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Review paper

Different approaches in reformulation of dry fermented sausages

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A R T I C L E I N F O

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A B S T R A C T

Salting is considered to be one of the oldest food preservation techniques. In the past, salt that was used in meat product formulations always contained different types of impurities, among which were sodium nitrite and potassium nitrite. Today, nitrite salts are considered to be one of the most important additives in processed meat production. Due to their negative impact on human health, however, the industry has a challenge to find suitable ways to reduce nitrite content in meat products or to remove nitrites altogether from meat product formulations. Another challenge facing the meat industry is the formulation of low-fat products or products containing vegetable oils, which have more favourable n-3/n-6 fatty acid ratios than animal fats. Next, since excessive intake of sodium chloride was found to have negative effects on human health, the industry has to find a way to reduce the amount of this salt in meat products or to replace sodium chloride with different salts in order to meet consumer demands. In this paper, we review different approaches to reducing nitrites, fatty tissue and sodium chloride in dry fermented sausages.

1. Introduction

Fermented sausage production originated around the Mediterranean, where ancient Egyptians and Greeks stuffed salted meat and fat into casings of slaughtered animals (*Zanardi & Novelli*, 2021). The Romans inherited these production methods, but they also introduced the use of spices, which led to fermented sausages becoming a group of products with very differentiated flavours. The popularity of fermented sausages can be attributed to their high nutritional value and long shelf life, which were especially important in the past. Despite technological development in the 20th century, methods for producing fermented sausages have not changed much. Generally, they are produced from meat, fatty tissue, salt and spices. Meat and fatty tissue are cut into small cubes, to which salt and spices are added. The mixture is stuffed into casings and subjected to fermentation and drying, and sometimes smoking.

The amount of fatty tissue used in sausage production depends on the traditional formulation of each specific type of sausage. In addition, cutting methods for both meat and fatty tissue have an impact on the sausage's specific technological and sensory parameters, especially on drying kinetics and taste. Fatty tissue has an great influence on the flavour and texture of sausages. Fat also acts as a medium for the dissolution of liposoluble substances, like vitamins and aromatic compounds. From the human health point of view, fat is very important as it provides a high amount of energy, and it is also good source of essential fatty acids. However, the modern human diet often involves excessive consumption of fat. That is why, in the last few decades, many health initiatives from food companies have been directed towards the reduction of fat content in their products or replacement of fats high in saturated fatty acids with those rich in unsaturated fatty acids (*Rajic et al.*, 2022).

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According to the World Health Organisation (WHO), consumption of saturated fatty acids, which occur in large amounts in animal fat, should not exceed 10% of daily total energy consumption (*WHO*, 2023). Excessive fat intake leads to a number of cardiovascular diseases, like elevated blood pressure, coronary heart disease, obesity and others (*WHO*, 2013).

Salting is considered to be one of the oldest food preservation techniques. The first written evidence of salted food trading dates back to the 12th century BCE, when salted fish was sold in the Eastern Mediterranean (*Binkerd & Kolari*, 1975). In the 9th century, salting, together with smoking of meat, was already considered to be an old practice (*Binkerd & Kolari*, 1975). Back then, the origin of salt consumed in a specific region was determined by its natural availability. Hence, in the Mediterranean area, salt usually originated from the sea, while in regions distant from the sea, salt was obtained from minerals that are naturally rich in sodium chloride. Technological procedures at that time were not advanced enough to ensure production of high purity sodium chloride. Hence, the salt that was used in the past contained different types of impurities, among which was potassium nitrite.

