Food Production, Processing and Nutrition

RESEARCH Open Access

Study of the volatile compounds of dry fermented sausage with salt reduction and its relationship with sensory acceptance

Marcio Aurélio de Almeida[1], Silvina G. Fadda[2], Miguel Ángel Sentandreu Vicente[3], Nilda Doris Montes Villanueva[4], Jair Sebastião da Silva Pinto[1*] and Carmen Josefina Contreras Castillo[1]

Abstract

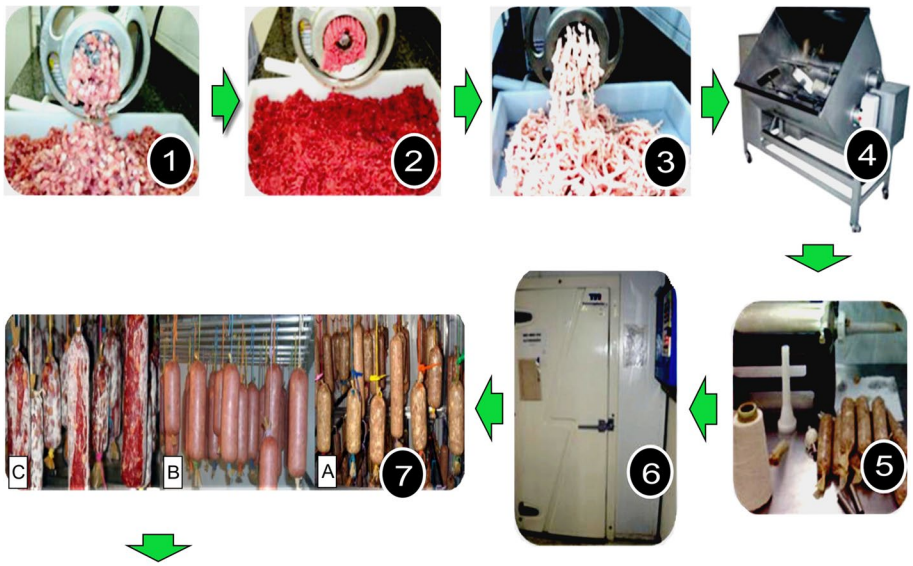
In many industrialised countries, sodium intake exceeds the nutritional guidelines. High sodium intake has been associated with health problems, such as arterial hypertension and, consequently, a higher health risk of cardiovascular disease. The aim of this study was to evaluate overall quality of salamis with reduced sodium content by analysing aldehydes, proteolysis, and lipolysis, as well as to verify the correlation between sensory acceptance and volatile compounds. Four formulations were prepared replacing NaCl by a mixture of KCl and $CaCl_2$. Two controls, one with a high level and the other with a low NaCl level were also included in the study. Water activity and pH levels were monitored in all samples. Volatile compounds were measured, in particular, hexanal, 2,4-decadienal and 3-methylbutanal. Finally, a sensory acceptance test was carried out using potential consumers. The results highlighted a relation between aldehyde concentration and sensory acceptance, hexanal/3-methybutanal ratio, that may be a marker of partial NaCl substitution in salami. Thus, our results may be used to guide the use of NaCl substitution by KCl/$CaCl_2$ in terms of volatile compounds.

Keywords Strecker reactions, Hexanal, 3-methylbutanal, Low sodium, SPME/HRGC-MS

*Correspondence:
Jair Sebastião da Silva Pinto
jsspinto@usp.br
Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Graphical Abstract



1- Grinding of pork
2- Grinding of beef
3- Grinding of fresh bacon
4- Mixing
5- Stuffing
6 - Fermentation chamber
7A- Pre-fermentation
7B- Post-fermentation
7C- End of the drying process
8 - Package final product

Introduction

Since 1960, there has been an ongoing and warm debate on the excessive consumption of sodium and its implications on public health (WHO 2012; Estevez et al. 2021). Brazil, because of the influence of European immigration, has adopted the habit of consuming of dry-fermented sausages and other processed meat-based products, and salami is one of the most consumed meat products consumed in Brazil.

