

EFFECT OF NITRATE-REDUCING BACTERIA AND CELERY ROOT (*APIUM GRAVEOLENS* VAR. *RAPACEUM*) ON THE SENSORY PROPERTIES OF DRIED MEAT "BILTONG"

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ABSTRACT

The objective of our study was to assess the effect of nitrate-reducing bacteria and natural nitrate source on the sensory properties of dried meat biltong. Appearance, colour on the cut, aroma, taste and juiciness, acidity, sweetness, and consistency were evaluated. The members of the panel gave their ratings between 1 and 5 according to the characteristics of the samples, with 1 being the lowest rating and 5 being the best rating. In the sensory evaluation of dried meat samples without oxygen absorber packaging, the group (P2) with bacterial culture, celery powder, and NaCl showed the highest scores for appearance, taste, juiciness, acidity, and consistency. Notably, P3 samples had significantly lower consistency scores compared to P2. Additionally, sample P1, with bacterial culture and NaCl, improved the colour on the cut compared to the negative control (C) with only NaCl. The best aroma and sweetness results were in the P3 group with curing salt, although these differences were not statistically significant ($P \geq 0.05$). The sensory evaluation of dried meat samples packaged with an oxygen absorber revealed that the combination of bacterial culture, celery powder, and NaCl (AP2) had the most positive influence on appearance, colour on the cut, and consistency. The group with the absorber and curing salt (AP3) showed improved aroma, taste, and juiciness. Acidity was primarily affected by the addition of bacterial culture and NaCl (AP1), as well as by AP2 and AP3. The most pleasing sweetness was achieved by adding bacterial culture and NaCl to samples with the absorber (AP1). Samples with the best consistency when packaged with the absorber were those with bacterial culture and celery powder (AP2). In summary, among dried meat samples with oxygen absorber, those with added curing salt (AP3) had the best overall sensory evaluation compared to the negative control with NaCl (AC). For samples without an oxygen absorber, the highest overall sensory scores were in the group with bacterial culture and celery powder, surpassing the control with NaCl (C). The use of an oxygen absorber and curing salt proved to be effective in the production of biltong, but replacing the curing salt with NaCl and the addition of celery powder and bacterial culture significantly improved the sensory evaluation compared to the other groups (except AP3).

Keywords: *Staphylococcus*, celery powder, curing salt, bacterial culture

INTRODUCTION

Dried meat is a popular food all over the world due to its durability and nutrient content. Drying and preserving meat dates back several centuries as an effective way of preserving food, especially meat. Drying meat allows the moisture content of the meat to be reduced, resulting in a product with low water activity (a_w) and therefore a microbial safe and storable product, as less water is available for the growth of microorganisms (Karolenko *et al.*, 2020). Dried meat products are available worldwide and although their original purpose was to preserve perishable meat products, they are now recognized as a healthy meat "snack" with high protein content (Gavai *et al.*, 2022). Biltong is a type of dried beef product (Karolenko *et al.*, 2020). From the Dutch terms bil (meat or leg) and tong (strip or tongue) comes the name biltong (Senekal, 2020).

A popular "air-dried" meat product from South Africa, biltong, is produced according to several recipes and drying techniques, resulting in a variety of properties that have been documented in the literature (Muga, 2019; Wilkinson, 2023). Due to the industrialization of biltong production over the past decade, it is now sold in supermarkets in the USA, South Africa, Australia, the UK, the Netherlands, and France (Arnaud *et al.*, 2021). It is comparable to other dried meat products found around the world, including jerky from the USA, rou gan from China, carne seca from Mexico, and charque from South America (Muga, 2019). Nevertheless, biltong has a unique characteristic that sets it apart from other dried and cured meat products: vinegar is often added during the preparation process to acidify the meat. South African settlers used ostriches to make biltong, as well as game such as kudu, springbok, and gemsbok. Later, because of its abundance and favourable properties, beef became the main meat. The loins just below the kidneys, the back muscles on each side of the spine, and the rear legs are the most used cuts for making biltong (Senekal, 2020).