Nitrite salts are one of the most commonly used additives in processed meat production. These salts have positive effects on numerous quality characteristics of meat products. The most important effect of nitrites is development of the characteristic bright pink colour of cured meat. In addition, nitrites act as strong antioxidants by slowing down fatty acid oxidation, which is the main reason for the development of rancidity in processed meat. Some studies also point out the great importance of nitrites in the flavour development of meat products (*Simunovic et al.*, 2023) and in antimicrobial effects against specific types of bacteria (*Christiansen et al.*, 1973). Despite the advantages of using nitrite with respect to many quality parameters, the International Agency for Research on Cancer (IARC) classified nitrites in group 2A as probably cancerogenic substances. More precisely, it is believed that changes to which nitrites are subjected during production of meat products can lead to the formation of carcinogenic nitrosamines (*Simunovic et al.*, 2022a). In recent years, consumers have become more open to concept of "clean label" food, due to the increase in health concerns regarding processed food.

The aim of this manuscript was to summarize achievements regarding strategies for the reformulation of dry fermented sausages. These include: i) nitrite reduction or use of nitrite replacers; ii) fatty tissue reduction or replacement with vegetable oils and; iii) sodium chloride reduction or replacement, or application of saltiness-enhancing compounds.

2. Nitrites

In 1925, the United States Department of Agriculture permitted the use of sodium nitrite in the production of processed meats (*Binkerd & Kolari*, 1975). Levels of nitrites that can be added to meat products remained more or less the same to this day. Back then, sodium nitrite could be added up to 200 mg/kg of the product. Today, the European Regulation on food additives No 1333/2008 prescribes limits for the use of sodium or potassium nitrite that depend on the type of product, but generally range from 100 to 175 mg/kg of the product *(EC, 2008)*. These limits are the same in Serbia, as in 2018, the local regulation on food additives was harmonised with the European law *(Official Gazette of the Republic of Serbia, 2018)*.

As fermented sausages are usually low pH and low water activity (a_w) products, they are considered to be an undesirable environment for the growth of many pathogenic bacteria. It seems that the main problem in obtaining a long shelf life in traditional dry fermented sausages is lipid oxidation, i.e., oxidation of fatty acids that results in development of a rancid flavour. It is believed that this is the result of the formation of compounds such as aldehydes, ketones, alcohols, esters and others. When oxygen is present, it is only possible to slow down oxidation of fatty acids, but not to stop the process. This is where nitrites play a crucial role, by biding with haem that acts as a catalyst for lipid oxidation (*Karwowska et al.*, 2019).

Besides of their irreplaceable effect on the colour of cured meat products, some justification for the use of nitrites in processed meat production is found in their antimicrobial activity. However, some studies have questioned whether the use of nitrites in some types of meat products is justified regarding the control of botulinum toxin production. In the study of *Hospital et al.* (2016), spores of *Clostridium botulinum* were inoculated into the meat batter of *fuet*, a traditional Spanish fermented sausage, but *C. botulinum* was not capable of surviving the low pH and a_w found in this specific study's conditions. On the other hand, in the case of dry meat products, the use of nitrites has been banned in the production of traditional Parma ham for more than 30 years. During that period, there have been no cases of botulism connected with Parma ham, while at the same time, the characteristic red colour of the product is maintained thanks to the exchange of ions in the porphyrin ring of myoglobin (*Honikel*, 2008).

There have been numerous attempts to reduce the use of nitrites in meat products using a few different approaches. The first approach includes direct reduction of the nitrite level in product formulation, while still taking care that nitrite salts are added in amounts that are sufficient to ensure their positive effects on product quality. The second approach is by application of various compounds that act as nitrite alternatives. Since nitrites have multiple effects on a number of quality traits in meat products, separate compounds are usually used for colour enhancement, flavour development, stabilisation of lipids and antimicrobial activity. The third approach is the application of nitrate-rich ingredients. The main idea of this approach is that the activity of bacteria present in the meat batter of fermented sausages reduces nitrate to nitrite. Thus, there is indirect production of nitrite which can exert its influence on product quality.

