RISK ANALYSIS FOR THE PRESENCE OF SODIUM AND PHOSPHATES SALTS IN THE MODEL SYSTEMS OF ORGANIC COOKED SAUSAGE

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Abstract

Organic cooked sausages are a complex dispersion system in which inorganic phosphate salts are completely replaced by natural ingredients, while the presence of sodium salt is reduced to an acceptably low value. Therefore, the main objective of this paper was to analyze the risks to the presence of sodium and phosphate salts in model systems of organic cooked sausages in which they are reduced or replaced entirely by using natural ingredients.

Organic cooked sausage models have been developed in which the phosphate salts are replaced by a natural fiber, proteins and polysaccharides, while the presence of salt is reduced with KCl. Thus developed models were analyzed for the presence of sodium, Cl and phosphorus in the form of P_2O_5. Based on the results of chemical analysis was performed risk analysis for the presence of these salts in the tested models. The sodium content was determined by atomic absorption spectrophotometry (AAS), chloride by Vollhard method and phosphorus by spectrophotometry. A risk analysis was performed according to Health Safety Environment - HSE standard method. Data were subjected to analysis of variance (ANOVA) and means were separated by a T - Tukey multiple range test at ($p \leq 0.05$); ($p \leq 0.01$) and ($p \leq 0.001$) significance level.

It was found that the total sodium content significantly decreases ($p \leq 0,01$), and ranges from $0,74 \pm 0,03\%$ (M0) to $0,23 \pm 0,01\%$ (M12). In that way, the presence of sodium salt can be reduced by 40%, which leads to a decrease the risk to consumers' health. The average phosphorus content ranged from $0,046 \pm 0,003\%$ to $0,097 \pm 0,002\%$.

The risk analysis showed that the probability of occurrence of such salts in these models can be characterized as possible to rare while the hazard seriousness was between minor to moderate.

Key words: *Organic cooked sausage, Risk analysis, Sodium and phosphate salts.*

1. Introduction

Phosphates are additives which have been used in the production of all types of sausages and have a functional property of emulsifiers, thus they affect the increase of solubility of proteins from muscles in the way they influence increasing the ionic strength of the colloidal system in which there are found [1]. In the final products it usually adds 3 - 5 g of phosphate per kilogram in the final product, calculated as the content of phosphate pentoxide (P_2O_5) [2, 3]. According to the Regulations on the conditions for the use of food additives in foods intended for human consumption in Bosnia and Herzegovina [4], the maximum allowable concentrations (MAC) for phosphate is 5 g/kg [4]. They participate in the processes of water binding, improving texture, and stabilization of the product [5]. The use of large amounts of phosphate in the preparation of meat products, and thus the cooked sausages, can have negative effects on human health [6]. The risk assessment related to the amount of use phosphate and their effect on human health does not depend only on the quantity of phosphate entered into the body, but also on the impact of technological phosphate [7]. Salt (NaCl) is the oldest additive that is used in the preparation of cooked sausages. In synergy with the phosphate salt acts in a way to increase solubility of proteins by increasing the ionic strength of colloidal systems

in the meat emulsion [8]. Increased intake of salt can cause a series of health problems such as hypertension, osteoporosis and cancer of the digestive tract [9, 10].

According to USDA and regulations of the EU Commission, meat products which are declared as organic are classified in a special category, with the common characteristic, that is in products from this category it shouldn't be added inorganic salts, like phosphate, especially potassium or sodium nitrite and nitrate salts [11]. Consequently, the technological role of these salts is largely replaced with natural analogues that provide similar or the same technological properties in the final product [12]. Vegetable is a good source of natural protein and fiber, which can be used as a textural enhancer and replacement of the use of inorganic phosphate salts [11, 12]. The main condition for the use of such derivatives in organic production is that it is produced in accordance with the rules and requirements of organic food production [13, 14]. The most frequently used protein derivatives in the conventional production are the protein fractions obtained from soy [15], proteins obtained from different types of peas and beans [16], wheat proteins and proteins of other cereals represents a newer trend in the production of organic cooked sausages [17].