The meat is often sliced into thin slices 20-50 mm thick, 40-150 mm wide and 300-600 mm long, which are used to produce biltong by slicing along the muscle fibres (Muga *et al.*, 2020). The dimensions are critical as they affect the biltong properties and drying time. Water removal can take longer than usual if the slices are too thick, which increases production costs and time and reduces profitability.

Too thin slices cause over-drying of the meat and an unfavourable result. As many customers like fat, a strip of fat may be left on the edges, but this can cause lipid oxidation and rancidity. Fat also retards salt absorption and reduces water diffusion (Senekal, 2020).

Before drying, the meat slices are salted, seasoned (with black pepper or coriander) and then vinegar is added. The distinctive flavour of biltong meat comes from the combination of spices, vinegar, and salt in the marinating mixture (Muga, 2019). By reducing the a_w of the product, the salt prevents bacteria from growing. Although up to 4% salt can be added, 2.5% is often added to the meat prior to marinating, as this achieves a flavour that is acceptable over a wide range of moisture contents in the finished product. At the salting stage, vinegar (red wine vinegar, apple cider vinegar) is added at a ratio of 3 to 6%. Its primary purpose is to inhibit the growth of microorganisms and its secondary purpose is to improve flavour (Jones *et al.*, 2019).

Meat slices are traditionally dried for one to two weeks at ambient temperature (24 °C) and relative humidity (55%), or for 1 to 3 days in an industrial condition (Mirade *et al.*, 2020), while convection hot-air dryers are used by commercial companies (Muga, 2019). Dehumidifiers and fans, together with other airtight containers like dryers, produce a dry, circulating air environment at room temperature. As a result, the drying process may be better controlled and is shortened from two weeks to a few days (Senekal, 2020). To satisfy customer preferences, the weight of the meat used to make biltong is dried to a minimum of 50% (Muga, 2019). Water activity varies from 0.54 to 0.93 and moisture content from 10 to 50% (Muga *et al.*, 2020). Biltongs are categorized as moist (35-42%) or dry (21-25%) depending on the amount of moisture they contain. According to Muga (2019), biltong with higher moisture level is now preferred by consumers. Throughout South Africa, biltong is often offered packaged in a variety of conditions, using everything from brown paper bags to plastic bags. Biltong that is manufactured industrially is often sold under vacuum or in modified atmospheric packaging (Jones *et al.*, 2019).

The biltong drying technique can lead to the production of biltong with a high number of microorganisms and unevenly dried products. The meat product cannot be stored at room temperature if its a_w is higher than 0.91, as this prevents the

development of microorganisms. Since the development of microorganisms is also dependent on pH, the addition of vinegar to the biltong may affect it. The antibacterial and antioxidant properties of vinegar are attributed to the concentration of polyphenols and melanoidins in addition to the acetic acid content. It is known that pathogens including *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* can be eliminated by using marinades that contain salt and organic acids (Jones et al., 2019).

Although biltong is considered a safe commodity, the microbiological profile of biltong has attracted criticism in some studies. These studies have revealed that biltong sometimes contains pathogens in addition to high concentrations of potentially harmful bacteria. There may be a hidden risk associated with biltong consumption due to the presence of pathogenic bacteria and toxinogenic fungi such as *Bacillus cereus* and *Aspergillus niger* (Muga, 2019). Concerns regarding microorganisms associated with raw meat or the meat processing environment are raised by the absence of a thermal lethality phase in the biltong processing (Gavai et al., 2022). Foodborne pathogens such as salmonella, listeria, enterotoxigenic staphylococci, and *E. coli* O157:H7 can be transmitted through food. In the past, there have been reports of foodborne illness associated with biltong consumption. One death and at least two outbreaks of severe gastroenteritis associated with salmonella have been reported from South Africa, while in Botswana there have been 17 deaths associated with biltong contamination (Muga, 2019). In this case, strict processing control is needed to avoid problems with the stability of the final product (Arnaud et al., 2021).

