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Institute of meat hygiene and technology

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**Institute of Meat Hygiene and  
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11000 Belgrade, Kacanskog 13  
P.O. Box 33-49  
Phone 381 11 2650-655  
Fax 381 11 2651-825  
e-mail: [institut@inmes.rs](mailto:institut@inmes.rs)  
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# Determining mandatory nutritional parameters for Iberian meat products using a new method based on near infra-red reflectance spectroscopy and data mining

Daniel Caballero<sup>1,2,3\*</sup>, Maria Asensio<sup>1</sup>, Carlos Fernández<sup>1</sup>, Noelia Martín<sup>1</sup>, Antonio Silva<sup>1</sup>

**Abstract:** The new regulation about mandatory labelling on nutrition requires the declaration of specific parameters: protein, lipid, salt and carbohydrate contents. This study reports a fast, accurate method to determine the values of these mandatory nutritional parameters based on near infra-red reflectance spectroscopy (NIRs) technology and data mining techniques, used in an automatic way. For that, two batches of different Iberian pork meat products (dry-cured ham, dry-cured loin, dry-cured shoulder; dry-fermented Salchichón sausage, and dry-fermented Chorizo sausage) were used. One batch of each product was used to train the method and the remaining batch was used for validation. To develop the method, prediction equations were obtained from the NIRs, while nutritional data for the training batches were obtained by applying data mining techniques, and the prediction equations were evaluated against the NIRs data from the validation batch. The prediction equations achieved from very good to excellent degrees of relationship ( $R > 0.75$ ) and accurate results ( $MAE < 1$ ,  $RMSEC < 1$ ,  $RMSEP < 1$ ) from the training batch. These prediction equations were corroborated using the validation batch, which showed very good to excellent correlation coefficients ( $R > 0.75$ ). This new method is rapid, as it takes around 10 minutes in comparison with traditional methods that take around 6 days.

**Keywords:** pork, NIRs, data mining, labelling, Iberian meat products, nutritional information.

## Introduction

In the European Union (EU), mandatory labelling of nutritional information requires specific parameters (protein, lipid, saturated lipid, salt, carbohydrate and sugar contents) to be declared (*European Union*, 2011). This regulation aims to protect consumers' health by informing them of the food's nutritional content. Thus, it moderates the provision of useful, understandable and uniform information to consumers, allowing them to make coherent decisions and safe food choices (*Benoit et al.*, 2016).

In this regulation, the detection limits for the main nutritional parameters are 0.1 %, but if the value detected is less than this percentage, the amount can be declared as not detectable (*European Union*, 2011). In addition, this regulation aims that allergens or substances causing intolerances be declared, updating the guidance document under regulation 2000/13/EC (*European Union*, 2000). This regulation was active until 2014.

Consumers rate meat and meat products derived from Iberian pigs highly because of the products' unique sensory traits, which are a consequence of both the raw materials' characteristics, especially lipid-related ones, and their particular processing conditions. Among these products, the most appreciated are the expensive, dry-cured Iberian hams. These are derived from Iberian pigs reared outdoors and fed on natural resources, mainly acorn and grass (*Cava et al.*, 2000). The main nutritional parameters of protein, lipid, salt and carbohydrate contents of Iberian pork meat products derived from Iberian pigs were widely studied previously (*Cruz and Vieira*, 2017; *Muriel et al.*, 2004; *Utrilla et al.*, 2010; *Ventanas*, 2012).

Traditional physico-chemical analysis methods for nutritional information are tedious, time- and solvent-consuming and require the destruction of the samples. These analyses take around 6 days for results to be produced, delaying the response time. In this sense, the use of alternative techniques, such

<sup>1</sup>Animal Source Foodstuffs Innovation Service (SiPA), University of Extremadura, Av. Ciencias S/N, ES- 10003, Cáceres, Spain.

<sup>2</sup>Media Engineering Group, School of Technology, University of Extremadura, Av. Ciencias S/N, ES-10003, Cáceres, Spain.

<sup>3</sup>Chemometrics and Analytical Technology, Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 26, DK-1958, Frederiksberg C, Denmark.

\*Corresponding author: Daniel Caballero, [dcaballero@unex.es](mailto:dcaballero@unex.es)

as computerized tomography (CT), magnetic resonance imaging (MRI) and near infra-red reflectance spectroscopy (NIRs) were proposed for determining some nutritional parameters in these products.

CT was applied by some authors to characterize some nutritional parameters of meat products (Fulladosa et al., 2010; Picouet et al., 2013). MRI was proposed to monitor the ripening process of hams (Caballero et al., 2016) and to determine some quality parameters of Iberian dry-cured loins (Ávila et al., 2018; Caballero et al., 2017a; Caballero et al., 2017b; Pérez-Palacios et al., 2017).

Collell et al. (2011) applied NIRs to predict data on moisture, water activity and NaCl content at the surface of dry-cured ham during processing. The fatty acid compositions of dry-cured ham subcutaneous fat were predicted by NIRs (Pérez-Juan et al., 2010). In lamb or rabbit meat products (Cifuni et al., 2016; Cozzolino et al., 2000), NIRs was applied in order to determine some nutritional parameters. Moreover, in other meat products, NIRs was applied to obtain some physico-chemical parameters (González-Mohino et al., 2018; Pérez-Palacios et al., 2019; Zamora-Rojas et al., 2011).

Data mining is an important part of a larger process known as Knowledge Discovery in Databases (KDD) (Fayyad et al., 1996). The main goal of data mining consists of extracting hidden information from a data set. This can be achieved by the automatic analysis of large amounts of data, which allows the extraction of interesting and previously unknown patterns (Sayad, 2011). The development of robust and efficient algorithms to process data and the increase in computing power has enabled the use of intensive computational methods for data analysis (Hastie et al., 2001). The development and interest in data mining have recently grown because of the rapidly decreasing cost of large storage devices and increasing ease of data collection over networks (Mitchell, 1999).

There are some examples of data mining techniques applied to determine quality traits of different meats, like beef (Song et al., 2002) or lamb (Cortez et al., 2006). In the case of pork, several examples of application of data mining techniques have been published (Caballero et al., 2016; Caballero et al., 2017a; Caballero et al., 2017b; Caballero et al., 2019; Pérez-Palacios et al., 2014; Pérez-Palacios et al., 2017; Silva et al., 2013).

However, currently, there is not a fast and specified method to obtain the required nutritional information about Iberian pork products according to the EU regulation. Judprasong et al. (2013) studied the performance of laboratories in Thailand that

reported nutritional analyses according to ISO 13528 regulation. The analytical method proposed in the current paper is faster than the proposed method by Judprasong et al. (2013) and is faster than the official methods proposed by AOAC (2000). In fact, some industrial meat companies have applied this novel NIRs method of analysis successfully on their dry-cured meat products, and have confirmed it takes less time and money to obtain the nutritional parameters.

Therefore, the main objective of this study was to report a fast and accurate method to determine the values of the main nutritional parameters mandatory on the labelling of Iberian dry-cured and dry-fermented meat products based on NIRs technology and data mining techniques used in an automatic way.

## Materials and Methods

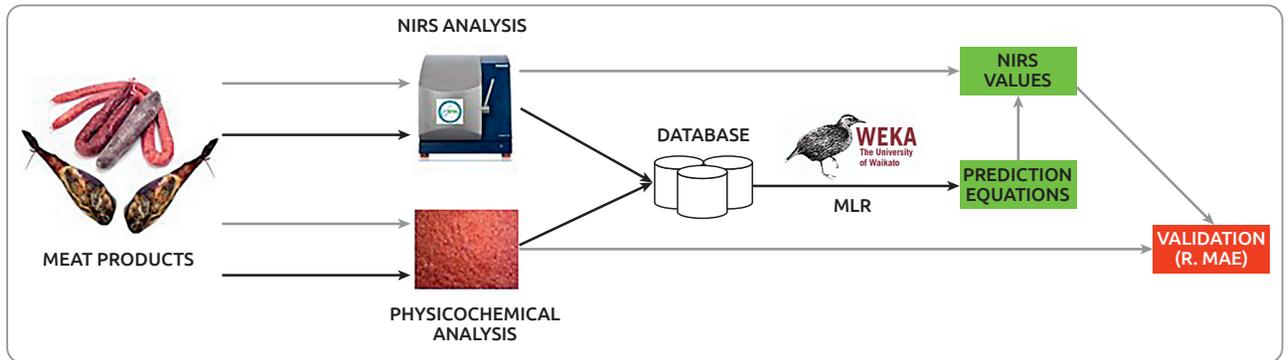
### Experimental design

Two batches of some Iberian meat products (dry-fermented Chorizo sausage, dry-cured ham, dry-cured loin, dry-fermented Salchichón sausage and dry-cured shoulders) were used.

The first batch was composed of 45 samples of each Iberian meat product for training the new method, while the second batch was composed of 50 samples of each product used to validate the new method. All these Iberian meat products were acquired in different supermarkets to maximise the sample variability for each product.

Firstly, the samples from the first training batch were analysed in two ways: i) by physico-chemical analyses to obtain the content of the main mandatory nutritional parameters and ii) by using a NIRs (FOSS FOODSCAN lab, FOSS analytics, Hillerød, Denmark) to acquire NIRs spectra. All these data were gathered in a database. In this database, Multiple Linear Regression (MLR) was applied to obtain prediction equations for the mandatory nutritional parameters for each Iberian dry-cured meat product as a function of the bandwidth values. Then, the samples from second, validation batch were analysed in the same two ways as were used for the training batch. Thus, the prediction equations obtained in the first, training batch were applied using the NIRs data obtained from second, validation batch, and for validating the prediction equations, the calculated values were evaluated with the data from physico-chemical analyses from samples comprising the second, validation batch.

Figure 1 shows the experimental design followed in this study.



**Figure 1.** Experimental design. The training batch (n=45) was analysed by the black line (---) to obtain the prediction equations. The validation batch (n=50) was analysed by the grey line (---) to validate the prediction equations.

### Physico-chemical analyses

The following physico-chemical analyses were performed in quintuplicate to determine the main nutritional parameters of each sample.

Lipid extraction of Iberian meat products was performed using the original extraction ratio of 20 parts of chloroform:methanol (2:1 v/v) to 1 part of sample. Briefly, 5 g of Iberian meat product sample were mixed with 100 ml of chloroform:methanol (2:1 v/v). The mixture was homogenised, centrifuged (10 min, 3000 rpm) and filtered. Subsequently, 5 ml of distilled water was added to the filtrate and the new mixture was shaken vigorously. The final biphasic system was separated by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroform phase was filtered through anhydrous sodium sulphate and collected. The lipid content was then gravimetrically determined after chloroform was evaporated with a rotary evaporator under vacuum and the solvent was further evaporated under nitrogen (Pérez-Palacios *et al.*, 2008).

Salt content was determined by the official method for meat and meat products (AOAC, 2000, ref. 971.19). It consists of mixing the sample with water and ethyl alcohol. After successive centrifugations, the final extract is obtained and further measured using volumetric analysis by precipitation.

Protein content was determined by the official method (AOAC, 2000, ref. 981.10). It consists of determining the nitrogen content by the Kjeldahl digestion method based on volumetric analysis, and then deriving the protein content by multiplying the nitrogen content by a factor of 6.25.

Carbohydrate content was determined by difference (Equation 1), according to the official method (AOAC, 2000, Item 85).

$$\text{Carbohydrate} = 100 - (\text{proteins} + \text{moisture} + \text{lipids} + \text{ash} + \text{salt} + \text{fibres}) \quad (1)$$

### NIRs analyses

Approximately 20 g of each sample of Iberian meat product were minced using a commercial meat mincer (Moulinex A327R1, Moulinex, Alençon, France) and analysed by NIRs spectrometer (FOSS FOODSCAN lab, FOSS analytics, Hillerød, Denmark) using a wavelength range from 850 nm to 1048 nm and taking 45 s for each spectra. This spectrometer has a wavelength accuracy of 0.5 nm and a wavelength precision of 0.1 nm. For each sample, five spectra were acquired.

Next, spectral data were imported using WinISI III (FOSS analytics, Hillerød, Denmark) to extract the numerical data from each NIRs spectrum. Then, the noise was eliminated by MSC filter (Martens and Naes, 1989). This correction allows a measured spectrum to be compared against a reference spectrum, the spectrum can be corrected using the slope of this fit, and, consequently, any outlier spectra can be removed.

Finally, values for 2 nm bandwidths were calculated from the NIRs spectra. Therefore, for each NIRs spectrum, two hundred values were calculated, extracted and gathered into the database.

### Data mining analyses

Predictive data mining techniques were applied to the database constructed with results from the physico-chemical and NIRs analyses. Future models can be predicted from current data using trend analysis (Wu *et al.*, 2008). Therefore, for each main nutritional parameter of each Iberian meat product, predictive equations as a function of NIRs data were obtained. Thus, the main nutritional parameters could be calculated as a function of NIRs data.

The free software WEKA (Waikato Environment for Knowledge Analysis v. 3.8.1, University of Waikato, Hamilton, New Zealand) was used to perform the predictive analyses. MLR models the linear relationship between a target variable and multiple independent variables. It produces a linear regression equation that can be used to predict future values (Hastie et al., 2001). For the selection of attributes, the M5 method was applied. This method is based on stepping through the attributes, the one with the smallest standardized coefficient being removed until no further improvement is observed in the error estimation (Kira and Rendell, 1992). A ridge value of  $1 \times 10^{-4}$  was applied. The estimation procedure was a 10-fold cross validation (Dietterich, 1998), where the data were divided into 10 subsets of equal size. One subset was tested each time and the remaining data were used for fitting the model. The process was repeated sequentially until all subsets were tested. Therefore, all data subsets were used for both training and testing. However, since this method requires 10 analyses (i.e. with the 10 different data subsets), it is a robust method (Grossman et al., 2010).

The correlation coefficient (R; equation 2) was used for evaluating the goodness of the prediction and for its validation. According to the Colton rules (Colton, 1974), R from 0 to 0.25 is considered as little to no association, from 0.25 to 0.50 indicates a weak degree of relationship, from 0.50 to 0.75 indicates a moderate to good degree of relationship and from 0.75 to 1 indicates a very good to excellent degree of relationship.

$$R = \frac{\sqrt{\sum_{i=1}^n (f_i - \bar{y})^2}}{\sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (2)$$

where  $f_i$  is the predicted value,  $y_i$  is the real value and mean ( $\bar{y}$ ) is the average value.

Moreover, the mean absolute error (MAE; equation 3), root mean square error of calibration (RMSEC; equation 4) and root mean square error of prediction (RMSEP; equation 5) (Hyndman, 2006) were also used to validate the prediction results. The MAE measures the difference between real values and predicted ones. Values of MAE lower than 2 are appropriate (Hartemink and Minasny, 2016).

$$MAE = \frac{1}{n} \sum_{i=1}^n |f_i - y_i| \quad (3)$$

where  $f_i$  is the predicted value and  $y_i$  is the real value.

The RMSEP measures the relative difference between real values and predicted ones. This measure is commonly used to assess the predictive ability

of the models, since is a constant measure for prediction. Values of RMSEP lower than 5 are appropriate (Hartemink and Minasny, 2016).

$$RMSEP (\%) = \sqrt{\frac{1}{n} \sum_{i=1}^n (f_i - y_i)^2} \times 100 \quad (4)$$

where  $f_i$  is the predicted value and  $y_i$  is the real value.

The RMSEC measures the goodness of fit between real data and the data from the calibration model. Depending on the type of data, models and their application can be subject to huge optimistic bias due to over-fitting compared to the results when applying the calibration (Austin and Tu, 2004). Values of RMSEC lower than 5 are appropriate (Hartemink and Minasny, 2016).

$$RMSEC (\%) = \sqrt{\frac{1}{n} \sum_{i=1}^n (f_i - y_i)^2} \times 100 \quad (5)$$

where  $f_i$  is the real value and  $y_i$  is the value obtained by the calibration model.

### Comparison of analytical methods

The compatibility index (En) (Beilby, 1972) was applied to evaluate the compatibility of the official methods of analyses and the new NIRs method. This index was calculated by the following equation (Equation 6):

$$E_n = \frac{|X_1 - X_2|}{\sqrt{(\frac{S_1}{N_1})^2 + (\frac{S_2}{N_2})^2}} \quad (6)$$

where  $X_1$  is the average value according to the official method and  $X_2$  is the average value according to the new proposed method.  $S_1$  and  $S_2$  are the standard deviation for the official method and the new method, respectively.  $N_1$  is the number of samples for the official method and  $N_2$  is the number of samples for the new method. Values of  $E_n$  lower than 2 indicate the methods are compatible (Golnick et al., 2016).

### Statistical analyses

Groups of values of the main nutritional parameters calculated by the official and proposed methods from training and validation batches were compared using one-way analysis of variance (ANOVA) using the general linear model procedure. In addition, the effect between batches was compared by using ANOVA. Statistical analyses were performed using the SPSS package software (IBM SPSS v. 20.0, IBM Co, New York, New York, USA, 2011).

## Results and Discussion

### Results from training batch

The main nutritional parameters of the Iberian meat products were obtained by applying the traditional physico-chemical analyses on the training batch. Table 1 shows the results from these analyses.

The values for the main nutritional parameters were quite in concordance with previous studies on Iberian meat products (Caballero *et al.*, 2016; Caballero *et al.*, 2018; Cruz and Vieira, 2017; Lorenzo *et al.*, 2000; Muriel *et al.*, 2004; Utrilla *et al.*, 2010; Ventanas, 2012).

MLR was applied to the NIRs data from the training batch of Iberian meat products to obtain prediction equations for the main nutritional parameters (protein, lipid, salt and carbohydrate contents) of these meat products. Table 1 shows the predicted values based on the NIRs method and the correlation coefficient, p-value, RMSEP, RMSEC and MAE of the prediction equations for the main nutritional parameters of each Iberian meat product.

Note the correlation coefficient of the carbohydrate contents of dry-cured ham, dry-cured loin and dry-cured shoulder were not calculated because the values of the carbohydrates in these Iberian dry-cured

**Table 1.** Mean  $\pm$  standard error value, p-value, correlation coefficient (R), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and mean absolute error (MAE) between values obtained using the official and new NIRs methods to produce the parameters (lipid content (%), protein content (%), salt content (%) and carbohydrate content), the main mandatory items on nutritional labels, for the training batch of different Iberian dry-cured and dry-fermented meat products (chorizo, ham, loin, Salchichón and shoulder).

	Official method	NIRs	p-value	R	RMSEC	RMSEP	MAE
<b>LIPID CONTENT (%)</b>							
Chorizo	44.0 $\pm$ 0.17	44.2 $\pm$ 0.14	0.996	0.923	0.81	1.22	0.67
Ham	19.5 $\pm$ 0.21	19.4 $\pm$ 0.12	0.969	0.996	0.10	0.22	0.08
Loin	20.1 $\pm$ 0.32	20.0 $\pm$ 0.35	0.998	0.957	0.95	1.21	0.82
Salchichón	39.9 $\pm$ 0.28	39.8 $\pm$ 0.16	0.881	0.932	0.95	1.28	0.81
Shoulder	27.7 $\pm$ 0.23	27.6 $\pm$ 0.19	0.999	0.913	0.98	1.39	0.86
<b>PROTEIN CONTENT (%)</b>							
Chorizo	26.5 $\pm$ 0.21	26.6 $\pm$ 0.07	0.999	0.909	0.42	0.76	0.34
Ham	28.8 $\pm$ 0.18	28.7 $\pm$ 0.10	0.979	0.993	0.12	0.25	0.10
Loin	30.9 $\pm$ 0.39	32.9 $\pm$ 0.50	0.999	0.973	0.92	1.38	0.74
Salchichón	29.3 $\pm$ 0.14	29.4 $\pm$ 0.23	0.893	0.860	0.38	0.52	0.30
Shoulder	30.0 $\pm$ 0.49	29.8 $\pm$ 0.62	0.999	0.924	0.86	1.31	0.68
<b>SALT CONTENT (%)</b>							
Chorizo	3.76 $\pm$ 0.14	3.77 $\pm$ 0.02	0.992	0.910	0.02	0.04	0.02
Ham	4.06 $\pm$ 0.10	4.04 $\pm$ 0.19	0.948	0.972	0.05	0.08	0.03
Loin	4.42 $\pm$ 0.30	4.41 $\pm$ 0.42	0.996	0.989	0.13	0.23	0.09
Salchichón	3.42 $\pm$ 0.08	3.43 $\pm$ 0.02	0.789	0.875	0.05	0.07	0.04
Shoulder	3.06 $\pm$ 0.48	3.05 $\pm$ 0.55	0.999	0.981	0.30	0.42	0.23
<b>CARBOHYDRATE CONTENT (%)</b>							
Chorizo	2.17 $\pm$ 0.04	2.19 $\pm$ 0.04	0.997	0.934	0.21	0.39	0.16
Ham	< 0.10	-----	-----	-----	-----	-----	-----
Loin	< 0.10	-----	-----	-----	-----	-----	-----
Salchichón	2.19 $\pm$ 0.05	2.17 $\pm$ 0.04	0.862	0.912	0.27	0.35	0.22
Shoulder	< 0.10	-----	-----	-----	-----	-----	-----

**Table 2.** Mean ± standard error value, the p-value, the correlation coefficient (R), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) and mean absolute error (MAE) between values obtained using the official and new NIRs methods to produce the parameters (lipid content (%), protein content (%), salt content (%) and carbohydrate content), the main mandatory items on nutritional labels, for the validation batch of different Iberian dry-cured and dry-fermented meat products (chorizo, ham, loin, Salchichón and shoulder).

	Official method	NIRs	p-value	R	RMSEC	RMSEP	MAE
<b>LIPIDS CONTENT (%)</b>							
Chorizo	43.6 ± 0.12	43.8 ± 0.14	0.459	0.808	1.13	1.27	1.09
Ham	20.1 ± 0.24	20.4 ± 0.27	0.635	0.929	0.96	1.23	0.88
Loin	18.8 ± 0.22	19.1 ± 0.23	0.525	0.905	1.17	1.31	1.05
Salchichón	38.5 ± 0.09	38.6 ± 0.19	0.961	0.896	0.96	1.02	0.93
Shoulder	28.2 ± 0.15	28.3 ± 0.23	0.197	0.864	1.41	1.53	1.27
<b>PROTEINS (%)</b>							
Chorizo	25.7 ± 0.14	25.6 ± 0.16	0.826	0.843	0.72	0.81	0.66
Ham	27.7 ± 0.12	27.6 ± 0.13	0.983	0.783	1.16	1.40	1.10
Loin	32.6 ± 0.26	32.8 ± 0.27	0.774	0.956	0.97	1.11	0.85
Salchichón	29.3 ± 0.09	29.2 ± 0.19	0.782	0.847	0.71	0.78	0.64
Shoulder	27.3 ± 0.22	28.1 ± 0.24	0.695	0.870	1.47	1.64	1.40
<b>SALT CONTENT (%)</b>							
Chorizo	3.76 ± 0.14	3.77 ± 0.02	0.321	0.865	0.11	0.15	0.08
Ham	4.06 ± 0.10	4.04 ± 0.19	0.203	0.828	0.14	0.18	0.12
Loin	4.42 ± 0.40	4.41 ± 0.62	0.989	0.927	0.26	0.35	0.21
Salchichón	3.42 ± 0.08	3.43 ± 0.02	0.987	0.816	0.18	0.23	0.14
Shoulder	3.06 ± 0.48	3.05 ± 0.55	0.725	0.955	0.35	0.45	0.31
<b>CARBOHYDRATES (%)</b>							
Chorizo	2.21 ± 0.03	2.29 ± 0.04	0.314	0.779	0.32	0.47	0.28
Ham	< 0.10	-----	-----	-----	-----	-----	-----
Loin	< 0.10	-----	-----	-----	-----	-----	-----
Salchichón	2.00 ± 0.03	2.04 ± 0.04	0.665	0.818	0.32	0.39	0.27
Shoulder	< 0.10	-----	-----	-----	-----	-----	-----

meat products is considered minimum (<0.100%) and at undetectable levels according to the current regulation (European Union, 2012; Ventanas, 2012). Therefore, for the new NIRs method proposed in this study, the values of carbohydrates for these meat products is shown as lower than 0.100 % (not detectable) in all samples of dry-cured ham, dry-cured loin and dry-cured shoulder.

Prediction by means of NIRs data and values for nutritional analyses for the remaining cases achieved very good to excellent degrees of relationship according to the rules given by Colton (Colton, 1974) (R >0.75). R values were R >0.97 for the dry-cured

ham and R >0.95 for the dry-cured loin, for all nutritional parameters. For MAE, for all prediction equations, the values obtained were lower than 1, which is an appropriate value for MAE (Hyndman, 2006). For RMSEP and RMSEC, for all prediction equations, the values obtained were lower than 1.50, which are appropriate values for RMSEP and RMSEC (Hartemink and Minasny, 2016). However, the most accurate results were obtained for the dry-cured ham (MAE < 0.15, RMSEC < 0.15, RMSEP < 0.30). Moreover, no significant differences were found between values from official methods and the new method based on prediction equations.

These results indicate the ability of the proposed model to calculate the main nutritional parameters based on NIRs and data mining are comparable with the results obtained by the official methods of analysis. Thus, the NIRs method could be proposed as an alternative method to estimate the values of these nutritional parameters with similar accuracy as the official methods. Previous studies obtained similar results ( $R > 0.75$ ) for the nutritional parameters salt and lipid contents (Collell *et al.*, 2011; González-Mohino *et al.*, 2018; Pérez-Juan *et al.*, 2010; Pérez-Palacios *et al.*, 2019; Zamora-Rojas *et al.*, 2011). MLR was evaluated to predict some quality parameters of Iberian dry-cured meat products, dry-cured ham or dry-cured loin, in previous studies (Caballero *et al.*, 2016; Caballero *et al.*, 2017a; Caballero *et al.*, 2017b; Caballero *et al.*, 2018; Pérez-Palacios *et al.*, 2014; Pérez-Palacios *et al.*, 2017).

The ability of this proposed method to calculate the main nutritional parameters of different Iberian dry-cured meat products was next evaluated using the validation batch.

#### Results from validation batch

Taking a step forward, for validating the models obtained from the training batch, the prediction equations obtained from the training batch were applied to the NIRs data from validation batch of the different Iberian meat products.

Table 2 shows the values from the official methods of analyses and the values from the new method based on NIRs, the correlation coefficients, RMSEC, RMSEP and MAE of the results obtained from NIRs data and data from the official analytical methods for the main nutritional parameters (lipid, protein, salt and carbohydrate contents) of the studied Iberian meat products. As previously stated, the

values of carbohydrates for some dry-cured products (ham, loin and shoulder) were labelled as not detectable according to the current regulation (European Union, 2012).

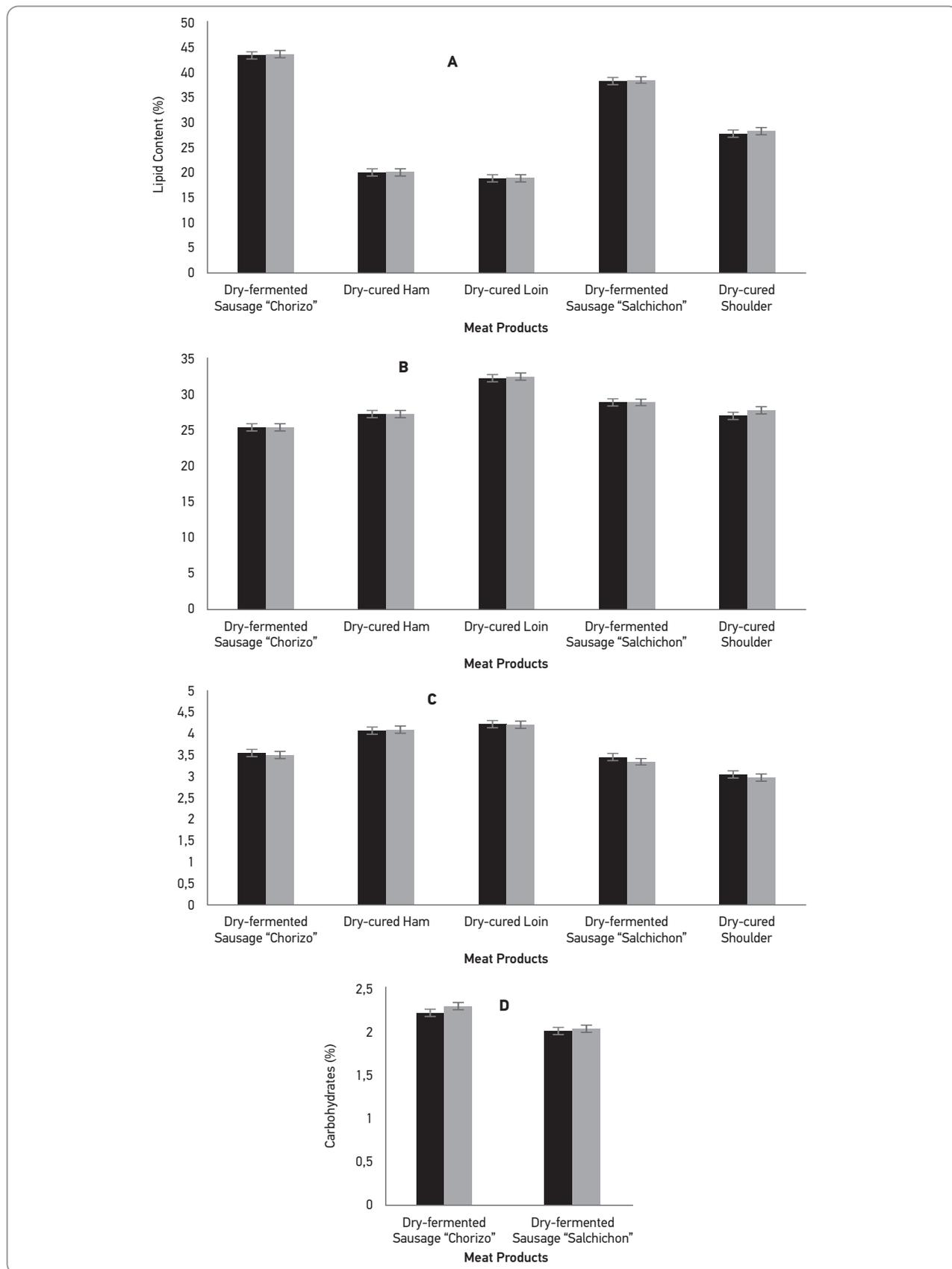
The values obtained from the official methods and from the new method of analysis (Table 2) were similar to the results obtained from the training batch (Table 1), and they are in agreement with the results obtained in previous studies (Caballero *et al.*, 2016; Caballero *et al.*, 2018; Cruz and Vieira, 2017; Lorenzo *et al.*, 2000; Muriel *et al.*, 2004; Utrilla *et al.*, 2010; Ventanas, 2012).

The values obtained for the NIRs method and the official methods for all studied cases achieved very good to excellent correlation coefficients according to the rules given by Colton (1974) ( $R > 0.75$ ). The strongest degrees of relationships were achieved for dry-cured loin ( $R > 0.90$ ) and dry-cured shoulder ( $R > 0.85$ ). Regarding the nutritional parameters, the highest correlation coefficients were found for the lipid and salt contents ( $R > 0.80$ ) from all Iberian meat products. In relation to the MAE, in all cases, the values obtained were lower than 1.50, which is a good value for MAE (Hyndman, 2006), and for RMSEP and RMSEC, all values were lower than 2, which is a very good value for RMSEP and RMSEC (Hartemink and Minasny, 2016). However, the most accurate results were obtained for the Salchichón dry-fermented sausage (MAE < 1, RMSEC < 1 and RMSEP < 1.10).

Evaluating the values obtained for the compatibility index (Table 3), all values obtained were lower than 2 ( $En < 2$ ), indicating the analytical methods used in this study were compatible (Golnick *et al.*, 2016). For some products, their compatibility indices were lower than 1: dry-cured ham ( $En < 0.75$ ), dry-fermented Chorizo sausage ( $En < 0.85$ ) and dry-cured loin ( $En < 0.95$ ). These results support the

**Table 3.** Compatibility index (En) results, comparing the official methods and the new NIRs method of analysis analysing the main parameters on mandatory nutritional labels for the validation batch of different Iberian dry-fermented and dry-cured meat products.

	Chorizo	Ham	Loin	Salchichón	Shoulder
Lipid content (%)	0.816	0.727	0.910	0.077	0.535
Protein content (%)	0.263	0.125	0.438	0.214	1.898
Salt content (%)	0.463	0.482	0.011	1.089	0.341
Carbohydrate content (%)	0.583	-----	-----	0.241	-----



**Figure 2.** Results for the main mandatory nutritional parameters (mean value  $\pm$  standard error) of the validation batch determined by official methods (black) and estimated from the new method based on NIRs data (grey) for a) lipid content (%), b) protein content (%), c) salt content (%) and d) carbohydrate content (%).

compatibility of the newly-developed method based on NIRs and data mining with the official methods of analysis.