2.1. Nitrite reduction

In a recent study, *Simunovic et al.* (2022a) produced traditionally fermented *kulen* sausage with 50% nitrite reduction and without nitrite. The goal of the study was to evaluate the influence of sodium nitrite on various quality parameters of the product. *Kulen* is traditionally produced using high levels of red paprika powder, which has an important influence on the sausage colour. The study revealed that colour parameters remained the same, regardless of the level of nitrite used in *kulen* formulation. With respect to oxidative stability, nitrite proved to be powerful antioxidant, as *kulen* produced without sodium nitrite had significantly higher thiobarbituric acid reactive substance (TBARS) levels, which were used as a measure of the development of secondary lipid oxidation products (*Simunovic et al.*, 2023). On the other hand, TBARS levels were similar when the sodium nitrite content in *kulen* formulation was reduced from 110 mg/kg to 55 mg/kg. Since fatty acids can be oxidised, it is very important to determine levels of free fatty acids. Levels of free fatty acids were the highest in sausages formulated without nitrites, but similar between sausages formulated with 110 mg/kg or 55 mg/kg of sodium nitrite, which is in accordance with the levels of TBARS. The study of *Simunovic et al.* (2022a) revealed that oral processing parameters differed, depending on the level of nitrite. More precisely, the consumption time for one bite(s) and the number of chewing strokes were significantly lower in sausages formulated without nitrites. These results are consistent with the results obtained by texture profile analysis, by which it was found that values of hardness, gumminess and chewiness of nitrite-free *kulen* were significantly lower than those of nitrite-formulated sausages. The cohesiveness of nitrite-free sausages was numerically lower than for nitrite-formulated *kulen*, but was not statistically significantly different. *Simunovic et al.* (2023) showed that sensory panel scores for aroma, taste, consistency and sensory acceptability of nitrite-free *kulen* were significantly lower than those of *kulen* formulated with sodium nitrite, regardless of concentration. In general, the results of these two studies showed there is no negative effect from reducing sodium nitrite in *kulen* from 110 mg/kg to 55 mg/kg, according to all analysed quality parameters. On the other hand, complete removal of nitrite from the formulation resulted in reduced oxidative stability, different texture and lower sensory acceptability of *kulen* compared with nitrite-formulated *kulen*.

2.2. Nitrate alternatives

In fermented sausages, the main idea of utilising vegetables that are rich in nitrates is their conversion to nitrites during sausage ripening. Since an important effect of nitrites is the formation of a characteristic pink, cured meat colour, most of the studies regarding nitrite substitutes focused in the first place on colour. *Ozaki et al.* (2021a) evaluated the possibility of using beetroot and radish powders as nitrite alternatives. The idea of using beetroot and radish lies in their high levels of nitrates, which according to authors are around 16,000 and 14,000 mg/kg in dry powder form. Beetroot and radish powders were separately added to fermented sausages at concentrations of 0.5% and 1%. The most promising results in terms of product colour were obtained using 0.5% radish powder. The study showed that during drying of sausage with added beetroot and radish powders, nitrite was formed after seven days. At the end of processing, sausages formulated without nitrites but added beetroot and radish powders, regardless of concentration, had significantly lower TBARS values, indicating less oxidative stability, than nitrite-formulated sausages. However, at the end of the storage period that lasted for 60 days, TBARS values of all batches were similar with the exception of the control no. 2 that was produced without nitrites, beetroot or radish powder. *Ozaki et al.* (2021a) evaluated nitrite replacement with a combination of radish powder and oregano essential oil in cooked fermented sausages. They produced two combinations, one that contained 0.5% radish powder and 100 mg/kg of oregano essential oil, and a second that was formulated with 1% radish powder and 100 mg/kg of oregano essential oil. Sensory analysis showed no difference between control sausages and those formulated with radish powder and oregano essential oil. In addition, sausages produced with the combination of 1% radish powder and 100 mg/kg of oregano essential oil showed similar TBARS values as the control that was formulated with 150 mg/kg of sodium nitrite. Similarly to their previous study (*Ozaki et al*., 2021a), nitrite was formed after eight days of drying, probably as a result of nitrate reduction to nitrite (*Ozaki et al.*, 2021b).

