Changes in chemical attributes during ripening of traditional fermented sausage, “Pirot ironed”

S Simunovic¹, V Djordjevic¹, S Bogdanovic², I Dimkic³, S Stankovic⁴, S Novakovic⁴ and I Tomasevic⁴

¹ Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade, Serbia
² College of Agriculture and Food Technology, Cirila and Metodija 1, Prokuplje, Serbia
³ Department of Microbiology, Faculty of Biology, University of Belgrade, Studentski trg 16, Belgrade, Serbia
⁴ Animal Source Food Technology Department, Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade, Republic of Serbia

E-mail: stefan.simunovic@inmes.rs

Abstract. “Pirot ironed” is a traditional Serbian dry-fermented sausage manufactured in the south-east of Serbia. The changes in the chemical attributes of Pirot ironed sausage were followed during ripening. Samples were taken on the processing days 0, 7, 14, 21 and 28. Pirot ironed sausage was produced from the most valuable cuts of beef and chevon, without addition of starter cultures or fat tissues. Sausages were manufactured in a traditional drying/ripening chamber, where they were pressed every two days to acquire the typical flat form and to speed up the drying. The final water activity was 0.839. The lowest pH value recorded was 5.30 on the processing day 28. During ripening, the water content decreased significantly from 74.72% to 40.32%, while the protein and the fat amounts increased significantly from 19.12% to 45.79% and from 1.22% to 6.21%, respectively. Up to now, the properties of Pirot ironed sausage have not been recognized or published in scientific literature in spite of the long tradition and popularity of this meat product in Serbia.

1. Introduction

Traditional dry-fermented meat products constitute a diverse group of food products. Originating from distinct geographic regions, they bear characteristic sensorial properties gathered in high-quality meat products [1]. “Pirot ironed” (flattened) is a dry-fermented sausage manufactured in the municipality of Pirot (south-eastern Serbia). The sausage is traditionally made by mincing and mixing beef, chevon and mutton. Pirot ironed is an artisan Serbian sausage made only from meat and spices (sodium chloride, garlic, hot ground paprika and black pepper) without any additives or starter cultures. After mixing, the meat batter is stuffed into bovine small intestine in units of approximately 4 cm in diameter and 35 cm in length. The product has a characteristic flavour, which is mostly achieved by adding garlic. In the process
of drying, the sausage is pressed with a glass bottle every two days to acquire its typical flat form and to speed up the process. The microbial stability of the sausage is ensured by drying, low winter temperatures and the salt content. The risk in fermented meats is generally considered low because the bacteria numbers decline constantly under the conditions of ripening [2].

Most dry fermented sausages are products with a relatively high fat content, mostly due to addition of pork and fat tissues. The quality of the sausage depends on several factors, among which the meat to fat ratio plays a critical role. Low fat and high protein content of Pirot ironed sausage is achieved by using beef, chevon and mutton of the most valuable meat cuts and with no addition of fat. The sausage is typically manufactured by traditional means in small processing units, so therefore, their chemical attributes can vary. In order to protect the traditional aspect of the product, it is essential to understand the dynamics of changes in chemical parameters during ripening. The objective of this study was to investigate the effect of ripening time on the chemical attributes of Pirot ironed sausage. Up to now, the properties of this type of sausages have not been recognized or published in scientific literature in spite of the long tradition and popularity of this sausage in Serbia.

2. Materials and methods

2.1. Dry fermented sausages

The study was carried out on 15 dry sausages manufactured in a small-scale facility in Pirot (south-eastern Serbia). Pirot ironed sausage was produced by mincing and mixing the most valuable meat cuts of beef and chevon (50:50). They were trimmed of visible fat. During mixing, the following ingredients were added: sodium chloride, garlic, hot ground paprika and black pepper. The meat batter was stuffed into 37-40 mm diameter bovine small intestine to make horseshoe-shaped sausages of 450-500 g. The sausages were transferred to a traditional drying/ripening chamber where they were kept for 28 days. During the entirety of the drying/ripening period, the sausages were pressed every two days with a glass bottle to acquire the flat form. Once collected, the samples were vacuum-packed and then transported to the laboratory in a refrigerated box. Within this study frame, samples of the sausages were taken on the processing days: 0, 7, 14, 21 and 28. At each processing stage, three samples were analysed in triplicate.