The batch effect on the main mandatory nutritional parameters of the studied Iberian meat products (dry-fermented Chorizo sausage, dry-cured ham, dry-cured loin, dry-fermented Salchichón sausage and dry-cured shoulder) was studied. No significant differences were found between training and validation batches for the main mandatory nutritional parameters. These results indicated the assignment of samples into batches had no influence on the results obtained, and therefore, the results are batch-independent. In a previous study, the batch effect acted like a random effect (Herbert *et al.*, 1974), but in our case, the batch did not influence the results.

Average values for the main mandatory nutritional parameters determined by official methods and our new method based on NIRs are shown in Figure 2. Thus, the accuracy of the new method based on NIRs was corroborated for lipid (Figure 2A), protein (Figure 2B), salt (Figure 2C) and carbohydrate (Figure 2D) contents. No significant differences were found between values from official methods and the new method based on NIRs. These results are in accordance with the results showed in Table 3.

## Conclusion

A new, fast and accurate analytical method was studied, which should be suitable to analyse the content of the main nutritional parameters required for mandatory labelling according to the new EU regulation. The new method is based on prediction equations obtained from the combination of NIRs spectra and data mining techniques. In other proposed methods in ISO 13528, the lead-in time to produce results is around 5 or 6 days, with similar accuracy to the official methods (although these analyses were conducted on rice, not on meat and meat products). However, the new NIRs method proposed in this study is faster and more accurate than the previous methods for the required nutritional analyses in comparison with the traditional methods. Additionally, only a small number of samples is required before the results obtained are accurate. Thus, the new NIRs method produces the main nutritional parameters for each dry-cured meat product in around 10 minutes per sample, using a NIRs spectrometer and a conventional laptop in an automated way. This method could be of interest to inspection agencies in order to evaluate the nutritional labelling of Iberian meat products in a timely manner.

# Određivanje obaveznih nutritivnih parametara Iberijskih mesnih proizvoda korišćenjem nove metode zasnovane na NIR i pretraživanju podataka

Daniel Caballero, Maria Asensio, Carlos Fernández, Noelia Martín, Antonio Silva

*Apstrakt:* Nova uredba o obaveznom označavanju nutritivnih sastojaka zahteva deklarisanje određenih parametara, kao što su proteini, sadržaj lipida, sadržaj soli ili ugljenih hidrata. Ovo istraživanje ima za cilj da razvije brz i tačan metod da se na automatski način utvrde vrednosti obaveznih nutritivnih parametara na osnovu NIR tehnologije (bliska infracrvena spektroskopija) i tehnika pretraživanja podataka. U ovu svrhu su korišćene dve serije različitih proizvoda od svinjskog mesa (suva pršuta, suva slabina, suva fermentisana kobasica „Salchichón“ i suva fermentisana kobasica „Chorizo“). Jedna serija je korišćena kako bi se trenirala metoda, a druga serija za svrhu validacije. Da bi se razvio postupak, jednačine predviđanja dobijene su iz NIR i nutritivnih podataka prve serije primenom tehnika pretraživanja podataka, a zatim su jednačinee procenjivane sa NIR podacima iz serije validacije. Štaviše, jednačine predviđanja postigle su vrlo dobar do odličan stepen korelacije ( $R > 0,75$ ) i tačnih rezultata ( $MAE < 1$ ,  $RMSEC < 1$ ,  $RMSEP < 1$ ) sa prvom, trenaznom serijom. Ove jednačine predviđanja potkrepljene se u grupi za validaciju, koja je pokazala vrlo dobre do odlične koeficijente korelacije ( $R > 0,75$ ). Prema tome, ova nova metoda traje oko 10 minuta u poređenju sa tradicionalnom metodom koja traje oko 6 dana.

**Ključne reči:** svinjsko meso, NIR, pretraživanje podataka, deklarisanje, Iberijski mesni proizvodi, nutritivne informacije.

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# Evaluation of sensory and chemical parameters of fermented sausages

Ivana Brankovic Lazic<sup>1\*</sup>, Jelena Jovanovic<sup>1</sup>, Stefan Simunovic<sup>1</sup>, Mladen Raseta<sup>1</sup>, Dejana Trbovic<sup>1</sup>, Tatjana Baltic<sup>1</sup>, Jelena Ciric<sup>1</sup>

**Abstract:** Meat preservation became essential for transportation of meat for long distances without spoilage of texture, colour and nutritional value after the development and rapid growth of supermarkets. The most ancient methods of preserving meat are drying and fermentation of meat products. Fermented sausage is mostly industrially produced, although not standardized, but it is acceptable to the majority of the population. In this study, the sensory proprieties and chemical characteristics of kulen, a type of dry fermented sausage, were examined. Two groups of kulen sausages had differing percentages of pork meat and sweet and cayenne pepper, while the amount of solid fat tissue and other ingredients added at manufacturing were the same. The results obtained show the ingredients used might have an influence on kulen's sensory properties, in this case especially on odour, taste, cross-section colour and overall acceptability average scores. Chemical analyses showed that kulen group 1 had a higher protein content (24.52%) than kulen group 2, which contained 23.00% meat protein, while the content of collagen in the meat protein was 7.02% (group 1) and 9.16% (group 2). Moisture in all these kulen sausages was less than 35%, while the values of other parameters were similar between the two kulen groups. Therefore, the raw materials and the other ingredients used in the technological production of kulen fermented sausage can influence the final product quality.

**Keywords:** fermented sausage, sensory analysis, chemical analysis.

## Introduction

Meat contains protein of high biological value and important micronutrients needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fat (William, 2007). Meat preservation became essential for transportation of meat for long distances without spoilage of texture, colour and nutritional value after the development and rapid growth of supermarkets (Nythes *et al.*, 2008). The most ancient methods of preserving meat are drying and fermentation of meat products. There are many historical accounts about the production and consumption of sausages in ancient civilizations, going back thousands of years.

However, there is no specific date for when sausage was first produced, because this dates from the period before written history (Savic, 1985). During the Middle Ages, great migrations led to the mixing of different cultures and customs, and therefore, knowledge of food conservation was transmitted worldwide more rapidly. After the Second World

War, development and modernization of product technology and equipment for fermented meat products continued (Babic and Babic, 2000). This kind of product has a specific microbiota that is typical of the region or area where they are produced. Naturally present or added lactic acid bacteria in fermented sausage produce acid, which has a positive effect on safety, sensory characteristics and shelf life of sausages (Comi *et al.*, 2005; Petäjä-Kanninen and Puolanne, 2007; Zukál and Incze, 2010).

Fermented sausages are high-quality products. Traditionally, fermented sausages were considered healthy and safe foods. More recently, eating fermented sausages has been associated with health hazards caused by the high contents of saturated fats and NaCl, the presence of nitrite and degradation products such as nitrosamines, and use of smoking that can lead to toxic compounds such as polycyclic aromatic hydrocarbons in the products. Hazards can also be both of direct microbiological nature, the sausages potentially being contaminated with foodborne pathogens, and of indirect microbiological nature by the metabolic activity of

<sup>1</sup>Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia.

\*Corresponding author: Brankovic Lazic Ivana [ivana.brankovic@inmes.rs](mailto:ivana.brankovic@inmes.rs)

microorganisms causing the presence of biogenic amines and mycotoxins. The organoleptic and other properties of fermented sausages depend not only upon the products of bacterial fermentation of sugar, but are also strongly influenced by biochemical and physical changes occurring during the long drying or ageing process. The use of starter cultures for this category of raw sausages is less successful than for the semidry varieties. The lengths of production, either with or without smoking, and the drying period depend upon a multiplicity of factors, such as diameter and physical properties of casings, sausage formulation, choice and methods of preparing meat, drying conditions etc., but overall processing time can require up to 90 days. The final pH of dry sausages is usually slightly higher (5.0–5.5) than that of semidry sausages, and it increases during the second part of this long ageing process. Dry sausages are made from selected, mainly coarsely chopped, meat (some Italian salamis, some types of sudzuk); often they are moderately chopped (the majority of small-diameter dry sausages), and very occasionally they are finely chopped. They are cut in thin slices, their water content is <35%, but normally is <30%. Most varieties of dry sausage undergo cold smoking (12 to 18°C) but sometimes not; in some countries they are often heavily spiced with red pepper or garlic or sometimes are heavily smoked and strongly salted. In principle, they are processed by long, continuous air-drying, sometimes after a comparatively short period of smoking. The formulation, degree of grinding, level of fermentation, smoking intensity, temperature of ageing and type and size of casing as well as other factors determine the properties of the final product. Dry sausages are stuffed into natural or artificial casings of different diameters. Dry sausages are usually sold as moderately dry (about 30% weight loss) and dry sausages (about 40% weight loss) (FAO, 2013).

There is a difference in fermented sausage technology between the United States and the European countries. US methods rely on rapid acid production (lowering the pH) through a fast fermentation in order to stabilize the sausage against spoilage bacteria. Fast acting starter cultures such as *Lactobacillus plantarum* and *Pediococcus acidilactici* are used at high temperatures up to 40°C. As a result, pH drops to 4.6, the sausage is stable but the flavour suffers and the product is sour and tangy. In European countries, the temperatures of 22–26°C are used and the drying, instead of the acidity (pH) is the main hurdle against spoilage bacteria; this favours better flavour development. The final acidity

of a traditionally-made salami is low (high pH) and there is no sour taste. There are different types of fermented sausages in hosts retail market such as kulen, winter salami, Srem sausage, sudzuk and tea sausage, but other types of related products also exist. In Serbia, this type of sausage is mostly industrially produced, which means the quality of this product is not standardised, but it is acceptable for the majority of the population because it is characterised by an attractive appearance, good grinding ability and pleasant aroma. Today, the national market offers dry fermented sausages with similar sensory properties (Petrohilou and Rantsios, 2005; Veskovic Moracanin et al., 2011)

According to Serbian regulation on the quality of ground meat, meat preparations and meat products (Serbia, 2015; 2017), fermented sausage can legally contain category 1 or 2 domestic pork, beef or equine meat, category 1 poultry meat or game meat, solid fat tissue, and additives. These ingredients are mixed, and after filling into casings, the sausages are preserved by drying and fermentation, with or without smoking. The drying process is carried out at a low temperature, and only then does sausage get its characteristic, spicy aroma, solid consistency and extended shelf life during the ripening process (Vukovic, 2012). Additives for fermented sausages, according to Serbian regulations, can be salt, curing salt, spices, spice extracts, sugars, additives, starter culture and beverages (wine and others). Fermented dry sausages must contain less than 35% water.

The aim of this paper was to point out the existence of differences in the sensory and chemical parameters of kulen, a dry fermented sausage, produced using two different recipes. These recipes differ in the quality of raw pork meat and the amount of added ingredients.

## Materials and Methods

Fermented dry kulen sausage was produced according to two recipes, which differed in the category and quality pork meat and the amount of some added ingredients. Technological production processes were the same for both sausage groups. Kulen group 1 sausage included slightly better quality raw pork meat than the kulen group 2. The other differences between those two groups were in their contents of sweet and cayenne pepper. Table 1 shows the percentage distribution of the ingredients used to manufacture the two groups of kulen.

The technological processing of the sausages took place under industrial conditions. The raw

**Table 1.** The percentage distribution (%) of ingredients used to produce two groups of kulen dry, fermented sausage

Raw material	Group 1 kulen percentage (%)	Group 2 kulen percentage (%)
Pork meat category 1	60	55
Pork meat category 2	10.9	15.9
Solid fat tissue	25	25
Nitrite salt	2.5	2.5
RADA <sub>ferm</sub>	0.05	0.05
Dextrose	0.2	0.2
Ascorbic acid	0.05	0.05
Sweet pepper (oleoresin)	0.7	0.8
Cayenne pepper (oleoresin)	0.3	0.2
Garlic	0.3	0.3

materials, pork meat (−4.3°C) and solid fat tissue (−5.2°C) were minced in a cutter (CFS master). After that, other ingredients were added, while starter culture was added at the end of the process. The homogenization was carried out until an 8 mm granulation mosaic was obtained. Mixed sausage meats were stuffed using a vacuum filler into collagen casings, diameter  $\varnothing$ 55. Sausages were then hung on horizontal bars of drying racks and left in the ante-room of the automatic air conditioning chamber for about 4 to 6 h. This procedure was used to optimize the process of fermentation/ripening, as the temperature of the filling needs to be raised as near as possible to the optimal temperature (recommendation: to achieve at least 18–19°C, and ideally, 22–24°C) before the fermentation process starts (Brankovic et al., 2019). This ensures optimal conditions for the metabolism of starter cultures (Brankovic et al., 2019). The production process (fermentation/drying and smoking, ripening) was a combination of automatic air conditioning chamber and traditional smoke chamber. This process lasted for 26 days.

**Table 2.** Numerical descriptive scale for the assessment of sensory properties

Number rating	Descriptive rating
5	extremely acceptable
4	very acceptable
3	acceptable
2	at the margin of acceptability
1	unacceptable

### Laboratory analyses

After production, the sausages were analysed in sensory and chemical laboratories accredited according to SRPS ISO/IEC 17025:2005.

### Sensory analyses

Sensory properties of sausages (appearance, surface colour, cross-section colour, cross-section, odour, taste, consistency, salinity, seasoning, overall acceptability) were assessed using a quantitative-descriptive test (SRPS ISO, 2001b), with a grading scale from one to five (1 = unacceptable, 5 = extremely acceptable) (Table 2). A five-person trained panel was assembled in order to evaluate the sensory properties. Panellists were previously tested for detection and recognition of various tastes (SRPS ISO, 2001a) and odours (SRPS ISO, 2002b). Sensory property results were the median value given by the five panellists.

### Chemical analyses

After sensory evaluation, samples from each sausage were taken for chemical composition analysis. Total fat content (SRPS ISO, 1998b), NaCl (SRPS ISO, 1999) hydroxyproline content (SRPS ISO, 2002), moisture content (SRPS ISO, 1998a) and pH (SRPS ISO, 2004) were determined using standard reference methods. Nitrogen content was determined by an in-house method, the Kjeldahl method, and protein content was determined by multiplying the nitrogen content by 6.25 (Kjeltec Auto 1030

Analyzer, Tecator, Sweden), while the NaCl content was determined by SRPS ISO, 1999.

### Statistical analysis

The statistical analysis was performed using the GraphPad Prism version 7.00 software. The results were expressed as mean value and standard deviation and were subjected to analysis of variance (one-way ANOVA). The parameters were analysed using the Student's t-test at the probability of 0.05. Pearson's correlation analysis was applied to examine the relationship between chemical characteristics and sensory properties of the two groups of kulen fermented sausages.

## Results and Discussion

### Sensory Properties

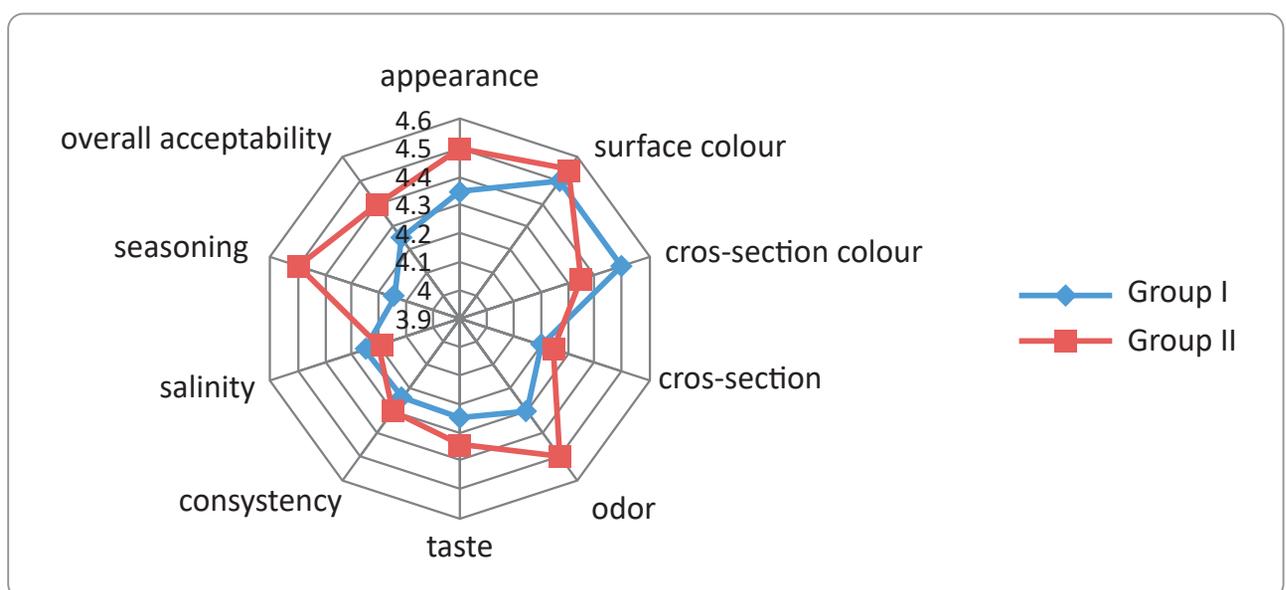
Two variants of kulen with slightly different categories of pork meat and different contents of sweet and cayenne pepper were prepared. The results of sensory analyses by professionally trained assessors are presented in Figure 1.

The obtained results showed the average scores for all tested sensory properties were similar between these two sausage groups. A higher rating was given to the group 2 kulen, which contained a slightly higher amount of category 2 pork meat. Also, this group of sausages contained a slightly higher percentage of red sweet pepper (oleoresin), but less

cayenne pepper (oleoresin). Higher scores reflected the better odour, taste and consistency of group 2 kulen. Differences in amounts of sweet and cayenne pepper between these two groups of sausages affected scores for seasoning and overall acceptability, which were better in group 2 sausages, although the cross-section colour was slightly better in group 1 kulen, which had more cayenne pepper and category 1 pork meat. The odour and taste, as well as other sensory properties of fermented products were influenced by the quality of raw material, ingredients, the metabolic activity of the microbiota present, the physicochemical changes due the drying and ripening processes, and enzymatic degradation of proteins and fats (Virgili *et al.*, 1999; Vukovic *et al.*, 2009). Sausages with a smaller content of fatty tissue are less juicy, have a more solid consistency, and the surface is uneven and wrinkled (Mendoza *et al.*, 2001).

### Chemical characteristics

The chemical composition of the two different groups of kulen sausages are shown in Table 3. The results obtained show that group 1 kulen had a higher protein content (24.52%) than sausages in group 2, which had 23.00% meat protein, while the content of collagen in meat protein in group 2 kulen was 9.16%. This was more collagen in meat protein than the group 1 kulen contained (7.02% of collagen in meat protein). The final protein contents in these sausages were similar to the majority of reported protein



**Figure 1.** Sensory properties of kulen fermented sausage (group 1 and group 2) (Significant differences were found between taste, odour and overall acceptability between the two groups of sausages ( $p < 0.05$ ))

**Table 3.** Chemical composition of kulen fermented sausages (mean±standard deviation)

Traits	Group 1	Group 2
Protein (%)	24.52±1.02 <sup>a</sup>	23.00±1.00 <sup>a</sup>
Collagen (%)	7.02±0.90	9.16±0.90
Ash (%)	3.81±0.05	4.32±0.07
Water (%)	31.47±2.40	23.93±1.90
Fat (%)	36.99±3.10 <sup>a</sup>	39.59±3.90 <sup>a</sup>
NaCl (%)	2.81±0.01 <sup>a</sup>	3.37±0.01 <sup>a</sup>
pH	5.31±0.90	5.33±0.80
a <sub>w</sub>	0.841±0.001	0.856±0.001

**Legend:** The same letters in a row show statistically significant differences between the two groups of sausages,  $p < 0.05$

contents in a range of different fermented sausages (Comi et al, 2005; Saldago et al., 2005). Fermented sausages are meat products with high fat content, approximately about 35%, but this value can rise to about 45–60%. (Gomez and Lorenzo, 2013). Some sensory properties, such as hardness, juiciness and flavour depend on fat content, and the high fat content (40–50%) is essential for these sensory properties (Wirth, 1988). The naturally fermented dry sausages from the Mediterranean region are generally characterized by low acidity with a final pH ranging from 5.2 to 6.4 (Comi et al, 2005; Fista et al., 2004), which matches our results. According to Heinze and Hautzinger (2007) the water activity (a<sub>w</sub>) of fermented dry sausage is in the range from 0.70 to 0.96. In our study, a<sub>w</sub> was 0.841 and 0.856 for group 1 and

group 2 kulen, respectively. Moisture contents in our sausages were less than 35%. The two main factors contributing to the safety and stability of these products are low pH and reduced a<sub>w</sub>. In general, dry sausages have a final pH of 5.0–5.3, the moisture loss is between 25–50% and the final % moisture is around <35% with a<sub>w</sub> ranging from <0.85 to 0.91. Significant differences were found in fat and protein contents between the two groups of kulen sausages ( $p < 0.05$ ). Table 4 and 5 show the correlations between chemical composition and sensory properties of the two groups of kulen sausages. We found very good and positive correlation between protein content and overall acceptability (according to Colton, 1974). Similarly, very good and positive correlation between was found between fat and taste.

**Table 4.** Correlations among some parameters for the group 1 kulen fermented sausages (Pearson correlation coefficients)

Parameter	Overall acceptability	Consistency	Taste	Odour
Protein	0.962	0.241	0.735	0.455
Collagen	0.433	0.251	0.408	0.216
Fat	0.407	0.726	0.805	0.637

**Table 5.** Correlations among some of the parameters for the group 2 kulen fermented sausages (Pearson correlation coefficients)

Parameter	Overall acceptability	Consistency	Taste	Odour
Protein	0.861	0.240	0.635	0.450
Collagen	0.420	0.230	0.390	0.200
Fat	0.400	0.710	0.800	0.620

## Conclusion

The results of this study shows that different quantities and qualities of pork meat and different amounts of sweet and cayenne pepper can affect the sensory properties of kulen fermented sausages. In this case, the odour, taste, cross-section colour and overall

acceptability were especially affected. However, the differences in sausage composition resulted in dissimilar protein, collagen in meat protein and fat contents in the final products. Therefore, the raw materials and the other ingredients used in the technological production of this fermented sausage can affect the quality of the final kulen sausage product.

# Ocena senzorskih i hemijskih parametara fermentisanih kobasica

Ivana Branković Lazić, Jelena Jovanović, Stefan Simunović, Mladen Rašeta, Dejana Trbović, Tatjana Baltić, Jelena Ćirić

*Apstrakt:* Fermentisane kobasice su visokokvalitetni proizvodi industrije mesa i kao takve su veoma cenjene i tražene. Ove vrste kobasica u današnje vreme se najviše vezuje za industrijski način proizvodnje, što znači da kvalitet proizvoda nije standardizovan, ali je prihvatljiv za većinu stanovništva, jer ga karakteriše privlačan spoljašnji izgled i prijatna aroma. Cilj ovog rada jeste da se ukaže na postojanje razlika u senzorskim i hemijskim parametrima, između dve grupe fermentisanih kobasica tipa kulena za čiju proizvodnju su korišćene dve recepture koje su se razlikovale prema upotrebljenoj kategoriji mesa svinja, kao i količini slatke i ljute paprike. Dobijeni rezultati su ukazali na postojanje razlika u senzorskim osobinama proizvoda, mirisu, ukusu i ukupnoj prihvatljivosti proizvoda, dok su hemijskim ispitivanjima utvrđene razlike u sadržaju proteina mesa, koji se kretao od 23.00% (grupa 2) do 24.52% (grupa 1) i sadržaju kolagena u proteinima mesa 7.02% (grupa 1) i 9.16% (grupa 2). Sve ukazuje da izbor sirovine i upotrebljenih sastojaka može imati uticaja na senzorska i hemijska svojstva ovakvog tipa proizvoda.

**Cljučne reči:** fermentisane kobasice, senzorska analiza, hemijska analiza.

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# Quality and labelling of meat preparations on the Serbian market according to the new regulations

Jelena Budincevic<sup>1</sup>, Zoltan Szabo<sup>1</sup>, Nedjeljko Karabasil<sup>2</sup>, Mirjana Dimitrijevic<sup>2</sup>, Dragan Vasilev<sup>2\*</sup>

**Abstract:** The chemical quality parameters of meat preparations sampled from the domestic market were analysed from the aspect of regulations that define the quality of meat products and product labelling. A total of 30 meat preparations were investigated. They were classified in six groups as: minced meat with ingredients; ćevapčići; pljeskavica; barbecue meat; fresh sausages, and; fresh sausages under another name. The highest percentage of non-compliant meat preparations in terms of meat protein content and the content of collagen in meat protein was determined in fresh sausages (33% were non-compliant). The highest percentage of the label non-compliances (60%) concerned a missing nutritive declaration, but another serious problem was inadequate protein specification, as 54% of label errors concerned proteins; these errors included missing information about meat protein content, use of non-meat protein sources in meat preparations where these are banned, and inappropriate protein content designation. Other errors, including improper designation of the product group and additives, as well as missing information about the producer, occurred to a lesser extent.

**Keywords:** meat products, quality, labelling, minced meat with ingredients, fresh sausages, protein content.

## Introduction

Meat preparations are obtained from fresh meat and are intended for consumption after heat treatment, with the exception of steak tartare and carpaccio. During the production of meat preparations, addition of connective tissue, offal, processed meat, nitrites, nitrates, sulphur dioxide and sulphites is banned (*Official Gazette RS*, 2019). Allowed additives in meat preparations are defined by a special regulation concerning food additives (*Official Gazette RS*, 2018), and they can only be used in packed meat preparations in accordance with food labelling regulations (*Official Gazette RS*, 2017 and 2018). Meat preparations should be stored chilled between 0 and 4°C, or frozen at least or below -18°C. Thawed meat preparations are not allowed to be frozen again (*Official Gazette RS*, 2019).

Meat preparations are produced and placed on the market according to the subdivision defined by the regulation (*Serbia*, 2019), as follows: 1) minced meat with ingredients (ćevapčići/ćevapi, pljeskavica, hamburger/burger); 2) barbecue meat; 3) fresh sausages; 4) marinated meat, and; 5) aged meat and steaks. *Minced meat with ingredients* is non-cured ground fresh meat and fatty tissue, containing only

table salt and spices as ingredients, but when marketed as packaged food, it can contain additives in accordance with the special regulation on food additives. *Barbecue meat* is produced from ground fresh meat, fatty tissue and a wide range of ingredients which, besides table salt and spices, also includes sugars, water, fibre, starch, starch products, protein products, milk, milk products, eggs, egg products, oils, fats, other food of plant or animal origin, strong alcoholic drinks and natural aromas. *Fresh sausages* are meat preparations stuffed in natural (sheep small intestine) or artificial edible casings. Fresh sausages can be produced and marketed under the prescribed name “fresh sausage”, which besides meat and fatty tissue, can contain table salt, spices, spice extracts, sugars, additives and water as ingredients, as well as “fresh sausage under another name”, which is given by the producer; this latter product can contain all the same ingredients as fresh sausage, and all other ingredients as specified for barbecue meat. *Marinated meat* is a meat preparation obtained by marinating meat, bone-in or boneless, with its fatty and connective tissue. *Aged meat and steaks* are obtained by exposing meat to a ripening process, which includes the use of meat enzymes or added enzymes.

<sup>1</sup>Veterinary Specialist Institute, Segedinski put 88, 24000 Subotica, Serbia;

<sup>2</sup>University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia.

\*Corresponding author: Vasilev Dragan, [vasilevd@vet.bg.ac.rs](mailto:vasilevd@vet.bg.ac.rs)

Quality parameters for meat preparations are defined by regulation (*Official Gazette RS*, 2019), which determines raw materials, sensory properties and chemical parameters such as minimal content of meat protein (for minced meat with ingredients and fresh sausages) or total protein (barbecue meat). The meat or total protein content should be at least 14%, the relative collagen content of meat protein or total protein should be at most 15%, while in poultry meat preparations, relative collagen cannot exceed 10%. Labelling of meat preparations is defined by local regulations (*Official Gazette RS*, 2017 and 2018), which are harmonized with European Union regulations (*European Union*, 2011). Some special labelling requirements concerning meat products are defined by the Regulation on the Quality of Minced Meat, Meat Preparations and Meat Products (*Official Gazette RS*, 2019).

The aim of this study was to investigate the chemical quality parameters and the label content accuracy of meat preparations, within the scope of this new local legislation.

## Materials and Methods

The investigation was conducted on 30 meat products collected from the retail market: Minced meat with ingredients (n=3); Čevapčići (n=3); Pljeskavica (n=8); Barbecue meat (n=7); Fresh sausage (n=3), and; Fresh sausage under another name (n=6). Each sample (200 g) was transported at 2±1°C to the laboratory and analysed during the 24 h after arrival.

Chemical quality parameters were investigated by standard methods as follows: protein content was analysed according to the reference method SRPS ISO 937:1992; collagen content was analysed according to the reference method SRPS ISO 3496:2002). The relative content of collagen in meat protein was calculated by the following formula:

$$\text{Collagen content in protein} = \frac{\text{Collagen content (\%)} \times 100}{\text{Protein content (\%)}}$$

The labels were investigated according the requirements of the Regulations on Food Declaration, Labeling and Advertising (*Serbia*, 2017 and 2018), so label information was collected on the product name, ingredient list, allergen info, additives, nett weight, expiration date, storage conditions, guide for use, data about the food business operator registered in the Republic of Serbia, country of origin, nutritive declaration, and series or lot number. According to

the Regulation on the Quality of Minced Meat, Meat Preparations and Meat Products (*Official Gazette*, 2019), the name of the product group or subgroup, the meat protein content or total protein content and the collagen content in meat protein or in total protein must also be provided on the label. The proper use of additives was checked in accordance with the requirements of the Regulation for Food Additives (*Official Gazette RS*, 2018).

## Results and Discussion

The results (Table 1) showed the highest average content of meat protein was found in čevapčići (16.29%) and the lowest in pljeskavica (14.16%). However, concerning the individual meat preparations, the highest meat protein content was found in a sample of pljeskavica (18.87%) and the lowest in a fresh sausage (10.94%); this contained significantly below the minimum allowed meat protein content (14%). Considering the total protein content, which includes the meat proteins and proteins from non-meat sources together (*Official Gazette RS*, 2019), the average total protein was similar in fresh sausage under another name (16.01%) and barbecue meat (15.85%). Concerning the individual meat preparations, the highest total protein content (19.25%) was found in a preparation of fresh sausage under another name.

The meat preparation group with the highest percentage of non-compliant samples, which did not meet the regulatory requirements of 14% meat protein or the total protein content (*Official Gazette RS*, 2019), was fresh sausage (33% were non-compliant). This is a named meat preparation prescribed by regulations and is expected to be a high quality product. Barbecue meat and fresh sausage under another name are generally considered as lower quality meat preparations, since non-meat protein and many other non-meat ingredients are allowed. In spite of the producers' ability to add protein, the total protein content was non-compliant for relatively high percentages of barbecue meat and fresh sausage under another name (14.3 and 16.6%, respectively).

Meat proteins are highly digestible (about 95%), which is significantly higher than the proteins in beans (78%) and wheat (86%) (*Bhutta*, 1999). From the nutritive and quality points of view, meat proteins are divided into muscle tissue protein and connective tissue protein (collagen). As collagen contains almost half the essential amino acids that muscle tissue protein contains, so it has half the nutritive value (*Teodorovic et al.*, 2015). Therefore, the relative collagen content

**Table 1.** Protein content in the grouped meat preparations

Meat preparation	$\bar{X} \pm SD$	Minimum	Maximum	CV (%)	Non-compliant* (%)
Minced meat with ingredients, MP	16.06 ± 0.66	15.59	16.83	4.1	–
Cevapcici, MP	16.29±0.49	15.76	16.74	3.0	–
Pljeskavica, MP	14.16±2.64	14.10	18.87	18.6	–
Barbecue meat, TP	15.85±2.44	12.18	17.61	15.3	14.3
Fresh sausage, MP	14.28±3.15	10.94	17.81	22.11	33.3
Fresh sausage under another name, TP	16.01±2.08	12.75	19.25	13.04	16.6

**Legend:**  $\bar{X} \pm SD$  — mean±standard deviation; CV — coefficient of variation; \* Does not meet the regulatory requirements (Official Gazette RS, 2019); MP — meat protein; TP — total protein

in protein (meat protein or total protein) content has been introduced as another important quality parameter, defined by regulation, and which must not be higher than 15%. The collagen content in the meat preparations are shown in Table 2.