Traditional South African biltong differs from American jerky mainly because it does not have a heat rating for lethality. Different ingredients are needed to produce a microbially safe product because biltong production does not include a heat lethality rating; instead, it uses low temperature and humidity conditions to dry the meat for long periods of time. The main ingredients used in the production of biltong are salt, pepper and vinegar, which add flavour and prevent the growth of microorganisms. On the other hand, to produce a microbially safe product, heating, salting, smoking, and drying methods are usually used instead of vinegar in the production of jerky. Ultimately, both processes must demonstrate that they can provide a product that is safe for consumers (Karolenko et al., 2020).

One of the most popular substances for preserving dried meat products such as biltong is nitrite. Nitrites can be reduced from nitrates added in the form of a nitrate salting mixture or applied to meat products as curing salt. Their use is valued by the food sector because they significantly improve the organoleptic properties of meat and have antibacterial properties. In preservation processes, nitrites add red colour to meat products, aid flavour development and provide oxidative stability. In addition, they play a role in inhibiting the growth of harmful micro-organisms such as *Clostridium botulinum*. However, over the years, serious concerns have been raised, that consumers are being exposed to potentially dangerous substances that may develop in meat and meat products both during and after processing. When it was discovered in the 1960s that nitrites could combine with secondary amines to form carcinogenic nitrosamines in a mildly acidic environment, the use of nitrites to preserve meat became a serious concern. Therefore, attempts have been made to minimize their use as much as possible from a health perspective (Rodríguez-Daza et al., 2019).

Current trends are reflected in ingredients that naturally contain nitrates. Nitrates and nitrites can be added to foods through vegetables. Examples are the families Asteraceae (lettuce), Apiaceae (celery, parsley), Chenopodiaceae (beetroot, spinach) and Brassicaceae (arugula, radish, and mustard). Celery, which is recognized as a significant food allergen in Europe, is often used as a substitute for nitrates and nitrites. The conversion of nitrates to nitrites is an important stage in this process. Microorganisms can be used to represent this process. A possible strategy is to use safe nitrate-reducing bacteria to produce nitrate, which can then be used as a substrate for nitrite synthesis. The group of nitrate-reducing bacteria is well known due to their food tolerance as well as the fact that they represent an enzymatic repertoire for this activity (Rodríguez-Daza et al., 2019).

The objective of our study was to assess the effect of nitrate-reducing bacteria and natural nitrate source on the sensory properties of dried meat biltong.

MATERIAL AND METHODS

Characteristics of nitrate-reducing bacteria

From the group of nitrate-reducing bacteria, commercial species *Staphylococcus xylosus* (*S. xylosus*) and *Staphylococcus carnosus* (*S. carnosus*) (Indasia, Germany) were evaluated. For the meat fermentation, 0.2 g of nitrate-reducing bacteria per kilogram of meat was used.

Preparation of celery powder

Fresh celery root (*Apium graveolens* var. *rapaceum*) was purchased at a local store. The celery root was cleaned, peeled, and salted. The method of salting was carried out by creating a "salt crust" by mixing the salt with a small amount of water to form a compatible mass that was easy to work with. The mass formed was then applied to the raw material, which was wrapped in two layers of gauze. This process was followed by a drying process. The celery root was cut into thin slices

which were spread in a thin layer on sieves and left to dry for 1 month in a natural way (dark room with a constant temperature of 30 °C - summer season with natural air flow). After the main drying, the raw materials were dried at 50 °C for 20 minutes. The raw materials were then ground into a celery powder.