2.2. Chemical analysis (pH, water activity, fat, moisture and protein)

In order to prepare the samples for analysis, the sausages were homogenised using a bowl chopper (Blixer 2, Robot Coupe, France). The pH of samples was measured using a digital pH-meter (CyberScan pH 510, Eutech, Singapore). Water activity (a_w) was determined using a Fast-lab (Gbx, Romans sur Isére Cédex, France) water activity meter, previously calibrated with sodium chloride and potassium sulphate. Fat content was determined using the ISO method 1444:1996 [3]. Moisture content (%) was determined by weight loss of the sample maintained in an oven (Lenton WF 200, Hope Valley, England) at 105 °C until constant weight (ISO 1442:1997) [4]. Total protein content was determined using the Kjeldahl method (ISO 937:1978) [5] that made use of an Unit 20 digestion block (Tecator™, Foss Analytical AB, Höganäs, Sweden) and automated distillation & titration device (Kjeltec™ 8400, Foss Analytical AB, Höganäs, Sweden). Triplicate determinations of chemical parameters were performed on each tested sausage.

2.3. Statistical analysis

For the analysis of the results of chemical traits, one way analysis of variance (ANOVA) was conducted using SPSS package (SPSS 23.0, Chicago, IL, USA). To distinguish statistical differences between the data, Tukey’s post hoc test was performed with statistical significance being set at P<0.05. Testing of normal distribution for datasets was evaluated by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Correlations between variables were determined using the Pearson’s linear correlation coefficient.
3. Results and discussion

During the 28 days of ripening, a decrease of pH, from 5.78 to 5.30, was observed (Table 1). The pH values showed significant differences (P<0.05) during manufacture. A decrease of pH during the manufacture is also reported for most other fermented sausages such as Salchichón, Chorizo, Horse Salami and other [6-13]. The initial pH value of Pirot ironed sausage was similar to those found in Salchichón, Chorizo and Horse Salami [6-8]. The decrease of the pH is probably a consequence of the conversion of sugar into lactic acid due to the action of bacteria [14]. At the end of the drying/ripening, pH values of sausages were higher than those reported for Chorizo de cebolla, Chorizo, Salchichón, Salami [15, 16], but lower than those reported for Fuet, Salame Felino and Sremska [16-18], which indicate that fermentation was limited.

| Processing time (days) | 0      | 7      | 14     | 21     | 28     |
|------------------------|--------|--------|--------|--------|--------|
| Chemical composition   |        |        |        |        |        |
| pH                     | 5.78±0.01<sup>a</sup> | 5.71±0.01<sup>b</sup> | 5.69±0.01<sup>b</sup> | 5.58±0.06<sup>c</sup> | 5.30±0.05<sup>d</sup> |
| a<sub>w</sub>           | 0.956±0.00<sup>a</sup> | 0.945±0.00<sup>b</sup> | 0.905±0.00<sup>c</sup> | 0.868±0.00<sup>d</sup> | 0.839±0.00<sup>e</sup> |
| Moisture               | 74.72±1.0<sup>a</sup> | 65.59±1.40<sup>b</sup> | 54.19±1.31<sup>c</sup> | 43.18±1.08<sup>d</sup> | 40.32±0.70<sup>e</sup> |
| Protein                | 19.12±0.13<sup>a</sup> | 25.24±1.61<sup>b</sup> | 33.82±0.73<sup>c</sup> | 42.71±0.55<sup>d</sup> | 45.79±0.91<sup>e</sup> |
| Fat                    | 1.22±0.7<sup>a</sup>   | 1.49±0.14<sup>a</sup>   | 3.88±0.37<sup>b</sup>   | 3.94±0.33<sup>b</sup>   | 6.21±2.36<sup>e</sup>   |

<sup>a</sup>-<sup>e</sup> Values in the same row followed by different letters are significantly different (P<0.05).