The results showed that the highest average content of collagen in meat protein (C/MP) was found in fresh sausage (13.87%) and the lowest in ćevapčići and pljeskavica (9.88 and 9.98%, respectively). However, concerning the individual samples, the highest content of collagen in meat protein was found in a fresh sausage (16.33%) and the lowest in a sample of pljeskavica (6.71%), while 33% of fresh sausages did not meet the regulatory requirement that C/MP must not be higher than 15%. Considering the collagen content in total protein

content, (C/TP) the highest C/TP was determined in a barbecue meat (19.16). Altogether, 14.2% of all samples of this product did not meet this regulatory requirement, while all fresh sausages under another name complied with the regulation.

Meat preparations containing higher amounts of collagen have a lower nutritive value and worse sensory properties (Vukovic, 2012). This is usually a consequence of the presence of connective tissue contained in muscle fascicles (*endomysium*, *perimysium*, and *epimysium*) and tendons (Moon, 2006), which should be thoroughly trimmed while preparing meat for the production of meat preparations. Connective tissue has a strong negative influence on sensory properties, making product tougher (Cross *et al.*, 1973).

**Table 2.** Collagen content in protein in the grouped meat preparations

Meat preparation	$\bar{X} \pm SD$	Minimum	Maximum	CV (%)	Non-compliant* (%)
Minced meat with ingredients (C/MP)	10.47±3.87	6.75	14.48	36.98	–
Cevapcici (C/MP)	9.88±0.92	8.96	10.80	9.31	–
Pljeskavica (C/MP)	9.98±2.58	6.71	14.68	25.91	–
Barbecue meat (C/TP)	12.24±3.38	9.00	19.16	27.64	14.2
Fresh sausage (C/MP)	13.87±2.51	11.31	16.33	18.11	33.3
Fresh sausage under another name (C/TP)	10.18±2.67	7.10	14.02	26.25	–

**Legend:**  $\bar{X} \pm SD$  — mean±standard deviation; CV — coefficient of variation; \* Does not meet the regulation requirements (Official Gazette RS, 2019); C/MP — collagen content in meat protein; C/TP — collagen content in total protein

**Table 3.** Number and percentage of non-compliant labels of the grouped meat preparations

Meat preparation	Number of samples	Non-compliant (n)	Non-compliant* (%)
Minced meat with ingredients	3	3	100
Cevapcici	3	2	66.7
Pljeskavica	8	6	75
Barbecue meat	7	5	71.4
Fresh sausage	3	3	100
Fresh sausage under another name	6	5	83.3

\* Does not meet the regulation requirements (Serbia, 2017 and 2018)

The results of the label content investigation showed there were issues concerning labelling that did not meet the regulatory requirements. The number of investigated and non-compliant meat preparation labels is shown in Table 3.

The results show that every group of investigated meat preparations had some error in the labels, and the percentage of non-compliant labels was very high over all groups (from 66.7 to 100%). For minced meat with ingredients and fresh sausage, the labels of every product studied contained error(s). According to the literature, some investigations in the European Union show deviations in food labels of up to 50% from the legal requirements, mostly including smaller mismatches, but sometimes bigger or even fraudulent cases are dealt with (Arayess & Hendrickx, 2016).

The main reasons for the label non-compliances are presented in Table 4, and we note 30 of the investigated meat preparations had more than one labelling error.

The highest percentage of the label non-compliances (60%) concerned a missing nutritive declaration, but inadequate specification of proteins was also an extremely serious problem, since 54% of label errors referred to proteins, such as missing information about meat protein content (20%), use of non-meat protein sources in preparations where this is not allowed (17%) and inappropriate protein content designation (17%). Other errors occurred to a lesser extent, and included improper designation of the product group and additives, as well as missing information about the producer.

Proper labelling is of great importance, giving information to consumers about the composition and main properties of food, and must not be misleading or confusing. As food labelling is a complex requirement, the general demands are regulated by law (Henderikx, 2017). Serbian regulations concerning labelling (Official Gazette RS, 2017 and 2018) are harmonized with the European Union

**Table 4.** Main reasons for label non-compliance in meat preparations (n=30)

Label error	N*	(%)
Missing nutritive declaration	18	60
Missing information about meat protein content	6	20
Use of non-meat protein sources where it is not allowed	5	17
Inappropriate protein content designation	5	17
Inappropriate product group	5	17
Inappropriate name or designation of additives	2	7
Use of additives which are not allowed	2	7
Missing data about food business operator	2	7

\*N — number of meat preparation labels that contained the error

regulations (European Union, 2011), and an obligatory nutritive declaration as a part of the label was recently introduced. This could be considered as the main reason that missing nutritive declarations were the most frequent label error. Among meat preparations, ćevapčići and pljeskavica, because of their sensory properties, are especially appreciated by consumers. This opens some possibilities for fraud by unallowed use of non-meat protein sources in these products. In order to overcome these issues, such problems should be appropriately addressed by the food business operators and the competent authorities. However, consumers also need to be informed, so they can understand the information stated on the labels (Henderikx, 2017).

## Conclusion

The highest meat protein content was found in a sample of pljeskavica (18.87%) and the lowest in a fresh sausage (10.94%). The group of meat products with highest percentage of non-compliant samples was the group of fresh sausages (33%). For barbecue meat and fresh sausage under another name, the total protein content was non-compliant for a relatively

high percentage of each group (14.3 and 16.6%, respectively). The highest content of collagen in meat protein was determined in a fresh sausage (16.33%) and the lowest in a sample of pljeskavica (6.71%), while 33% of fresh sausages did not meet the regulatory requirements for collagen. The highest collagen content in total protein content was determined in a sample of barbecue meat (19.16% of collagen in protein), while 14.2% of all samples of this product did not meet the regulatory requirement. Every group of investigated meat preparations had some labelling error(s), and the percentage of non-compliant labels was very high among the different groups of meat preparations (from 66.7 to 100%). The highest percentage of the label non-compliances (60%) concerned a missing nutritive declaration, but inadequate specification of proteins is also a serious problem, since 54% of label errors concerned protein declarations. These errors included missing information about meat protein content, use of non-meat protein sources in preparations where this is not allowed and inappropriate protein content designation. Other errors occurred to a lesser extent and included improper designation of the product group and additives, as well as missing information about the producer.

# Kvalitet i deklarisanje poluproizvoda od mesa na tržištu Srbije u skladu sa novim propisima

Jelena Budinčević, Zoltan Sabo, Nedjeljko Karabasil, Mirjana Dimitrijević, Dragan Vasilev

*Apstrakt:* U radu su prikazani rezultati ispitivanja hemijskih parametara kvaliteta poluproizvoda od mesa uzorkovanih na domaćem tržištu, koji su analizirani sa aspekta propisa koji definišu kvalitet proizvoda od mesa i deklarisanje hrane. Ukupno je ispitano 30 uzoraka poluproizvoda od mesa uključujući Usitnjeno meso sa dodacima, Ćevapčiče, Pljeskavice, Roštilj meso, Svežu kobasicu i sveže kobasice pod drugim nazivom. Najveći procenat uzoraka koji nisu ispunjavali odredbe u pogledu sadržaja proteina mesa i udela kolagena u proteinima mesa utvrđen je kod Sveže kobasice (33%). Najveći procenat neusaglašenosti deklaracija (60%) bio je nedostatak nutritivne deklaracije, ali veliki problem predstavlja i neadekvatno navođenje proteina (54%) u smislu ne navođenja sadržaja proteina, upotrebe proteina koji ne potiču od mesa tamo gde to nije dozvoljeno i neadekvatno navođenje sadržaja proteina. Ostali nedostaci se odnose na neadekvatno navođenje pripadnosti grupi i aditiva, kao i nedostatak podataka o proizvođaču.

**Cljučne reči:** poluproizvodi od mesa, kvalitet, deklarisanje.

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# Optimization of liver pate sterilization from the aspect of preserving nutritional value and ensuring food safety

Mladen Raseta<sup>1\*</sup>, Ivana Brankovic-Lazic<sup>1</sup>, Boris Mrdovic<sup>1</sup>, Branislav Baltic<sup>1</sup>, Becskei Zsolt<sup>2</sup>, Vesna Djordjevic<sup>1</sup>

**Abstract:** Sterilization is a physical method of food preservation that, through high temperature over 100°C which destroys microorganisms and inactivates tissue enzymes. Safety and shelf-life of the canned liver pate (75 and 150 grams) is ensured with determined  $F_0$  values  $\geq 3$ . Commercial sterility was achieved, but treatment intensity was too high ( $F_0=12,07-15,93$  and  $F_0=9,71-13,17$ ) thus reduced the nutritional value of the food ( $C_0=111-160$  and  $C_0=123-180$ ). Based on the measurements, under defined heat treatment conditions (20' heating time and 121.1° C autoclave medium under 2.5 bar pressure followed by 20–25' cooling time) it is suggested that effective sterilization should be reduced by 5 and 10 minutes, respectively. This way the safety of the product would not be compromised, negative impact of heat on the nutritional value would be reduced, whereby production efficiency would be increased and energy consumption would be reduced.

**Keywords:** Liver pate, Sterilization,  $F_0$  value,  $C_0$  value, food safety, thermal treatment.

## Introduction

Sterilization is the process of heat treatment of a can at a temperature higher than 100° C, whereby a lethal value of at least  $F_0 = 3$  must be achieved in the thermal center of the product (Anonymous, 2019). The shelf-life of the can depends primarily on the degree of bacterial destruction (biological sterility), which is difficult to achieve due to the overly harsh heat treatment regime on the product's sensory properties. The sterilization mode is therefore adjusted to achieve commercial sterility (FSIS, 2005). Commercial sterility is achieved by applying heat of sufficient intensity to independently or in combination with other elements of importance (degree of initial contamination of the stuffing, stuffing weight, stuffing temperature, packaging) to make the product safe by eliminating the existing microbiological risk, while preventing the microbial growth by preventing them from following the label regarding the intended storage conditions (sterilized can is stored at temperatures up to + 25°C (FAO).

The characteristic of commercially sterilized food is adequate heat treatment which eliminates all pathogenic microorganisms and reduce presence of defect-causing micro-organisms, to a level

where they do not pose a threat to consumer health or affect the quality and degree of acceptability of the final product. Can is a commercially sterilized food contained in hermetically sealed container. "Hermetically sealed container" means a container designed to prevent the entry of hazardous substances (Anonymous, 2004).

Prior to filling at the production line, the prepared cans must be clean, free from irregularities and damage which can impair the integrity of the can. Also during the closing phase, attention should be paid to the proper execution of the procedure itself — visual and manual damage control (Codex alimentarius, 2011). The filled cans shall be sealed in such a way as to ensure good sealing and prevent recontamination of the contents after heat treatment, as well as during storage, distribution and sale. The higher the temperature of sterilization the greater is the rate of thermal destruction. The rate of thermal destruction is also affected by the nature of the product (liquids heat faster than solids) and the container size (FAO).

Liver and fat as a basic raw material for liver pate must have optimal hygienic and technological quality that can be ensured by measures such as the selection of an animal, adequate preparation for slaughter and the performance of slaughter operations, and product processing, until it is closed

<sup>1</sup>Institute for Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia;

<sup>2</sup>University of Belgrade, Faculty of Veterinary medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Republic of Serbia.

\*Corresponding author: Raseta Mladen, [mladen.raseta@inmes.rs](mailto:mladen.raseta@inmes.rs)

into the can. Used components are regularly stuffed in metal containers. For this purpose, cans of white tin and aluminum are used and containers of refined aluminum thin strip. The can is made up of a body, a bottom and a cover of different shape and size.

For the purpose of reliable control of the efficiency of the heat treatment process, a unit of lethality of the heat treatment process has been introduced, which designates it as F value. When heated in a humid environment, microorganisms die out in logarithmic cycles, where the total lethality or lethal capacity of the heat treatment process is expressed by F value. It has been defined that the sterilization process should be such as to provide 12 decimal reductions in the number of *Clostridium botulinum* spores at sterilization temperature — 121.1°C (Vukovic, 2012). Because greatest danger for meat cans stored at room temperature represent spores of mesophilic types A and B *Clostridium botulinum*, the F value for the sterilization procedure, according to the Regulations, is calculated according to this microorganism (Anon, 2019; FAO, Codex alimentarius 2011; FSIS, 2005).

As a consequence of heat treatment, meat products lose more or less nutritional value, which depends on the temperature and the length of its action. The higher the temperature and the time of action, the greater the loss of nutritional value (Amit et al., 2017). In addition to ensuring the safety and shelf-life of food, the equally important task of heat treatment is to preserve the biological value of meat and meat products (Vukovic, 2012). During heat treatment of the liver, irreversible changes in the protein ferritin occurs at temperatures above 80°C (Prochaska et al. 2000).

C<sub>0</sub> value (Cooking value) expresses the reduction in the nutritional value of heat treated products.

For C<sub>0</sub> value there are no standards and recommendations as well as for the F-value, and as the optimal heat treatment process is one that for a given value of F<sub>0</sub>, has a smaller value of C<sub>0</sub> (Vukovic, 2012).

Heat treatment provides food with desirable sensory properties and it becomes more digestible, but too intense heat treatment leads to a loss of nutritional value. For this reason, aim of this paper is to determine optimum between the conservation effect of heat treatment and the preservation of the nutritional value of canned liver pate.

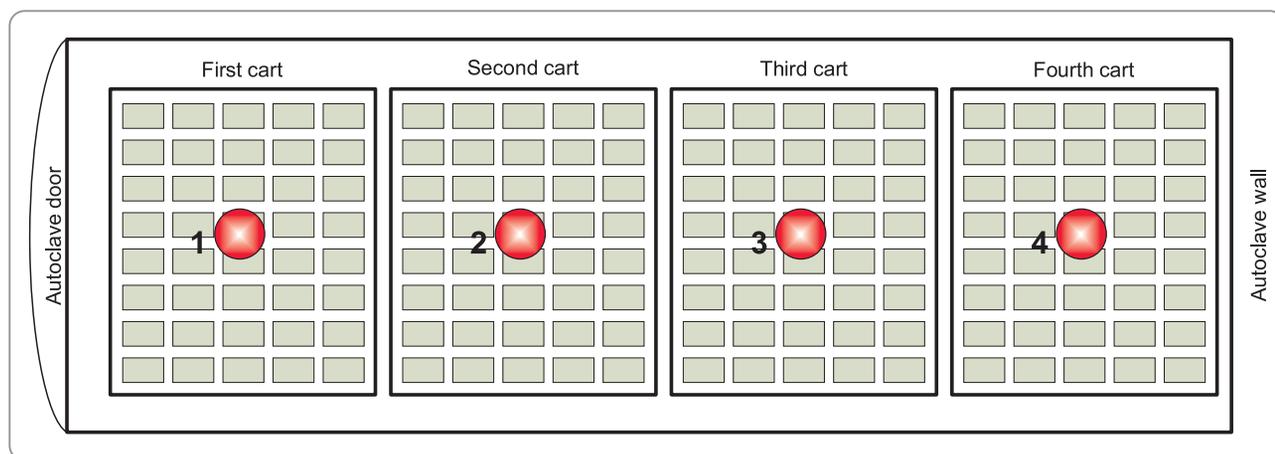
### Materials and methods

During period February-April 2018, in domestic meat industry, during regular production, systematic monitoring of sterilization process of liver pate (a product belonging to the group of “cooked sausages”, which is sterilized in can, in accordance with legal provisions — Anon, 2019) was implemented. Sterilization monitoring was performed on same autoclave (horizontal overpressure autoclave that can accommodate four carts) on two types of same product with different weights and packaging methods:

1. Liver pate 75 g in two-piece soft aluminum sheet
2. Liver pate 150 g in a two-part hard-drawn tray

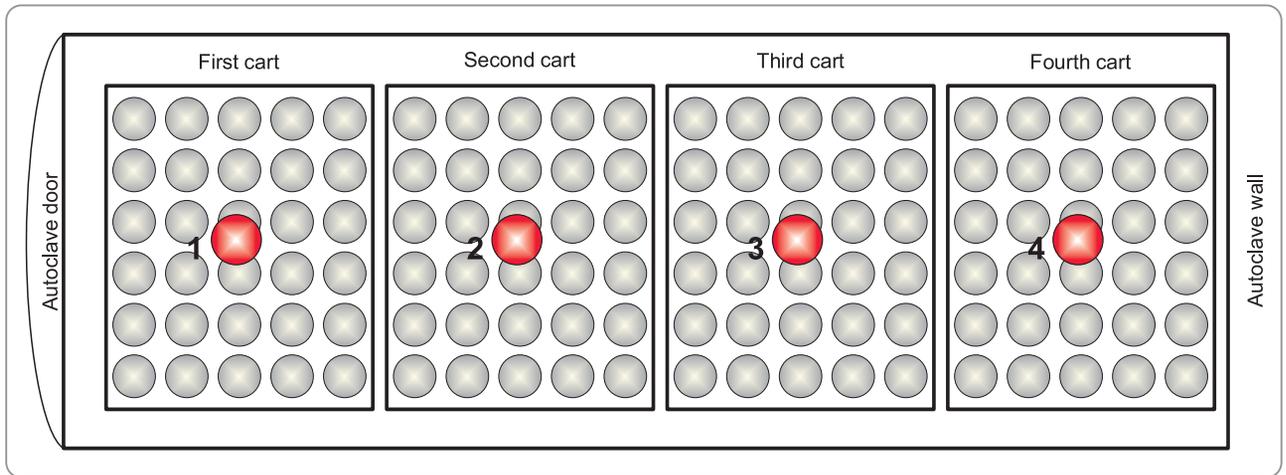
For each product, an existing heat treatment plan is verified and ways of optimizing the production process are discussed.

Measurements were made by a thermocouple type ‘Ellab’ model E Val-Pro. Thermocouples with compensating cables were used, 4 probes which were



**Scheme 1.** Positions of the placed probes in the autoclave viewed from the side:

**Legend:** — Probe placed in the geothermal center of the product



**Scheme 2.** Positions of the placed probes in the autoclave viewed from above:

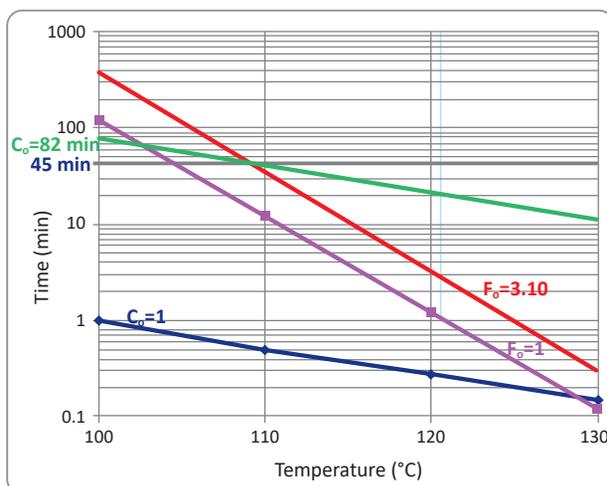
**Legend:**  — Probe placed in the geothermal center of the product

placed in the geothermal center of canned liver pate. Position on used probes were represented graphically (Schemes 1 and 2) and was identical at each check.

The obtained data were processed at the Meat Hygiene and Technology Institute, Belgrade, using commercial computer programs.

$F_0$  values were automatically read in real time on a thermocouple E Val-Pro (manufacturer Ellab Danmark, 2018. year), compared to temperature readings in the geothermal center of the product and recorded every 2–3 minutes.

$C_0$  values were determined graphically by means of the semi-logarithmic TDT diagram (Chart 1), in which the previously constructed of two straight lines:  $C_0 = 1$  (1 minute at 100°C, with  $a = 33^\circ\text{C}$ ) and  $F_0 = 1$  (1 minute at 121, 1°C, with  $a = 10^\circ\text{C}$ ). In order to



**Chart 1.** Semi-logarithmic Thermal Death Time (TDT) diagram (Rašeta et al., 2018)

determine the  $C_0$  value, the  $F_0$  value of the sterilization process should be known and how long in minutes lasted the temperature equal to or higher than 100°C in the thermal center of the product.  $C_0$  value was obtained by connecting a point which is parallel with a line  $C_0 = 1$ , and which passes through a point of intersection lines which are plotted based on the data of  $F_0$  value (which is parallel with the line of  $F_0 = 1$ ) and the straight line is drawn on the basis of the time of operation temperature greater than 100°C for a given mode of heat treatment (Vukovic, 2012; Raseta et al., 2018).

## Results

### *Sterilization of canned liver pate 75 g in two-piece soft aluminum sheet*

The results of sterilization validation are presented on the charts 1–2. Validation were performed during the regular production of sterilized liver pate. The established results (temperature in the geothermal center and  $F_0$  value) are presented in charts (2–3). A concise measurement comment is provided for each measurement.

Temperature change in geothermal center of canned liver pate 75 grams package in two-piece soft aluminum sheet, during sterilization is presented on chart 1. Determined  $F_0$  values in the geothermal center of canned liver pate 75 g package is presented on chart 2.

- Chart 2. Temperature change in geothermal center of canned liver pate 75 g
- Chart 3. Determined  $F_0$  values in the geothermal center of canned liver pate 75 g

Sterilization of canned liver pate 75 grams packaged in two-piece soft aluminum sheet, with a glued lid, lasted 1 hour and 19 minutes. The heat treatment formula is:

$$T_o = 20' + \frac{35'}{121.1^{\circ}\text{C}/2.5 \text{ bar}} + 24'$$

Sterilization temperature of 121.1°C and a pressure of 2.5 bars has been reached for 20 minutes in the autoclave medium. The effective sterilization time was 35 minutes and cooling time was 24 minutes. In the geothermal center of the product, in all four inspection sites, where probes were placed, F<sub>o</sub> values were over 3, which is the sterilization minimum, and ranged from 12.07 to 15.93.

The obtained F<sub>o</sub> values during sterilization of liver Pate 75 g, in two-piece soft aluminum sheet, ensured safety of the final product.

*Sterilization of canned liver pate 150 g in a two-part hard-drawn tray*

The results of sterilization validation are presented on the charts 3–4. Validation were performed during the regular production of sterilized liver pate. The established results (temperature in the geothermal center and F<sub>o</sub> value) are presented in charts (4–5). A concise measurement comment is provided for each measurement.

Temperature change in geothermal center of canned liver pate 150 grams package in two-part hard-drawn tray, during sterilization is presented on chart 3. Determined F<sub>o</sub> values in the geothermal center of canned liver pate 150 g package is presented on chart 4.

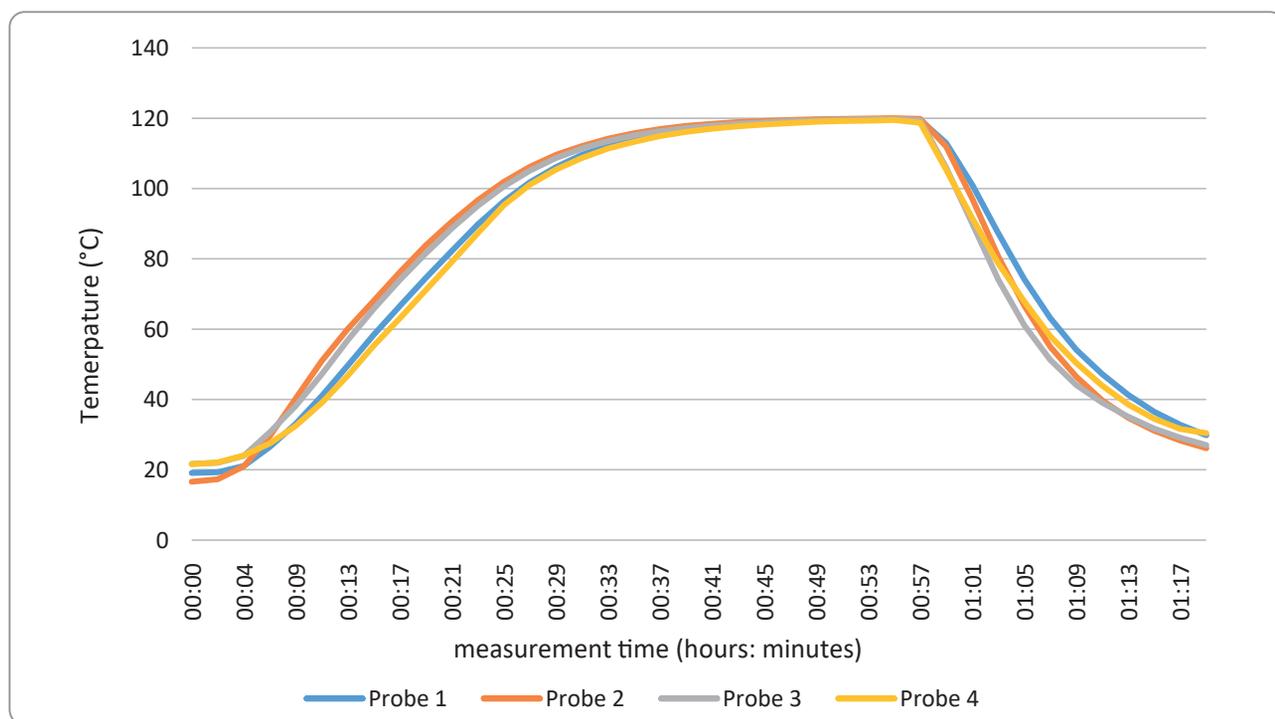
- Chart 4. Temperature change in geothermal center of canned liver pate 150 g
- Chart 5. Determined F<sub>o</sub> values in the geothermal center of canned liver pate 150 g

Sterilization of canned Liver pâté 150 grams packaged in two-part hard-drawn tray, lasted 1 hour and 36 minutes. The heat treatment formula is:

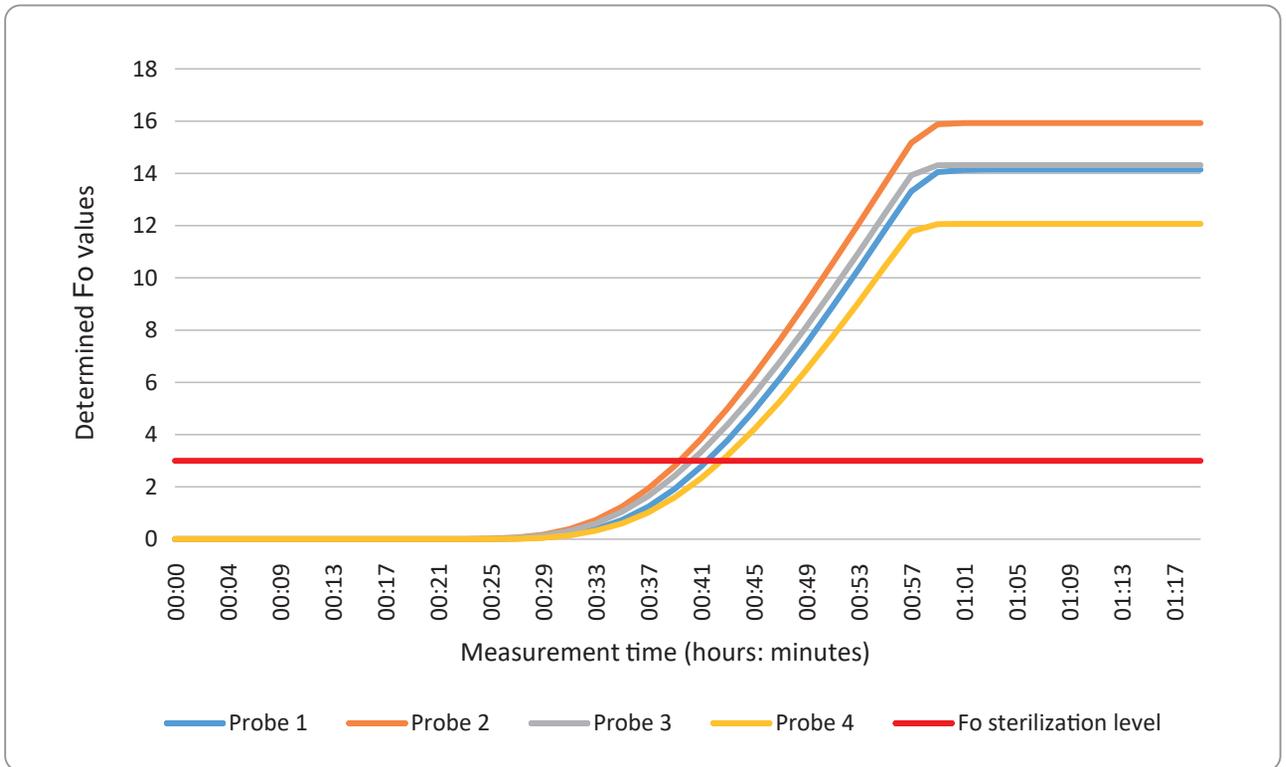
$$T_o = 18' + \frac{50'}{121.1^{\circ}\text{C}/2.5 \text{ bar}} + 28'$$

Sterilization temperature of 121.1°C and a pressure of 2.5 bars has been reached for 18 minutes in the autoclave medium. The effective sterilization time was 50 minutes and cooling time was 28 minutes. In the geothermal center of the product, in all four inspection sites, where probes were placed, F<sub>o</sub> values were over 3, which is the sterilization minimum, and ranged from 9.71 to 13.17.

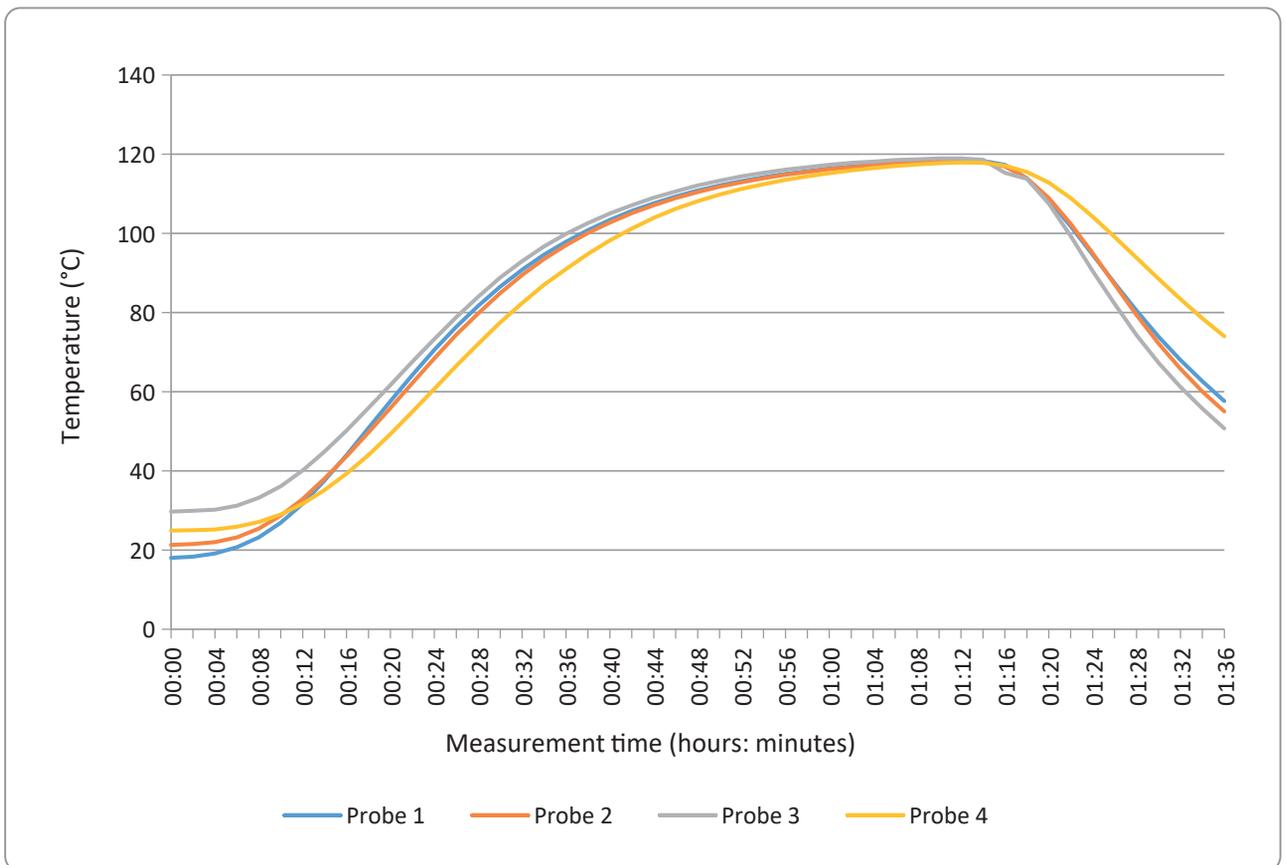
The obtained F<sub>o</sub> values during sterilization of liver Pate 150 g, in two-part hard-drawn tray, ensured safety of the final product.