Natural fiber from grains, fruits and vegetables are very suitable for incorporation into organic meat products, especially cooked products such as cooked sausages, reduced cook loses, increase water holding capacity and other textural properties [18]. As polysaccharide for improving the textural properties of different food products, starch and its allotropic modifications are often used [19]. Besides the texture enhancers of vegetable origin, in the production of organic cooked sausages, proteins of animal origin can be used, usually egg and milk proteins [20]. The most commonly used additive in organic products which is declared as natural and organic is sea salt [11, 20]. Sea salt contains a small amount of nitrate, so in additives it is used as an additional source of smaller amount of nitrate, and nitrite salts. Mediterranean sea salt contains 1.1 ppm nitrate and 1.2 ppm nitrite [21]. Sea salt, which is used in the diet must be in accordance with the Regulations, which regulate this field and actually it is salt that contains at least 97.5% NaCl, with specific limits on calcium/magnesium content, as well as the content of arsenic and heavy metals [22].

Besides sea salt, an important ingredient which is used in the preparation of organic cooked meat products is raw sugar, also known as brown sugar [12], [23]. In the meat products it is mainly used as a source of carbohydrates, which is necessary to ensure proper functioning of the starter culture, and it is mostly used when it is necessary to ensure proper fermentation [23]. Further applications as additive in meat products refer to the reduction of salt content, adding other

salts as potassium chloride to mask undesirable flavors that carry supplements of other salts [24].

In accordance with the previously, the aim of this paper was to conduct a risk analysis for the presence of sodium and phosphate salts in model systems of organic cooked sausages, in which the presence of these salts is reduced or fully replaced with natural additives, according to the regulations that regulate the field of organic food production, where they are treated as a potential hazard.

2. Materials and Methods

2.1 Procedure of model creation

The procedure of making the tested model (M0 to M13) of organic cooked sausages is shown by the following scheme (Figure 1):

Experimental studies were carried out on 14 models (M0 to M13 - Tables 1 - 4), with the first model M0 which is the reference model. In the models, M1 to M5 it was reduced the content of sodium salt for values up to 35% of its presence in the model M5 . Salt was modified using sea salt combined with brown sugar and KCl, as shown in Table 1. In the models, M6 to M13 inorganic phosphate salts were replaced entirely by using natural texture modifiers (oat and barley fibers, milk proteins, albumin, soy proteins, bean proteins, starch and maltodextrin).
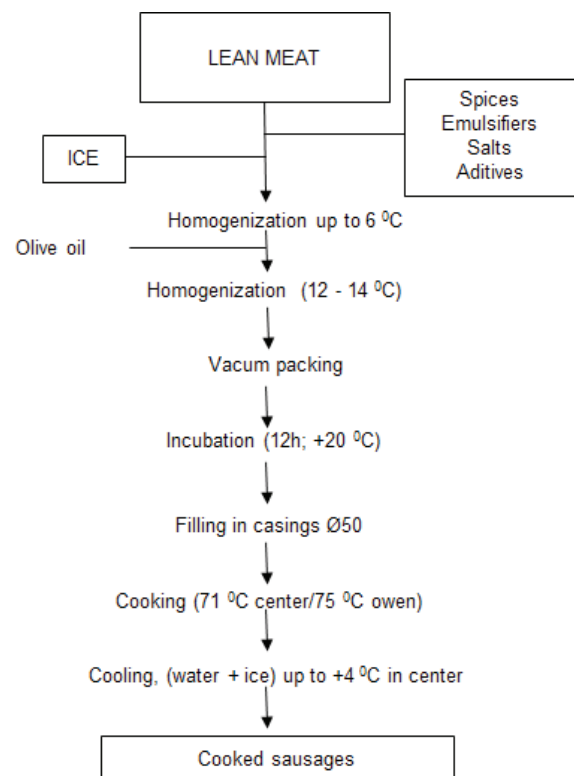

