Chemical quality parameters (water, protein, fat, NaCl ash and nitrites) in fermented sausage with the addition of *Yersinia enterocolitica*

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Abstract. The connection between diet and health was known to the oldest civilizations. Meat processing has influenced the increase of meat consumption, protein utilization and its energy value. Fermented sausages which are prepared in a safe way are rich in animal proteins and are an important part of the diet. Meat and adipose tissue are the basic ingredients in the production of fermented sausages, along with spices and salts, and other additives depending on the specifics of the product. Ripening is a process that separates raw sausages from all other types of sausages, and the quality of raw materials and their treatment directly affects the quality of the finished product, because the process of fermented sausage production is dominated by biological and biochemical processes. Different types of foods can cause food-borne illnesses, one of which is zoonotic yersiniosis caused by *Yersinia enterocolitica*. Due to the importance of *Yersinia* in meat, the European Food Safety Authority (EFSA) recommended that in addition to controlling the presence of *Salmonella*, it is mandatory to examine pork carcasses for the presence of *Y. enterocolitica*.

1. Introduction

The genus *Yersinia* belongs to the family *Enterobacteriaceae*. In this genus, 3 of the 12 species are pathogens, among them *Y. enterocolitica*, for which 6 biotypes are recognised (5 pathogenic and 1 non-pathogenic). This Gram negative, facultative anaerobe grows in a wide range of temperatures: optimal 28-29°C, minimum -2°C. *Y. enterocolitica* can thrive in cool temperatures as well as warmer ones, so it can grow easily in refrigerated environments. This bacterium has the ability to grow from -2°C to 42°C. The sources of this pathogen include contaminated water and the intestinal tracts of any infected humans or animals. It is frequently found in pigs. The foods associated with this pathogen are water, unpasteurized milk, meat (especially pork), and seafood. Disease symptoms can last for 1 to 3 weeks [1-3].
To avoid exposure to *Y. enterocolitica*, a number of procedures should be followed. Be sure to wash your hands after handling raw meat, especially pork. Avoid cross-contamination, and make sure you always drink water from a clean source. Avoid drinking milk that has not been pasteurized. Coming in contact with the bodily liquids of any infected animal can also spread the bacteria, so you should be careful and wash your hands after disposing of animal waste. Also, be sure to cook any meat products thoroughly to a safe temperature. [4-6].

2. Fermented sausages

Some meat products are very stable and do not need to be stored in refrigerators. Such are fermented products, in which the growth of microorganisms is inhibited by a very low moisture content. This type of meat product is not subject to heat treatment and is, therefore, often referred to as raw fermented sausages [1,3]. They are still of great importance in regions and conditions where it is difficult to provide a cold chain for transport and storage [1]. The production of dry, fermented sausages in Europe dates back to ancient Rome, and from the Mediterranean area [7,8], from where it spread to Germany, Hungary and other countries including the USA, Argentina and Australia [7]. In our region, dry fermented sausages arrived at the beginning of the 19th century from Italy, across the Pannonian plain [2,3].

Traditional production of fermented raw sausage in households in Serbia takes place under uncontrolled conditions (temperature, humidity, and fermentation) during colder seasons [8]. This process relies on the activity of fermentative bacteria that are naturally present in the meat and the environment of the production area [7,9-13]. Preservation of traditional production and authentic products can help small producers and local economies, but it is also important to preserve the traditional knowledge, cultural heritage and regional identity in what are often small and underdeveloped areas [5,8,10,14,15].

Today, in industrial production, these conditions are provided in air-conditioned plants and production is no longer conditioned by climatic conditions.

3. Basic chemical parameters of quality properties (water, protein, fat, NaCl, ash and nitrites)

   *in fermented sausage with inoculated Yersinia enterocolitica*

In Central and Northern Europe, the reduction of pH in fermented products is much more important for conservation, while in Mediterranean countries, the reduction of water activity (a_w) is more significant [16,17]. In foods which have a neutral pH, stored at a temperature of 5 °C, there is a possibility that the number of *Y. enterocolitica* will increase in a short period of time [12]. The presence of organic acid reduces the ability of *Y. enterocolitica*. *Y. enterocolitica* has the potential to multiply during storage of meat and meat products. *Y. enterocolitica* can grow at refrigeration temperature, in vacuum packaging or in modified atmosphere packaging. Foods that are rich in protein can be spoiled with bacteria which create hydrogen peroxide [18].

During ripening, the content of protein, fat, ash and salt increases due to drying (due to the loss of moisture). The content of nitrates and nitrites constantly decreases due to bacteria that reduce them to nitrogen monoxide. The influence of table salt on the microbiological processes in sausage is related to the influence of salt on the amount of water available to microorganisms, and also salt is used as a carrier for nitrite and sodium nitrite [1,3]. The amount of table salt added to fermented sausages is from 2.4 to 3.0% [3], and usually is between 2.8 and 3.2% [2]. In addition to the direct effect on the taste of the product, the table salt participates in the aromatic properties of other meat ingredients, colour and affects the microbiological processes in sausage [1-3]. The chemical composition of sausage fillings is on average: water content 55.36%, fat 25.71%, protein 15.45%, ash 3.54% and water 30.1% [15].