**Chart 2.** Temperature change in geothermal center of canned liver pate 75 g



**Chart 3.** Determined Fo values in the geothermal center of canned liver pate 75 g



**Chart 4.** Temperature change in geothermal center of canned liver pate 150 g

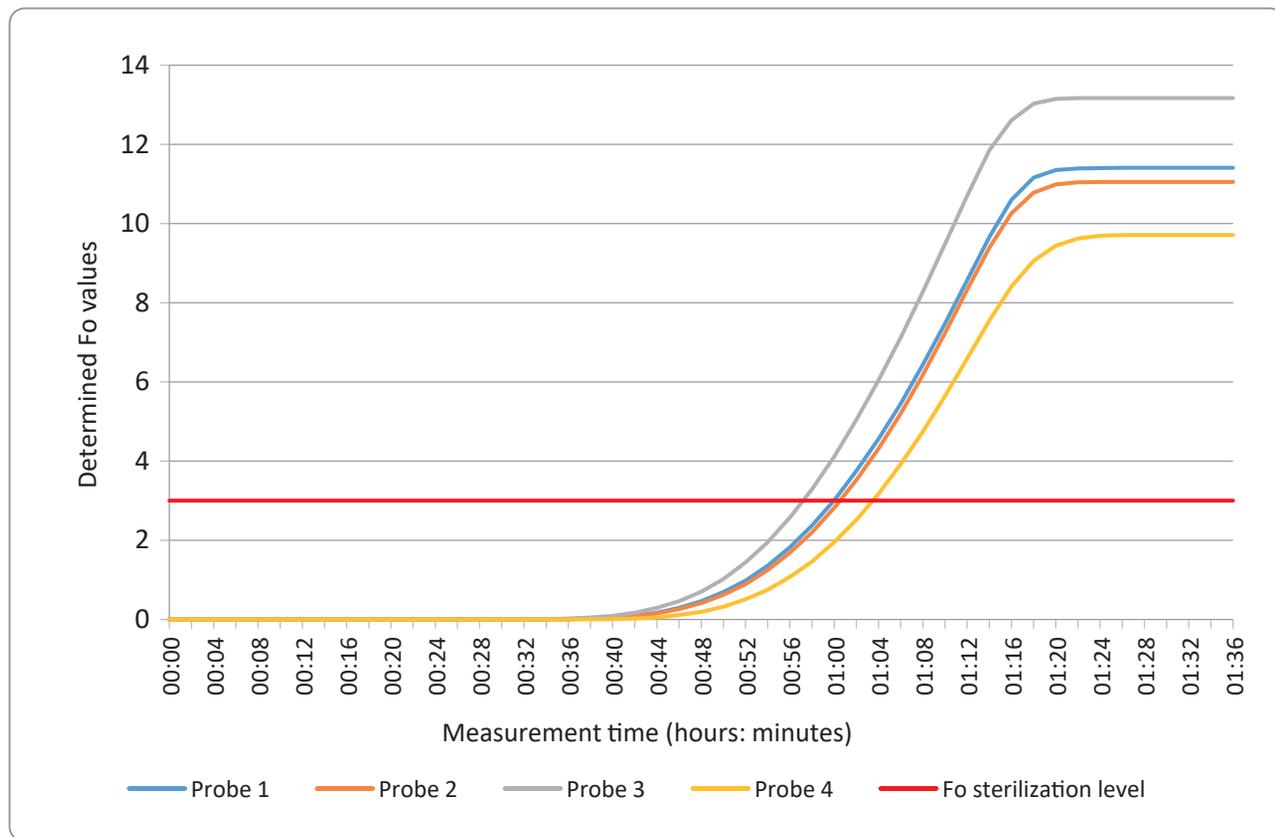


Chart 5. Determined Fo values in the geothermal center of canned liver pate 150 g

Summary table of heat treatment programs with determined  $F_o$  and  $C_o$  values

In order to adequately assess the sterilization process, in Table 1 heat treatments regimes of canned liver pate, with minimum and maximum determined  $F_o$  values and calculated  $C_o$  values are listed. In this way the process of commercial sterilization of liver pate was outlined. Commercial sterility  $F_o$  value of  $\geq 4$  for sterilized meat products in cans essential from the aspect of quality. Therefore, there is always a current tendency that produces thermal processing as low as possible.

Summary table of current heat treatment programs with optimization proposal

Although the variation in sterilization process dynamics exist, there is the possibility of shortening the effective sterilization time. Only after the autoclave work standardization and elimination of the current present variations it is indicated by access validation of the proposed optimized regime. Then it will be possible to perceive the maximum effect of the preservation of the biological value of the cans.

Table 1. Display of sterilization regimes, and determined  $F_o$  and  $C_o$  values during heat treatment of canned liver pate 75 and 150 grams

Product and package	Heat treatment plan	Fo values		Co values (min)	
		Min	Max	Min	Max
Liver pate 75 g in two-piece soft aluminum sheet	$T_o = 20' + \frac{35'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 24'$	12,07	15,93	111	160
Liver pate 150 g in a two-part hard-drawn tray	$T_o = 18' + \frac{50'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 28'$	9,71	13,17	123	180

**Table 2.** Proposed heat treatment plan corrections for tested products

Product and package	Current heat treatment mode	Proposed heat treatment regime
Liver pate 75 g in two-piece soft aluminum sheet	$T_0 = 20' + \frac{35'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 24'$	$T_0 = 20' + \frac{30'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 20'$
Liver pate 150 g in a two-part hard-drawn tray	$T_0 = 18' + \frac{50'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 28'$	$T_0 = 20' + \frac{40'}{121.1^\circ\text{C}/2.5 \text{ bar}} + 25'$

## Discussion

In the system of integrated control of production and processing of meat according to the rules of HACCP concept (Hazard Analysis Critical Control Points) a special place belongs to the control of heat treatment. In all HACCP plans, heat treatment is designated as a Critical Control Point (CCP) and is a place where the existing hazard is eliminated or minimized (Surak and Wilson, 2014). Safety level for canned food, for continental climate conditions, are defined in domestic legislative (Anonymous, 2019) as statutory minimum of  $F_0 \geq 3$ , and then it is considered that spores of thermoresistant type A and B *Clostridium botulinum* which may be present, are with certainty destroyed (Vukovic, 2012). According to FAO recommendations (FAO, 2019) based on microbiological risk assessment, sterilization of canned meat products should achieve  $F_0$  values of 4–5.5, while the temperature should be in range 117–130°C, depending on characteristics of the products.

The sterilization temperature used is exactly proportional to the antimicrobial effect (Vukovic, 2012). For example, spores of mesophilic types *Clostridium botulinum* are destroyed at 100°C after 330 minutes and at 120°C in four minutes (FAO, 2019).

Optimal thermal regimes should satisfy the conditions of lethality necessary to destroy harmful microorganisms and to minimize the biological value of the product (Codex alimentarius, 2011). High temperature necessary for the destruction / inactivation of thermoresistant spores, repercussions negatively on sensory properties and promotes the occurrence of abiotic changes in the can during storage. Thermal processing results in thermal denaturation of the protein, resulting in a decrease in volume, release of water, the formation of coarse and tough consistency, as well as a loss of the meat's ability to bind water.

In domestic meat industry, it is often the case that microbiologically flawless, practically sterile meat cans filling have a softer consistency, more or less pronounced brown color and a burnt aroma. This is practiced by manufacturers who, in excessive heat treatment rather than in hygiene mode and good manufacturing practice, find a solution for the good sustainability of their products. Products treated in this way have less biological value, and due to higher energy consumption, the profitability of production is always lower.

During sterilization process monitoring in autoclave, uneven dynamics is noticeable on different autoclave medium spots and considerable variation  $F_0$  value was achieved during measurement (12,07–15,93 for liver pate 75 g packed in two-piece soft aluminium sheet and 9,71–13,17 for 150 g package of the same product in two-part hard-drawn tray). To fully comprehend the qualitative changes in cans differently positioned in the medium of the autoclave and to evaluate nutritive value of the product,  $C_0$  values were determined using TDT diagram (Chart 1). Obtained results showed variations presented in Table (111–160 minutes for liver pate 75 grams and 123–180 minutes for liver pate 150 grams). Longer effective sterilization time of liver pate of 150 grams (50 minutes), compared with sterilization of liver pate of 75 grams (35 minutes), resulted in higher  $C_0$  values, although  $F_0$  values are lower.

The established  $F_0$  values in sterilization of liver pate of 150 g (9,71–13,17) are higher than in other studies (7,24–8,58) that had a longer effective sterilization time (55 minutes), lower temperature of autoclave medium (114°C), and higher pressure (3.2 bar) (Raseta et al., 2016). A high-quality and well-maintained autoclave and HACCP based everyday work practice, can provide us with the opportunity for further optimization and reduction of  $F_0$  value in sterilization of liver pate to average values of  $3.81 \pm 0,5$  gained on effective sterilization time of 45 minutes. Reducing the effective sterilization time

by ten minutes, resulted in a 19.6% increase in the cooking value of the product. The average  $C_0$  value for regular sterilization regime was  $109,83 \pm 1,33$ , while for the optimized regime, it was  $88,67 \pm 4,27$  minutes (Raseta et al., 2018). It is necessary to stabilize dynamics of the autoclave work, in order to reduce the existing sterilization dynamics variation.

The table 2 shows a proposal of a sterilization process optimization, which involves settling of autoclave, identical at each sterilization conditions (temperature of  $121.1^\circ\text{C}$  and pressure of 2.5 bar), whereby the heat-up time should be the same, or with minimal fluctuation, in each round.

Food safety can be improved with preventive approach, and appropriate management during processing of liver pate (raw material selection, adequate temperature regime and storage conditions, within the framework of the food security system that has been a legal obligation in our country since 2009. year (Anonymous, 2009). Determining the degree of initial contamination of the liver pate stuffing before heat treatment, can provide us with information on the hygiene of the raw material and the production process itself (Raseta et al., 2016). Since the main responsibility for food safety has food business operator, it's obligation is to define and implement appropriate measures of good hygiene practices (GHP) and Good Manufacturing Practice (GMP) and methods based on the principles of Hazard Analysis and Critical Control Points (HACCP), in order to achieve the objectives of food safety (Food Safety Objectives) defined in food regulations (Anonymous 2002, 2004, 2004a, 2004b, 2004c). Based on the risk analysis, the existing risk should be recognized and eliminated or reduced to an acceptable level.

Optimal thermal regimes should satisfy the conditions of lethality necessary for the destruction of harmful micro-organisms and to minimize the biological value of the product (Codex alimentarius, 2011). High-temperature cooking methods generate compounds that may contribute to carcinogenic risk, but their role is not yet fully understood (WHO,

2015). Due to high amounts of fat and non-haeme iron as well as the manufacturing process itself, liver pate is highly susceptible to lipid oxidation (Lorenzo & Pateiro M, 2013). However, fat is an important source of fatty acids and is important to balance the ratio between saturated and unsaturated fatty acids, rather than exclude it from the diet.

Success of the sterilization process is determined by reaching minimum  $F_0 = 3$  in geothermal center of the canned meat product, upon completion of the heat treatment process. Then it is considered that the spores of thermosetting type A and B *Clostridium botulinum* which may be present, are with certainty destroyed (Anon, 2019; FAO, Codex alimentarius 2011).

## Conclusion

Monitoring of the process of sterilization of liver pate showed that the determined  $F_0$  values were 12.07–15.93 (liver pate 75 g) and 9.71–13.17 (liver pate 150 g) which ensured the food safety of product and  $C_0$  values were 111–160 (liver pate 75 g) and 123–180 (liver pate 150 g).

Considering the determined  $F_0$  and  $C_0$  values, sterilization of canned liver pate 75 g and 150 g is needlessly high and sterilization process should be optimized by reducing effective sterilization time and reducing the  $C_0$  value..

Reducing the effective sterilization time by 5 (liver pate 75 g) and 10 minutes (liver pate 150 g) per production batch did not endanger the safety of the product, while reducing unnecessary energy consumption, could open up the space for increased production and improved the nutritional properties of the product.

Optimized heat treatment regime with reduced effective sterilization time can be applied in regular production, but it is necessary to ensure that sterilization dynamics in all parts of medium of the autoclave should be carried out as uniformly as possible. It requires regular maintenance and control of the equipment.

# Optimizacija postupka sterilizacije jetrene paštete sa aspekta osiguranja bezbednosti i očuvanja biološke vrednosti

Mladen Rašeta, Ivana Branković-Lazić, Boris Mrdović, Branislav Baltić, Becskei Zsolt, Vesna Đorđević

*Apstrakt:* Sterilizacija je fizički metod konzervisanja hrane, koji delovanjem temperature preko 100°C uništava mikroorganizme i inaktivira tkivne enzime. Bezbednost i održivost jetrene paštete u konzervi (pakovanja 75g i 150g) je osigurana utvrđivanjem  $F_0$  vrednosti, koja mora da bude iznad 3. Pri proveru režima sterilizacije utvrđen je tretman intenzivniji od potrebnog ( $F_0=12,07-15,93$  and  $F_0=9,71-13,17$ ) koji je osiguravao bezbednost proizvoda, ali je imao za posledicu smanjenje biološke vrednosti ( $C_0=111-160$  and  $C_0=123-180$ ). Na osnovu sprovedenih merenja i u skladu sa definisanim uslovima sterilizacije (20' zagrevanja do dostizanja efektivne temperature sterilizacije od 121,1°C i pritiska autoklava 2,5 bar-a, efektivno vreme sterilizacije 35–50' i vreme hlađenja 20–25') predlaže se smanjenje efektivnog vremena sterilizacije za 5–10 minuta. Time bezbednost proizvoda nije ugrožena, dok se smanjuje negativni uticaj na biološku vrednost, pri čemu se povećava efikasnost proizvodnje uz smanjenje utroška energije

**Ključne reči:** Jetrena pašteta, Sterilizacija,  $F_0$  vrednost,  $C_0$  vrednost, Bezbednost hrane, Toplotni tretman.

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# Biological hazards in the pork chain continuum: Risk mitigation strategy

Nikola Betic<sup>1</sup>, Ivana Brankovic Lazic<sup>1</sup>, Ivan Nastasijevic<sup>1\*</sup>

**Abstract:** The volume of pork meat production is continuously growing in the EU over previous years due to lower food prices, higher number of reproduction sows and increased volume of pork exports to China. Consumer choices toward pork meat depend on culture, place of residence and social opportunities, as well as their perception regarding safety and quality of pork meat/meat products. The main biological hazards associated with pork meat/meat products important from the public health perspective are zoonotic food borne pathogens, bacteria and/or parasites, e.g. *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Trichinella* spp., *Toxoplasma gondii* and Verotoxin-producing *Escherichia coli* (VTEC), by decreasing order, including associated antimicrobial resistance (AMR). Pathways of infection and contamination of pork meat differ, taking into consideration the multiple entry routes for zoonotic biological hazards along the pork meat chain, from farm to the final product. Therefore, the defined level of safety of pork meat/meat products should be achieved by synergistic action of control measures effectively applied at different points along the pork meat chain and supported by the integrated risk-based food (meat) safety management system in major modules of the meat chain: pre-harvest (farm), harvest (slaughterhouse), post-harvest (meat processing, distribution, retail, consumers), as well as identification and traceability. The integrated meat safety management system should be based on good hygienic practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) encompassing the science based hazard analysis and risk characterization, as well as identifying the most effective control options and risk mitigation strategies in the pork meat chain continuum.

**Keywords:** pork meat, biological hazards, food safety, integrated approach.

## 1. Introduction

### 1.1. Current status in the European Union

The total number of pigs in Europe is 147.2 million. The volume of pig meat production increased by 1.3% in Europe from 2015 to 2016, and annual production of pig meat was 23.4 million tons. This growth trend is due to several reasons: lower food prices, higher number of reproduction sows and increased volume of pork exports to China (Eurostat, 2017). Consumption of pork per capita per annum in the European Union (EU) is 40.9 kg and growing (AHDB, 2015)

Consumer choices are certainly different depending on culture, place of residence and social opportunities. A basic rule for ethical food consumption is that the consumer knows what they are eating or purchasing. Therefore, product must carry a clear declaration where description of product ingredients, the information about manufacturer, the product processing method and the suitable storage conditions are stated (among other things) according

to the EU regulation (EU, 2011). Labels must be also clear and appropriate for the type of food, in accordance with the usual mode of food use, provide instructions for preparing the food and take account local customs. However, many declarations in the meat market show major and/or minor deviations from the prescribed rules. For such issues, food business operators (FBOs) are directly responsible, as are the competent authorities (EU, 2002). In addition, the consumers also take over their level of responsibility regarding food safety and it is beneficial to provide the proper training to consumers, so that they are able to understand and apply all information given on label, in particular, regarding the intended use of the food product and food preparation in the kitchen (Henderikx, 2017).

### 1.2. Epidemiology of pork meat-associated illnesses in the EU

In 2016, campylobacteriosis was the most commonly reported zoonosis in the EU, accounting for almost 70% of reported cases. Other bacterial

<sup>1</sup>Institute of Meat Hygiene and Technology, Kacanskog 13, 11040 Belgrade, Republic of Serbia.

\*Corresponding author: Ivan Nastasijevic, [ivan.nastasijevic@inmes.rs](mailto:ivan.nastasijevic@inmes.rs)

diseases reported to a lesser extent were: salmonellosis, yersiniosis, verotoxin-producing *Escherichia coli* (VTEC) infections, and listeriosis. The most important biological hazards that affect human health associated with pork meat/meat products are *Salmonella* spp., *Yersinia enterocolitica*, *Trichinella* spp. and *Toxoplasma gondii* (Figure 1) (EFSA, 2017).

**Salmonella:** About 2,600 serovars of *Salmonella* spp. have been described so far. The five most important serovars that caused alimentary disorders in humans are *S. Enteritidis*, *S. Typhimurium*, monophasic *S. Typhimurium*, *S. Infantis* and *S. Derby*. The serovars that are the most common causes of human diseases associated with pork meat include: *S. Typhimurium*, and *S. Derby*. Within the EU, 25,049 units of fresh pig meat were examined microbiologically in 2016, of which 2.38% were positive for *Salmonella* spp.; 8,641 samples of meat prepared for use in the form of chopped meat were tested, with detected *Salmonella* prevalence of 1.93%. In 2016, there were 94,530 confirmed cases of salmonellosis reported in the EU, with an incidence of 20.4 per 100,000 inhabitants (EFSA, 2017).

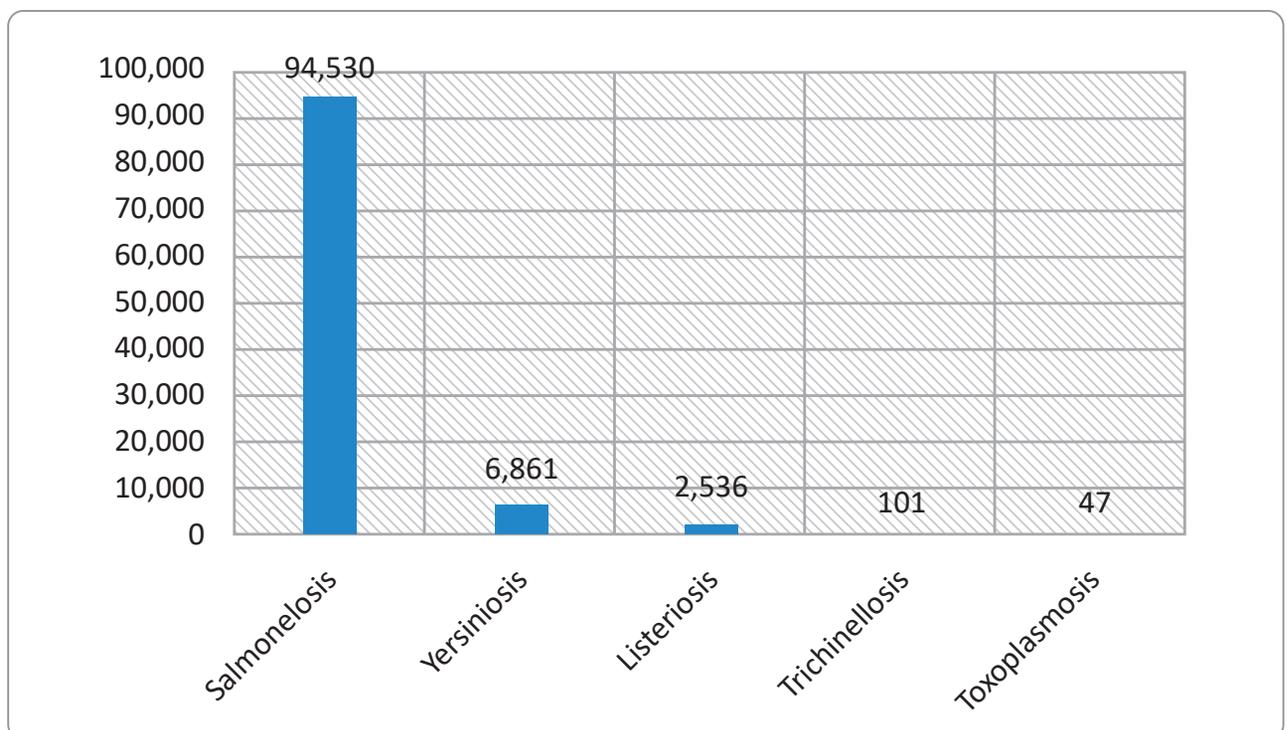
**Yersinia.** Twenty-six EU member states reported 6,861 confirmed cases of yersiniosis in 2016, with 1.82 reported cases per 100,000 inhabitants (EFSA, 2107). According to reports, *Yersinia enterocolitica*

is the most reported *Yersinia* species in all countries, accounting for 99.1% of yersiniosis in the EU. A slightly higher yersiniosis incidence was reported in the period from May to August (EFSA, 2107).

**Listeria.** Based on the severity of zoonosis, listeriosis is one of the top-ranked as it can be associated with the fatal outcome. *L. monocytogenes* is a significant public health pathogen, because it is often found in foods. During 2016, 2,536 cases of listeriosis were reported and confirmed in the EU, i.e. 0.47 cases per 100 000 inhabitants. The mortality rate among cases was 16.2%, and it often occurred in people older than 64 years old, while the especially sensitive population are those older than 84 years. During 2016, in a survey carried out in the EU regarding different food categories, *L. monocytogenes* was detected in pig meat products (not including fermented sausage) in 3.1% of samples (EFSA, 2017).

**Trichinella.** In 2016, 101 cases of trichinellosis in humans, i.e., 0.02 cases per 100,000 inhabitants, were reported and confirmed in the EU, which was a decrease of 26.5% compared to the previous year. This is the lowest number of cases and the lowest rate of trichinellosis since implementation of EU-level reporting (EFSA, 2017).

**Toxoplasma.** One of the most recognized human and animal parasites is *T. gondii*, which has a global presence. Ingestion of viable cysts via



**Figure 1.** The incidence of food borne diseases caused by the main biological hazards associated with pork meat/meat products (EFSA, 2017)

consumption of insufficiently thermally processed meat is considered one of the dominant ways human are infected with this parasite. During 2016, 47 cases of congenital toxoplasmosis were reported and confirmed in 19 EU Member States (MSs), with rate of 1.57 cases per 100,000 new-borns. It is not possible to estimate the prevalence of non-congenital toxoplasmosis, because only three MSs have an active control system for this disease (EFSA, 2017).

### 1.3. Antimicrobial resistance (AMR)

Excessive veterinary use of antimicrobial agents in animal species for food production, as well as the use in human population, contributes to the spread of AMR. Major zoonotic pathogens such as *Salmonella* spp. and *Campylobacter*, including those with AMR, can spread to humans by food and water consumption or by direct contact with animals. Several *Salmonella* spp. serotypes of public health significance were showed a high resistance rate to sulfisoxazole, tetracycline, ampicillin, ofloxacin, isolated from retail pork meat (Zhang et al., 2018). Commensal bacteria (*E. coli*, *Enterococcus* spp.) can also develop genetic resistance to antimicrobials, which can facilitate transmission of AMR to pathogenic bacteria that can cause diseases in humans and animals. The seriousness of this problem is the fact that more than 25,000 people die each year in the EU from diseases caused by antibiotic resistant bacteria. The use of antibiotics in food production systems has to be reduced, and compensated for by improvement of animal health through preventive measures and good hygiene and management practices (Nulty et al., 2016).

### 1.4. Microbiological criteria for pork carcasses/meat products

To date, the best approach to ensure the food safety is a preventive, integrated approach, by managing all processes in food production, from primary production to the consumer. The main responsibility regarding food safety is related to Food Business Operators (FBOs), who define and implement appropriate measures for good hygienic and manufacturing practice, as well as other procedures based on Hazard Analysis and Critical Control Point (HACCP) principles, in order to achieve the food safety objectives (FSO) defined in food regulations. The numbers of aerobic bacteria (aerobic colony count/ACC) and *Enterobacteriaceae* (EC) and the presence/absence of *Salmonella* spp. are the process

hygiene criteria defined for pig carcasses, after dressing-before chilling. ACC and *E. coli* counts are the process hygiene criteria for minced pork and pig meat preparations, at the end of the manufacturing process. The presence/absence of *Salmonella* spp. is also a food safety criterion, defining the counts/limits for *L. monocytogenes* during the shelf-life of minced meat or meat preparations intended to be eaten cooked (EU, 2005). There are also food safety criteria for *L. monocytogenes* in ready-to-eat (RTE) foods, which include cooked sausages, canned meats, and raw, dry fermented sausages. Therefore, pork meat/processed pork meat product placed on the market must, throughout its shelf-life, comply with food safety criteria that are clearly defined by the EU Regulation 2073/2005 (Table 1).

## 2. Overview of biological hazards in the pork meat chain and risk ranking

Hazards are defined as the biological, chemical or physical agents that can lead to illness or injury of consumers if adequate control measures are not in place. Biological hazards are organisms or agents of biological origin whose presence can make the product inappropriate or dangerous for consumption. Biological hazards include microorganisms (bacteria, viruses, fungi), parasites and prions. It is generally accepted that biological hazards are the major risk for meat consumers, especially due to their short term effects (Lawley et al., 2008).

### 2.1. Farm

The farm is the first link in the pig meat production chain. At the farm, biosecurity measures should be implemented and the principles of animal welfare should be fulfilled. Biosecurity measures should be part of a general strategy, developed in a close and continuous cooperation between the owner, employees and animal health expert. This cooperation should enable everyone in the team to be informed of relevant health questions at local, national and international levels.

Farm animals can be the primary source of human infection, directly through consumption of raw products or insufficiently thermally processed food derived from infected animals, or indirectly by spreading pathogens to plant products through fertilisers originating from infected animals. Therefore, it is very important to have knowledge of pathways of infection from the farm to final food products.

**Table 1.** Food safety criteria and process hygiene criteria for minced meat, meat preparations and ready-to-eat (EU, 2005; Serbia, 2011)

Food category	Microorgan-isms	Sampling plan		Limits		Analytical reference method	Stage where the criterion is applied	Action in case of unsatisfactory results
		n	c	m	M			
Process hygiene criteria								
Carcasses of pigs	Aerobic colony count			4,0* (3,3)** log CFU/cm <sup>2</sup>	5,0* (4,3)** log CFU/cm <sup>2</sup>	ISO 4833	Carcasses after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
	<i>Enterobacteriaceae</i>			2,0* (1,3)** log CFU/cm <sup>2</sup>	3,0* (2,3)** log CFU/cm <sup>2</sup>	ISO 21528–2		
	<i>Salmonella</i>	50	5	Absence in the area tested per carcass		EN/ISO 6579		
Minced meat	Aerobic colony count	5	2	5×10 <sup>5</sup>	5×10 <sup>6</sup>	ISO 4833	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
	<i>E. coli</i>	5	2	50 CFU/g	500 CFU/g	ISO 16649–1		
Meat preparations	<i>E. coli</i>	5	2	500 CFU/g	5 000 CFU/g	ISO 16649–1		
Food safety criteria								
Food category	Microorg-anisms	Sampling plan		Limits		Analytical reference method	Stage where the criterion applied	
		n	c	m	M			
Minced meat and meat preparations intended to be eaten raw	<i>Salmonella</i>	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf-life	
Minced meat and meat preparations made from other species than poultry intended to be eaten cooked	<i>Salmonella</i>	5	0	Absence in 10 g		EN/ISO 6579		
Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purpose	<i>L.monocyt-ogenes</i>	5	0	100 CFU/g		EN/ISO 11290–2	Before the food has left the immediate control of the FBOs, who has produced it	
				Absence in 25 g		EN/ISO 11290–1		

**Legend:** \* Destructive method of swabbing; \*\*Non-destructive method of swabbing; n = number of units comprising the sample; c = number of sample units giving values between m and M

Pigs are sensitive to a wide spectrum of *Salmonella* spp., and young animals are more sensitive than older ones. Livestock can often be infected without any evident clinical symptoms of disease. Salmonellosis in pigs with clinical manifestations was often caused by *S. Choleraesuis* in the past, but with monitoring and control measures, the occurrence of this serotype on farms has been significantly reduced. *S. Typhimurium* is mostly transmitted among animals on the farm, while other serotypes mostly originate from food or the environment. (EFSA, 2017).

Among domestic animals, pigs are considered as the main reservoir of *Y. enterocolitica*, and they are asymptomatic carriers of this bacterium. Regarding control of food and animals, only a low number of EU member states reported data for 2016, which disables wider conclusions about its prevalence (EFSA, 2017). The prevalence of *Y. enterocolitica* on some farms in the EU is ranging from 4%–93%; some regional variations were also detected, which indicates the possibility of *Yersinia* control in pigs (Fredriksson-Ahomaa et al., 2000).

Livestock production, including pig farming in developed countries, is continuing to go through significant structural changes, including a significant reduction in the number of farms and a corresponding increase in capacity in closed systems, due to better efficiency and economic policy. However, at the same time, the number of smaller farms for growing pigs in the open is increasing. In farms with the open holding systems there is an increased risk of infection from *T. gondii* (Gamble et al., 1999; García-Bocanegra et al., 2010). Indeed, the trend of breeding pigs in the open could have caused increases in pig seroprevalence for *T. gondii*. Also, in poorly managed systems, where pigs are bred in less controlled conditions, seroprevalence in pigs was as high as 68% (Gamble et al., 1999). It was suggested that low or negligible seroprevalence of *T. gondii* at farm level can be used as an indicator of good hygienic practice (van Knapen et al., 1995).

Consumers, especially residents of Europe and North America, often prefer organic meat from breeding systems that also recognise the animal welfare requirements. The consumer impression is that food produced by these principles can be automatically considered a safe food; however, according to the scientific research the situation is quite different. For example, keeping pigs in the open implies exposure of domestic pigs to increased risk of *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudintermedius* infections originated from wildlife reservoirs (Burke et al., 2008).

## 2.2. Slaughterhouse

At slaughter, pathways of microbiological contamination are numerous and they can be categorised as internal or external. Meat originating from healthy, rested animals is normally sterile, but in stressed pigs, bacteraemia (bacteria in the bloodstream) can occur more easily (an internal contamination pathway). The external pathways of contaminating carcasses or pig meat can be direct, usually from skin of slaughtered animals, or indirect, e.g., skin-knife/equipment-meat.

Since the important source of *Salmonella* spp. in the meat production chain are the animals themselves, the prevalence of these bacteria on farm must be closely monitored (Korsak et al., 2003). Obviously, the *Salmonella* spp. prevalence in fresh meat is directly related to its prevalence in the animals, although the prevalence of this pathogen on/in pork meat in stages also depends on the further technological processing that meat undergoes as food.

Inadequate hygiene conditions and lack of sanitation procedures during transport of animals can contribute to the presence of bacteria on pig carcasses. Inadequately washed, dirty pigs originating from farms with poor hygiene contributes, can also increase the overall prevalence of microbes (e.g. ACC), including pathogens on/in pork meat. *EC* are also very widespread in the environment, and they are also an integral part of the gastrointestinal microbiota of humans and animals. One of the most important places for contamination of pork skin with *EC* is the stunning box, which each pig touches. The technology of pig skin removal after slaughter also carries a high risk of contaminating carcasses/meat with *EC* (Aslam et al., 2003). In addition, there is a high risk of meat contamination with gastrointestinal tract content during pig evisceration. Evisceration is the processing step that most contributes to bacterial contamination on carcass surfaces, because afterwards, there is no primary treatment that could reduce the number of bacteria. Inadequate procedures during technological operations at slaughter line (e.g. failure of workers to comply with work procedures, inadequate equipment, dirty work clothing, inadequate sanitation during work, not preventing cross-contamination) can lead to contamination of pig carcasses (Raseta et al., 2015). Cross-contamination at slaughter line is also a recognized issue from the perspective of meat safety, as confirmed by the increased prevalence of *S. enterica* from farm to slaughterhouse (De Busser et al., 2011; Karabasil et al., 2012). Although the contamination/infection of pigs with *Salmonella* spp. can happen at any point from the farm to the slaughterhouse,

it should be emphasised that the slaughterhouse has an important role in this process. The surfaces in the lairage and in the stunning box are almost always contaminated with *Salmonella* and they can be sources of cross contamination, ultimately increasing *Salmonella* prevalences on carcasses at slaughter line (Nulty *et al.*, 2016).

