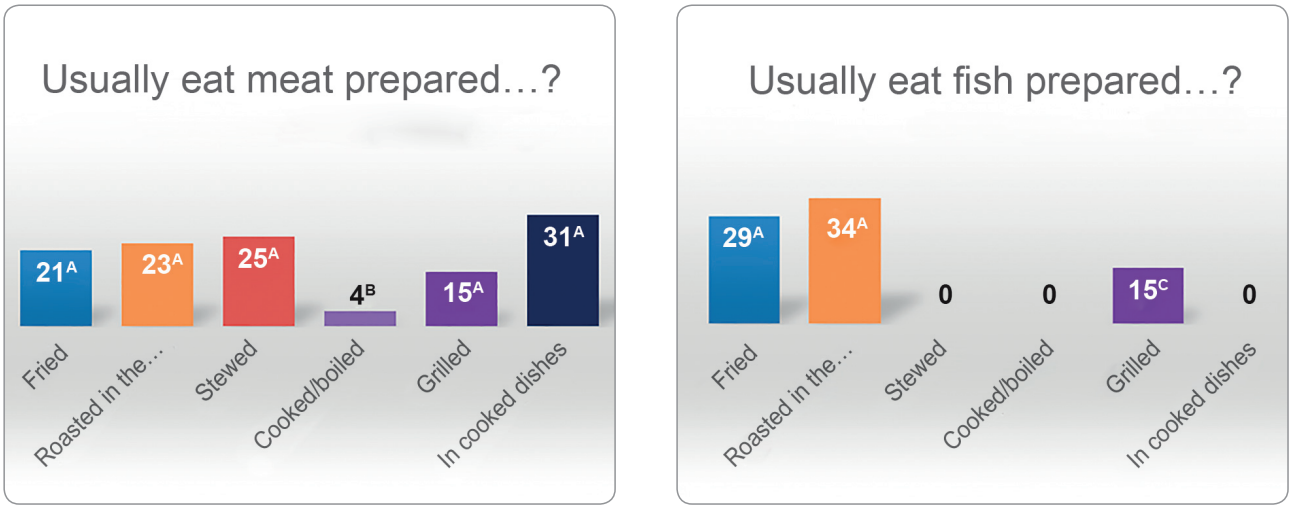



**Figure 11** Method of food preparation for meat and fish consumed by preschool children (different letters indicate statistical significance, P<0.05)

Results of ANOVA showed that no statistical differences were observed for the method of meat preparation, except for boiling, which was rarely used. The preferred kinds of fish preparation were roasting in the oven and frying, less favoured was grilling, while stewing, boiling and utilisation in complex dishes containing non-fish ingredients were not used at all.

## Conclusion

Parental influence on preschool children's attitudes and habits with regard to consumption of meat and fish is predominant and hence in some part interpretation of survey results should be considered with caution. According to the results of our survey, we can conclude:

- Preschool children consumed meat rather than fish;
- Fish is not consumed as often as is recommended by European Union authorities;
- Preschool children consumed river fish rather than sea fish;
- For preschool children, meals were prepared in 100% of the homes, which should give parents the opportunity to provide education on proper nutrition. The impact of restaurants and fast food was negligible.

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## References

**Bowen, J., Baird, D., Syrette J., Noakes M. & Baghurst, K. (2012).** Consumption of beef/veal /lamb in Australian children: Intake, nutrient contribution and comparison with other meat, poultry and fish categories. *Nutrition and Dietetics, 69* (2), 1−16.

**Brewis, A. & Gartin, M. 2006.** Biocultural construction of obesogenic ecologies childhood: parent feeding versus child eating strategies. *American Journal of Human Biology*, *18*, 203−213.

**Dietz, W. (1994).** Critical period in childhood for the development of obesity. *American Journal of Clinical Nutrition*, *59,* 995–999.

**FAO 2008.** The state of the world fisheries and aquaculture. Rome, Italy: FAO fisheries and aquaculture development, Food and Agriculture Organization of United Nations.

**Kleder, S., Perry, C., Klepp, K. & Lytle, L. (1994).** Longitudinal tracking of adolescent smoking, physical activity and food choice behaviors. *American Journal of Public Health, 84,* 1121−1126.

**Nicklas, T. A. & Hayes, D. (2008).** Position of the American Dietetic Association: Nutrition guidance for healthy children ages 2 to 11 years. *Journal of American Dietetic Association*, *108*, 1038−1044; 1046−1047.

**Nicklaus, S., Boggio, V., Chabanet, C. & Issanchou, S. (2004).** A prospective study of food preferences in childhood. *Food Quality and Preferences, 15* (7), 805–818.

**Sarcevic, D., Djordjevic, V., Petronijevic R., Matekalo-Sverak, V., Karabasil, N., Popovic, Lj. & Jankovic, V. (2013).** The attitudes and habits of Serbian schoolchildren in consumption of meat. *Tehnologija Mesa*, *54*, 2, 160−167.

**Singer, M., Moore, L., Garrahie, E. & Ellison, R. (1995).** The tracking of nutrient intake in young children: the Framingham children's study. *American Journal of Public Health*, *85,* 1673–1677.

**Vereecken, C., Keukelier, E. & Maers, L. (2004).** Influence of mother's education level of food parenting practices and food habits of young children. *Appetite*, *43*, 93−103.

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Numbers in-text and in tables/figures. Use decimal points, not decimal commas. Avoid starting and ending sentences with numbers (re-write the sentence).