Figure 1. The procedure of making a model system of organic cooked sausages

Table 1. Reference model (M0) was formulated with the use of inorganic salts and standard amount of salt

Model	M 0	
Content	(%)	Comment
Lean pork meat	34.59	
Lean beef meat	11/59	
Olive oil	8/.9	
Ice	37.26	
Citrus fiber	5.79	Emulsifier
NaCl	1.80	
NaNO$_2$	0.014	
Pepper	0.18	
Coriander	0.02	Spices
Cardamom	0.02	
Sodium ascorbate	0.05	Antioxidants
Total	100	

Table 2. Models (M1 to M5) in which the content of sodium salt is reduced

Models	M 1	M 2	M 3	M 4	M 5	
Incubation			+20 °C/12h			
Content	(%)	(%)	(%)	(%)	(%)	
Lean pork meat	34.28	34.47	34.28	34.28	33.17	
Lean beef meat	11.59	11.59	11.59	11.59	11.09	
Olive oil	8.69	8.69	8.69	8.69	8.69	
Ice	37.26	37.26	37.26	37.26	36.26	
Citrus fiber	5.79	5.79	5.79	5.79	5.79	
Sea salt (87%) + KCl (10%) + brown sugar (3%)	1.80					
Sea salt (74%) + KCl (20%) + brown sugar (6%)		1.80				
Sea salt (61%) + KCl (30%) + brown sugar (9%)			1.80			Sodium salt reduction
Sea salt (48%) + KCl (40%) + brown sugar (12%)				1.80		
Sea salt (35%) + KCl (50%) + brown sugar (15%)					1.80	
Celery powder	0.34					
Beetroot powder		0.15				
Carrot powder			0.34			
Spinach powder				0.34		Color generators
Liquid					3	
Pepper	0.18	0.18	0.18	0.18	0.18	
Coriander	0.02	0.02	0.02	0.02	0.02	
Cardamom	0.02	0.02	0.02	0.02	0.02	
Starter culture	0.03	0.03	0.03	0.03	0.03	
Total	100	100	100	100	100	

Table 3. Models (M6 to M9) in which fibers of oats and barley, milk protein and albumin were used as a substitute for inorganic phosphate salts

Models	M 6	M 7	M 8	M 9	
Incubation	+20 ⁰C/12h				
Content	(%)	(%)	(%)	(%)	Comment
Lean pork meat	34.01	34.01	34.01	34.01	
Lean beef meat	11.59	11.59	11.59	11.59	
Olive oil	8.69	8.69	8.69	8.69	
Ice	37.01	37.01	37.01	37.01	
Oat fiber	5.79	-	-	-	Textural modifiers
Barley fiber	-	5.79	-	-	
Milk proteins	-	-	5.79	-	
Albumin	-	-	-	5.79	
Sea salt (61%) + KCl (30%) + brown sugar (9%)	1.80	1.80	1.80	1.80	
Celery powder Beetroot powder	0.34 0.26	0.34 0.26	0.34 0.26	0.34 0.26	Color generators
Pepper	0.18	0.18	0.18	0.18	Spices
Coriander	0.02	0.02	0.02	0.02	
Cardamom	0.02	0.02	0.02	0.02	
Acerola powder	0.26	0.26	0.26	0.26	Antioxidants
Starter culture	0.03	0.03	0.03	0.03	
Total	100	100	100	100	

Table 4. Models (M10 to M13) in which proteins of soy and beans were used as well as tapioca starch and maltodextrin as a substitute for inorganic phosphate salts