### 2.3. Meat processing

Meat products include products obtained by processing meat or further processing of such products, so that observation of a cross-sectional area indicates the product no longer has the characteristics of fresh meat. Depending on production methods, meat products can be classified into those produced without heat treatment and those produced with heat treatment. For example, fermented sausages are meat products produced without heat treatment, while pasteurised meat products are produced with heat treatment.

#### 2.3.1. Fermented sausages

Fermented sausages are not heat treated, so after meat and fat tissue chopping and mixing, addition of ingredients (additives and spices), filling the mixture into casings, they are preserved by fermentation and drying, with or without smoking. The shelf-life of fermented sausages is determined by their low pH and water activities ( $a_w$ ), and therefore, they can be stored at higher (i.e., not chill) temperatures. Antimicrobial factors of importance for the safety and shelf-life of fermented sausages are: low  $a_w$  of 0.80–0.90, salt content of 2.4–2.8%, pH of 5.3–6.0 (Teodorović *et al.*, 2015). As such, fermented sausage can be stored at a temperature of up to 5°C.

For the production of fermented sausages, the meat of older animals is more suitable (older fattened pigs, sows excluded), because it contains more dry matter and more myoglobin pigment than young animals. pH has a very important role in the selection of pork cuts for fermented sausages, with recommended pH < 6.0. It is easier for meat with the lower pH values to release water, to dry easier, and which allows effective salt penetration. Much attention is given to selection of fat tissue, the most suitable of which is subcutaneous tissue of the neck and back (loin).

Starters are microbial cultures used to promote and conduct the fermentation of meat products. Bacteria, particularly lactic acid bacteria and coagulase-negative staphylococci, as well as yeasts and molds, may be used as starters (Laranjo *et al.*, 2019). These are selected microorganisms that participate in the ripening of fermented sausages, and

they are responsible for typical sensory properties of the final products. In fermented sausages, starter cultures ferment sugars to produce lactic acid and also have an important function in sausage maturation, including their protective role, e.g. micrococci produce the enzyme catalase, lactic acid bacteria produce antimicrobial substances. Catalase helps prevent oxidation because it decomposes hydrogen peroxide to water and molecular oxygen. Lactic acid bacteria produce organic acids, ethanol, hydrogen peroxide, carbon dioxide and bacteriocins, all of which can act antimicrobially (Laranjo *et al.*, 2019).

Fermented dry sausages are mainly considered as a generally safe products from the microbiological aspect and their safety relies on these multiple antimicrobial properties, the so-called 'hurdle concept', e.g. pH,  $a_w$ , redox potential (Leistner, 1994). During the past decade in the EU countries, epidemiological research showed the occurrence of disease outbreaks that were associated with the consumption of fermented sausages. The main bacterial hazards associated with this type of pork product are *Salmonella* spp., *E. Coli* and *L. monocytogenes* (Toldra, 2010). During production of dry fermented sausages, high initial contamination of raw meat or possibly contaminated sausage ingredients, as well as inadequate processing conditions and/or contamination after processing, can cause a risk of salmonellosis (Gieraltowsky *et al.*, 2013). On the other hand, *L. monocytogenes* is of less concern if process hygiene is maintained at high levels during sausage production. Although contamination with this pathogen can occur in any phase of the sausage production process, it is more frequent in the latter stages of fermentation/ripening (Thevenot *et al.*, 2005). Ensuring process hygiene during production is a key element for controlling this pathogen in food.

#### 2.3.2. Pasteurised meat products

Pasteurised, heat-treated products include cooked sausages. Cooked sausages include numerous products that differ in diameter and fineness of their emulsified filling. Since they have a high pH (6.0–6.5) and  $a_w$  (0.95–0.98), their shelf-life depends on appropriate heat treatment and storage temperature. Common to all cooked sausages is the meat emulsion that forms the basis of their stuffing, and which is filled into casings, their heat treatment at pasteurisation temperature, with or without smoke, or at boiling or sterilisation temperatures. Cooked sausages are most often processed by hanging in a controllable chamber containing steam at 75–85°C so that the core product temperature reaches at least 70°C for 20 minutes (Teodorović *et al.*, 2015). These products

are stored at < 4°C because this heat treatment cannot destroy all microorganisms, but only the vegetative forms of mesophilic and psychrophilic microorganisms.

2.3.3. Canned meats

Canned meats, like cooked sausages, usually have high pH 6,0–6,5 and a<sub>w</sub> 0,96–0,98 and their shelf-life also depends on appropriate heat treatment (commercial sterilization), hermeticity and storage temperatures. For canned meat production, meat with higher pH values is needed (Teodorović et al., 2015). While the use of warm meat would be ideal, a chilled meat is most commonly used in industrial

production settings. Salting and chilling of meat are effective steps to reduce the number of aerobic bacteria, including *Salmonella* spp., but are less effective for *E. coli* (Sukumaran et al., 2018). Results in investigation Gabriel and Nakano (2009) about resistance among *E. coli*, *L. monocytogenes* and *S. Enteritidis*, it has been established that the *L. monocytogenes* is generally less susceptible to inactivation, and is even able to grow in the conditions found in many meat products, with the rate depending on different environmental factors (e.g., temperature, a<sub>w</sub>). Temperature proved to be the most effective environmental factor for the growth and death of *L. monocytogenes*.

**Table 2.** Preliminary qualitative biohazard prioritisation in the pig meat chain (EFSA, 2012)

Preliminary qualitative evaluation of the risk level: probability of occurrence against severity of consequences	Severity of consequences			
	Medium severity of consequences: human cases 1–10/100000, case fatality <0.1%	Low severity of consequences:		
High severity of consequences: human cases >10/100000, case fatality <0.1%	Human cases <1/100000, case fatality >0.1	Human cases <1/100000, case fatality <0.1%		
High probability: Prevalence on chilled carcasses >5%	High risk: <i>Salmonella</i>			
Medium probability: Prevalence on chilled carcasses 0.1–5%	Medium risk: <i>Campylobacter</i>	Medium risk: <i>Yersinia enterocolitica</i>	Medium risk: <i>L. monocytogenes</i> , VTEC	Low risk: <i>Toxoplasma</i>
Low probability: Prevalence on chilled carcasses <0.1%			Low risk: <i>Cl. Botulinum</i>	Low risk: <i>Sarcocystis suihominis</i> , <i>T. solium cysticercus</i> , <i>Trichinella</i> , <i>Cl. difficile</i> , <i>Cl. perfringens</i> , <i>Mycobacterium</i> , <i>S. aureus</i> , HEV

#### 2.4. Distribution/retail of meat and meat products

Food safety is an imperative in international trade, so World Trade Organization (WTO) member states apply clearly defined sanitary and phytosanitary measures (SPS Agreement), based on appropriate risk assessment, to ensure that food safety and quality will not be compromised (WTO, 1995).

Phases of the meat distribution chain that are important in terms of transmission of pathogens include: 1) transport and storage between processing and wholesale or retail markets, 2) food handling and storage at retail, 3) transport from retail to home, as well as 4) handling and storage of food at home.

Special attention is given to the storage of foods. During storage, optimal conditions are provided to ensure a method of protection against air particle contamination, weather, animals (e.g., insects, birds) and sunlight, and maintaining hygienic conditions. Application of HACCP principles and good distribution practices should ensure conservation of food at the prescribed storage temperature (e.g. < 7°C for fresh meats, while recommended temperature for thermally processed meat products should be < 4°C) (EU, 2004; FDA, 2018). The storage room should be designed to ensure efficient cleaning and maintenance and prevent microbial, chemical and physical cross-contamination.

Fresh meat is highly perishable and has a short shelf-life, which means the time available for product distribution is also short (Nastasijevic et al., 2017). If fresh meat and meat products are not kept in adequate, controlled temperatures, they can be good environments for the growth of pathogenic and other bacteria. The complexity of a global meat supply, in one country, between countries or between continents, requires cold chain solutions since product is acceptable to consumers only if it has the appropriate level of freshness and safety. Participants in the cold chain must cooperate, and they must have in mind the practices of previous and future participants. Therefore, the cold chain process must be documented. Although the importance of monitoring the correct cold chain temperatures is well known, this segment in the integrated meat supply chain is still a challenge. From recently, several tools impacting the cold chain have become recognized: biopreservation, ionising radiation, high hydrostatic pressure, active packaging, and wireless sensors connected to database software (Nastasijevic et al., 2017).

In the retail and consumer phases, the following risk factors can occur: inadequate storage, poor

personal hygiene, contaminated equipment, and chemical residues. As for the consumer phase, other main risk factors include: inadequate cooking, and food from undocumented sources (FSIS, 2004).

#### 2.5. Ranking and prioritisation of biological hazards in the pork meat chain

The prioritisation of biological hazards is made by taking into consideration public health data. For risk ranking biological hazards, i.e., prioritising them as of high, medium or low importance, the following data were used by European Food Safety Authority (EFSA, 2012): (i) Human incidence (EFSA/ECDC, 2011), (ii) Number of cases with fatal outcomes, (iii) Prevalence on pig carcasses (EFSA, 2009).

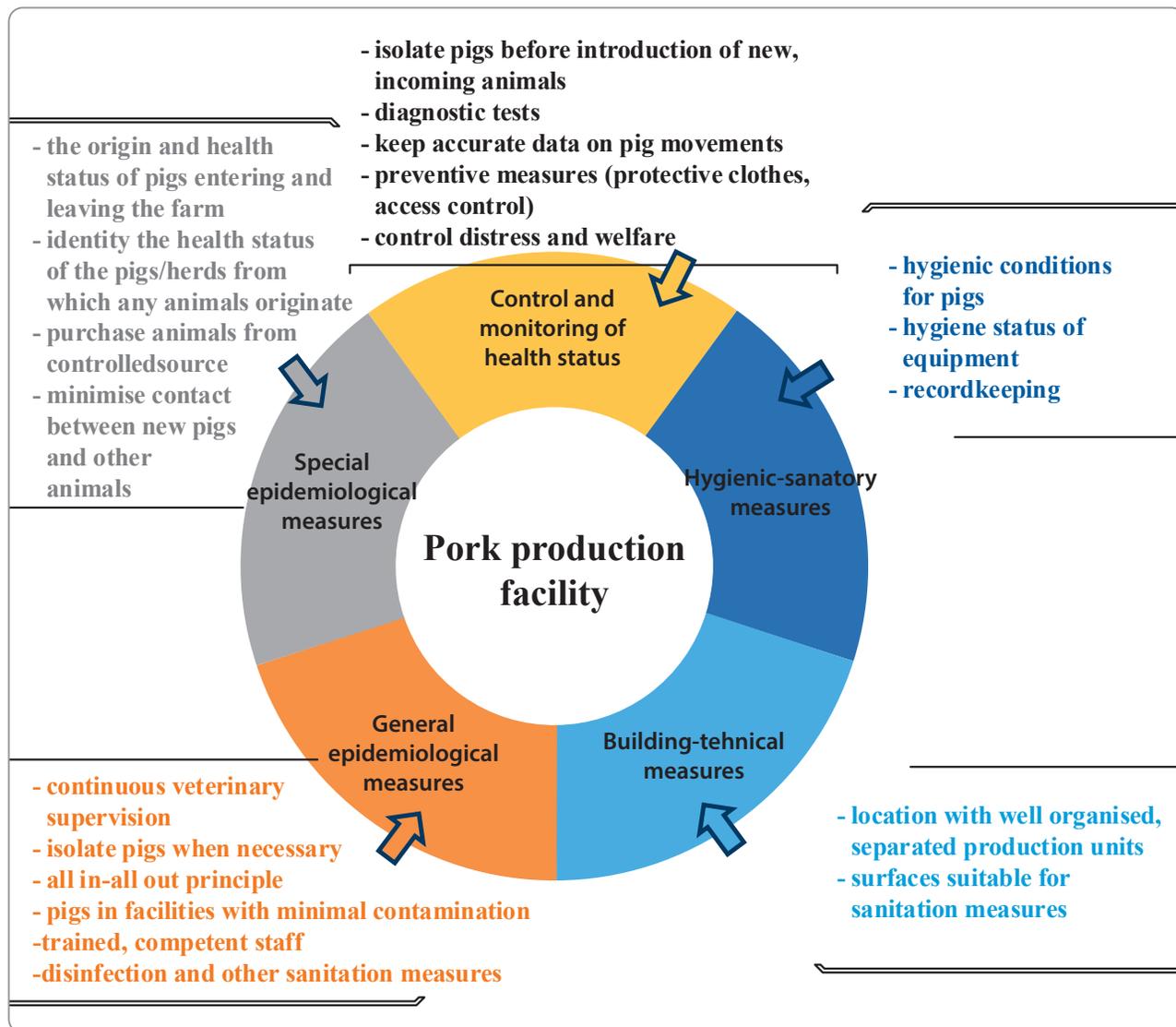
A qualitative risk assessment of biological hazards in the pig chain was conducted using chilled carcass' prevalence data, incidence and seriousness of the disease in humans and the attribution of hazards originating from pigs, in the EU. Based on this estimation, *Salmonella* spp. is considered as the main biological hazard originated from pigs, *Y. enterocolitica*, *L. monocytogenes*, VTEC and *T. gondii* are considered as medium risk hazards, while *Trichinella* spp. is of low risk (Table 2) (EFSA, 2012).

### 3. Control measures for biological hazards in the pork meat chain

Control measures are any actions or activities that are used to prevent or eliminate food safety hazards (Codex Alimentarius, 2005). The purpose of control measures is the production of food which is safe and suitable for human consumption. FBOs must be achieved by the implementation of the risk based meat safety assurance system (GHP/HACCP).

#### 3.1. Control measures on farm

On-farm biosecurity measures include all measures and systems that prevent, eliminate or reduce biohazards. Effective on-farm biosecurity contributes to better animal health, higher productivity and profitability, food safety and environmental protection. Such measures also contribute to the better reputation of animal production in the country and affect international traffic of animals and products of animal origin (Figure 2) (Stanković and Hristov, 2010). On-farm risk factors are unique for each farm, and, thus, each biosecurity plan should be farm-specific.



**Figure 2.** Overview of generic biosecurity measures on pig farm

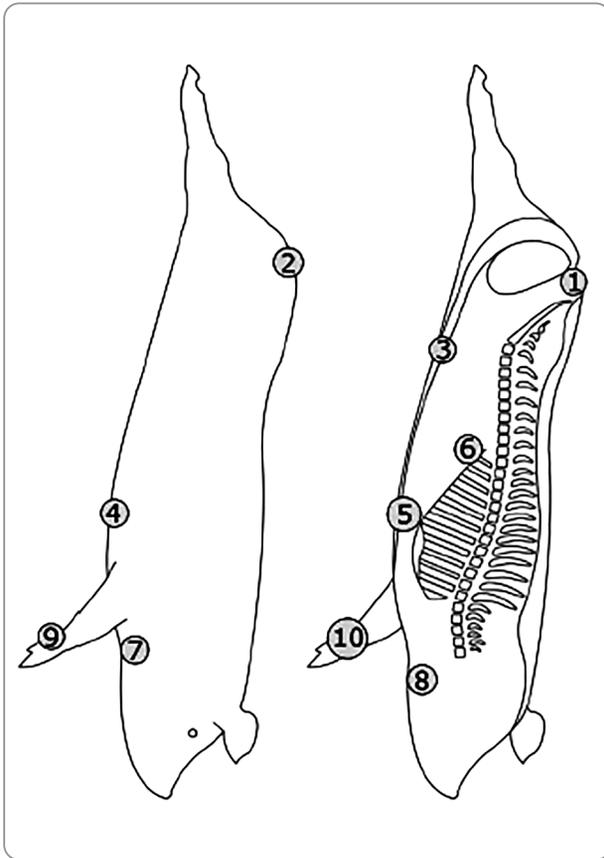
### 3.2. Control measures in slaughterhouse

The competent authority or delegated inspection authority is obliged to check the Food Business Operator’s (FBO) documentation and the applied self-control plans, including the set up microbiological criteria. The number or presence/absence of microorganisms on selected carcass surface sites of slaughtered pigs (Figure 3) is determined according to the standard methods (ISO, 2015). Time and frequency of sampling are regulated according to the: hygienic practice and technology for each slaughterhouse, design of risk-based process control or harmonised monitoring programmes, production volume, as well as epidemiological status of the area from which the animals originate.

The carcass sites from which samples are taken must be described in the self-control plan, which

is defined by the FBO. Since the purpose is to examine those carcass sites where the probability of contamination is the greatest, standard sampling sites on pig carcasses are recommended, as shown in Figure 3 (ISO, 2015).

If Food Business Operator (FBO) decides to sample different carcass sites than those in Figure 3, they are required to validate their sampling method, i.e., to confirm that it achieves at least the same effect in monitoring carcass contamination that is achieved by using the recommended system. Importantly, the defined carcass sites are sampled over time in order to monitor trends of the results obtained. EU regulation (EU, 2005) requires that FBOs analyse test result trends to enable appropriate measures to be taken without delay in the case of unsatisfactory trends. This is in order to limit or prevent the occurrence of

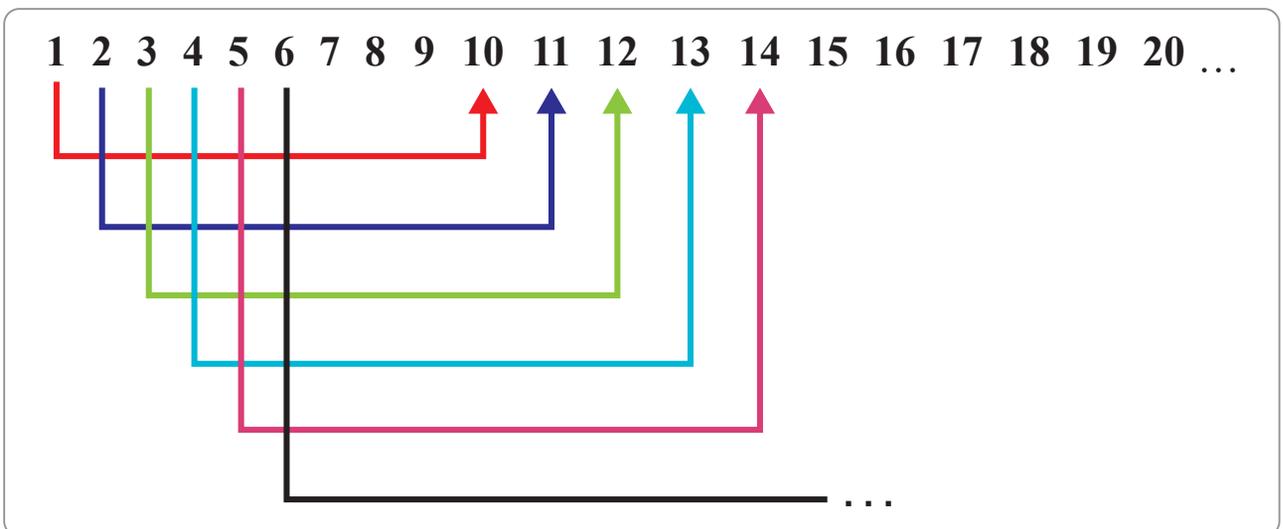


**Figure 3.** Suitable sites for taking samples from pig carcasses (ISO, 2015). Sites are: 1) Pelvic channel internal, 2) Pelvic channel external, 3) Abdominal, 4) Xiphoid external, 5) U Xiphoid internal, 6) Pillar of diaphragm, 7) Submaxillary external, 8) Submaxillary internal, 9) Forefoot external aspect, 10) Forefoot internal aspect

microbiological hazards. Slaughterhouses must conduct self-control checks every week, and the day of sampling must be changed every week, to ensure coverage of every day of the week. In case that results were satisfactory within the six consecutive weeks in a row (ACC, EC), FBOs can reduce sampling at once per two weeks/fortnightly (Serbia, 2011).

Samples are taken from the suitable sites on carcass surfaces by destructive or non-destructive sampling methods, after carcasses are washed, but before chilling. Destructive methods disturb the integrity of carcasses, by cutting and removing tissue samples from the depth of meat. Non-destructive methods involve swabbing carcass surfaces without disturbing the integrity of carcasses. Destructive methods give more precise results and show a higher level of carcass contamination than non-destructive methods. However, the destructive methods have negative consequences on carcass values, so the use of these techniques is limited. Certainly, non-destructive methods are more practical and economical in field conditions and are the most common sampling methods to monitor hygiene of pig carcass production processes.

The microbiological criteria for the hygiene of the production process for pig carcasses are: the aerobic colony count (EN ISO 4833), *Enterobacteriaceae* count (EN ISO 25528–2) and the presence/absence of *Salmonella* spp (EN ISO 6579) (Table 1). To obtain the aerobic colony count and number of *Enterobacteriaceae*, laboratory test results are shown as the number of colony forming units per cm (CFU/cm<sup>2</sup>) for each collective sample taken from one carcass. The daily average logarithmic value is obtained by calculating the logarithm (log<sub>10</sub>) of each individual laboratory test result/per



**Figure 4.** Sampling on the principle of a moving window (Serbia, 2011)

carcass site, and then calculating the average of these logarithmic values. The limit values for pig carcasses are shown in Table 1. In the case of unacceptable test results, improvement of slaughter hygiene and a review of process control must be undertaken.

The criterion for *Salmonella* spp. on pig carcasses is defined in the food safety criteria, as well as in the criteria for process hygiene (EU, 2005; Table 1). To determine the presence/absence of *Salmonella* spp. on slaughtered pig carcasses, a non-destructive method of sampling with abrasive sponges is normally used. The sampling area must cover at least 400 cm<sup>2</sup>, and five samples are taken from the pre-determined sampling sites. The serial trend results of *Salmonella* spp. presence/absence determination on pig carcasses are calculated from 50 samples collected over 10 consecutive samplings. This means the number of samples that contained *Salmonella* spp. is calculated after 10 consecutive weeks from the five samples taken each week (Table 1). The second series include samples taken from 2<sup>nd</sup> to the 11<sup>th</sup> week, the third from the 3<sup>rd</sup> to the 12<sup>th</sup> week, etc. Estimation of the trend of the successive sampling is based on the rolling window principle (Figure 4).

Corrective measures in the case of unacceptable trend results encompass the improvement of slaughter hygiene and the review of process control, as well as the origin of the pigs, including the improvement of biosecurity measures on farm of origin.

### 3.3. Control measures for pasteurised and fermented pork meat products

Food safety criteria are applied to meat and meat products (food) placed on the market and are applicable throughout the food's shelf-life (EU, 2005). When defining the microbiological criteria that are applied to a particular type of meat product, the way in which the product is consumed is taken into consideration, but it is also important to consider the specific (vulnerable) groups of consumers for whom it is intended, and the fate of the defined hazard in the food, e.g. YOPI (young, old, pregnant, immunocompromised).

Ready-to-eat (RTE) meat products do not require further thermal or other processing that would eliminate or reduce to an acceptable level the number of microorganisms defined as potential hazards in the product. However, RTE meat products that could support the growth of *L. monocytogenes* could pose a risk to human health. In such cases, the FBO must conduct a product shelf-life study, to determine the compliance with the microbiological criteria for *L. monocytogenes* during the product shelf-life (EU, 2005).

In the EU, to select the appropriate food safety criterion for *L. monocytogenes* in ready-to-eat meats, the food category must first be determined. RTE foods are classified into two categories based on the growth of *L. monocytogenes* in the food: a) RTE food that supports growth of *L. monocytogenes*; b) RTE food that does not support growth of *L. monocytogenes* (EU, 2005). To define the category b), it is considered that food should have any of the three following properties which means that such product does not support the growth of *L. monocytogenes*: 1) pH < 4.4 or a<sub>w</sub> ≤ 0.92; 2) pH < 5.0 or a<sub>w</sub> ≤ 0.94; 3) shelf-life < 5 days.

In case of food that supports growth of *L. monocytogenes* (category a), FBO should validate the production process for respective products applying 'double' criterion: (i) confirmation of *L. monocytogenes* absence in the product, in five 25g samples, before the food has left the immediate control of the FBOs, and (ii) confirmation that numbers of *L. monocytogenes* in the product will not exceed 100 CFU/g throughout the product shelf-life, where the *L. monocytogenes* criterion of fewer than 100 CFU/g in each of five samples of product is applied. In these foods, the FBO can determine temporary limit values to use during processing, which must be low enough to guarantee the number of *L. monocytogenes* will not exceed 100 CFU/g throughout the product shelf-life.

In the EU, criteria for *Salmonella* spp. in pork meat products and pig carcasses are applied (i.e. food safety and process hygiene criteria, respectively). For pork meat products, the FBO must conduct a sampling plan in accordance with the food safety criteria for: a) minced meat and meat preparations made from other species than poultry intended to be eaten cooked, and; b) meat products intended to be eaten raw, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk. *Salmonella* spp. must not be found in five samples, each being 25g of product and the criteria are applied to the products throughout their shelf-life (Table 1). Process hygiene criterion regarding *Salmonella* for pig carcasses is described in Table 1 and Figure 4. The results of the test show the microbiological validity of the examined series and can be used to demonstrate the effectiveness of the HACCP system or good hygienic practice of the process (EU, 2005).

In cases where the results of the test are unsatisfactory, the FBO is obliged to take corrective measures: pull-back or recall of food, identify and remove the causes, check that the process is under control again, reassess defined and applicable risk prevention and other available risk management measures.

### 3.4. Control measures in the distribution/retail chain

To maintain food safety, it is important that the cold chain is interrupted after pig slaughter and processing, and throughout all stages of the processing/distribution/retail chain (Nastasijevic *et al.*, 2017). Meat and meat products must be distributed to retailers by vehicles dedicated for that purpose, and which must comply with the above conditions. After delivery, meat and meat products are stored at an adequate chill temperature and in an appropriate manner that prevents cross-contamination with foodborne pathogens. At the consumer level, the general hygiene principles recommended by the World Health Organization (WHO) which should be applied are five keys for safer foods: maintain personal hygiene (hand hygiene), separate raw food from thermally processed food, cook food thoroughly ( $>70^{\circ}\text{C}$ ), store food at safe temperatures (below  $5^{\circ}\text{C}$  or above  $60^{\circ}\text{C}$ ), use potable water for food preparation and use safe materials for food preparation (WHO, 2006). Continuing education for FBOs and consumers is necessary, as well. In Serbia, following the official introduction of the new legislation that required HACCP implementation (Serbia, 2005; 2009), the process hygiene in food establishments was significantly improved compared to the hygiene levels in the period prior to HACCP implementation (Tomasevic *et al.*, 2016).

## 4. Risk mitigation strategy for biological hazards in the pork meat chain

*Salmonella* spp., *Y. enterocolitica*, *T. gondii*, and *Trichinella* spp. are recognised as the most important biological hazards originated from pork meat that can affect human health (EFSA, 2012). To obtain adequate level of safety of pork meat products and reduce foodborne outbreaks originated from pork meat/products, the pork meat production chain must comply with some well-described principles. Those principles are related to the integrated control along the whole meat production chain in the context of Longitudinally Integrated Safety Assurance (LISA concept), which include synergistic implementation of control measures in the farm–distribution continuum (Nastasijevic *et al.*, 2016).

**Farm.** On-farm biosecurity measures include all measures and systems on-farm that prevent, eliminate or reduce biohazards. Biosecurity measures must be implemented on pig farms, as their effective use contributes to better animal health, higher productivity and profitability, food safety and environmental protection.

**Slaughterhouse.** Adequate conditions must be allowed during loading, transporting and unloading pigs from farm to slaughterhouse. Pigs should be washed, clean and originate from farms where suitable biosecurity measures are applied, as there may be cross-contamination in the slaughterhouse, which is a significant problem from the aspect of food safety. The lairage surfaces and the stunning box are regularly contaminated with *Salmonella* spp. and can be sources of cross-contamination of animals, as well as the carcasses on the pig slaughter line. *Enterobacteriaceae*, as part of the gastrointestinal tract, are very widespread in the environment, and importantly, often contaminate the stunning box. Therefore, the principles of good hygiene (GHP) and good manufacturing practice (GMP) must be applied. The production process hygiene is determined using microbiological criteria for pig carcasses (Table 1): the aerobic colony count, *Enterobacteriaceae* count and presence/absence of *Salmonella* spp.

**Meat processing.** Products obtained by meat processing, and which no longer have the characteristics of fresh meat, must be produced using good hygienic/manufacturing practices. Initial contamination of raw materials with pathogenic bacteria must be limited, while processing conditions must be adequate and/or contamination after processing must be prevented, in order not to impair the product safety. Criteria are clearly defined for pork meat and meat products by determining the growth or presence/absence of pathogenic bacteria in products during their shelf-life (Table 1).

**Distribution.** To maintain meat and meat product safety from slaughterhouse to the consumer, the continuity of the cold chain should be maintained to encompass the consumer phase (Codex Alimentarius, 2005). In addition to adequate temperature, good distribution practices must be implemented and cross-contamination of pig meat with biohazards be prevented.

## 5. Conclusion

As world trade increases and the population grows up, food safety is more important than ever, and states must comply with clearly defined conditions for food production. Pork meat production increased by 1.3% in Europe in 2016, and annual production was 23.4 million tons. Significant numbers of foodborne illnesses have been associated with this large production volume of pork meat. The biohazards, *Salmonella* spp., *Y. enterocolitica*, *T. gondii*, and *Trichinella* spp. are recognised as the most important hazards that can affect human health and can originate

from pigs/pork meat. Integrated monitoring and control of biohazards, along the pork meat chain, including associated AMR, commensal and zoonotic bacteria in humans, animals and food are necessary as an important source of information for improving food safety and consumer protection. Infection and contamination pathways of pork meat are different, but biohazards can reach the food in each step from farm until consumption. Pigs can be the primary source of infection of humans, directly via raw pork or insufficiently thermally processed meat products from infected pigs or contaminated meat, or indirectly via spread of biohazards on plants via fertilisers that originate from infected pigs. On-farm biosecurity measures contribute to improved animal health, increased production and profitability, food safety and environmental protection. Microbiological contamination of pork meat can have a source and/or occur at multiple stages along the meat chain, which are generally divided into internal (distress on-farm/in transportation/lairage which may lead to increased faecal shedding of major zoonotic food borne pathogens) and external factors (dirtiness/cleanliness of incoming animals, meat handlers, tools, equipment, air). The following microbiological indicators are used to assess slaughter process hygiene for pig carcasses: aerobic colony counts (ACC) — indicate the level of general hygiene, *Enterobacteriaceae* counts (EC) — indicate the faecal

contamination and presence/absence of *Salmonella* spp. — indicate the occurrence of pathogen on farm. In the EU, FBOs are required to analyse process hygiene trends; when a trend is unsatisfactory, appropriate corrective measures must be applied without delay to prevent the unacceptable occurrence of microbiological hazards. EU legislation requires slaughterhouses to perform self-controls every week, with sampling days changing constantly to ensure coverage of all days in the week. Food safety management must be based on a good production/hygienic practices and effective risk-based food safety management system (HACCP), which requires the FBO to recognise, control and/or eliminate relevant hazards that could compromise product safety in proactive manner. The main purpose of laboratory examination of final products is to provide the validation whether food safety management systems operates effectively. The distribution chain requires that meat products should be protected from the contamination, so dedicated vehicles must be properly cleaned, washed and disinfected. For temperature-sensitive meat/meat products, a vigorous cold chain must be maintained from the producer to the consumer. Interruption of one or more components in the distribution chain may provoke consequent damage of the meat/meat products and, therefore, affect the consumers' health, as well as excessive economic damage due to food recalls.

## Integrirani pristup upravljanju biološkim opasnostima u lancu proizvodnje svinjskog mesa i proizvoda od svinjskog mesa u kontinuumu farma–klanica–prerada mesa–distribucija

Nikola Betić, Ivana Branković Lazić, Ivan Nastasijević

*A p s t r a k t:* Obim proizvodnje svinjskog mesa ima trend rasta na teritoriji EU, uz trend rasta proizvodnje pristune su i biološke opasnosti koje utiču na bezbednost hrane. *Salmonella* spp, *Yersinia enterocolitica*, *VTEC*, *Toxoplasma gondii*, *Trichinella* spp. i *L. monocytogenes* su prepoznati kao najvažniji biološki agensi koji utiču na zdravlje ljudi, poreklom od svinjskog mesa i proizvoda od svinjskog mesa. Neophodan je integrirani monitoring i kontrola bioloških opasnosti, kao i kontrola antimikrobne rezistencije (AMR) komensalnih i zoonotskih bakterija kod ljudi, životinja i hrane kao važan izvor informacija za poboljšanje bezbednosti hrane i zaštite potrošača. Putevi infekcije i kontaminacije svinjskog mesa su različiti i mogu se desiti u svim procesima proizvodnje od farme do finalnog proizvoda. Bezbednost hrane bi trebalo bazirati na dobroj proizvodjačkoj/higijenskoj praksi i HACCP sistemu, koji od subjekta u poslovanju hranom zahteva da prepozna opasnost koja može da utiče na bezbednost hrane, da opasnost kontroliše i eliminiše. Integrirani sistem u proizvodnji svinjskog mesa i proizvoda od svinjskog mesa mora da se bazira na identifikaciji i sledljivosti u kontinuumu farma–klanica–prerada–distribucija.