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Tables are numbered consecutively with cardinal Arabic numerals (Table 1; Table 2; Table 3 etc.). Each table is referred to in the text using consecutive cardinal Arabic numerals. Table titles are placed above the tables, centre aligned (in sentence case with the table number in bold, no full-stop after the table number, the first word in the title capitalised). Tables have only three full horizontal lines, one at the top, one at the bottom and one under the column headings. Use superscript letters for table footnotes. Tables should be fully understandable without reference to the text. Figures and tables are submitted separately, in an appendix.

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The author should apply the International System of Units (SI system) and current regulation on measuring units and measuring instruments. Symbols for units derived by division are given as negative exponents, e.g. 10 g $L^{-1}$; 250 V $cm^{-2}$.

Common abbreviations:

- CFU colony forming units, capitalised, common and so is never spelled out
- kg kilogram, common and so is never spelled out
- L litre, common and so is never spelled out
- *Longissimus dorsi* (LD) is redundant and so is not used. For the whole muscle, use *Longissimus thoracis et lumborum* (LTL). The correct terms for the two parts of this muscle are *Longissimus thoracis* (LT) or *Longissimus lumborum* (LL).
- mL millilitre, common and so is never spelled out
- µm micrometre, common and so is never spelled out
- mol mole, common and so is never spelled out
- M molar, common and so is never spelled out
- PCR polymerase chain reaction, common and so is never spelled out
- SD standard deviation, capitalised, common and so is never spelled out
- SE standard error, capitalised, common and so is never spelled out
- sp. species (singular), common and so is never spelled out (not capitalised, full-stop)
- spp. species (plural), common and so is never spelled out (not capitalised, full-stop)
- UV ultraviolet, capitalised, common and so is never spelled out
- $a_w$ water activity
- h hour(s)
- min minute(s)
- 25°C (no gap after the numeral)
- 20±1°C (no gaps between numbers, sign and unit in-text and in tables/figures)
- $p<0.05$, $p\leq0.01$ (not italicised, not capitalised, no gaps)
- n=120 (no gaps between the letter, sign and numerals in-text and in tables/figures)
- found in 20.05% of cats…(no gap in-text)

**Conclusion** This section provides a review of the most important facts obtained during the research.

**Acknowledgement** This should contain the number of the project under which the research was conducted, as well as the name of the institution that funded the project or program. The acknowledgement is written after the conclusion, before the references.

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If there are two authors of the publication, surnames of authors and year of publication is written in the brackets (Thomas and Fenwick, 2008).

If there are three or more authors, the surname of the first author is stated in the brackets, followed by abbreviation "et al." and year of publication (Thomas et al., 2008).

If multiple references are cited within the same brackets, citations should be in chronological order, and then in alphabetical order if necessary.

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The reference list (Times New Roman font size 12 pt) should include recent international publications. If the original literature cited is not available, the authors should quote the source used. The references should be sorted in alphabetical order and should be cited exactly the way they appear in the original publication. Sources, volume and issue numbers should be written in italics.

Examples:

*Journals:*

**Givens, D. I., Kliem, K. E., Gibbs, R. A. (2006)**. The role of meat as a source of n3 polyunsaturated fatty acids in the human diet. *Meat Science*, *74 (1)*, 209218.

*Books:*

**Bao, Y., Fenwick, R. (2004).** Phytochemicals in Health and Disease, CRC Press, Los Angeles.

*Books with authored chapters:*

**Marasas, W. F. O. (1996).** Fumonisins: History, worldwide occurrence and impact. In Fumonisins in food, advances in experimental medicine and biology. Eds. L. S. Jackson, J. W. DeVries, L. B. Bullerman, Plenum Press, New York, pp. 118.

*PhD and MSc theses:*

**Radeka, S. (2005)**. Grape mash maceration and varietal aroma of Malvazija istarska wine, PhD Thesis, Faculty of Agriculture, University of Zagreb, Croatia.

*Laws, regulations, decrees:*

**Serbia. (2010).** Regulation on general and special conditions of hygiene of food at any stage of production, processing and transport. Official Gazette of the Republic of Serbia, 72.

**European Union. (2013).** Amending annex I to regulation EC No2073/2005 as regards histamine in fishery products. Regulation 1019/2013/ EU. Commission Regulation EU No 1019/2013. Official Journal of the European Union. L 282, 46–47.

*Symposiums, Congresses:*

**Harvey, J. (1992).** Changing waste protein from a waste disposal problem to a valuable feed protein source: a role for enzymes in processing offal, feathers and dead birds. Alltech's 8th Annual Symposium, Nichdasville, Kentucky, Proceedings, 109−119.

*Citations with organisations as authors:*

**Food and Drug Administration. (1995).** Decomposition and histamine-raw frozen tuna and mahi-mahi; canned tuna; and related species; availability of revised compliance policy guide, Federal Registration, 60, 1, 39754–39756.

**European Food Safety Authority. (2011).** Scientific opinion on risk based control of biogenic amine formation in fermented foods, EFSA Journal, 9, 10, 2393.

*Software:*

**STATISTICA (Data Analysis Software System) (2006).** v.7.1., StatSoft, Inc., USA (www.statsoft.com).

*Websites:*

**Technical report on the Food Standards Agency project G010008 (2002).** Evaluating the risks associated with using GMOs in human foods, University of Newcastle, UK (http://www.foodsafetynetwork.ca/gmo/gmnewcastlereport.pdf).

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