Models	M 10	M 11	M 12	M 13	
Incubation	+20 ⁰C/12h				
Content	(%)	(%)	(%)	(%)	Comment
Lean pork meat	34.01	34.01	34.01	34.01	
Lean beef meat	11.59	11.59	11.59	11.59	
Olive oil	8.69	8.69	8.69	8.69	
Ice	37.01	37.01	37.01	37.01	
Soy proteins	5.79	-	-	-	Textural modifiers
Bean proteins	-	5.79	-	-	
Tapioca starch	-	-	5.79	-	
Maltodextrin	-	-	-	5.79	
Sea salt (61%) + KCl (30%) + brown sugar (9%)	1.80	1.80	1.80	1.80	
Celery powder Beetroot powder	0.34 0.26	0.34 0.26	0.34 0.26	0.34 0.26	Color generators
Pepper	0.18	0.18	0.18	0.18	Spices
Coriander	0.02	0.02	0.02	0.02	
Cardamom	0.02	0.02	0.02	0.02	
Acerola powder	0.26	0.26	0.26	0.26	Antioxidants
Starter culture	0.03	0.03	0.03	0.03	
Total	100	100	100	100	

2.2 Determination of total phosphate, chloride and sodium in models of organic cooked sausages

The total phosphates were determined by the method of ISO 13730 [25]. According to the method, presented inorganic phosphates make with acid molybdenum a complex phosphomolybdic acid, which color is yellow and its intensity is determined spectrophotometrically at 430 nm to the blank solution. The intensity of the developed color is directly proportional to the phosphate content in the examined sample.

Total content of chloride in the tested models of organic cooked sausages is determined by volumetric method of Volhard. The determination has been defined by the international standard ISO 1841-1 [26]. The basic principle of this method is based on the releasing of chloride salts after the destruction of organic compounds with nitric acid and potassium permanganate. The released chlorides are precipitated with a solution of silver nitrate in exuberance, and the superfluous silver ions are retitrited with a solution of ammonium rhodanide which concentration is known, in the presence of ferric ammonium sulfate as an indicator. The obtained result is expressed as a percentage of total chloride salt by weight.

The sodium content was determined by atomic absorption spectrophotometry (AAS) according to the method ISO 2366 [27]. The total sodium content is expressed in ppm.

2.3 Risk analysis for the presence of inorganic salts in models of organic cooked sausages

Risk analysis for the presence of organic salts (sodium salt and phosphate salt) in the observed models of organic cooked sausages was made according to the Health Service Executive (HSE) method of risk assessment in the matrix system of 5 x 5 [28]. As elements of risk assessment, the seriousness of the threat versus the probability of occurrence of danger were observed, as it was shown in the following Table 5.

Table 5. Matrix of probability of occurrence of inorganic salts in the tested models of organic cooked sausages

LEVEL OF RISK ASSESSMENT					
Probability of hazard occurrence	**Hazard seriousness**				
	I	II	III	IV	V
	Negligible	Minor	Moderate	Major	Extreme
Rare/ Remote	**I**	1	2	3	4	5
Unlikely	**II**	2	4	6	8	10
Possible	**III**	3	6	9	12	15
Likely	**IV**	4	8	12	16	20
Almost Certain	**V**	5	10	15	20	25

Based on the previous Table 5, it was determined the rank and description of the probability of occurrence of inorganic salts in the models of organic cooked sausages, as shown in the following Table 6.

Table 6. Matrix rank of probability of occurrence of inorganic salts in models of organic cooked sausages

Measure of the probability of occurrence of inorganic salts	Qualitative description of probability	Quantitative rang of probability of occurrence of inorganic salts
1, 2	Improbable	R1
3, 4, 5	Possible	R2
6, 8, 9	Almost possible	R3
10, 12, 15, 16	Probable	R4
20, 25	Highly probable	R5

2.4 Statistical analysis of the experimental data

All experiments were conducted on a model of minimum three repetitions. Analysis of variance (ANOVA) was carried out on all the variables in the general linear model. Statistically significant differences in mean values of obtaining experimental data were calculated using T - Tukey test with statistical significance from ($p \leq 0.05$) and ($p \leq 0.01$). In the histograms different letter codes a, b, c, d, etc., indicate the existence of those differences of significance, and the same letter codes indicate that there are no differences.