**Ključne reči:** biološke opasnosti, lanac bezbednosti hrane, proizvodnja svinjskog mesa, integrirani pristup.

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# Element contents in muscle tissue of Prussian carp from different lakes in an urban area

Dragoljub Jovanovic<sup>1</sup>, Vlado Teodorovic<sup>1</sup>, Radmila Markovic<sup>1</sup>, Milena Krstic<sup>1</sup>, Jelena Ciric<sup>2\*</sup>, Milan Z. Baltic<sup>1</sup>, Branislav Baltic<sup>2</sup>, Dragan Sefer<sup>1</sup>

**Abstract:** The aim of this study was to determine the content of some elements (Pb, Cd, Hg, Fe, Cu, Zn, As) in muscle tissue of Prussian carp (*Carassius auratus gibelio*) from seven different lakes in the Belgrade region, Serbia. Concentrations of Pb, Hg and As in fish muscle tissues from all examined lakes were under the maximum residual levels prescribed by the European Union (EU) and the maximum allowed concentrations (MAC) for Serbia. In all investigated fish, levels of Cd exceeded maximum allowed concentrations (0.05 mg kg<sup>-1</sup>). Data on the finding of elements in fish speak concurrently about the safety of fish as food and are good indicators of environmental pollution.

**Keywords:** Elements, Prussian carp, Lakes, Contamination.

## Introduction

In human diets, fish present a significant place as a biologically valuable food. Because of their nutritional value, fish meat and fish products have an important role in proper nutrition and health protection for all categories of people (Sidhu, 2003). Fish's nutritional value is primarily reflected in easily digestible proteins that have a high content of essential amino acids, the high content of  $\omega$ -3 polyunsaturated fatty acids, and low fat and cholesterol contents. Fish also contains other nutritional ingredients (vitamins, minerals) important for human nutrition (Kminkova, 2001). On the one hand, fish has favorable influences on brain activity, prevention and reduction of cardiovascular diseases, inflammatory joint processes, and prevention and treatment of cancer (Mayneris-Perxachs et al., 2010). On the other hand, fish, like other foods, can endanger consumers' health when it contains harmful chemical compounds. Hazards from the group of industrial-chemical pollutants include toxic metals and organic chemical contaminants (Elia et al., 2007). In order to protect consumer health, the amount of toxic metals in food and in fish is limited by regulation in most countries. The regulations primarily refer to mercury, lead, cadmium and arsenic, and in some

cases to other elements such as zinc, tin, copper and iron, the quantities of which are limited in in canned fish products. However, industrial development and inappropriate environmental measures have resulted in increased presence of toxic metals in the environment (Kosior et al., 2018). Increased concentrations of metals, mostly mercury, lead and cadmium, were recorded in freshwater fish from open waters, as a result of the fact that metal concentrations in water are positively correlated with their concentrations in fish tissue (Castro-Gonzalez et al., 2008). Numerous studies indicate that the concentrations of these metals in different types of fish are beyond permitted levels (Has-Schön et al., 2015, Andreji et al., 2006, Jovanovic et al., 2017), and so could endanger human health. High contents of toxic metals in fish can reduce the cardiovascular effect of fish consumption, and these compounds are associated with serious adverse effects on the health of children and adults (Yoshizawa et al., 2002).

The aims of this study were to determine the contents of seven elements in the muscle tissue of Prussian carp caught from lakes near Belgrade, Serbia, to determine whether the fish was safe as a food for human consumption, and to consider the state of the ecosystems with respect to the metals studied.

<sup>1</sup>University of Belgrade Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Republic of Serbia;

<sup>2</sup>Institute of Meat Hygiene and Technology, Kačanskog 13, Belgrade 11000, Republic of Serbia.

\*Corresponding author: Jelena Ciric, [jelena.ciric@inmes.rs](mailto:jelena.ciric@inmes.rs)

## Materials and methods

### Study Area and Sampling

Fish were collected from seven different lakes near Belgrade, Serbia. Lakes Rabrovac, Markovac, Ocaga and Grabovac are located to the south of the city, while Becmen, Veliko Blato and Mokri Sebes are positioned along the geographical longitude north of Belgrade. These locations were selected because of their proximity to important European rivers, the Sava and Danube (which both carry substantial quantities of wastewater), as well as proximity to the urban zone of Belgrade (Figure 1). The fish were caught by professional fishermen at the end of 2017.

### Analysis

In order to determine elements in 49 (seven from each lake) muscle tissue samples of Prussian carp (*Carassius auratus gibelio*), all individual fish in each fisherman's haul were identified to species level, and a random sub-sample of 7 individuals at each location was used for metal analysis. Fish were euthanized with an overdose of MS 222 (ethyl ester of p-amino benzoic acid, Sigma Aldrich), and then transported to the laboratory in a refrigerated truck. Fish were dissected, and muscle (ca. 200 g) below the dorsal fin was stored for metal analyses in

polypropylene vials previously pre-cleaned with nitric acid (10%) and rinsed three times in deionized water, and then quickly frozen and stored at  $-20^{\circ}\text{C}$ . Fish muscles were defrosted at room temperature and mechanically homogenized.

For the determination of elements (Pb, Cd, Hg, As, Cu, Fe and Zn), fish muscle portions of  $1\pm 0.001$  g were weighed out. All chemicals used were of analytical grade purity. Fish muscle was digested using a microwave closed system (MW 3000, Anton Paar GmbH, Graz, Austria). Digestion was carried out with a program suitable for preparing samples of fish meat. After digestion, the content of elements was determined by the atomic absorption spectrometry (Perkin Elmer Analyst 700 with the MHS system, Shelton, USA). Quality of analyses was controlled using the certified reference material, ERMBB422 fish muscle. The concentrations determined in the reference material were within the tolerances specified in the delivered certificate.

The element concentrations in fish meat (i.e., muscle) were compared with the maximum allowed concentrations (MAC) in fish meat for utilization in human diets, as established by the European Union (European Commission Regulation, 2006) and the Republic of Serbia (*Official Gazette of RS*, 2014). According to the EU legislation (*European Commission Regulation*, 2006), the MACs for Pb, Cd,

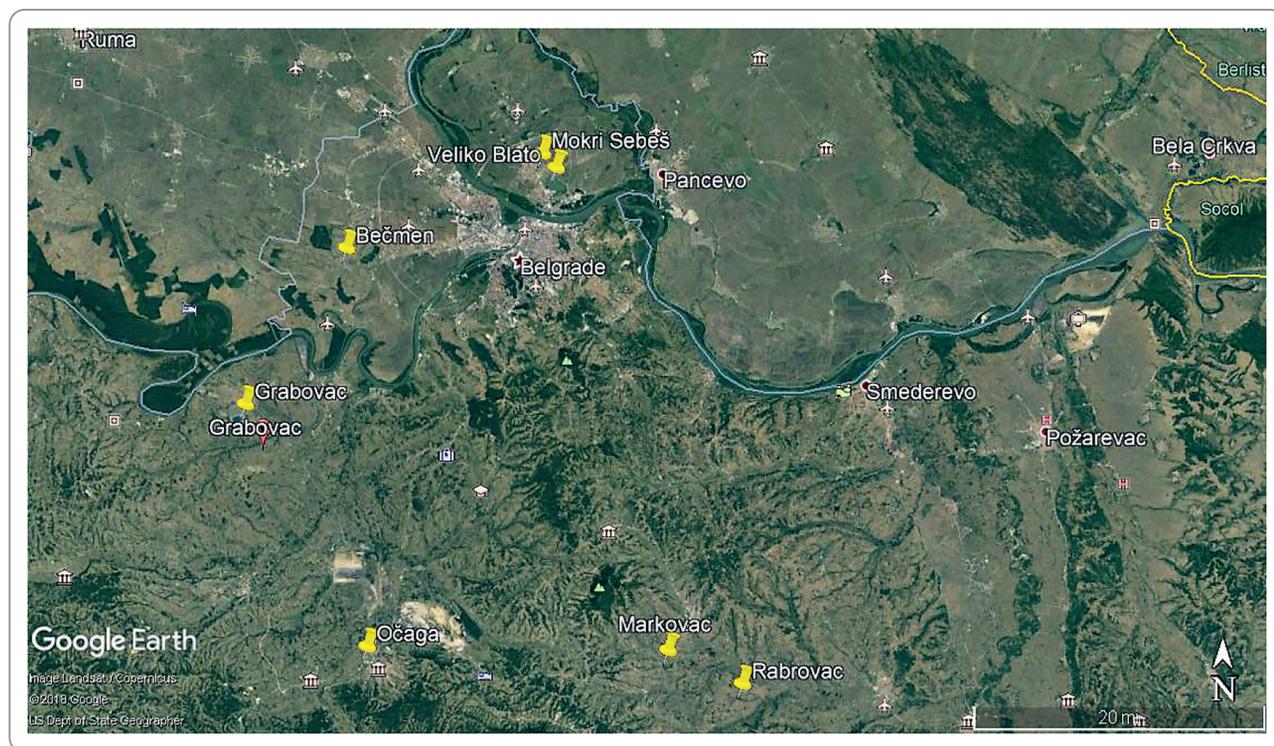


Figure 1. Map of fish collection sites ([www. https://earth.google.com](https://earth.google.com))

Hg are 0.30, 0.05 and 0.5, mg kg<sup>-1</sup> w/w, respectively. The national legislation (*Official Gazette of RS*, 2014) is in accordance with the European Commission regulation although it has an additional regulatory MAC for As, with MACs for Pb, Cd, Hg, and As being 0.30, 0.05, 0.5, and 2.0 mg kg<sup>-1</sup> w/w, respectively.

All fish muscles were collected and analyzed in duplicate, and the results are expressed as mean±standard deviation. Statistical analysis was elaborated using GraphPad Prism version 7.00 software. Statistical analysis was performed using Student's t-test to determine the significance of differences between means. A significance level of P<0.05 was applied.

## Results and Discussion

Element concentrations in muscle tissue of Prussian carp caught from three lakes north of Belgrade are presented in Table 1. The concentrations

of Pb, Hg and Cu was significantly higher in muscle tissue of Prussian carp from Veliko Blato than the other sites (P<0.05). Levels of Fe were not significant different between Becmen, Veliko Blato and Mokri Sebes (P>0.05). The Zn concentration was significantly different (P<0.05) in different sites and ranged between 5.93±0.10 mg kg<sup>-1</sup> (Mokri Sebes) and 7.91±0.26 mg kg<sup>-1</sup> (Becmen).

The concentration of lead, cadmium, mercury, arsenic, copper, iron and zinc in Prussian carp caught from Lakes Rabrovac, Markovac, Očaga and Grabovac to the south of Belgrade are shown in Table 2. Concentrations of Pb, Cd, Hg, As, Cu, Fe and Zn in muscle tissue of the Prussian carp from Rabrovac were 0.068±0.004, 0.066±0.002, 0.126±0.005, 0.202±0.005, 0.714±0.010, 7.72±0.12 and 6.44±0.10 mg kg<sup>-1</sup>, respectively. Similar metal concentrations were found in fish muscle from Lake Markovac. No significant differences were found in muscle tissue of the Prussian carp from Rabrovac

**Table 1.** Element concentrations (mg kg<sup>-1</sup>;  $\bar{X}\pm Sd$ ) in muscle tissue of Prussian carp from three examined lakes north of Belgrade

Metal concentration,	Lake		
	Bečmen	Veliko Blato	Mokri Sebeš
Pb	0.036 <sup>a</sup> ±0.004	0.043 <sup>b</sup> ±0.003	0.038 <sup>a</sup> ±0.002
Cd	0.066 <sup>a</sup> ±0.004	0.065 <sup>a</sup> ±0.005	0.059 <sup>b</sup> ±0.004
Hg	0.148 <sup>a</sup> ±0.023	0.223 <sup>b</sup> ±0.017	0.194 <sup>c</sup> ±0.008
As	0.240 <sup>a</sup> ±0.006	0.255 <sup>a</sup> ±0.033	0.276 <sup>b</sup> ±0.015
Cu	0.868 <sup>a</sup> ±0.018	0.875 <sup>a</sup> ±0.014	0.792 <sup>b</sup> ±0.012
Fe	7.76 <sup>a</sup> ±0.27	7.42 <sup>a</sup> ±0.25	7.26 <sup>a</sup> ±0.10
Zn	7.91 <sup>a</sup> ±0.26	6.45 <sup>b</sup> ±0.31	5.93 <sup>c</sup> ±0.10

**Legend:** Means (between the same elements from the different sites) within a row with the same letter are not significantly different. a, b, c P<0.05

**Table 2.** Element concentrations (mg kg<sup>-1</sup>;  $\bar{X}\pm Sd$ ) in muscle tissue of Prussian carp from four examined lakes south of Belgrade

Metal	Lake			
	Rabrovac	Markovac	Očaga	Grabovac
Pb	0.068 <sup>a</sup> ±0.004	0.064 <sup>a</sup> ±0.005	0.026 <sup>b</sup> ±0.005	0.035 <sup>c</sup> ±0.004
Cd	0.066 <sup>a</sup> ±0.002	0.061 <sup>a</sup> ±0.002	0.053 <sup>b</sup> ±0.003	0.061 <sup>a</sup> ±0.009
Hg	0.126 <sup>a</sup> ±0.005	0.119 <sup>a</sup> ±0.008	0.096 <sup>b</sup> ±0.005	0.331 <sup>c</sup> ±0.025
As	0.202 <sup>a</sup> ±0.005	0.190 <sup>a</sup> ±0.004	0.135 <sup>a</sup> ±0.003	0.278 <sup>a</sup> ±0.029
Cu	0.714 <sup>a</sup> ±0.010	0.707 <sup>a</sup> ±0.011	0.789 <sup>a</sup> ±0.021	0.667 <sup>b</sup> ±0.044
Fe	7.72 <sup>a</sup> ±0.12	7.30 <sup>a</sup> ±0.08	7.48 <sup>a</sup> ±0.68	7.00 <sup>a</sup> ±0.14
Zn	6.44 <sup>a</sup> ±0.10	6.29 <sup>a</sup> ±0.16	10.24 <sup>b</sup> ±0.45	5.32 <sup>c</sup> ±0.40

**Legend:** Means (between the same elements from the different sites) within a row with the same letter are not significantly different. a, b, c P<0.05

and Markovac ( $P > 0.05$ ). The concentrations of Pb, Cd, Hg and As were significantly lower in muscle tissue of the Prussian carp from Ocaga than concentrations of these metals from Grabovac, Markovac i Rabrovac. The major pollutants from these localities were Fe and Zn ( $P < 0.05$ ).

The level of element bioaccumulation in fish tissues depends on various biotic and abiotic factors, such as fish biological habits, chemical form of metal in the water, water temperature, water pH, concentration of dissolved oxygen in the water, as well as sex, age, body weight and physiological condition of fish (Has-Schön et al., 2015). The content of elements in fish tissues varies, and is most often examined in muscle tissue, gills, liver, digestive tract and kidneys. We decided to determine the contents of elements in fish muscle tissue because it is of the greatest interest due to its use in human nutrition, and it also speaks to the state of the environment. Additionally, the Prussian carp, as an omnivorous fish, is an excellent biological indicator of environmental pollution (Zrncic et al., 2013).

Lead in the Prussian carp muscle tissue, from fish landed from four investigated lakes south of Belgrade, showed the greatest variation among the examined elements. The lead content in the Prussian carp meat probably depends, to a large extent, on the catch location of these fish, and it was almost three-fold greater in fish from two of the southern lakes than in the carp meat from the two other southern lakes. On the other hand, north of Belgrade, the lead content in the carp meat did not vary greatly between the lakes. The lowest lead content recorded in the muscle of locally-harvested carp was from the Danube upstream (west) from Belgrade, while similar levels as ours were found in muscle tissue of carp caught in the Danube downstream of Belgrade ( $0.014 \text{ mg kg}^{-1}$ ;  $0.048 \text{ mg kg}^{-1}$ , respectively) (Milanov et al., 2016, Ivanovic et al., 2016). All measured lead contents in fish meat from the Belgrade region in the current study were significantly below the MACs of  $0.30 \text{ mg kg}^{-1}$  (Official Gazette of RS, 2014, European Commission Regulation, 2006). Higher contents ( $0.95\text{--}1.30 \text{ mg kg}^{-1}$ ) of lead were recorded in carp muscle tissue from Busko blato Lake in Bosnia and Herzegovina (Has-Schön et al., 2015), and such high levels indicate that the living world in lakes can be a good indicator of the state of the environment. High lead levels in the environment are most often derived from metallurgical fabricators, textile industry, battery production, gasoline additive (tetraethyl lead) and insecticides (based on lead arsenates). The high content of lead in the muscle tissue of all sorts of fish

can be explained by their feeding habits, as they reside at the bottom and feed on benthic organisms. Therefore, the fish are in constant contact with sediment and accumulate a relatively high amount of elements that reliably reflect the ecological state of the water environment (Wei et al., 2014).

The highest content of cadmium was determined in the muscle tissue of Prussian carp caught from Lakes Rabrovac and Becmen, and these levels were significantly higher than in carp from Lake Ocaga. All mean levels of cadmium in carp meat were above the MACs of  $0.050 \text{ mg kg}^{-1}$  (Official Gazette of RS, 2014, European Commission Regulation, 2006). Lower concentrations of cadmium than ours were found in the muscle tissue of Prussian carp caught in a lake in Bosnia and Herzegovina, and in the muscle tissue of Prussian carp from the Neretva river, in the order of  $0.01 \text{ mg kg}^{-1}$  and  $0.045 \text{ mg kg}^{-1}$ , respectively (Has-Schön et al., 2015). Almost identical cadmium levels,  $0.058 \text{ mg kg}^{-1}$  to  $0.067 \text{ mg kg}^{-1}$ , were determined in the muscle tissue of omnivorous fish from fishponds around Belgrade (Janjic et al., 2015). The high content of cadmium in fish muscle can be attributed to the migration of this elements from sludge to fish, because it is known that bottom-feeding fish looking for food plunge into sludge. This is particularly pronounced if cadmium is released from industrial systems such as those located near Belgrade, i.e., Obrenovac south, and Pancevo north of Belgrade, which can lead to high contents of metals like cadmium both in water and in living organisms including fish. Also, as cadmium is present in fertilizers (especially phosphate), and is an integral part of fungicide (in the form of cadmium succinate), increased cadmium content in muscle tissue of fish can be of plant origin and is due to agricultural activities near water (Dubovina et al., 2018). Cadmium accumulates at all levels of the food chain and its occurrence in the environment causes pollution of water and soil, and enters the food chain through the root of the plants (Galal, 1993). The cadmium levels obtained in our current study from the Belgrade region varied widely, indicating the current concentrations of this element could be increasing, and it would be useful to introduce cadmium monitoring in fish, rivers and sediments around Belgrade.

In all examined Prussian carp, mercury contents varied only slightly, except for fish from Lake Grabovac ( $0.331 \pm 0.025 \text{ mg kg}^{-1}$ ), south of Belgrade. As with lead, larger variations in mercury were observed in fish from the southern lakes than in those from the northern lakes. In other recent

research on mercury in Prussian carp muscle, in the Danube, upstream from Belgrade, higher mercury levels ( $0.327 \pm 0.110 \text{ mg kg}^{-1}$ ) were established (Milanov *et al.*, 2016). Also, in the Danube downstream of Belgrade, similar mercury concentrations ( $0.24 \pm 0.05 \text{ mg kg}^{-1}$ ) to ours were recorded in the muscle tissue of omnivorous fish, which indicates that the location of the catch affects mercury levels in fish (Ivanovic *et al.*, 2016). Regardless of the moderate or low level of contamination of these waters with mercury, the content of this element can be significant in fish meat. This can be interpreted as reflecting the ability of mercury to concentrate in fish flesh. The biological regulation of mercury in fish and its detoxification is such that the content of mercury in fish increases mainly with the age, so the meat of older fish tends to have accumulated larger amounts of mercury. Increased mercury levels in the environment are mainly of anthropogenic origin and due to industrial discharges. Although mercury is one of the most toxic metals in the environment, in all our examined samples of fish meat, recorded concentrations were below the MACs prescribed (Official Gazette of RS, 2014, European Commission Regulation, 2006).

The highest concentrations of arsenic in Prussian carp muscle were found in fish from lakes north of Belgrade, except Lake Grabovac, and these levels were statistically significantly higher ( $P < 0.01$ ) than in fish from the lakes south of Belgrade. It is interesting to note that arsenic and mercury concentrations in the fish from the four lakes south of Belgrade decrease in the same order (Grabovac > Rabrovac > Markovac > Ocaga). On the other hand, muscle tissue of carp from a lake near Belgrade contained a proximate level of  $0.378 \text{ mg kg}^{-1}$  of arsenic (Janjic *et al.*, 2015). We noticed dissipation of our established arsenic concentrations in the carp, indicating the catch location has an impact on the arsenic in muscle tissue of the fish.

Comparing copper concentrations in the fish, no statistically significant differences were found, although higher concentrations were measured in fish from northern lakes than in those from southern lakes. This uniformity of copper levels could be linked to the important role this metal plays in all living organisms, including fish. The copper contents in Danubian Prussian carp muscle were almost identical to ours and were in the interval  $0.809 \text{ mg kg}^{-1}$  to  $0.824 \text{ mg kg}^{-1}$  (Jovanovic *et al.*, 2017).

Zinc and copper were present in muscle of Prussian carp from lakes south of Belgrade in decreasing concentrations in the order Ocaga >

Rabrovac > Markovac > Grabovac. The highest concentrations of zinc ( $10.24 \pm 0.45 \text{ mg kg}^{-1}$ ) were in Prussian carp muscle from Lake Ocaga, and concentrations here were significantly higher ( $P < 0.01$ ) than in fish from other lakes. The zinc contents show that catch location has a major influence on the greatly differing content of this metal in the fish meat. Multiple-fold higher zinc concentrations were found in muscle tissue of Prussian carp ( $82.5 \pm 2.0 \text{ mg kg}^{-1}$ ) and bream ( $62.5 \pm 1.3 \text{ mg kg}^{-1}$ ) compared to our results (Pantelica *et al.*, 2012). Higher zinc concentrations were recorded in muscle tissue of Prussian carp from the Danube near Belgrade, with levels ranging from  $10.26 \text{ mg kg}^{-1}$  to  $11.16 \text{ mg kg}^{-1}$  (Jovanovic *et al.*, 2017). Zinc in the environment most often comes from industrial zones, and in the Earth's crust, it is a constituent of rock, minerals and certain carbonate sediments.

Iron concentrations, regardless of the location of the catch, did not greatly vary in our Prussian carp muscle tissue. Iron concentrations were in the range of  $7.00 \pm 0.14 \text{ mg kg}^{-1}$  (Grabovac) to  $7.76 \pm 0.27 \text{ mg kg}^{-1}$  (Becmen), and there were no statistically significant differences between lakes. Proximate iron contents in muscle tissue of Prussian carp from the Danube near Belgrade ranged from  $7.25 \text{ mg kg}^{-1}$  to  $8.05 \text{ mg kg}^{-1}$  (Jovanovic *et al.*, 2017). Iron, copper and zinc do not have defined MACs in fresh fish meat in Serbia or the EU (EU Regulations, Regulation of Republic of Serbia).

## Conclusions

The high concentrations of non-essential elements (cadmium) can be explained by anthropological influences, largely industrial development, discharge of waste water, and the subsequent increased element content in sewage sludge. On the other hand, the contents of lead, mercury and arsenic in fish muscle were below the MACs prescribed in Serbia and the EU. The Official Gazette of the Republic of Serbia does not define MACs for Cu, Fe or Zn in fresh fish, so the results obtained by quantifying these metals can be interpreted freely. Results of this study show that continuous monitoring of the state of aquatic ecosystems is required, along with the introduction of efficient wastewater treatment and control of potential industrial polluters. On the territory of Belgrade and throughout Serbia, these necessary environmental protections are basic measures that would improve the existing situation in the water ecosystems of lakes near Belgrade that are important for fishing.

# Sadržaj elemenata u mišićnom tkivu babuške iz različitih jezera sa urbanog područja

Dragoljub Jovanović, Vlado Teodorović, Radmila Marković, Milena Krstić, Jelena Ćirić, Milan Ž. Baltić, Branislav Baltić, Dragan Šefer

*Apstrakt:* Cilj ovog ispitivanja bio je da se utvrdi sadržaj pojedinih elemenata (Pb, Cd, Hg, Fe, Cu, Zn, As) u mišićnom tkivu babuške (*Carassius auratus gibelio*) iz sedam različitih jezera u regionu Beograda, Srbija. Koncentracije Pb, Hg i As u mišićnom tkivu ribe iz svih ispitivanih jezera bile su ispod maksimalno dozvoljenih novoo propisanih od strane Evropske unije (EU) i maksimalno dozvoljenih koncentracija (MAC) za Srbiju. Kod svih ispitivanih riba, nivo Cd je bio iznad maksimalno dozvoljene koncentracije (0,05 mg kg<sup>-1</sup>). Podaci o sadržaju elemenata u mišićnom tkivu riba istovremeno govore o bezbednosti ribe kao hrane i dobar su pokazatelj zagađenja životne sredine.

**Ključne reči:** Elementi, babuška, jezera, kontaminacija.

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# Consumer attitude and quality assessment of cooked sausages in Serbia

Vesna Djordjevic<sup>1</sup>, Tatjana Baltic<sup>1</sup>, Danijela Sarcevic<sup>1</sup>, Ivana Brankovic Lazic<sup>1</sup>, Jelena Ciric<sup>1\*</sup>

**Abstract:** The aim of this study was to determine consumer attitude and quality assessment of cooked sausages in Serbia. An eleven question survey was developed on an online survey website to analyse consumer attitude and quality assessment of cooked sausages. The questionnaire was completed by 1,959 respondents from October to December 2018. All participants were interviewed using a standardized questionnaire. The questions were related to consumption and preference for cooked sausages (hot dog, chicken frankfurters, Srpska sausages, Tirolska sausages, Pariska sausages, Ekstra sausages, Mortadella sausages and Šunkarica sausages). The results show the majority of Serbian consumers consume chicken frankfurters about once a month or less and Šunkarica sausages about once a week or less (61.85% and 28.48%, respectively). Consumers tended to strongly agree that they consume cooked sausages on any day of the week. Generally, consumers believe there are significant differences in the quality of imported and domestic cooked sausages.

**Keywords:** sausages, Serbia, consumption, consumer knowledge.

## Introduction

Consumer eating habits in Serbia suggest frequent use of cooked meat products in people's diets (Janjic *et al.*, 2016). In the group of cooked sausages, there are a large number of products that differ by composition, the comminution grade of the stuffing, and the type and diameter of the casings. Based on the comminution grade of the stuffing, raw and cooked sausages are divided into finely chopped cooked sausages, coarsely chopped cooked sausages, cooked sausages with meat pieces and meat loaves (Vukovic, 2006). According to the current regulation on the quality of ground meat, meat preparations and meat products (*Official Gazette of the Republic of Serbia*, 2015; 2017), coarsely chopped cooked sausages are produced and placed on the Serbian market under the names Srpska sausage, Pariska sausage, Ekstra sausage, Tirolska sausage, Sausage of the Kranjska type, Mortadella and ham.

In addition to local products, cooked sausages from foreign markets, which consumers often consume, are also available on the market in Serbia. This affects the competitiveness of local meat industry producers. Serbian companies engaged in meat production and processing face numerous problems, such as expensive raw materials on the domestic

market, constant price and livestock availability fluctuations, and serious competition from EU-based companies subsidized by their own countries. These factors have led to the fact that local meat industry companies are uncompetitive and exposed both on the domestic and world markets (Mitic *et al.*, 2018).

The most important target for the product manufacturer is the production of competitive products with high consumer acceptance. One of the ways to achieve the set goal is to implement modern methods of sensory analysis which, in addition to the product quality evaluation, can also be successfully used for correction of its organoleptic properties according to consumer preferences. Thus, sensory examinations are carried out using the methods of consumer evaluation based on the study of consumer impressions of organoleptic characteristics of a given product (Meilgaard *et al.*, 1999; Sarcevic *et al.*, 2013). Various methodologies of the approach to investigating consumer preferences and analysis of the obtained results help, not only to understand the consumer attitudes toward a product, but also to reveal the main purchase motivation (Resano *et al.*, 2009; Santa Cruz *et al.*, 2003).

In the last few years, however, these topics have received even more attention. We are currently witnessing many debates on the quality of imported

<sup>1</sup>Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade 11000, Republic of Serbia.

\*Corresponding author: Jelena Ciric, [jelena.ciric@inmes.rs](mailto:jelena.ciric@inmes.rs)

food, especially, around the presumption that imported meat and meat products are of lower quality than local products. There is no doubt that developing the meat products and processing sector will both improve the quality of food and increase the confidence of consumers (Mitic *et al.*, 2018).

The objective of this paper was to identify the core issues affecting consumer's confidence when buying cooked sausages and to explore the impact of the same imported products on consumers' purchasing and consumption behaviour. The results of this investigation can influence the competitiveness of local meat producers on the market in Serbia.

## Materials and methods

### *Research design and sampling*

Data were collected from October to December 2018 in Serbia. The final sample included 1,959 participants. All participants consumed any of the cooked sausages (i.e. hot dog, chicken frankfurters, Srpska sausages, Tirolska sausages, Pariska sausages, Ekstra sausages, mortadella sausages and Šunkarica sausages) at least one to five times every six months. Participants were recruited randomly. They were briefly informed about the aim of the study. Data were collected with the consent of family members and from consumers who had volunteered to participate in this research. Consumers were not offered any compensation for their participation in the survey, and the obtained answers and collected data were anonymous.

In order to gain an insight into how best to construct and conduct this survey, the team met with several researchers. The questionnaire was prepared in consultation with researchers who have experience in the collecting and processing of data obtained by questionnaire. Researchers discussed the survey structure, analysed the sample questions, which had already been drafted, and the process by which the survey should be conducted.

### *Questionnaire and scaling*

For this study, a standardized questionnaire was used in the form of an interview. The revised questionnaire was divided into three sections:

1. Demographic data of respondents (gender, age, education, dwelling, employment status, number of people in household, number of children under 10 years of age);
2. Unless otherwise specified, all the items mentioned below were measured on a five-point scale ranging from 'strongly disagree' (=1) to 'strongly agree' (=5). Also, consumption frequency of cooked sausages was measured. Consumer attitudes towards cooked sausages consisted of three distinct factors: (1) Cooked sausages are an important part of my family nutrition, (2) Domestic cooked sausages are of poor quality and (3) I believe that there are significant differences in the quality of imported and domestic cooked sausages.
3. Reported purchase behaviour was based on these pre-chosen factors: price, quality, colour, freshness, fat content, salt and country of origin. For each of these factors, consumers were asked to score on a 5-point scale from strongly agree to strongly disagree that it affects their purchasing decisions at retail.

### *Statistical analysis*

Statistical analysis was elaborated using GraphPad Prism version 7.00 software. Statistical analysis was performed using Student's t-test to determine the significance of differences between means. A significance level of  $P < 0.05$  was applied.

## Results and discussion

Table 1 summarizes the sociodemographic profile of the respondents and Table 2 provides an overview of the habits of cooked sausage consumption in the respondents' households. Among the 1,959 respondents, 61.87% were males and 38.13% females. The largest proportion (45.02%) of the interviewed householders was aged 35–49 years. Almost 37.82% of the consumers had university undergraduate education, while 4.13% of those interviewed were employed in meat production.

The respondents' cooked sausage consumption habits/frequency in households are presented in Figure 1. The figure shows that consumers strongly agree and agree that they eat cooked sausages at any day of the week and at least once a week. Significant differences ( $p < 0.5$ ) were found between answers.