Sucu & Turp (2018) added beetroot powder into the traditional Turkish fermented sausage, *sucuk.* Four batches were made in total. The first was produced as a control and contained 150 mg/kg of sodium nitrite, the second was produced using the combination of 100 mg/kg of sodium nitrite and 0.12% beetroot powder, the third was formulated using 50 mg/kg of sodium nitrite and 0.24% beetroot powder, while the fourth contained only 0.35% beetroot powder. Residual nitrite content was similar during the entirety of the ripening, regardless of the initial nitrite levels in sausage formulation (*Sucu & Turp*, 2018). On the other hand, nitrite addition showed a significant effect on lactic acid bacteria, as the highest number of these bacteria was found in nitrite-free sausages, i.e., sausages formulated with the addition of 0.35% beetroot powder. In terms of colour, all batches of *sucuk* produced with beetroot powder had a significantly higher a^* value, while neither nitrite reduction nor beetroot application effected changes in L^* or b^* values. From the consumer point of view, beetroot use in the sausages did not compromise the overall acceptability of *sucuk,* since all analysed batches had similar scores for outside appearance, inside appearance, outside colour, inside colour, texture and overall acceptability (*Sucu & Turp*, 2018).

3. Fat

In the production of pork fermented sausages, firm fatty tissue, which is naturally located on M. *longissimus dorsi*, is used. Firm fatty tissue has higher levels of saturated fatty acids and consequently a higher melting point than other animal fats. Because of this, cutting and achieving the desired granulation of fat in the meat batter is easier. Granulation and the distribution of fatty tissue in the sausage affects drying kinetics of the product (*Lorenzo & Franco*, 2012).

In the study of *Simunovic et al.* (2022b), traditional Serbian tea sausage was produced with three different levels of pork fatty tissue. The first batch was used as a control and contained 25% pork firm fatty tissue. The second batch contained around 17.5% of this tissue, while the third batch was produced using 10% fat. At the beginning of production, sausage formulated with only 10% fat had a significantly higher moisture content than other sausage types, as was expected, since meat contains more moisture than does fatty tissue. However, at the end of drying, more precisely after 35 days of ripening, sausage formulated with 10% fatty tissue had the lowest moisture content among the sausage types. These results indicate the great importance of fat level on the drying kinetics of fermented sausages. The higher level of fat used in the tea sausage was found to slow down the ripening process, which helps in achieving the characteristic speed of drying. *Simunovic et al.* (2022b) indicated that fat level does not affect colour parameters of the sausages, as had been indicated by other authors. This disagreement is because in most of the studies in the past, the colour of fat on the sausage cross section was not independently measured (*Lorenzo & Franco*, 2012). In terms of texture, *Simunovic et al.* (2022b) showed that by decreasing the level of fat in the sausage formulation, the sausage became harder, as measured using a texture analyser. Similarly, values of chewiness and gumminess increased with the increasing level of meat in the formulation. The numbers of total viable bacteria and lactic acid bacteria were similar in all analysed batches of tea sausage. TBARS value, which was used as a measure of the level of lipid oxidation, was not affected by fat reduction. On the other hand, sensory parameters, which were evaluated by sensory panel, were significantly different among the sausage types. The reduced-fat tea sausage was assessed by the method of oral processing, which is relatively new in sensory analysis. The results showed significant differences among the batches in terms of values obtained for consumption time of one bite(s), chewing rate (chew/s), eating rate (g/s) and fat intake rate (fat/s). Sausages produced with 10% fatty tissue obtained significantly lower scores than the other sausage types for overall acceptability after both 28 and 35 days of ripening. On the other hand, sausages produced with 10% and with 17.5% fat had similar values for overall acceptability after 28 days of ripening. The results showed that if drying the 17.5% fat sausages continued until 35 days, overall acceptability scores significantly decreased.