3. Results and Discussion

3.1 The contents of total phosphorus, chlorine and sodium

The total content of chloride, phosphate and sodium in the tested model (M0 to M13) is shown in Figure 2 and Figure 3.
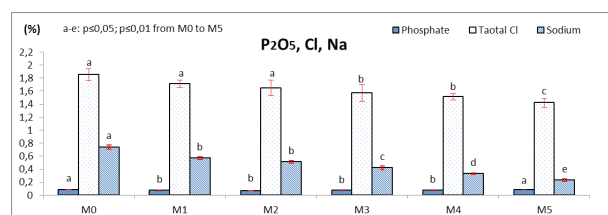

Figure 2. The content of total phosphate, Cl and Na in the tested models with reduced salt content (M0 to M5)
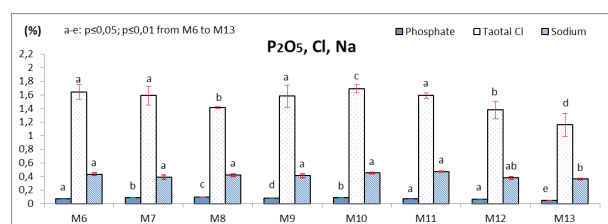

Figure 3. Content of total phosphate, Cl and Na of the group of test models in which the phosphate salts were replaced with natural additives (M6 to M13)

In the first group of models (M1 to M5), wherein the content of sodium salt is reduced, inorganic phosphate salts during the process of production are not added, so the amount of phosphates which is shown in the Figure 2 refers to phosphates which are naturally present in meat and other additives which are added during the process of the model formulation. Chloride content significantly decreases ($p \leq 0.05$) with a reduced content of salt, which is partly replaced with KCl and partly with unrefined brown sugar. From the given results it can be noted that the main source of chloride is sodium salt and KCl as its replacement. The total sodium content significantly decreases ($p \leq 0.01$) with the reduction of salt and moves with the initial value of $0.74 \pm 0.03\%$ (M0) to final $0.23 \pm 0.01\%$ (M5). Similarly to chlorides, from the presented results it can be noted that the content of Na^+ ions in the analyzed models is directly related to the function of the amount of sodium salt. Sodium as other microelements are naturally present in meat and used additives in preparation a model system of organic cooked sausages, but not in the amount that could significantly affect the final sodium content if a significant amount of salt is used [29]. The total content of chloride salts was significantly lower ($p \leq 0.01$), in the models M12 and M13 compared to other model from this group (Figure 3). The content of phosphor ranged on average determined in the previous group of models from $0.046 \pm 0.003\%$ (M13) to $0.097 \pm 0.002\%$ (M8), whereas in this group of models instead of inorganic phosphate salts various natural textural enhancers were added. Sodium content significantly varies ($p \leq 0.05$), the smallest amount was measured in models in which the smallest amount of chloride salt was measured (M12 and M13).

Food rich in protein, such as meat and meat products, naturally contains phosphor in the form of phospholipids, nucleotides, and orthophosphate [30]. The high content of naturally present orthophosphate, which amounts in meat products can range from 0.020% to 0.11%, can make more difficult analytical determination of phosphate, which were subsequently added during the production of cooked meat products [31, 32].

The dominant source of chloride and sodium in meat products is added chloride salts, such as NaCl, which is necessary to satisfy textural and sensory properties of the final product [29, 33].

As it can be seen from the given results, results related to the content of inorganic salts are largely consistent with previously published studies.