The average moisture content of the sausages decreased progressively during the entirety of the drying/ripening period. The average initial moisture content of the sausages (74.72%) was higher than those found by other authors in Chorizo de cebolla, Salame Felino and Linguíça [15, 17, 19]. This is because Pirot ironed sausage is made with muscle-only meat batters that will, consequently, have more water than other types of fermented sausages made with the addition of fat tissues.

Microbiological indicators of process hygiene in Serbian meat processing establishments are improving after the mandatory adoption of HACCP [20, 21], and likewise, the level of food safety knowledge among Serbian meat handlers is also improving [22]. When using fermentation as a main safety hurdle, most fermented meat products are microbiologically stable when pH 5.3 or lower is obtained within a relatively short period of time. Traditional meat fermentation is anything but fast because it relies on lactic acid bacteria which naturally occur in fresh meat and the environment to initiate the process. While this practice today is perceived as an “art form” in itself, it is highly unreliable as a single meat safety measure. The Pirot ironed sausage has a final pH $\leq$ 5.6, which coupled with the $a_w \leq$ 0.88 achieved during drying, ensures growth of Listeria monocytogenes and Staphylococcus aureus is unlikely on this product [23].

Weight loss of sausages is mostly caused by moisture evaporating. Muguerza et al. [24] investigated effects of fat level on processing and quality characteristics of fermented sausages and found that weight loss is significantly affected by the fat level (P<0.001), so the higher the fat level, the lower the weight losses over the same processing time. In consonance with these findings, moisture content of Pirot ironed sausages in our investigation decreased more rapidly during the 28 days of ripening than the values reported for fermented sausages made with addition of pork fat such as Salchichón, Horse Salami, Chorizo de cebolla and Salame Felino [6, 8, 15, 17]. This is because of the low fat content in our samples and the fact that muscles are more capable of losing water, taking it less time to evaporate the same amount of.
moisture compared to fat [25]. The other reason for rapid moisture loss is the flattening of the sausages with a glass bottle, making its surface to volume ratio much more favourable for water evaporation. At the end of the ripening process, moisture content values were similar to those found in Salchichón and Salame Felino after 28 days of ripening [6, 17], but higher then those reported for Horse Salami, Chorizo de cebolla and Androlla [8, 15, 26]. As a result of drying, the \( a_w \) gradually decreased to 0.839. Moisture content showed a positive correlation with \( a_w \) values \((r=0.95, P<0.01)\) (Figure 1), as also reported for Salchichón [6].

Reduction in moisture during ripening caused the increase in fat and protein contents [27], as also observed in our study. The average initial fat content of sausages was lower than those indicated in the literature for Horse Salami, Chorizo de cebolla, Salame Felino, and Androlla [8, 15, 17, 26] and for some traditional Serbian dry-fermented sausages such as Sremska and Sudzuk [18]. At the end of the drying/ripening period, total fat content in the sausage was lower than those reported for Chorizo de cebolla, Salchichón, Fuet, Salami, Salame Felino and Kulen [15-17, 28]. This is a result of addition of well-trimmed beef and chevon and no addition of fat tissues. In consonance with the low fat values, the Pirot ironed sausage showed a very high content of protein, with values higher than those found by other authors for Chorizo de cebolla, Salame Felino, [15, 17] and typical Serbian fermented sausages such as Sremska, Sudzuk and Kulen [18, 28].

![Figure 1. Correlation between water activity (\( a_w \)) and moisture content (%) during ripening of Pirot ironed sausage.](image)

4. Conclusion
The microbiological safety of Pirot ironed sausage is ensured by low \( a_w \) values. Use of well-trimmed beef and chevon and no addition of fat tissues results in high protein and moisture contents and, consequently, in low fat content of the sausage. High protein content and flattening of the sausage probably influences the more rapid evaporation of moisture compared to other types of dry-fermented sausages. Consumer awareness of the health benefits of low-fat diets is constantly growing, and therefore, Pirot ironed sausage can be recognized as a low-fat product, which fits their need. Identification and characterization of autochthonous starter cultures is needed, due to the possibility of industrial production in future.
References