Manufacture of dried meat

The beef samples (low sirloin) were marinated for 7 days at 4 °C after the addition of the spices (sugar, coriander, apple vinegar) and tested ingredients (NaCl, curing salt, bacterial culture, celery powder). After that time, the samples were dried in an air-conditioned chamber at 25 °C and 73% humidity. After drying, the samples were cut into thin slices and vacuum packed together with and without an oxygen absorber. Sensory evaluation of the samples was conducted after 6 months. The individual experimental groups were labelled as follows:

- **C** - Negative control with the addition of NaCl (2%),
- **P1** - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%),
- **P2** - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%),
- **P3** - Experimental group with the addition of curing salt (2%),
- **AC** - Negative control with absorber and the addition of NaCl (2%),
- **AP1** - Experimental group with absorber and the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%),
- **AP2** - Experimental group with absorber and the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%),
- **AP3** - Experimental group with absorber and the addition of curing salt (2%).

The evaluation was repeated 3 times.

Sensory evaluation

The sensory evaluation was conducted in the sensory laboratory by 5 sensory panellists. Appearance, colour on the cut, aroma, taste and juiciness, acidity, sweetness, and consistency were evaluated. The members of the panel gave their ratings between 1 and 5 according to the characteristics of the samples, with 1 being the lowest rating and 5 being the best rating.

Statistical analysis

Data of the individual analysed groups were subjected to analysis of variance (ANOVA) using SAS software (version 9.3, Enterprise Guide 4.2, USA) and the level of significance α was set to 0.05.

RESULTS AND DISCUSSION

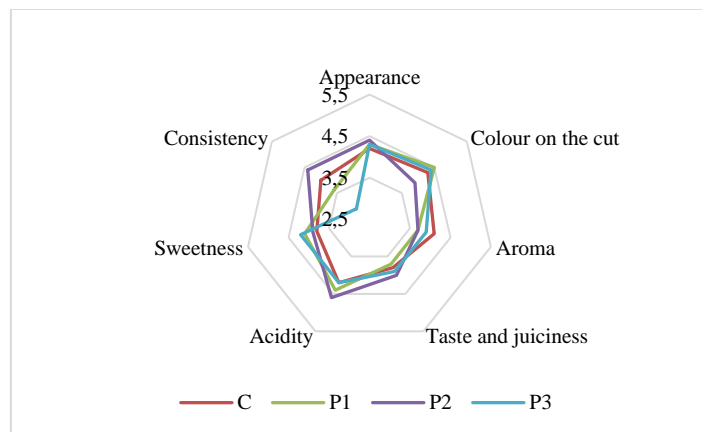


Figure 1 Sensory evaluation of dried meat samples packaged under conditions without oxygen absorber

Note: **C** - Negative control with the addition of NaCl (2%); **P1** - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%); **P2** - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%); **P3** - Experimental group with the addition of curing salt (2%).

For the sensory evaluation (Figure 1) of dried meat samples packaged under conditions without oxygen absorber the highest sensory scores for appearance, taste and juiciness, acidity, consistency, were obtained for the experimental group with the addition of bacterial culture, celery powder and NaCl (P2). Significantly the lowest scores in consistency were found in the P3 samples in comparison to the P2 samples. Experimental group with the addition of bacterial culture and NaCl (P1) favourably influenced the colour on the cut of the samples in comparison to the negative control with the addition of NaCl (C). In terms of aroma and sweetness the best results were obtained in the experimental group with the addition of curing

salt (P3). However, differences between the observed results were not significant ($P \geq 0.05$).