3.2 Risk analysis for the presence of sodium salt

Considering the results of the chemical analysis given in Figure 2 and Figure 3, it was shown that the sodium salt could be reduced up to 40%, and still keep satisfied sensory characteristics and technological needs of the observed models of organic cooked sausages [33]. Sodium salt was reduced using KCl salt and unrefined brown sugar as an additive to mask the bitter taste of KCl. In this way it is reducing the amount of daily intake of sodium salts consuming organic cooked sausages, which for a long period of time reduces the severity of the impact of sodium salt on health [12, 33]. Therefore, in the following matrix (Table 7) taking into account the percentage of possible reduction of the sodium salt presence in model systems of organic sausages, the probability of occurrence of sodium salt can be labeled as "Possible" (III), and the hazard seriousness as "Minor" (6) to "Moderate" (9), compared to the standard content of sodium salt in the model M0.

Table 7. Matrix probability of occurrence of sodium salt (NaCl) in the tested models of organic cooked sausages

LEVEL OF RISK ASSESSMENT						
Probability of hazard occurrence		**Hazard seriousness**				
		I	II	III	IV	V
		Negligible	Minor	Moderate	Major	Extreme
Rare/ Remote	I	1	2	3	4	5
Unlikely	II	2	4	6	8	10
Possible	III	3	6	9	12	15
Likely	IV	4	8	12	16	20
Almost Certain	V	5	10	15	20	25

Based on previous Table 7 it was designed range, qualitative and quantitative description of probability of occurrence of sodium salt in the tested models of organic cooked sausages, as shown in the following Table 8.

Table 8. Matrix rank of the probability of occurrence of sodium salt (NaCl) in models of organic sausages

Measure of the probability of occurrence of inorganic salts	Qualitative description of probability	Quantitative range of probability of inorganic salts occurrence
1, 2	Improbable	R1
3, 4, 5	Possible	R2
6, 8, 9	Almost possible	R3
10, 12, 15, 16	Probable	R4
20, 25	Highly probable	R5

According to the (HSE) protocol applied to the risk analysis, due to the seriousness of the hazards characterized in Table 7, as a minor to moderate, which is assigned to measure probability from 6 to 9 index units, qualitative description of probability, therefore, is characterized as "Almost possible", while a quantitative ranking of danger is (R3) (Table 8).

3.3 Risk analysis for the presence of phosphate salts

As it can be seen from the chemical analysis carried out (Figure 2 and Figure 3) on the observed models of organic sausages, the presence of phosphate salts is very small and is referred to the naturally occurred phosphates in meat, spices and used natural additives [12, 13, 34]. In the technological process of making a model of the observed organic sausages, inorganic phosphate salts are not added additionally, but their presence is replaced with additives of natural origin as textural enhancers (proteins, carbohydrates and natural fibers). Therefore, in the following matrix (Table 9), the probability of occurrence of phosphate salts can be labeled as "Rare/Remote" (I), and the severity of the hazards "Moderate" (3) with respect to the percentage of phosphate salts naturally presented in the tested model systems.

Table 9. Matrix probability of occurrence of phosphate salts in the tested models of organic cooked sausages

LEVEL OF RISK ASSESSMENT						
Probability of hazard occurrence	**Hazard seriousness**					
	I	II	III	IV	V	
	Negligible	Minor	Moderate	Major	Extreme	
Rare/Remote **I**	1	2	(3)	4	5	
Unlikely **II**	2	4	6	8	10	
Possible **III**	3	6	9	12	15	
Likely **IV**	4	8	12	16	20	
Almost Certain **V**	5	10	15	20	25	

Based on the Table 9, in the following Table 10 it is derived rank and description of probability of occurrence of phosphate salts in the observed model systems of organic cooked sausages.

Table 10. Matrix rank of probability of occurrence of phosphate salts in models of organic cooked sausages

Measure of the probability of occurrence of inorganic salts	Qualitative description of probability	Quantitative rang of probability of inorganic salts occurrence
1, 2	Improbable	R1
3, 4, 5	Possible	R2
6, 8, 9	Almost possible	R3
10, 12, 15, 16	Probable	R4
20, 25	Highly probable	R5

As it can be seen from the above Table 10 qualitative descriptions of probability is thus characterized as "Possible" with its quantitative risk ranking (R2).