Lorenzo & Franco (2012) evaluated fat's effect on various quality properties of dry foal sausage. In total, three batches were made, each containing different levels of firm fatty tissue. The first was formulated with 20%, the second with 10% and the third with 5% pork back fat. In terms of bacterial counts, lactic acid bacteria, *Micrococcaceae* and total viable count, fat reduction showed no significant effect. However, sausages formulated with the lowest level of fat had significantly higher pH than the other sausages. This is in accordance with the results of *Simunovic et al.* (2022b), who reported that with a decreasing content of fatty tissue in tea sausage formulation, the pH increased. *Lorenzo & Franco* (2012) conducted a sensory study in which thirteen sensory traits of reduced fat foal sausages were assessed. Sausages formulated with 5% pork fatty tissue had significantly lower scores for juiciness, fat distribution and fat/lean ratio than higher fat sausages. On the other hand, scores for hardness, taste intensity, black pepper odour and cohesiveness were higher for low-fat sausage. With regard to texture, the results of *Lorenzo & Franco* (2012) were in accordance with those later reported by *Simunovic et al.* (2022b), who found that reducing fat in fermented sausage results in increasing hardness.

The second approach in formulating low-fat products is to replace pork fat with various types of vegetable oils (*Bolumar et al.*, 2015; *Stajić et al.*, 2018). Since oils are mostly liquid at the room temperature, it is crucial to stabilize them. One of the most common ways to do that is to form oleogels (*Zampouni et al.*, 2022). *Stajić et al.* (2018) added flaxseed oil into the formulation of fermented sausages. Sausages enriched with flaxseed oil contained a more favourable n-3/n-6 fatty acid ratio. However, sensory scores for colour, odour, aroma, taste, texture and overall acceptability were significantly lower in sausages enriched with flaxseed oil than those scores for control sausages. Importantly, encapsulation of oil resulted in significantly higher (more acceptable) sensory scores in comparison with scores obtained for sausages in which the liquid form of flaxseed oil was added (*Stajić et al.*, 2018). This finding supported *Bolumar et al.* (2015), as those authors reported that sausages enriched with olive oil were unable to keep oil within the meat batter.

4. Salt

Salt is an essential part of the human diet, as it is necessary for maintenance of normal functions of the human body. On the other hand, if consumed in excessive amounts, salt is dangerous to human health. High intake of salt can lead to various health conditions, such as high blood pressure, different types of cardiovascular diseases, cancer, obesity and osteoporosis (*WHO*, 2023). According to WHO (*WHO*, 2023), the recommended daily adult intake