The data presented in Figure 2 demonstrate that consumers strongly disagree that cooked sausages are important part of their nutrition. On the other hand, most consumers strongly agree there are significant differences in the quality of imported and domestic cooked sausages. The quality of cooked sausages was the primary attribute

**Table 1.** Consumer characteristics (% of respondents, n=1,959)

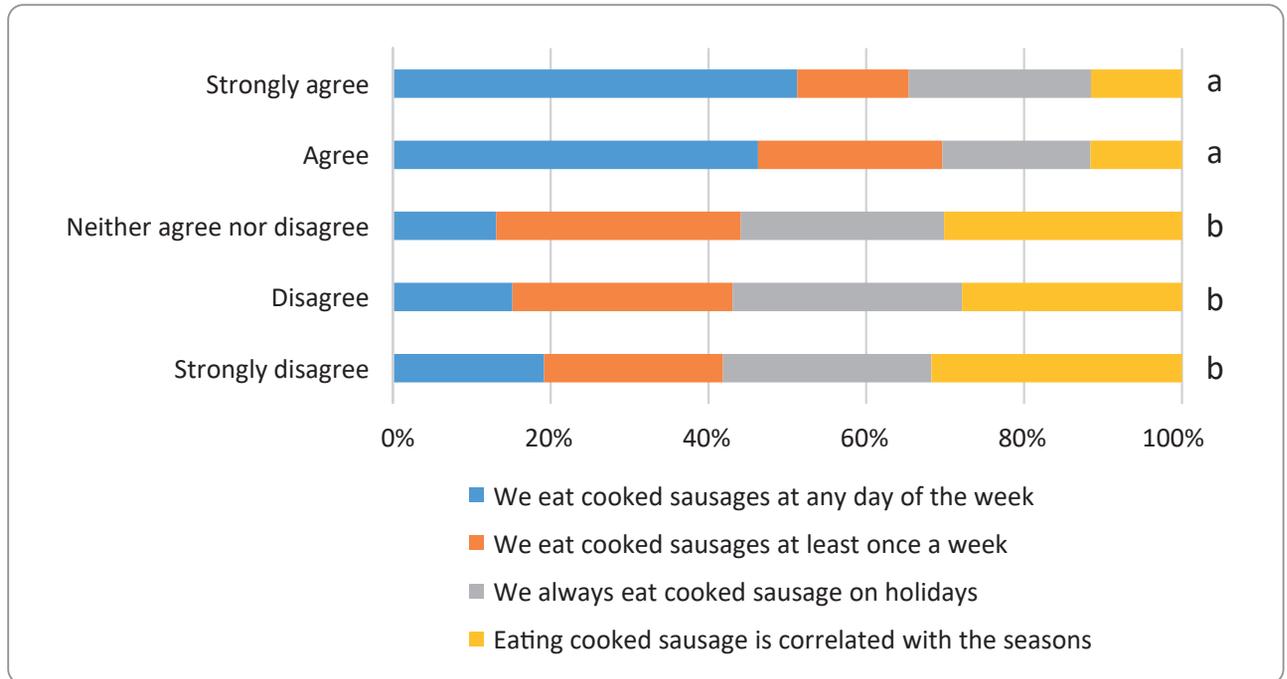
<b>Gender</b>	Female	38.13
	Male	61.87
<b>Age</b>	13–17 years	0.15
	18–24 years	6.74
	25–34 years	40.89
	35–49 years	45.02
	50–64 years	6.13
	≥ 65 years	1.07
<b>Education</b>	Elementary school or equivalent	1.23
	High school education	29.86
	Short-cycle higher education	14.24
	Undergraduate	37.82
	Master	15.01
	PhD	1.84
<b>Dwelling</b>	City	88.21
	Rural	11.79
<b>Profession (Employed in meat production)</b>	Yes	4.13
	No	95.87
<b>Number of children in the household</b>	1–6 years	35.07
	7–14 years	23.43
	No children	41.50
<b>Number of person in the household</b>	1	7.66
	2–3	48.70
	4–5	39.36
	5 or above	4.59

**Table 2.** Consumption frequency of cooked sausages in Serbian household (% of respondents, n=1,959)

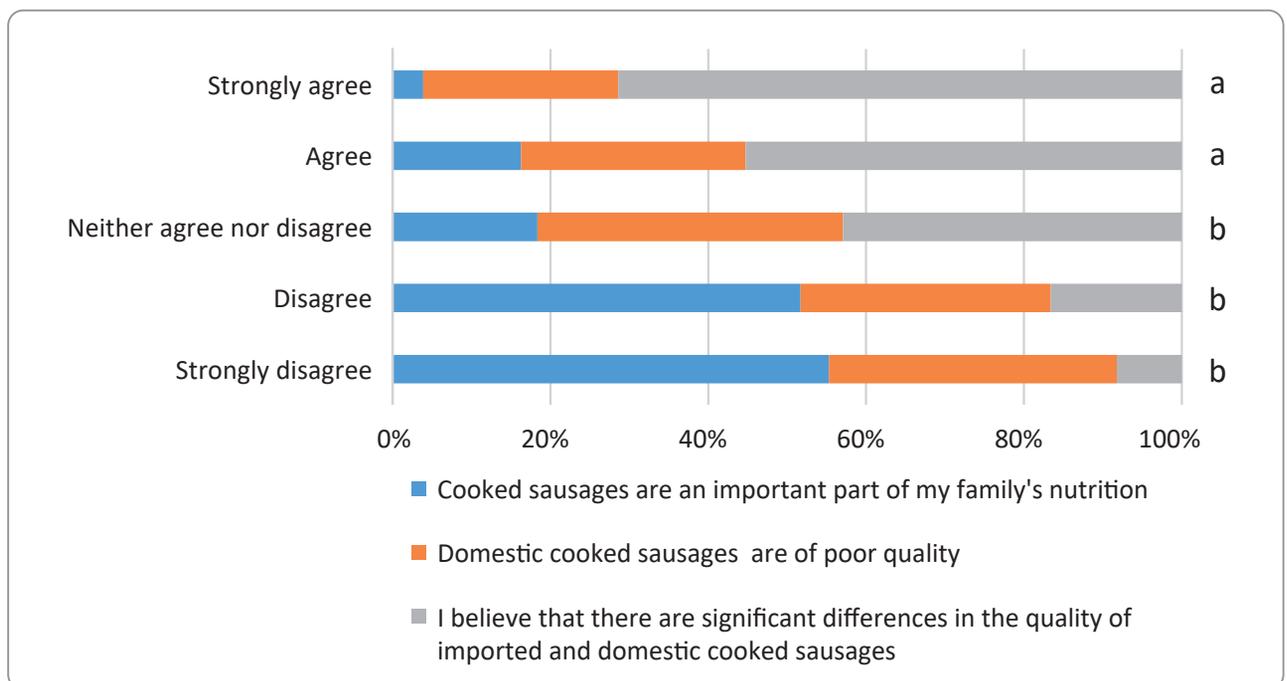
	Type of cooked sausages							
	Hot dog	Chicken frankfurters	Srpska sausages	Tirolska sausages	Pariska sausages	Ekstra sausages	Mortadella sausages	Šunkarica sausages
Never	32.62	3.68	54.21	70.90	68.91	72.43	42.27	20.52
Less than once a month	36.45	61.85	31.24	20.52	20.21	19.75	33.69	33.38
Less than once a week	24.04	24.81	13.02	7.20	9.19	7.20	20.21	28.48
At least 1–2 a week	5.30	6.13	1.23	1.00	1.50	0.40	3.52	14.70
At least once in two days	1.59	3.53	0.30	0.38	0.19	0.22	0.31	2.92

for consumers' buying behaviour (Figure 3). Also, Figure 3 shows the consumers perceived different factors were important when considering the purchase of cooked sausages. The majority of the consumers strongly disagreed that price is an important parameter.

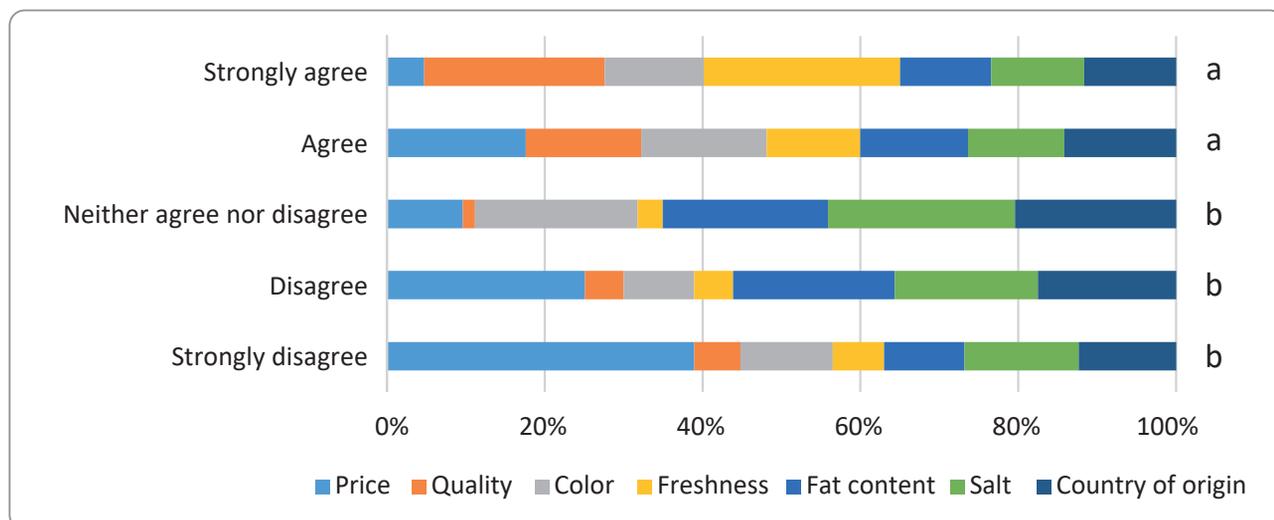
In particular, in terms of sensory properties, quality and freshness were the most important factors followed by colour and salt content (Girolami *et al.*, 2014). These attributes were also rated as the most influential in a previous study conducted on consumer liking of other cooked sausages (Mitic *et*



**Figure 1.** Frequency of cooked sausage consumption in households (n=1,959). The different superscripts <sup>a-b</sup> indicate significantly different levels of agreement at the 0.05 level.



**Figure 2.** Commitment to buying cooked sausages (n=1,959). The different superscripts <sup>a-b</sup> indicate significantly different levels of agreement at the 0.05 level.



**Figure 3.** Reported buying behaviour for cooked sausages (n=1,959). The different superscripts <sup>a-b</sup> indicate significantly different levels of agreement at the 0.05 level.

al., 2018). According to Moskowitz (1995), it is not sufficient to motivate consumers with high quality standard information without adequate sensory properties in the sausage they are purchasing. Numerous authors (e.g. Pieniak et al., 2009) showed that the traditional character or image of local food is a relevant element affecting consumer purchasing decisions. Accordingly, in this study the geographical origin was an important consumer behaviour determinant.

## Conclusion

The three most influential aspects affecting sausage consumer choice were perceived quality, salt content and freshness. This research could provide a novel approach (i.e. identification of the main aspects affecting consumers' purchase decisions and verification of product compliance to the identified determinants) to be followed for the study and promotion of other cooked sausages worldwide.

## Stav potrošača i procena kvaliteta barenih kobasica u Srbiji

Vesna Đorđević, Tatjana Baltić, Danijela Šarčević, Ivana Branković Lazić, Jelena Ćirić

**Apstrakt:** Cilj ove studije je bio utvrditi stav potrošača i procenu kvaliteta barenih kobasica u Srbiji. Istraživanje sa 9 pitanja u vidu ankete bilo je dostupno preko internet stranice, kako bi se analizirao stav potrošača o kvalitetu barenih kobasica. Upitnik je od oktobra do decembra 2018. godine popunilo 1.959 ispitanika. Pitanja su se odnosila na barene kobasice (hrenovke, pileće viršle, srpske kobasice, tirolske, pariske kobasice, Ekstra kobasice, kobasice Mortadela i kobasice Šunkarica); zatim o navikama konzumiranja barenih kobasica; tri pitanja su se odnosila na kupovinu barenih kobasica i jedno pitanje se odnosilo na ponašanja potrošača za kupovinu barenih kobasica. Rezultati pokazuju da većina srpskih potrošača konzumira pileće viršle otprilike jednom mesečno ili manje, a Šunkaricu otprilike jednom nedeljno ili manje (61.85% i 28.48%, pojedinačno). Potrošači su se u potpunosti slagali da konzumiraju barene kobasice bilo kog dana u nedelji. Potrošači su se uglavnom izjasnili da postoje značajne razlike u kvalitetu barenih kobasica iz uvoza i sa domaćeg tržišta.

**Ključne reči:** kobasice, Srbija, potrošnja, poznavanje potrošača.

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# Food waste management — reducing and managing food waste in hospitality

Natasa Kilibarda<sup>1</sup>, Filip Djokovic<sup>1</sup>, Radmila Suzic<sup>1</sup>

*Abstract:* Food wastage occurs along the entire food chain, from field to table. As much as it is an ethical issue on one hand, it also leads to economic losses and has negative impacts on the environment. Food wastage is, therefore, a significant problem for modern society and the first step in solving it is to identify and understand the reasons for its emergence in each part of the food chain and specific sectors — in this case, the hospitality sector. In order to create practices and recommendations aimed primarily at preventing food wastage, food waste categorization and quantification is essential. This is not that simple, especially in the hospitality sector, given the uneven production of food, and the specific and diverse ways of running a business in this sector. What is certain is that food waste management should be an integral part of management in the hospitality sector; primarily because an effective food safety management system is the starting point for implementing most of the practices that lead to both safe final product and reduction of food waste.

**Keywords:** food wastage, sustainability, food service, measurement, prevention.

## Introduction

The global challenges the world of today faces are population growth, climate change and land use pressure (Philippidis *et al.*, 2019). According to the United Nations Food and Agriculture Organization (FAO), the amount of food discarded daily around the world is one-third of the total food produced for human consumption, which is approximately 1.3 billion tonnes of food per year (Gustavsson *et al.*, 2011). In monetary terms, this is equivalent to US\$ 750 billion. This information bears even greater significance if the fact that in 2018 there were more than 820 million hungry people in the world is taken into account (FAO, IFAD, UNICEF, WFP and WHO, 2019). However, food wastage is not only a complex ethical issue for individuals, as was believed until recently, but it is also an environmental problem which has a negative impact on the global economy. It has been confirmed that food wastage leads to increased emissions of harmful gases, and to water and soil wastage, subsequently impairing biodiversity (FAO, 2013a; FAO, 2014; Betz *et al.*, 2015; Wang *et al.*, 2017). For example, intensive agriculture, without the possibility of field restoration, decreases soil fertility, which in turn leads to more frequent use of artificial fertilizers, increasing the level of environmental pollution and resulting in the loss

of arable land. In 2007, about 1.4 billion hectares of land (corresponding in size to Canada and India together) were used to produce food on a global level. Since one third of the food produced is wasted, one third of the total arable land is unnecessarily exposed to exploitation by intensive agriculture, which undeniably, leads to soil degradation. Food wastage also affects climate change, since none of the stages of food production, from fertilizer production to transport of produced food, can occur without the use of fossil fuels (oil). Furthermore, when discarded into landfill, food decomposes under anaerobic conditions, followed by the emission of the greenhouse gas, methane. In 2007, the global carbon footprint of food wastage was estimated at 3.3 Gtonnes of CO<sub>2</sub>eq, which is twice the amount of greenhouse gas emissions of all US road transportation in 2010 (FAO, 2013b). This means food wastage accounted for 8% of global greenhouse gas emissions that year (FAO, 2013b).

Food wastage is also one of the factors with a strong impact on biodiversity, which indirectly increases the need for new farmland. Due to the need for more food (despite food wastage), there is increasing deforestation (74% of total global annual deforestation) and formation of farmland in wilderness areas, which leads to extinction of wildlife. Additionally, to offset the need for

<sup>1</sup>Singidunum University, 32 Danijelova st., 11010 Belgrade, Republic of Serbia.

**Corresponding author:** Kilibarda Natasa, [nkilibarda@singidunum.ac.rs](mailto:nkilibarda@singidunum.ac.rs)

agriculturally-produced food, water resources are being depleted through uncontrolled fishing (FAO, 2013b).

Given the multiple negative impacts of food wastage on global development, it is clear this impact can only be reduced by implementing strategies to limit the amount of food waste generated. First of all, this requires the development of awareness among individuals, the application of best practices and responsible behaviour, and the involvement of the scientific community and competent authorities (Janjić *et al.*, 2019). Also, food business entities must develop an awareness of the amount of food wastage they produce and its impact at the global level. A concerted effort by all involved would contribute to the development of a society that meets human needs by utilizing the available resources, without further compromising natural systems and the environment. This is the only way to ensure the long-term existence of human society and its environment (Tekin and Ilyasov, 2017).

Food wastage exists along the entire food chain, starting with agricultural production, post-harvest treatment and storage, and it continues through food production, distribution, consumption and end-of-life (FAO, 2013b), i.e. it covers everything from field to table, including the hospitality sector. However, in order to understand the scope of this problem, the terms food loss and food waste must be distinguished. According to FAO, (2013a): “Food loss refers to the decrease in mass (dry matter quantity) or nutritional value (quality) of food that was originally intended for human consumption”, while “food waste refers to food deemed appropriate for human consumption being discarded, regardless of whether it is kept beyond its expiry date, or left to spoil”. That is why, when it comes to food waste in general, regardless of its occurrence and its place in the food chain, the term food wastage is used. “Food wastage refers to any food lost by deterioration or by being discarded”. Thus, the term “wastage” encompasses both food loss and food waste (FAO, 2013a). Food loss occurs mostly during the earlier phases of the food chain, mainly in developing countries. This is due to the limited post-harvest infrastructure and underdeveloped technologies (Parfitt *et al.*, 2010; Dorward, 2012; Kummu *et al.*, 2012; Wang *et al.*, 2017). In contrast, food waste occurs mainly in developed countries and in the consumption phase, so primarily is the responsibility of consumers, reflecting their behaviour and attitude towards food (Stenmarck *et al.*, 2016; Reynolds *et al.*, 2019). For instance, in sub-Saharan Africa, more than 90%

of food waste occurs in the pre-consumption phase (Gustavsson *et al.*, 2011), while, on the other hand, in Europe, up to 53% of food waste is from households at the consumption phase (Stenmarck *et al.*, 2016). The household sector contributes most to the generation of food waste (47 million tonnes  $\pm$  4 million tonnes), followed by the food processing sector (17 million tonnes  $\pm$  13 million tonnes). These two sectors are said to be responsible for 72% of EU food waste. The remaining 28% of food waste is comprised of 11 million tonnes (12%) of waste that comes from food service, 9 million tonnes (10%) from primary production, and 5 million tonnes (5%) from wholesale and retail (Stenmarck *et al.*, 2016). The food service, which includes hospitality sector, is third in terms of the amount of food waste produced, and so is a significant contributor to this problem that can and must be influenced.

### Food Waste Generation in the Hospitality Sector

Due to globalization, there has been a worldwide increase in living standards and an increase in *per capita* earnings, leading to growth in the hospitality sector (Pirani and Arafat, 2014; Kaur, 2017; World Tourism Organization, 2019). Food preparation businesses in the hospitality sector include hotels, restaurants, cafes, bars, sandwich shops and similar businesses, providing takeaway food and/or sit-down meals on the premises (Pirani and Arafat, 2014). With the development of the hospitality sector, the number of food preparation businesses has also increased, proportionately leading to a greater amount of food waste generated (Ball and Taleb, 2011; Pirani and Arafat, 2016; Filimonau and De Coteau, 2019). The 21<sup>st</sup> century, on the other hand, has led to changes in consumers’ life styles and habits (Sarcevic *et al.*, 2018). Due to the lack of time to prepare meals at home, people increasingly resolve to purchase takeaway meals or dine outside the home in restaurants (von Massow and McAdams, 2015; Kilibarda, 2019a).

The only way to solve the problem of food waste, which has become a problem of modern society, is to understand and identify the reasons for its occurrence in every part of the food chain, and identify the specific sectors which contribute to its generation. For this reason, numerous studies have been conducted with the aim of categorizing and quantifying food waste.

Food waste generated in the hospitality sector can be divided into two categories, depending

on whether it is generated before or after the food is consumed. Pre-consumer food waste occurs during the procurement and storage of purchased raw materials (storage/purchase waste), then during the food preparation (preparation waste), and food exposure (as a result of overproduction) (Baldwin, 2015; Betz et al. 2015; Costello et al., 2016; Pirani and Arafat, 2016, Kilibarda, 2019b). Post-consumer waste refers to leftovers on the plate itself. Waste generated at this stage is also called plate food waste (Costello et al., 2016) and is defined as food purchased by a consumer and subsequently not eaten (Costello et al., 2016). Overproduction waste could also be seen as post-consumer waste since it refers to food that, although not sold to the consumer, is prepared with this intention (Costello et al. 2016). Food waste can also be classified depending on whether it is edible or inedible, and whether it can be prevented or not (WRAP, 2009; Parfitt et al., 2010). Edible food waste refers to the waste resulting from excessive amounts of prepared food, spoiled food, improper processing of food, expired food, or food leftovers. This avoidable food waste results from preparing or serving food in larger quantities than necessary; it can be the result of overcooking or random mistakes (i.e. in recipes) during food preparation, which can put at risk the required quality that is expected or demanded (Betz et al., 2015; Papargyropoulou et al., 2016; Kilibarda, 2019b). The largest amount of edible, and therefore, avoidable food waste is buffet leftovers (Betz et al., 2015; Papargyropoulou et al., 2016). In the hospitality sector, avoidable food waste accounts for 56% or more of the total food waste (Papargyropoulou et al., 2016). On the other hand, inedible food waste refers to food residues such as eggshells, inedible parts of fruits and vegetables, animal bones and seafood shells, all resulting from the mechanical processing and preparation of food (Betz et al., 2015; Papargyropoulou et al., 2016). This waste type is unavoidable (Betz et al., 2015; Papargyropoulou et al., 2016). Plate food waste is most often a mix of inedible food chunks and edible surplus (Papargyropoulou et al., 2016).

In the hospitality sector, the amount of food discarded by restaurants depends on the type of service or the type of restaurant (Kilibarda, 2019b). The largest amount of superfluous food is derived from buffet-style restaurants rather than **à la carte restaurants** (Papargyropoulou et al., 2016; Pirani and Arafat, 2016). Post-consumer (plate) food waste is usually composed of side dishes (accompaniments) like salads and starchy foods such as potato, rice, pasta and bread, while main dish leftovers

are uncommon. In the process of food preparation, fruits and vegetables are the most commonly wasted foods (Silvennoinen et al., 2012; Betz et al., 2015; Papargyropoulou et al., 2016; Pirani and Arafat, 2016).

The reasons for food waste generation differ depending on the phase in which the waste is generated. In the phase of procurement and storage of basic food preparation ingredients, the most common reason for waste generation is non-compliance with good hygiene and good manufacturing practices (Kilibarda, 2019b). These defined procedures ensure safe handling of food and the food's safety and convenience, but non-compliance with temperature regimes or non-application of the first in-first out principle can generate food waste (Engstrom and Carlsson-Kanyama, 2004). Lump sum ordering can lead to poorly planned procurement or bulk procurement of food, so accumulated food, if not used up, expires and must be discarded (Baldwin, 2015). During the food preparation phase, unavoidable inedible food waste is usually generated through mechanical processing. The amount of this food waste can be larger than acceptable, especially if such processing is carried out with inadequate equipment or by unskilled personnel (Baldwin, 2015; Papargyropoulou et al., 2016). Employees' misjudgement of the expected number of customers often leads to overproduction in the food preparation phase. Therefore, cooperation and exchange of information about the number and type of customers among all hospitality sectors is very important. Lack of communication and poor coordination among sectors involved in purchase, preparation (kitchen) and serving (wait staff) also lead to generation of excessive waste (Papargyropoulou et al., 2016; Priefer et al., 2016; Kilibarda, 2019b). In the serving phase, the largest amount of discarded food occurs after food has been used in buffet style service (Silvennoinen et al., 2012; Betz et al., 2015; Papargyropoulou et al., 2016; Pirani and Arafat, 2016). This is because the rules regarding the maximum duration and temperature regime of food exposure at buffet tables are not adequately followed (Papargyropoulou et al., 2016). Also, the amount of food offered is plentiful and the price is fixed (Priefer et al., 2016), factors that stimulate consumers to overfill their plates with more food than they can eat. However, management would rather waste food than lose customers; that is, by displaying excessive quantities of food they aim to please customers and exceed their expectations (Papargyropoulou et al., 2016). All of this leads to post-consumer food

waste, which is most often caused by oversized portions (Pirani and Arafat, 2014; Priefer et al., 2016; Reynolds et al., 2019). Oversized portions require larger quantities of ingredients and are mostly the result of non-compliance with standard defined portion sizes. This plate waste also occurs when a customer judges an ordered dish does not have the expected and/or required quality (Baldwin, 2015). Finally, the amount of waste generated in the hospitality sector also depends on the season, the number of customers, days of the week, the location where the waste is generated, etc. (Merchant and Cloy, 2017).

### Food Waste Management in Hospitality

The concept of sustainable development in contemporary business conditions has become a framework for strategic decision-making in hospitality (Djokovic, 2018). It rests on three pillars of sustainability: economic, environmental and social. The economic pillar stems from the *raison d'être* of every business, to make a profit. The environmental pillar is based on conservation of the environment and all the resources available in the hospitality sector's location. The social pillar refers to all the factors that affect the quality of life and well-being of the population. All pillars of the hospitality sector's sustainable development are interdependent, since business success should be derived from the environmental protection of a particular destination (region) and improvement of the quality of life of people directly (employed in the hospitality sector) and indirectly (employed in other segments of the tourist industry, tourists and other consumers) related to this sector. At the same time, life today is characterized by the fact that the modern consumer's demands on the services offered by the hospitality sector are becoming more sophisticated and complex. According to the UNWTO (2019), tourists' motives are directly correlated with the principles of sustainable development. The hospitality sector, in accordance with these requirements, has started to adapt to the concept of sustainable development. In order for this concept to have practical implications, it is essential the strategic orientation of the hospitality sector towards environmental protection is reduced to operational management mechanisms. The state should, by a legal framework, encourage hospitality sector entities to use their businesses to improve an environmentally friendly ambience. The key areas of hospitality management, taking into the account the concept of sustainable development, include energy efficiency, the use of renewable energy

sources and waste management. Energy efficiency and the use of renewable energy sources are a priority in hospitality management due to the lowering of operating costs and reducing local pollution.

As has been pointed out, global food production leads to losses that are associated with consumers and food consumption, especially in developed countries (Racz et al., 2018). Therefore, the hospitality sector can play an important role in raising awareness of the value of food in order to globally reduce food wastage. However, waste management has become an unresolved issue for most hospitality sector entities, including those in Serbia. For example, hotels are large waste producers due to their intensive business activities. In practice, there is a daily problem of waste disposal and storage, especially in tourist destinations with a distinctive seasonal character. The most common wastes the hospitality sector produces are food and packaging (cardboard, plastic and glass packaging containing food, beverages, cleaning and cosmetic products) wastes. For hospitality sector management, the demands are great, as consumers expect satisfaction with both service and food. Simultaneously, the government's food safety standards must be fulfilled. Then, management expects increasing profits. Finally, there is growing concern for environmental conservation (Rodgers, 2005), creating yet another challenge — food waste management — for hospitality management.

### Food Waste from Prevention to Disposal

It is important for management in the hospitality sector to identify and define the reasons for the occurrence of food waste in order to create and provide practices and procedures that will prevent or reduce this waste.

The Community Strategy for Waste Management (European Parliament Council, 1989) defines the food waste hierarchy. Its basic objective is sustainable food management, the first and most desirable step of which is prevention (Papargyropoulou et al., 2014). The desirability of the other proposed steps towards achieving the sustainable objectives decreases in the order specified in the guidance. The following proposed steps include: reusing, diverting unused food for human or animal consumption, and recycling food waste *via* composting or renewable energy generation. However, the last step, and the least desirable one, is disposal of food waste in landfills (Papargyropoulou et al., 2014; Baldwin, 2015; HOTREC, 2017).

As the prevention or reduction of food waste is a priority activity in the process of waste reduction, specific recommendations should be implemented in the hospitality sector. They also integrate the principles of sustainable development in managing food waste. With the aim of responsible behaviour, the following stages should be included:

- procurement of groceries — base procurement procedures on needs, and select suppliers that can meet those needs;
- inventory management — meet appropriate best conditions for storing groceries, monitor the dynamics of grocery consumption, and keep special, dedicated records on the types and amounts of food waste generated (*Silvennoinen et al., 2012; Baldwin, 2015; Priefer et al., 2016*);
- portion control — use groceries according to food norms and efficiently use food residues from previous meals; train kitchen staff appropriately to reduce food waste. Reduce portion sizes, especially for those types of foods known to make up the largest part of plate food waste (*Engstrom and Carlsson-Kanyama, 2004; Betz et al., 2015; Papargyropoulou et al., 2016*).
- serving meals to guests — focus on the quality of the service and the competence of the wait staff. For buffet presentation, reduce the amount of displayed food, and take into account the time/temperature of warm meal exposure (*Engstrom and Carlsson-Kanyama; Betz et al., 2015; Priefer et al., 2016*);
- raise consumers' awareness of the negative effects of food waste (*Engstrom and Carlsson-Kanyama, 2004; Betz et al., 2015; Pirani and Arafat, 2016; Priefer et al., 2016; HOTREC, 2017*). Encourage consumers to take their own leftovers home for later consumption in specially prepared packages (*Zuraikat et al., 2018*);
- manage leftover food — establish procedures to ensure rational management of leftovers to minimize waste generation (give leftovers to animals, prepare and sort leftovers for employees, donate food to soup kitchens and similar institutions);
- disposal — ensure any final food waste is used in aerobic composting and establish procedures for quarterly analysis to improve waste disposal (*Awasthi et al., 2018*).

An effective food safety management system must be the basis for implementing the large number of stages mentioned. ISO 22000 is an international standard for food safety management systems that

defines the requirements of a food safety management system. This standard is based on the principles of the hazard analysis and critical control point (HACCP) system and the requirements of the ISO 9001 standard. This standard combines the recognized key elements of food safety such as interactive communication, system management, process control, HACCP principles and prerequisite programs (*Kilibarda, 2019a*). In the USA, 55–60% of all foodborne outbreaks of disease were from restaurants (*Thaivalappil et al., 2018*). In the EU Member States, the hospitality sector ranks as second in terms of foodborne disease, as confirmed by *EFSA* data (2018). In this regard, implementing ISO 22000 standard, with its primary objective of producing safe food, has a two-fold and very important benefit, since food waste is also greatly reduced when the requirements of this standard are met. Also, this standard deals with predictions and analysis of a large number of external factors and hazards that not only can endanger food safety, but can also adversely affect the production process and finances of companies and employees. Bearing in mind the need to integrate the concept of sustainable development in the hospitality sector, we emphasize the ISO 14000 (environmental management system; EMS) standards related to environmental protection. ISO 14000 is a risk management standard, dedicated to controlling the risks of environmental pollution. The EMS establishes mechanisms that, over time, reduce these risks and the number of incidents, and enhance the business's reliability in meeting its legal and other environmental requirements. This is one of the preconditions of any business run in accordance with the sustainable development goals.

In the hospitality sector worldwide, there are currently over 100 voluntary eco-labels. These eco-labels refer to various aspects of environmental management activities, including food waste management. On a global scale, the Green Key is the most recognizable and widespread eco-label to be awarded in the hospitality sector. Green Key is supported by the World Tourism Organization and the United Nations Environment Program (UNEP). The problem with eco-labels is that they are voluntary and very expensive for individual entities. In Serbia, only four hotels currently hold a Green Key Certificate. The Certified Green Restaurant is a restaurant-only eco-label granted exclusively in the USA and Canada for the time being, although there are some indications that this type of certification will be extended to other countries (*Pirani and Arafat, 2014*).

## Food Operators in Hospitality and Their Attitude Towards Food Waste Management

In addition to achieving the UN's sustainable development goals, implementing more effective practices in food logistics, storage and preparation, and hospitality businesses can minimize the costs of food waste management (*Papargyropoulou et al.*, 2014). According to *WRAP* (2013), if currently generated waste were reduced by 5%, more than £250 million would be saved in the hospitality sector

Creating a hotel management model that includes food waste management depends largely on whether the hotel belongs to a chain or is independent. If a hotel belongs to a corporate hotel chain or is a part of consortium, the establishment of a food waste management system is integrated through the business standards of that chain. The construction and furnishing of the hotel provide the infrastructure for the implementation of standards, including the training of employees in the food and beverage sector. Independent hotels are able to create and enforce their own standards, negating the ability to create a single system that will oblige hotels to have a comprehensive food management system. Therefore, the biggest challenges are related to independent hotels, which, due to material and human resources, can have limited capacity to initiate and implement food waste management standards (*Djokovic*, 2018).

However, in the hospitality sector, managers of small hotels or food business entities are often not interested in adopting and implementing eco-friendly practices, since they believe the amount of food waste they produce is insignificant. This is the biggest problem regarding this waste's negative environmental impact, since all food waste from these small entities is generally directed to landfills, which is the least acceptable solution. This attitude could be due to the lack of official guidance on the importance of reducing the food waste they generate, meaning they do not think about the issue. Also, they lack the support of competent institutions and government bodies, and therefore, they do not want to spend either their time or their money, believing effective food waste management will only cause financial losses, and will not save material resources (*Pirani and Arafart*, 2014).