In order to satisfy textural, microbiological and sensory request of the meat products, chloride salts are added usually in the form of NaCl, which is therefore the main source of the increased presence of sodium ions and chloride salts, which increases the risk of developing a number of health problems, most commonly cardiovascular [12, 33].

Increased intake of salt is considered to be one of the most important factors in the pathogenesis of hypertension. It is also connected with an increased risk of developing kidney stones, cancer of the stomach and intestines, stroke, enlargement of left ventricular and some other medical disorders. Increased intake of salt can have harmful effects on the human skeletal system, due to the effects of sodium on calcium excretion and thus it represents a serious risk factor for developing osteoporosis [5, 12, 35, 36]. As the excessive consumption of salt is in direct relation with hypertension as one of the most common chronic diseases of the cardiovascular system, WHO recommended that the daily intake of salt must not be greater than 5 g of salt per day which is equivalent to about 2 g Na^+ ions [12, 37]. Consuming meat and meat products, 16% to 25% of the total amount of salt is taken daily, which places them in the second place just after bread and pastries [12, 37].

As it can be seen from the preliminary results presented in this paper NaCl can be successfully replaced with the use of KCl, which can significantly reduce the presence of Na^+ ions. This method therefore represents the most used and researched way of eliminating a part of salt until the present day [38]. The main disadvantage of this method is the appearance of mild bitter taste, which KCl has, that disturbs sensory properties of the final product. In order to avoid it in such models, it is usually added minimum one or more components that mask the negative bitter taste of KCl. This model represents currently the most researched and developed methods of replacing sodium salt in meat products [39].

The main problem of excessive consumption of phosphorus in daily diet is the appearance of nephrocalcinosis and hyperphosphatemia [40], which reflects the disruption of normal metabolic processes of calcium, magnesium and iron in the human body [41]. All these processes lead to secondary hyperparathyroidism, deformities of bone, loss of bone mass, and/or ectopic calcification [40, 41]. Increased intake of phosphorus causes a cardiac fibrosis, thickening of the artery walls and narrowing them. All of these problems lead to an increased risk of cardiovascular disease [42]. As a particularly vulnerable category of people who are susceptible to these problems are children, the elderly and people who suffer from diabetes and have problems with the normal functioning of the kidney. These negative impacts on human health caused by excessive intake of phosphate in the human body, as well as growing concern about increased emissions of

phosphates in waste water from the meat processing industry, justify the use of natural ingredients in the production of new meat products released in the presence of phosphate salts [41, 42, 43].

4. Conclusions

- According to the conducted chemical analyses for the presence of organic salts in models of organic cooked sausages, the content of phosphate salts was low and ranged from $0.046 \pm 0.003\%$ (M13) to $0.097 \pm 0.002\%$ (M8).

- The total content of phosphorus which was detected was related to the naturally occurring phosphate in meat in the form of phospholipids, nucleotides, and phosphorus, which is naturally present in the form of orthophosphate in spices and natural additives.

- Content of sodium and total chloride was significantly declined ($p \leq 0.05$) with the reduction of salt content in the tested models. Sodium content ranged from an average of $0.74 \pm 0.03\%$ (M0) to $0.23 \pm 0.01\%$ (M5).

- Conducted risk analysis for the presence of salt and phosphate salts was found that the presence of salt in these models can be reduced up to 40% compared to its presence in the reference model M0. Phosphate salts can be left out of usage completely and replaced with natural textural modifiers, such as fruit and vegetable dietary fiber and plant and animal proteins.

- Using natural products in these models it is possible to formulate models with significantly reduced content of phosphate salts. Accordingly, it was found that the quantitative risk ranking in terms of the presence of sodium salt is Almost possible (R3), and the presence of phosphate salts and their content is classified as Possible (R2). Such results show that the risk to the health of consumers is reduced by decreasing the salts amount, which is being increased with the presence of inorganic salts.

Acknowledgement

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