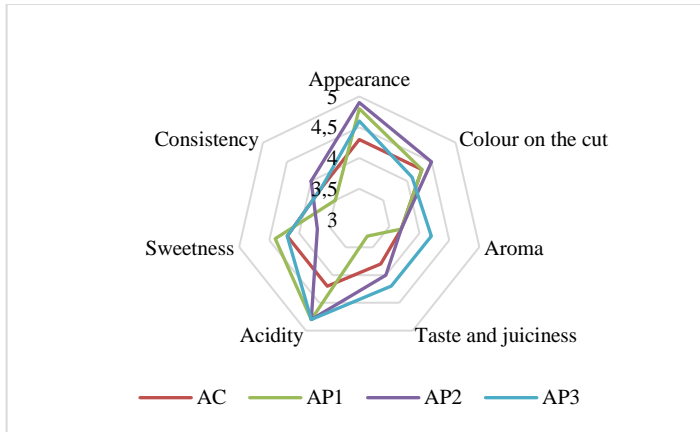


Figure 2 Sensory evaluation of dried meat samples packaged under conditions with oxygen absorber

Note: AC - Negative control with the addition of NaCl (2%); AP1 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%); AP2 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%); AP3 - Experimental group with the addition of curing salt (2%).

Based on the results of the sensory evaluation (Figure 2) of the dried meat samples packaged under conditions with oxygen absorber, it was found that the combination of bacterial culture, celery powder and NaCl (AP2) best influenced appearance, colour on the cut and consistency. Experimental group with absorber and the addition of curing salt (AP3) into the samples positively influenced aroma, taste, and juiciness. Acidity was most affected by the additions of bacterial culture and NaCl (AP1); bacterial culture, celery powder and NaCl (AP2) and curing salt (AP3) into the samples packaged together with absorber. The most pleasant sweetness was achieved by the addition of bacterial culture and NaCl into the samples with absorber (AP1). The consistency of the dried meat samples with absorber was best evaluated in the samples with the addition of bacterial culture and celery powder (AP2).

By combining vegetable juice or powder that is high in naturally occurring nitrates with a starting culture that reduces nitrates, "natural curing" can be achieved, leading to indirectly "cured" products (Sindelar et al., 2010). A nitrate-reducing bacterial culture is a necessary component for processed meats containing natural nitrate sources if the desired results are typical cured meat attributes. Since nitrate-reducing cultures have been commercially marketed for a while, it has long been understood that bacterial reduction of nitrate to nitrite is essential for meat curing. Most of these cultures have been employed in the manufacturing of dry sausages, where a culture's moderate flavour contributions are desirable and a long-term nitrite reservoir is needed (Olesen et al., 2004). The culture of nitrate-reducing bacteria, such as *Kocuria varians*, *S. xyloso* or *S. carnosus* is usually mixed with vegetable juices or powders. Nitrate may be converted to nitrite by these microorganisms. Temperatures between 15 and 40 °C are shown to produce the greatest results. Adequate growing conditions are necessary for the added culture (Wajdzik, 2016).

According to Sebranek & Bacus (2007), processed meats with characteristics like cured meat can be produced by using vegetable extracts as high-nitrate substitute ingredients alongside with a nitrate-reducing starter culture. It is important to monitor the amount of nitrite produced during this process. Because celery (*Apium graveolens* var. *dulce*) has a high nitrate and nitrite content, it has been used for many years as a flavouring and curing ingredient in meat products (Bacus, 1984). To organically cure meat products, processors add nitrate-producing substances, such as celery powder and a nitrate-reducing starter culture (Terns et al., 2011). Alternative cured meat products have been demonstrated to possess comparable sensory qualities to traditionally cured (Sindelar et al., 2007). However, the addition of celery and cherry powders may cause them to appear slightly yellow in colour (Redfield & Sullivan, 2015). Since celery has a moderate flavour and low vegetable pigment content, processors also typically employ celery powders as a natural source of nitrate and nitrite in meat products (Sebranek & Bacus, 2007; Sebranek et al., 2014). Its antioxidant qualities have also been linked to phenolic acids like pyrogallol and ellagic acid, as well as flavonoids like quercetin and hesperidin (Sorour et al., 2015). Furthermore, the synthesis of nitric acid might be affected favourably or unfavourably by other components of celery, including proteins, fibres, and carbohydrates. On the other side, bacteria like *Listeria monocytogenes* and *Clostridium botulinum* can be affected by nitric acid (Sebranek et al., 2014).