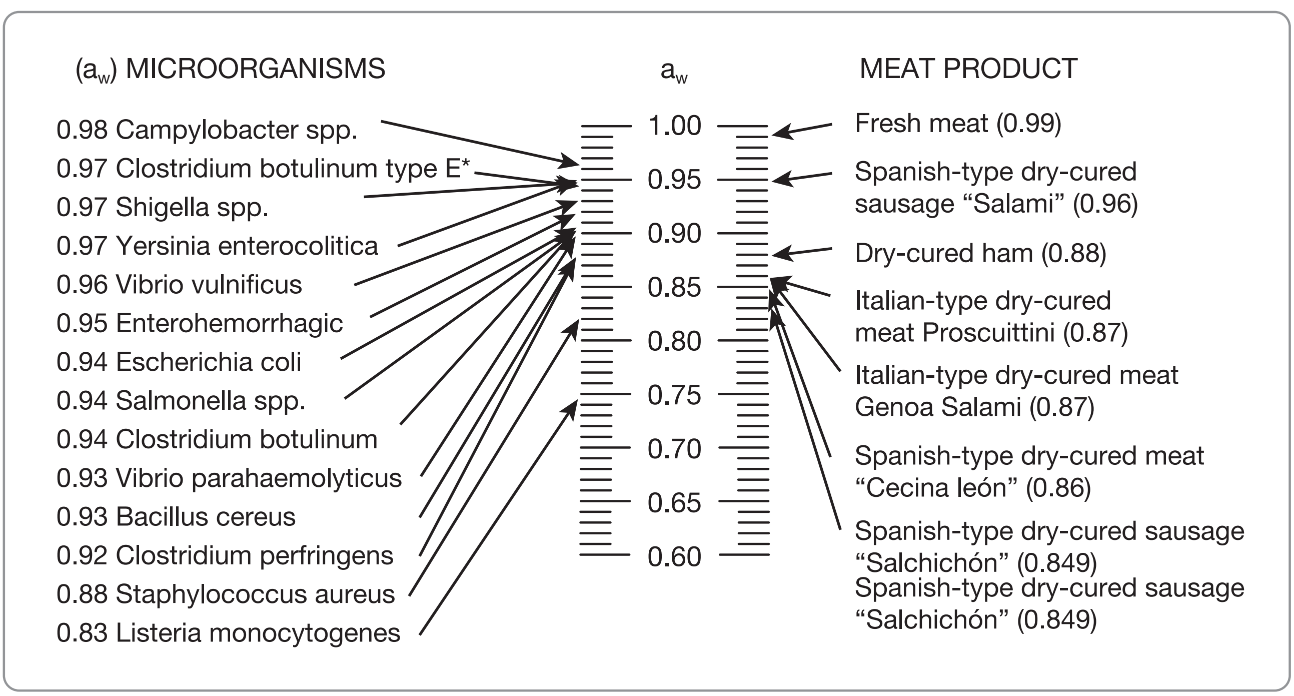

Figure 1. Minimum a_w values for growth of different bacteria and a_w values of popular meat products (*Grau et al.*, 2014).

of salt (measured as sodium equivalent) is less than less than 2 g of sodium/day; this corresponds to less than 5 g of sodium nitrite/day.

In recent decades, there have been numerous attempts to formulate foods with lower sodium levels. These include different approaches, among which two were the most common. The first is reducing sodium chloride in product formulations, while the second is replacing sodium chloride with other types of salt. In addition, a recent study has used saltiness-enhancing peptides in low-sodium meat products (*Chen et al*., 2023). In the production of dry fermented sausages, salt is of great importance for drying and maintaining low a_w. Figure 1 presents the lowest a_w values at which the specified bacteria can grow and typical a_w values of popular meat products.

Şimşek *et al.* (2023) partly replaced sodium chloride in *sucuk* with potassium chloride, magnesium chloride and calcium chloride, and they reported the most promising alternative was the combination of 50% sodium chloride, 30% potassium chloride and 20% magnesium chloride. Generally, potassium chloride causes a bitter taste to develop, which is why it is crucial to determine the extent to which this no-sodium salt can be added to a meat product formulation but not affect consumer acceptability.

Različiti pristupi u optimizaciji procesa proizvodnje fermentisanih kobasica

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INFORMACIJE O RADU

Ključne reči:
Fermentisane kobasice
Nitriti
Čvrsto masno tkivo
Smanjenje sadržaja masti

APSTRAKT

Soljenje se smatra jednom od najstarijih tehnika konzervisanja. U prošlosti, so koja se koristila u proizvodnji proizvoda od mesa sadržala je različite vrste nečistoća, od kojih su neke bili natrijum i kalijum nitrit. Danas se nitritne soli smatraju jednim od najvažnijih aditiva u proizvodnji proizvoda od mesa. Zbog njihovog negativnog uticaja na zdravlje ljudi, industrija ima izazov da pronađe odgovarajući način da smanji sadržaj nitrita ili pak da potpuno prestane sa njihovom upotrebom Drugi izazov sa kojim se suočava industrija mesa je formulisanje proizvoda sa niskim sadržajem masti ili proizvoda sa dodatim biljnim uljima koja imaju povoljniji odnos n-3/n-6 masnih kiselina. Dalje, pošto je utvrđeno da prekomereni unos natrijum-hlorida negativno utiče na zdravlje ljudi, industrija se suočava sa potražnjom proizvoda sa smanjenim sadržajem natrijum-hlorida i proizvodima u kojima se koriste zamene za natrijum-hlorid. U ovom radu dajemo pregled različitih pristupa u smanjenju nitrita, masnog tkiva i natrijum-hlorida u proizvodnji suvih fermentisanih kobasica.

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