[1] Tomić N, Tomašević I, Radovanović R and Rajković A 2008 J. Muscle Foods 19 237–46
[2] Casquete R, Benito M J, Martín A, Ruiz-Moyano S, Aranda E and Córdoba M G 2012 Food Control 24 191–8
[3] ISO 1444:1996 Meat and meat products - Determination of free fat content
[4] ISO 1442:1997 Meat and meat products - Determination of moisture content (Reference method)
[5] ISO 937:1978 Meat and meat products - Determination of nitrogen content (Reference method)
[6] Lorenzo J M, Tempérán S, Bermúdez R, Cobas N and Purriños L 2012 Meat Sci. 90 194–8
[7] Lois A L, Gutiérrez L M, Zumalacárregui J M and López A 1987 Meat Sci. 19 169–77
[8] Kovacevic D, Mastaenjevik K, Pleadin J and Frece J 2016 Ital. J. Food Sci. 28 96–106
[9] Gómez M and Lorenzo J M 2013 Meat Sci. 95 658–66
[10] Lorenzo J M, González-Rodríguez R M, Sánchez M, Amado I R and Franco D 2013 Food Res. Int. 54 611–20
[11] Lorenzo J M, Gómez M and Fonseca S 2014 Food Control 46 382–89
[12] Fonseca S, Gomez M, Domínguez R and Lorenzo J M 2015 Grasas Aceites 66 1–13
[13] Fonseca S, Gomez M, Domínguez R and Lorenzo J M 2015 Grasas Aceites 66 1–13
[14] Leite A, Rodrigues S, Pereira E, Paulos K, Oliveira A F, Lorenzo J M and Teixeira A 2015 Meat Sci. 105 114–20
[15] Andrés A, Barat J M, Grau R and Fito P 2007 Principles of Drying and Smoking. In: Toldrá F (ed.). Handbook of Fermented Meat and Poultry (Oxford: Blackwell Publishing Ltd.) pp 37–48
[16] Salgado A, García Fontán M, Franco I, López M and Carballo J 2005 Food Chem. 92 413–24
[17] Herrero A M, Ordóñez J A, de Avila R, Herranz B, de la Hoz L and Cambero M I 2007 Meat Sci. 77 331–8
[18] Saccani G, Fornelli G and Zanardi E 2013 Int. J. Food Prop. 16 1460–71
[19] Ducic M, Vranic D and Baltic M 2018 Meat Technol. 59 120–6
[20] Gonzales-Barron U, Cadavez V, Pereira A P, Gomes A, Araújo J P, Saavedra M J, Estevinho L, Butler F, Pires P and Dias T 2015 Food Res. In. 78 50–61
[21] Džekić I, Kuzmanović J, Andelković A, Saračević M, Stojanović M M and Tomasević I 2016 Food Control 60 131–7
[22] Tomasevic I, Kuzmanovic J, Andelkovic A, Saracevic M, Stojanovic M M and Dzekic I 2016 Meat Sci. 114 54–7
[23] Smigic N, Antic D, Blagojevic B, Tomasevic I and Dzekic I 2016 Br. Food J. 118 pp 9–25
[24] Tomašević I and Džekic I 2017 HACCP in fermented meat production. In: Zdolec N (ed.). Fermented Meat Products: Health Aspects (Boca Raton: CRC Press) pp 512–34
[25] Muguerza E, Fista G, Ansorena D, Astiasaran I and Bloukas J G 2002 Meat Sci. 61 397–404
[26] Incze K 2007 European Products. In: Toldrá F (ed.). Handbook of Fermented Meat and Poultry (Oxford: Blackwell Publishing Ltd.) pp 307–18
[27] Fanco I, Prieto B, Cruz J M, López M and Carballo J 2002 Food Chem. 78 339–45
[28] Olivares A, Navarro J L, Salvador A and Flores M 2010 Meat Sci. 86 251–7
[29] Parunović N, Petrović M, Matekalo-V Sverak V, Radojković D and Radović Č 2014 J. Food Process Pres. 38 2061–8