Regarding the fats, much attention is given to the relationship between meat consumption and cardiovascular diseases, cancer, diabetes and obesity [6]. However, adipose tissue is a traditional and necessary ingredient of sausages, because it helps to bind various ingredients and participates in creating the specific taste of sausage. Adipose tissue as a component of sausage stuffing affects the quality of fermented sausages. If the adipose tissue is fresher, with a lower content of polyunsaturated fatty acids,
the products have a better sensory quality [2]. Sausages that are produced with fatty tissue with a higher content of polyunsaturated fatty acids have less weight loss after drying [2,3]. During the long-term ripening of sausages, chemical changes can occur that can lead to rancidity of sausages [18]. The development of rancidity can be slowed down by proper selection of fat for production [1-3]. Peroxides, formed by the oxidation of fatty acids, oxidize myoglobin and prevent the formation of nitrosyl-myoglobin, a pigment which is responsible for the stable colour of fermented sausages [3]. It is believed that for the production of home-made sausages, the optimal amount of adipose tissue is from 10 to 20% [19]. The composition of fats of different animal species gives a characteristic aroma and taste of these products [16].

Inactivation of pathogenic bacteria during the ripening of sausages requires the control of their growth, so ripening is a key step in the production of fermented sausages [20,21]. In a study on fermented sausages that were experimentally contaminated with Y. enterocolitica [15], during maturation, this bacteria was not detected in control sausages. In narrow-diameter sausages that were contaminated with Y. enterocolitica, this pathogen was detected by the 7th day while in the wider diameter sausages it was detected by the 18th day of maturation [15]. The control group of sausage with wider and narrower diameters showed similar values of the chemical parameters as the sausages without the addition of starter culture (statistically significant difference p < 0.01). The water content of dry fermented sausages is always below 35%, but also less than 30% in many cases, which corresponds to an aw of 0.90; these low aw levels prolong the shelf life of the product [1,3]. At the end of the fermented sausage production process, no significant differences were found between the examined chemical quality parameters of sausage for both narrower and wider diameter sausages. Testing of the basic chemical composition of the sausages was done on day 0 and at the end of the production process, and standard analytical methods for testing were used. ISO reference methods were used to test the basic chemical composition and physicochemical properties.

3.1. Basic chemical quality parameters of the narrower diameter sausages
The chemical composition of the sausages of narrower diameter: the average water content of sausages of narrower diameter is from 28.19 ± 0.37% to 29.34 ± 0.13%, fat from 40.03 ± 0.34% to 40.87 ± 0, 52%, protein from 25.42 ± 0.23% to 25.67 ± 0.19%, ash from 5.17 ± 0.031% to 5.28 ± 0.045%, NaCl from 3.98 ± 0.036% to 4.09 ± 0.055%, nitrite from 0.05 ± 0.001% to 0.054 ± 0.001%. No statistically significant differences were found between the average values of the examined chemical quality parameters of the sausages of narrower diameter (difference between the average values is statistically significant at p <0.05).

3.2. Basic chemical quality parameters of the wider diameter sausages
The chemical composition of the sausages of wider diameter: the average water content of sausages of wider diameter was from 28.45 ± 0.20% to 29.96 ± 0.19%, fat from 39.51 ± 0.178% to 39.92 ± 0.23 %, protein from 25.33 ± 0.145% to 25.38 ± 0.17%, ash from 5.198 ± 0.10% to 5.29 ± 0.07%, NaCl from 3.99 ± 0.83% to 4.08 ± 0.07%, nitrite from 0.05 ± 0.001% to 0.054 ± 0.001%. No statistically significant differences were found between the average values of the examined chemical quality parameters of the sausages of wider diameter (difference between the average values is statistically significant at p <0.05).

4. Conclusion
During the production of the fermented sausages which are produced without heat treatment during ripening and drying, controlled conditions are used that stop the growth of pathogenic bacteria due to the simultaneous actions of several factors. There are numerous data in the literature on the chemical composition of fermented sausages [14,19,22-30]. The chemical composition of fermented sausages depends on the choice of raw materials, the proportions of muscle and fat tissue and their relationship. Dry fermented sausages should contain up to 35% water, meat protein content should be at least 20%, and collagen in meat proteins at most 15%, with the exception of poultry sausages and sausages placed on the market under a different trade name [31].
Our results show that all examined groups of sausages from this experiment meet this condition. In many European countries, increased demand for traditional products has been observed. However, the composition of fermented sausages differs between products and regions [32]. These products are foods with strong regional characteristics and origin, which should be protected and promoted as a part of the national traditions.

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