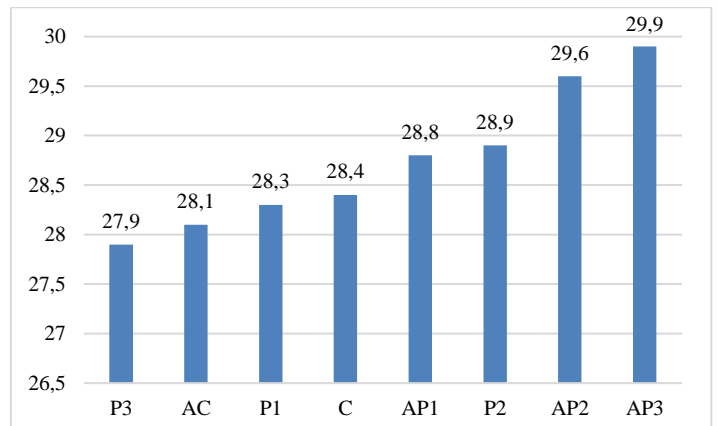


Figure 3 Overall sensory evaluation of dried meat samples packaged under conditions with and without oxygen absorber

Note: C - Negative control with the addition of NaCl (2%); P1 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%); P2 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%); P3 - Experimental group with the addition of curing salt (2%); AC - Negative control with the addition of NaCl (2%); AP1 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹) and NaCl (2%); AP2 - Experimental group with the addition of bacterial culture (0.2 g.kg⁻¹), celery powder (10 g.kg⁻¹) and NaCl (2%); AP3 - Experimental group with the addition of curing salt (2%).

Experimental group with oxygen absorber and the addition of curing salt (AP3) compared to the negative control with absorber and the addition of NaCl (AC) achieved the best overall sensory evaluation among the other dried meat samples packaged under conditions with oxygen absorber. From the dried meat samples packaged under conditions without oxygen absorber, the best overall sensory evaluation (Figure 3) was achieved in the experimental group with the addition of bacterial culture and celery powder in comparison with the negative control with the addition of NaCl (C). Regardless of the addition of the tested ingredients, the highest sensory scores were found in the samples packaged with the oxygen absorber (AP2 and AP3). In contrast, samples with the addition of curing salt packaged without oxygen absorber (P3) achieved demonstrably the lowest scores. Samples with added NaCl packaged with oxygen absorber (AC) achieved the second lowest scores in terms of the sensory descriptors investigated. In the production of dried meat "biltong", due to its sensory properties, the use of oxygen absorber and curing salt was found to be a suitable combination. However, by replacing curing salt with NaCl and adding celery powder as well as nitrate-reducing bacteria, a significantly higher sensory score was found compared to the other experimental groups (except for AP3).

Currently being studied for the manufacture of meat products are commercial species *S. xyloso* and *S. carnosus*, which belong to the nitrate-reducing group of bacteria (Wang et al., 2022). The ability of these microorganisms to convert nitrate to nitrite and produce nitrosyl myoglobin, which gives meat products their distinctive red colour, stabilizes colour, inhibits rancidity by breaking down hydrogen peroxide with the help of catalase activity, and develops flavour through lipolytic and proteolytic activities (Cebrián et al., 2020). For instance, it has been shown that dry sausages inoculated by *S. xyloso* had lower moisture content and a_w, higher a* values, and better texture. These results have been attributed to *S. xyloso* metabolites changing the characteristics of the meat proteins, which improves the product's quality. It was also examined at how *S. xyloso* affected the oxidation and hydrolysis of meat proteins. In a different investigation, *S. carnosus* shown proteolytic activity and secreted protease throughout the fermentation process (Wang et al., 2022).