In a hotel survey conducted by *Pirani and Arafart* (2014) a significant percentage of hotel kitchens (44%) were equipped with signs encouraging staff to minimize food waste. On the other hand, it is very interesting that signs of similar content, directing guests towards responsible behaviour and

awareness of food waste, cannot be found in hotel restaurants. In fact, only 7% of the surveyed hotels had conducted a campaign aimed at raising awareness among guests to act more responsibly regarding food waste generation. Also, 67% of hotels compost or plan to compost their food surpluses, while 47% of hotels donate both surplus and unused food. The most common reason for those who do not compost is the belief they do not generate enough waste, while those who do not donate admit that they were not aware of the existence of such charity programs, or they see obstacles to food donation in the food safety legislation. Food business entities, in this case, food operators in the hospitality sector, are responsible for food safety (according to General Food Law Regulation (EC) 178/2002). However, in the case of food donations, they do not wish to bear responsibility for the safety of food post-donation, which is beyond their control. For this reason, food operators actually feel forced to discard food in order to avoid being held accountable for its safety in the case of donations (*Priefer et al.*, 2016). A more flexible approach to food safety liability would reduce the amount of irreversibly discarded food in these situations. Shifting responsibilities wholly or partially from the management of food operators in the hospitality sector would help redirect surplus food to those who need it most.

## Modern Technology and Food Waste Management

The expansion of advanced technologies has enabled the development of various smartphone applications that facilitate quantification and categorization of food waste in hospitality sector kitchens. For example, there is a "Wise Up on Waste" application developed by Unilever Food Solutions (2017) that helps chefs measure, monitor and manage food waste in their kitchens. Research shows that adopting such technological innovations can significantly reduce food waste (*Gould*, 2016). From a consumer perspective, a smartphone application ("Too Good To Go") has been developed to help consumers buy prepared meals from restaurants at significantly lower prices at the end of the day, thus reducing waste and waste disposal costs, and increasing sales at the same time. According to their data, Too Good To Go is currently used in 14 European countries, numbering more than 26 million sold meals which is equivalent to 66,000,000 kg of prevented CO<sub>2</sub> emissions — about the same as taking >11,000 cars off the road for a year (*Filimonau and De Coteau*, 2019).

However, in Serbia, the implementation of such applications has not proved encouraging. The reason is that a large part of the population would, in fact, tend to exclusively buy meals from restaurants at the end of the business day. This would have a significant impact on restaurant earnings, since the number of visits would significantly decrease during the day (personal communication).

## Conclusion

It is clear that food wastage has an impact on global sustainable development, and the facts themselves support the extent to which the negative impact on the environment would be decreased if the amount of food waste on a global level were reduced. Due to the numerous operational activities in hospitality, it is not possible to completely eliminate food waste. However, new/revised procedures and regulations in the hospitality sector (and other sectors that produce food waste) will reduce the quantity of food waste (Kasavan et al., 2017). Food waste

management should be an integral part of hospitality management, especially since an effective food safety management system is the basis for implementing most of the practices that have led to the reduction of food waste in hospitality. This accomplishes an even more important food safety goal, which is to ensure the production of a safe product and the health of consumers. Positive food waste management practices in the hotel industry can have a stimulating effect on other hotels, hotel chains and associations. Sharing experiences increases the opportunities for involvement of stakeholders in food waste production, which can lead to greater organizational culture of businesses and environmental awareness of individuals. However, to successfully manage food waste, in addition to motivating management interest, the food waste generated by the hospitality sector must be initially categorized and quantified. There are numerous challenges for managers, as food waste management needs to be integrated into other business areas that include human resource training, enterprise software and technology.

# Upravljanje otpadom od hrane — smanjenje i prevencija nastanka otpada od hrane u ugostiteljstvu

Nataša Kilibarda, Filip Đoković, Radmila Suzić

*Apstrakt:* Otpad od hrane nastaje duž celog lanca hrane, od njive do trpeze. Bacanje hrane predstavlja etičko pitanje, zatim dovodi do ekonomskih gubitaka, ali i utiče negativno na životnu sredinu. Otpad od hrane je iz tog razloga značajan problem savremenog društva i prvi korak u njegovom rešavanju jeste identifikacija i razumevanje razloga njegovog nastanka u svakom delu lanca hrane i specifičnim sektorima kao što je ugostiteljstvo. Kako bi se kreirale prakse i preporuke koje bi za cilj imale, pre svega, sprečavanje nastanka otpada od hrane, neophodno je kategorisati i kvantifikovati otpad od hrane. U ugostiteljstvu to i nije tako jednostavan zadatak, uzimajući u obzir neujednačenu proizvodnju hrane i specifičan i raznolik način obavljanja delatnosti subjekata koji posluju hranom u ovom sektoru. Ono što je sigurno jeste da upravljanje otpadom od hrane treba da bude sasatvni deo menadžmenta u ugostiteljstvu, pre svega zbog toga, što efektivni sistem upravljanja bezbednošću hrane predstavlja polaznu osnovu za implementaciju većine praksi koje dovode, kako do bezbednog finalnog proizvoda, tako i do smanjenja otpada u ugostiteljstvu.

**Ključne reči:** otpad od hrane, održivost, ugostiteljstvo, kvantifikacija, prevencija.

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## Letter to the Editor

**RIBMINS**

**MORE THAN 150 MEAT SAFETY EXPERTS  
ACROSS EU TOGETHER TO IMPROVE  
MEAT INSPECTION**

The role of meat safety is to provide safe and wholesome meat for human consumption. Numerous control measures and tools are employed in the whole food chain to produce safe meat and one of them is meat inspection that represents supervision of animals and meat, mainly by official veterinarians. Despite the efforts taken, the EU is still witnessing meat scandals occasionally. Moreover, the hazards causing disease in humans, such as *Salmonella* or *Campylobacter*, cannot necessarily be seen at meat inspection. Consequently, meat or meat products may already have been sold and consumed, before the source of contamination is identified. Therefore, the current criteria of meat inspection are under revision in the light of the knowledge of food science.

The new EU COST action “Risk-based meat inspection and integrated meat safety assurance” (RIBMINS) has gathered more than 150 experts in the field, even outside of the EU, keen to put the accent on improving meat inspection in the next four years. To do this, food safety professionals are analysing the way in which meat inspection could be made more efficient and cost effective for governments and industries, but also for the benefit of the consumers. This could be in the form of development of meat safety assurance systems. This EU COST action is much in line with the European Food Safety Authority’s (EFSA) proposal to work on building modern, risk-based meat safety assurance system based on risk assessment and where control measures are applied at those points in the meat chain, where there is a high cost-effectiveness in reducing meat-borne risks.

This initiative for launching the EU COST action in meat inspection came from 37-year old Associate Professor Dr Bojan Blagojevic, European

specialist in veterinary public health from the University of Novi Sad (Serbia), who is now chairing the action: “More than a century-old system was for long time employed to tackle modern meat safety threats – with limited success. My idea was to gather the best experts from more than 35 countries to make the meat safety system more efficient. To tackle the main meat-borne hazards, meat inspection must be revised to be risk-based and as such to be a part of the modern, longitudinally integrated system that entails prevention and control throughout the meat chain with the main focus on farm and abattoir stages.”

The vice-chair of the action, Dr Lis Alban, is Adjunct Professor at the University of Copenhagen and Chief Scientist within the Danish Agriculture & Food Council. “Our EU COST action is in the interest of fair competition in the meat industry sector and more efficient regulatory controls across Europe. To speed up this process, we need to collaborate to find the best solutions – and only hereby will the producers and abattoirs be able to deliver safe meat to all consumers.” says Dr Alban, who organised a large conference within the EU COST action at University of Copenhagen from 6<sup>th</sup> until 8<sup>th</sup> November 2019.

The science communication manager of the EU COST action, Dr Boris Antunovic, Professor at the University of J.J. Strossmayer in Osijek in Croatia, says: “We have well established experts from different EU countries within the action. Some of them have never seen each other before and now they are able to work together in next four years and share their ideas and experiences in meat inspection. There is also an opportunity for young researchers to join the action by applying for short term scientific missions. This is very powerful tool.”

The meat industry has highly welcomed the new EU COST action RIBMINS. Francisco Requena is the Director of a 200-year old Spanish company with headquarters in Malaga, processing 25,000 pigs per week and exporting 50% of production to other EU countries and Asia. “Meat inspection is complex and, in the day-by-day operations, it brings a number of controversial problems to industries. The effect of using end-product laboratory testing as a substitute for meat inspection in detecting most relevant human health hazards is very limited — it is expensive and non-proactive while tested food samples may not be sufficiently representative due to the heterogeneous distribution of pathogens. In addition, the results may be delayed, depending on tests’ performances, and may relate only to the examined hazards. In overall, testing and negative results certainly do not guarantee the safety of meat.”, says Mr Requena.

Due to increased international trade of meat on the global level, the EU COST action has already attracted interest of experts outside the EU. Dr Mick Bosilevac, research microbiologist in the U.S. Meat Animal Research Center in Nebraska (USA) sees the EU COST action in meat inspection as an excellent opportunity for food safety and meat safety experts: “We are fortunate that at least in the beef community, food safety is considered a non-competitive arena. This means problems and solutions are openly shared and addressed. The public does not respond to an outbreak or recall by avoiding beef from just the company or outlet involved. Rather they avoid all beef products. The more we can get each commodity group to work from this point of view, the faster solutions can be identified and put into place.”

New EU legislation on official controls, which is to be applied from 14th December 2019 will ensure, among other things, the application of food law and rules on animal health and welfare, and finally, revised meat inspection procedures. The COST action RIBMINS is going to be executed just in time of adjusting national control systems to this new EU legislation. For the purpose of better coordination of the activities on national levels, RIBMINS national contact points have been established for each country.

The representatives of Serbia in this COST action are Dr. Ivan Nastasijevic, Senior Research Associate (member of the Management Committee member and co-leader of Working Group No. 5: training, communication and monitoring for the meat safety system) of the Institute of Meat Hygiene and Technology in Belgrade, Professor Dr. Nedeljko Karabasil (MC Substitutes) from the University of Belgrade, Faculty of Veterinary Medicine, Assistant Professor Ljiljana Kuruca (Member of the Management Committee) from the University of Novi Sad (Faculty of Agriculture, Department of Veterinary Medicine) and Dr. Dragan Ljubović Pelic, Senior Research Associate (MC substitute) Scientific Veterinary Institute “Novi Sad” in Novi Sad.

For Serbia, the role of national contact point is taken by professor Dr Nedjeljko Karabasil, from the University of Belgrade, Faculty of Veterinary Medicine.

**For more information visit:**

<https://ribmins.com>

**Prof. dr Nedjeljko Karabasil**  
University of Belgrade,  
Faculty of Veterinary medicine

## Писмо уреднику



**ВИШЕ ОД 150 СТРУЧЊАКА ЗА БЕЗБЕДНОСТ МЕСА У  
ЗАЈЕДНИЧКОЈ АКЦИЈИ ШИРОМ ЕВРОПСКЕ УНИЈЕ  
СА ЦИЉЕМ УНАПРЕЂЕЊА ИНСПЕКЦИЈЕ МЕСА**

Улога безбедности хране јесте да осигура употребљивост и безбедност меса за јавну потрошњу. Дуж ланца хране користе се бројне контролне мере са циљем производње безбедног меса. Једна од њих је и инспекција и преглед меса. Ово укључује надзор над животињама и производњом меса за јавну потрошњу од стране ветеринарске инспекције и овлашћених ветеринара. Упркос уложеним напорима у Европској унији, још увек смо повремено сведоци инцидента у вези са месом. Штавише, опасности које узрокују болести код људи, попут салмонела или кампилобактера, практично се не могу уочити приликом прегледа меса. Сходно томе, месо или производи од меса можда су већ продати и конзумирани, пре него што се утврди извор контаминације. Због тога су, у склопу науке о храни, у току ревизије тренутних критеријума инспекције и прегледа меса.

Нова COST акција Европске уније, у вези са прегледом меса заснованом на оцени ризика и интегрисаним системом контроле (“Risk-based meat inspection and integrated meat safety assurance” — RIBMINS), окупила је више од 150 стручњака из ове области, чак и ван Европске уније, који желе да у наредне четири године унапреде поступак прегледа меса. Да би то постигли, стручњаци за безбедност хране анализирају начин на који би инспекција меса могла бити ефективнија и ефикаснија за регулаторна тела и субјекте у пословању храном, као и за потрошаче. То укључује и развој система за потврду безбедности меса. Ова COST акција Европске уније у великој мери је усклађена с предлогом Европске агенције за безбедност хране (EFSA) и развоју модерног система осигурања безбедности меса

који се базира на оцени ризика и примени мера контроле у оним тачкама у ланцу производње где ће утврђени ризик бити ефективно елиминисан односно смањен на прихватљив ниво.

Иницијатива за покретање COST акције Европске уније у вези са инспекцијом меса стигла је од ванредног професора др Бојана Благојевића, европског стручњака за ветеринарско јавно здравство са Универзитета у Новом Саду (Србија), који сада председава акцијом: „Систем прегледа меса старији од једног века, дуго је служио за решавање савремених претњи безбедности меса — са ограниченим успехом. Моја идеја је била да окупим најбоље стручњаке из више од 35 земаља како би систем безбедности меса био ефикаснији. Да би се уклониле кључне опасности у вези са месом као вектором, поступак прегледа се мора ревидирати и бити заснован на ризику и део модерног, лонгитудиналног интегрисаног система који подразумева превенцију и контролу у читавом ланцу производње меса, са кључним фокусом на фарме и кланице.“

Потпредседник COST акције др Лиз Албан је професор на Универзитету у Копенхагену и водећи научник у оквиру Данског савета за пољопривреду и храну. „Наша COST акција у интересу је фер конкуренције у сектору индустрије меса и ефикасније регулаторне контроле широм Европе. Да бисмо убрзали овај процес, морамо сарађивати у проналажењу најбољих решења — и само на тај начин ће произвођачи и кланице моћи испоручити безбедно месо свим потрошачима.“ каже др Албан, која је организовала велику конференцију у оквиру COST акције на Универзитету у Копенхагену од 6. до 8. новембра 2019. године.

Руководилац за научну комуникацију у оквиру COST акције је др Борис Антуновић, професор са Универзитета J.J.Strossmayer из Осиека у Хрватској и каже: „У оквиру наше акције укључени су реномирани стручњаци из различитих земаља Европске уније. Неки од њих се никада раније нису срели, а сада имају прилику да раде заједно у наредне четири године и деле своје идеје и искуства из области инспекције и прегледа меса. Такође, млади истраживачи имају прилику да се придруже акцији путем пријаве за краткорочне студијске боравке. Ово представља једно веома моћно средство.“

Представници индустрије меса су позитивно реаговали на покренуту RIBMINS акцију Европске уније. Francisco Requena је директор 200 година старе шпанске компаније са седиштем у Малаги. Капацитет линије производње је од 25.000 свиња недељно и извозе 50% производње у друге земље Европе и Азије. „Инспекција меса је сложена активност и у свакодневном раду доноси низ контроверзних проблема субјектима у пословању храном. Ефекат резултата лабораторијских испитивања готових производа у откривању најрелевантнијих опасности по здравље људи је ограничен — скуп и није проактиван, док испитани узорци хране можда нису довољно репрезентативни због хетерогене дистрибуције патогених микроорганизама. Поред тога, резултати испитивања имају ограничења, у зависности од перформанси методе испитивања и односе се само на испитиване опасности. Самим тим, поступак испитивања и добијени чак и негативни резултати испитивања не гарантују безбедност меса.“, каже Requena.

Због сталног раста трговине месом на глобалном нивоу, RIBMINS COST акција је привукла стручњаке и ван Европске уније. Др Mick Bosilevac, микробиолог истраживач из америчког Центра за истраживање меса у Небраски (САД), ову COST акцију види као одличну прилику за стручњаке из области безбедности хране и меса: „Имамо среће да се бар у заједници произвођача меса говеда, безбедност хране сматра неконкурентном ареном. То значи да се проблеми и

решења отворено деле. Јавност не реагује само на конкретан инцидент и опозив производа од субјекта који је умешан, већ радије избегава све производе од меса говеда. Што више заинтересованих страна придобијемо да послује са ове тачке гледишта, решења ће се брже идентификовати и применити.

Ново законодавство Европске уније у вези поступка званичне контроле, које ће се примењивати од 14. децембра 2019. године, осигураће, између осталог, примену Закона о храни и правила о здрављу и добробити животиња, као и ревидиране процедуре инспекције меса. RIBMINS COST акција се реализује у време прилагођавања националних система контроле овом новом законодавству Европске уније. У циљу боље координације активности на националним нивоима, успостављене су националне контакт тачке RIBMINS за сваку земљу.

Представници Србије у овој COST акцији су др Иван Настасијевић, виши научни сарадник (члан менаџмент комитета — Management Committee member и ко-руководилац радне групе бр. 5: тренинг, комуникација и мониторинг за систем осигурања безбедности меса) са Института за хигијену и технологију меса у Београду, професор др Неђељко Карабасил (MC substitute) са Универзитета у Београду, Факултет ветеринарске медицине, доцент др Љиљана Куруца (члан менаџмент комитета) са Универзитета у Новом Саду (Пољопривредни факултет, Департаман за ветеринарску медицину) и др Драгана Љубојевић Пелић, виши научни сарадник (MC substitute) са Научног института за ветеринарство „Нови Сад“ у Новом Саду.

За Србију, улогу националне контакт тачке преузима професор др Неђељко Карабасил са Универзитета у Београду, Факултета ветеринарске медицине.

#### **За више информација:**

<https://ribmins.com>

*Проф. др Неђељко Карабасил  
Универзитет у Београду*

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## Guidelines for Authors

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„Meat Technology” (ISSN 0494–9846) is a scientific journal publishes:

Original scientific papers (papers which present previously unpublished results of authors’ own investigations using scientific methodology);

Review papers (papers which include original, detailed and critical overview of a research problem or an area to which the author has significantly contributed, as evidenced by auto citations);

Brief or preliminary papers (full-format original scientific papers or of preliminary character);

Reviews (of books, scientific conferences etc.)

Eligible for publishing are those papers, which have not been previously published, presented or considered for publication in another journal, except as abstracts presented at scientific conferences. The first author is both responsible for meeting these criteria and for obtaining agreement to publish from all of the co-authors.

### Procedure

Papers are subject to anonymous reviews (two at least), while the decision to accept the paper for publishing is reached by the editor-in-chief, together with subeditors and the members of the editorial board.

Accepted papers are subject to proofreading. The editorial board reserves the right to minor corrections of the manuscript. Where major corrections are necessary, the first author will be notified, and the paper sent for revision, with a set deadline. After all corrections, authors are requested to submit a „Statement authors“ on mail danijelas@inmesbgd.com.

### Language

Papers must be written on English (British English spelling).

### Editing of the manuscripts

The paper should be edited in Microsoft Word software, using Times New Roman font, size 12 pt, paragraph spacing 1.5 and margins of 2 cm. Papers are submitted in electronic form by email: danijelas@inmesbgd.com or institute@inmesbgd.com. The text should be clear, concise, grammatically correct and should contain the following sections:

The title (lowercase, bold, font size 14 pt). Below the title, names of the authors (first name, last name,

lowercase, italic, font size 12 pt). Numbers following names in superscript refer to the authors’ institution.

At the bottom of the first page, put affiliation according to the number in superscript, name and address of the institutions authors are employed in should be given (italic, font size 10 pt, the main words capitalized). In the new line, the name and e-mail of the corresponding author should be provided (font 12).

Abstract on English and Serbian should contain 150–250 words with keywords (maximum 5, italic, font 12). The English abstract should be typed below the title and names of the authors, and the Serbian below the conclusion.

The original scientific paper should contain the following chapters: introduction, material and methods, results and discussion (combined or separate), conclusion, notes (optional) and references. Chapter names are typed in lowercase, font size 12, bold.

**INTRODUCTION:** should contain clear description of the investigated subject and aim of the research with the short citations of the relevant literature (not more than 10 years old);

**MATERIAL AND METHODS:** this chapter describes material and methods used and outlines the design of the experiment;

**RESULTS AND DISCUSSION:** The results should be processed by statistical methods appropriate to the experiment; they should be clear and concise using tables, graphs, photographs, illustrations and other. The same result should not be presented through both table and graph. Discussion should be related to presented results avoiding repetitions of already stated facts, using comparison of obtained results and relevant literature data related to similar group of products, comparable analytical method et sim.

When in the text, literature is cited by giving author’s last name, last name with “and”, if the cited literature is published by two authors, or, in the case of more than two authors by “et al.” abbreviation after the surname of the first author (italic). Cited literature with the year of publishing should be in brackets.

Figures and illustrations are numerated with the same number as given in the text of the paper. Titles of the tables are written above the tables; titles of the graphs and illustrations are printed below (in lowercase). Tables, graphs and figures are submitted separately, in the appendix.

If tables, graphs or figures originate from other sources, the author is required to state the source of such data (author, year of publishing, journal etc.). Notes should be placed at the bottom of the page containing cited material.

The author should apply the International System of Units (SI system) and current regulation on measuring units and measuring instruments.

**CONCLUSION:** provides the review of the most important facts obtained during the research.

**Acknowledgement:** should contain title and number of the project i.e. title of the program from which is the research carried out and described in the paper, as well as the name of the institution that funded the project or program and should be written after conclusion, before references.

**REFERENCES:** (Times New Roman 12 pts) should include recent international publications. If the original literature cited has not been available, the authors should quote the source used. The references should be numerated 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 italic.

► **Example:**

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 more chapters:

**Marasas, W. F. O. (1996).** Fumonisin: History, worldwide occurrence and impact. In *Fumonisin 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 thesis:

**Radeka, S. (2005).** Grape mash maceration and varietal aroma of Malvazija istarska wine, PhD Thesis, Faculty of Agriculture, University of Zagreb, Croatia.

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 8<sup>th</sup> Annual Symposium, Nicholasville, Kentucky, Proceedings*, 109–119.

Software:

**STATISTICA (Data Analysis Software System) (2006).** v.7.1., StatSoft, Inc., USA ([www.statsoft.com](http://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>).

Each publication cited in the text must be listed in References. The citations in the text need to be arranged in the following way:

If there is only one author of the cited paper, the author's surname and the year of publication is stated in the brackets (Thomas, 2008). In case the same author has more publications in the same year, additional letters are added next to the year (Thomas, 2008a; Thomas, 2008b).

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 more references are cited within the same brackets, citations should be done in chronological order.

Papers belonging to the category other than original scientific papers can contain chapters titled by choice of the author.

Papers are submitted by e-mail:

- [danijelasarcevic@inmes.rs](mailto:danijelasarcevic@inmes.rs);
- [meat.technology@inmes.rs](mailto:meat.technology@inmes.rs);

or on

- [www.journalmeattechnology.com](http://www.journalmeattechnology.com)

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## LIST OF REVIEWERS

As an Editor in chief of scientific journal “Meat Technology”, I would like to express my gratitude to professors, scientists and researchers for their contribution of reviewing in our journal. In this volume we present the list of reviewers.

**Milan Z. Baltic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Department of Hygiene and Technology of Animal Origin

**Iva Steinhäuserová, PhD**

Faculty of Veterinary Hygiene and Ecology, Department of Meat Hygiene and Technology, Brno, Czech Republic

**Andrej Kirbis, PhD**

University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Republic of Slovenia

**Igor Tomasevic, PhD**

University in Belgrade, Faculty of Agriculture, Department for Technology of Animal Products, Belgrade, Republic of Serbia

**Vesna Z. Djordjevic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Tomaz Polak, PhD**

University in Ljubljana, Faculty of Biotechnology, Department of Meat Technology and Food Risk, Ljubljana, Republic of Slovenia

**Sava Buncic, PhD**

University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine, Novi Sad, Republic of Serbia

**Olgica Ceric, PhD**

Food and Drug Administration – FDA, Veterinary Laboratory Investigation and Response Network, New Hampshire, USA

**Luca Cocolin, PhD**

Università degli Studi di Torino, Faculty of Agriculture, Department of Agricultural, Forest and Food Sciences, Turin, Italy

**Galia Zamaratskia, PhD**

Swedish University of Agricultural Science, Department of Food Science, Uppsala, Sweden

**Lea Demšar, PhD**

University in Ljubljana, Faculty of Biotechnology, Department of Meat Technology and Food Risk, Ljubljana, Republic of Slovenia

**Aurelija Spiric, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Antonia Ricci, PhD**

National Laboratory for *Salmonella*, Department for Food Safety, Risk Analysis/OIE Referential Laboratory for *Salmonella*, Padua, Italy

**Irina Tchernukha, PhD**

The Gorbatov’s All Russian Meat Research Institute, Moscow, Russia

**Vlado Teodorovic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Olivera Djuragic, PhD**

Institute for Food Technology, Novi Sad, Republic of Serbia

**Dragan Momcilovic, PhD**

Food and Drug Administration – FDA, Centre for Veterinary Medicine, Rockville, USA

**Tomas Alter, PhD**

Faculty of Veterinary Medicine, Institute of Meat Hygiene and Technology, Institute of Food and Milk Hygiene, Berlin, Germany

**Lidija Peric, PhD**

University of Novi Sad, Faculty of Agriculture, Novi Sad, Republic of Serbia

**Marija Jokanovic, PhD**

University in Novi Sad, Faculty of Technological Science, Novi Sad, Republic of Serbia

**Petrovic Jelena, PhD**

Scientific Institute for Veterinary medicine, Novi Sad, Republic of Serbia

**Zorica Jugovic-Knezevic, PhD**

University in Belgrade, Faculty of Technological Science, Belgrade, Republic of Serbia

**Breda Jakovec-Strajn, PhD**

University in Ljubljana, Faculty of Veterinary Medicine, Ljubljana, Slovenia

**Rebeka Garsija, PhD**

Animal Plant Health Agency, Dartford, United Kingdom

---

---

**Vladimira Pistenkova, PhD**

Faculty of Veterinary Hygiene and Ecology,  
Brno, Czech Republic

**Snjezana Mandic, PhD**

University in Banja Luka, Technological Faculty,  
Banja Luka, Republic of Srpska

**Milenko Saric, PhD**

University in Banja Luka Faculty of Agriculture,  
Banja Luka, Republic of Srpska

**Meho Basic, PhD**

University in Tuzla, Faculty of technological Science,  
Bosnia and Herzegovina

**Dragan Vasilev, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Djordje Fira, PhD**

University in Belgrade, Faculty of Biological Science,  
Belgrade, Republic of Serbia

**Nedjeljko Karabasil, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Bozidar Zlender, PhD**

University in Ljubljana, Faculty of Biotechnical  
Science, Ljubljana, Republic of Slovenia

**Snezana Ivanovic, PhD**

Scientific Institute for Veterinary medicine,  
Belgrade, Republic of Serbia

**Gordana Uscebrka, PhD**

University of Novi Sad, Faculty of Agriculture,  
Department of Veterinary medicine, Novi Sad,  
Republic of Serbia

**Mirjana Dimitrijevic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Sabine Leroy, PhD**

National Institute for Agricultural Research,  
Research Centre Klermon-Feran, France

**Anne Leskoviz, PhD**

Ecole Nationale Supérieure Agronomique de Toulouse,  
Toulouse, France

**Martin Bouwknecht, PhD**

National Institute for Public Health and Environment,  
Eindhoven, Netherlands

**Jacques-Antoine Hennekinne, PhD**

Laboratory for Food Safety, ANSES-Agence  
Nationale Sécurité Sanitaire de l'Alimentation, de  
l'Environnement et du Travail, Maisons-Alfort, France

**Speranda Marcela, PhD**

University Josip Juraj Strossmayer in Osijek,  
Republic of Croatia

**Milorad Radakovic, PhD**

University of Cambridge, Department of Veterinary  
Medicine, Cambridge, United Kingdom

**Slaven Grbic, PhD**

Pan-European University Aperiion, Banja Luka,  
Republic of Srpska

**Natasa Kilibarda, PhD**

University Singidunum, Belgrade, Republic of Serbia

**Svetlana Stanisic, PhD**

University Singidunum, Belgrade, Republic of Serbia

**Mirjana Bojanic-Rasovic, PhD**

University in Montenegro, Faculty of Biotechnical  
Science, Podgorica Montenegro

**Jasna Djordjevic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Natasa Glamoclija, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Vladimir Tomovic, PhD**

University in Novi Sad, Faculty of Technology,  
Novi Sad, Republic of Serbia

**Milan M. Petrovic, PhD**

Institute for Animal Husbandry, Belgrade,  
Republic of Serbia

**Zorica Basic, PhD**

Military Medical Academy, Institute of Hygiene,  
Belgrade, Republic of Serbia

**Zora Colovic Saric, PhD**

University in Banja Luka, Faculty of Agricultural  
Science, Banja Luka, Republic of Srpska

**Milka Popovic, PhD**

University in Novi Sad, Faculty of Medical Science,  
Novi Sad, Republic of Serbia

**Ksenija Nesic, PhD**

Scientific Veterinary Institute – Belgrade,  
Belgrade, Republic of Serbia

**Bojan Blagojevic, PhD**

University of Novi Sad, Faculty of Agriculture,  
Department of Veterinary medicine,  
Novi Sad, Republic of Serbia

---

---

**Zdenka Skrbic, PhD**

Institute for Anima Husbrandy, Belgrade,  
Republic of Serbia

**Stamen Radulovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Lazo Pendovski, PhD**

Ss. Cyril and Methodius University in Skopje, Skopje,  
Republic of Macedonia

**Jelena Ciric, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Marija Dokmanovic-Starcevic, PhD**

Army of Serbia, Belgrade, Republic of Serbia

**Radomir Savic, PhD**

University in Belgrade, Faculty of Agriculture Science,  
Belgrade, Republic of Serbia

**Sladjana Sobajic, PhD**

University in Belgrade, Faculty of Farmaceutical  
Science, Belgrade, Republic of Serbia

**Slavisa Stajic, PhD**

University in Belgrade, Faculty of Agricultural  
Science, Belgrade, Republic of Serbia

**Radoslav Grujic, PhD**

University in Banja Luka, Faculty of Technological  
Science, Banja Luka, Republic of Srpska

**Slavica Grujic, PhD**

University in Banja Luka, Faculty of Technological  
Science, Banja Luka, Republic of Srpska

**Jelena Janjic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Goce Cilev, PhD**

University St. Kliment Ohridski, Veterinary Faculty,  
Bitola, Macedonia

**Milan P. Petrovic, PhD**

Institute for Anima Husbrandy, Belgrade,  
Republic of Serbia

**Snezana Bulajic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Radoslava Savic-Radovanovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Milica Laudanovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Milorad Mirilovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Zlatko Jusufhodzic, PhD**

Veterinary Institute Bihac, Bosnia nad Herzegovina

**Nikola Stanisic, PhD**

Institute for Anima Husbrandy, Belgrade,  
Republic of Serbia

**Ilija Djekic, PhD**

University in Belgrade, Faculty of Agriculture,  
Belgrade, Republic of Serbia

**Milica Petrovic, PhD**

University in Belgrade, Faculty of Agriculture,  
Belgrade, Republic of Serbia

**Silvana Stajkovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Branko Velebit, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Nenad Parunovic, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Brankica Lakicevic, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Radivoj Petronijevic, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Dejana Trbovic, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Sasa Jankovic, PhD**

Institute of Meat Hygiene and Technology,  
Belgrade, Republic of Serbia

**Marija Boskovic, PhD**

University in Belgrade, Faculty of Veterinary  
Medicine, Belgrade, Republic of Serbia

**Zeljko Sladojevic, PhD**

Veterinary Institute "Vaso Butozan" Banja Luka,  
Republic of Srpska

---

---

**Zoran Kulisic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Nevenka Maksimovic, PhD**

Institute for Anima Husbrandy, Belgrade, Republic of Serbia

**Zoran Markovic, PhD**

University in Belgrade, Faculty of Agriculture, Belgrade, Republic of Serbia

**Dragana Ljubojevic, PhD**

Veterinary Institute Novi Sad, Novi Sad, Republic of Serbia

**Radmila Markovic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Ivan Nastasijevic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Dragan Milicevic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Zoran Petrovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Vesna Jankovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Danijela Vranic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Srdjan Stefanovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Jasna Djinovic-Stojanovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Branka Borovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Milan Milijasevic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Tatjana Baltic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Drago Nedic, PhD**

Vetrinary Institute "Vaso Butozan" Banja Luka, Banja Luka, Republic of Srpska

**Katarina Savikin, PhD**

Institute for Research of Medical Herbs "Josif Pancic", Belgrade, Republic of Serbia

**Milena Krstic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Jelena Babic-Milijasevic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Radmila Mitrovic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Milos Petrovic, PhD**

Veterinary specialist institute Nis, Republic of Serbia

**Slobodan Lilic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Ivana Brankovic Lazic, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Slavica Veskovic-Moracanin, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

**Dejan Krnjaic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Tatjana Markovic, PhD**

Institute for Research of Medical Herbs "Josif Pancic", Belgrade, Republic of Serbia

**Dragoljub Jovanovic, PhD**

University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

**Mladen Raseta, PhD**

Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia

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