In one study, natural meat products were made with *S. carnosus* as starter culture and celery powder. The experimental group that included natural preservatives (plant-derived nitrate and *S. carnosus*) and the control group that had added nitrite were not different in their impact on the qualitative characteristics of the ham after 90 days of storage. After 4 weeks of cold storage at 4 °C, Jin et al. (2018) examined the impact of an experimental group containing 0.8% celery powder and a control group containing 100 ppm nitrite on the physicochemical parameters of salami. They concluded that the experimental group's residual nitrite level was 17 ppm, whereas the control groups was 29 ppm.

The two strains of *S. carnosus* that had different nitrate-reducing abilities were employed as starter cultures in a separate investigation to investigate the impact on colour development during raw ham curing. The high nitrate-reducing activity strain that was used to inoculate the cured ham resulted in less oxidative effects and a dried bright red colour. The fact that there was a notable variation in *S. carnosus* batches attests to the importance of nitrate-reducing activity in the development of colour. The L* (lightness), a* (redness), and b* (yellowness) values of a colorimeter or spectral analysis of the meat pigment may be used to assess the colour of natural meat products (Sun & Ning, 2021).

Pre-converted celery powder that is often used is thought to have 10,000–15,000 ppm of sodium nitrite (Sindelar et al., 2010). However, because off flavours can occur at levels higher than this, the addition of celery powder to processed meat is typically limited to 0.2–0.4% of the formulation weight (Sindelar et al., 2007). According to Ballmer-Weber et al. (2002), a further drawback of celery consumption is the frequency of allergic responses among users (Ballmer-Weber et al., 2002). Celery powder is a good substitute source of nitrite for meat, but only when combined with nitrate-reducing bacterial cultures to produce a traditional cured meat product, as it has a considerable quantity of naturally occurring nitrate. Celery powder, however, has little pigment and a mild taste that improves the flavour of meat (Alahakoon et al., 2015).

To enhance the quality and safety of cold-smoked sausages, Eisinaité et al. (2020) looked at the possibility of converting synthetic nitrates and nitrites to nitrates from freeze-dried celery. Sausages that were cold-smoked and included 2.58% freeze-dried celery (or 150 mg.kg⁻¹ nitrate) were produced using either *S. xyloso* alone or in combination with *Pediococcus pentosaceus*. Features including microbiological quality, physicochemical qualities, and relative changes in myoglobin types were assessed during fermentation and ageing. The current study's findings indicate that freeze-dried celery may be used in place of added nitrites in cold-smoked sausages without affecting the product's quality or safety. To guarantee controlled fermentation, it is crucial to choose the starter cultures appropriately. According to Eisinaité et al. (2016), compared to control sausages that do not contain any vegetables, the addition of 3% freeze-dried celery, parsley, and leek to dry fermented sausages does not significantly affect the changes in pH, a_w, the presence of lactic acid bacteria, or coagulase-positive staphylococci during the fermentation and ageing process of the dry fermented sausages. They indicated that the sausages with the addition of freeze-dried celery juice had the best sensory qualities. According to Jin et al. (2018), adding 0.8% of celery powder to cooked sausages successfully prevented quality degradation during storage. Additionally, natural nitrite sources work well to regulate the growth of microorganisms and the quality of meat products. Golden et al. (2014) reported that *L. monocytogenes* development in deli-style turkeys was inhibited by pre-converted celery powder (nitrite content: 80 mg.kg⁻¹).

CONCLUSION

In the production of dried meat biltong, due to its sensory properties, the use of an oxygen absorber and curing salt was found to be a suitable combination. However, by replacing curing salt with NaCl and adding celery powder as well as nitrate-reducing bacteria, a significantly higher sensory score was found compared to the other monitored groups (except for AP3). Based on our sensory analysis findings, we recommend using celery powder and nitrate-reducing bacteria in combination with NaCl to replace curing salt in the production of dried biltong meat. To achieve favourable sensory properties, packaging with the addition of an oxygen absorber is also recommended.

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