Italian salami has a characteristic flavour as a result of the balance not only between volatile organic compounds (alcohol, ketones, aldehydes and furans) and non-volatile compounds (amino acids, peptides, sugars and nucleotides), but also because of the addition of raw materials (meats, spices, nitrites and other additives), or compounds generated from biochemical reactions that occur during fermentation and ripening (Flores 2018; Holck et al. 2017; Kirkyol & Akköse 2022; Stahnke 2002). The non-volatile components enhance basic tastes, such as sweetness, saltiness, sourness, umami, and bitterness, as these compounds are produced during hydrolysis of meat proteins. Their presence provides the sensory characteristics of the final product. Salami has a high added value and constitutes an important source of NaCl and may reach up to 5.0% w/w (Cichoski et al. 2009). One possible alternative the decrease the use of NaCl is by replacing it by other chloride salts (KCl, $MgCl_2$ and $CaCl_2$). However, these inorganic salts have some practical limitations, such as they may influence the sensory quality of products. Therefore, the use of alternative salting agents is restricted, and consumers would have to adapt to the new flavour and aroma caused by the differences in the formulation (Pires & Noronha 2019; Raybaudi-Massilia et al. 2019). In dry-fermented meat products, the replacement of NaCl by different chloride salt combinations has shown satisfactory technological and microbiological NaCl reductions of 50–60%, but has negative impacts on the final quality of the salami (Almeida et al. 2015; Campagnol et al. 2012; da Silva et al. 2020; Santos et al. 2014). When developing low-sodium salami, additional substances should be added to improve its quality traits (Zhao et al. 2005).

In addition to enhance flavour, NaCl also reduces microbial growth and the enzymatic activity (Andrés et al. 2007; Muchaamba et al. 2021), as well as it contributes to lipid oxidation (Ordónez et al. 1999). All in one, reducing NaCl in salami will case changes in the lipid oxidation kinetics, peptidases and aminopeptidases activity, protein and peptide hydrolysis, as well as in the composition of generated volatile and non-volatile compounds.

Throughout processing, the degradation reactions of lipids and proteins and the formation of by-products occur simultaneously, depending on temperature, time, pH, and water activity. Lipids and phospholipids are hydrolyzed by lipases or phospholipases, leading to their respective free fatty acids, which are oxidized to form ketones, alkanes, alcohols, and mainly linear-chain aldehydes such as hexanal, along with intermediates that will react with free amino acids. At the same time, proteins are hydrolyzed by proteases into peptides, and these peptides are further broken down into amino acids by aminopeptidases. The free amino acids formed and aldehydes resulting from the oxidation of free fatty acids will react with each other, forming the products of the Strecker degradation reaction, i.e., the loss of amino acids that are related to the flavor and aroma of meat products.

Solid phase micro-extraction with high resolution gas chromatography coupled to mass spectrometry (SPME/HRGC-MS) has been used for the headspace analysis of volatile compounds in salami (Lorenzo et al. 2013; Polatoglu et al. 2017). This technique enables the accurate identification of emerging, intermediate and final volatile compounds derived from the reactions that impact the quality of salami such as lipolysis and Strecker degradation (del Pulgar et al. 2013). The main product of lipid oxidation is hexanal, which is an indicator of lipid degradation (Lorenzo et al. 2013). On the other hand, 3-methylbutanal is a by-product of Strecker degradation. Thus, it is of primary importance to monitor these aldehydes in order to understand some of the main mechanisms that impact the final quality of salami.

The objective of this study was to develop a new approach to produce low-sodium salami, linking lipolysis to Strecker degradation and observing the effects on proteolysis and consumers' sensory responses. We employed SPME/HRGC-MS as a high throughput approach to analyse the volatile compounds, specifically aldehydes, hexanal, 2,4-decadienal and 3-methylbutanal, and as a way to select the most adequate chloride substitutes to produce commercial salami with sensory appeal, it was evaluated the correlation between the volatile compounds profile and the sensory acceptance.

Materials and methods

Chemicals and reference compounds

Hexanal (> 97%) was obtained from Santa Cruz Biotechnology (Dallas, USA). C7-C30 Saturated Alkanes was obtained from Supelco (St. Louis, USA). n-Hexane (> 95%) HPLC Spectra was obtained from Tedia (Fairfield, USA) and used as a solvent for preparing the stock solutions. Sodium standard solution was obtained from Grupo Química GQ (São Paulo, Brazil). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, USA). Sodium Chlorite food grade was obtained from Ibrac Aditivos e Ingredientes (Rio Claro, Brazil). Potassium Chlorite (> 99%) and Calcium Chlorite (> 99%) all food grades were obtained from Êxodo Científica (Sumaré, Brazil).

Salamis

The experimental treatments were six salami formulations, four of them prepared using different concentrations of NaCl substituted by KCl and mixed with $CaCl_2$. Salami control (SC) samples were prepared using a standard NaCl concentration of 25 g kg^{-1} (SC 2.5). Other salami control samples were prepared using the concentration of 10 g kg^{-1} NaCl (SC 1.0) without any substitutes (Table 1). For each treatment, 30 samples per replicate were manufactured.

Manufacture of salami samples

Raw meat was acquired from a slaughterhouse under federal inspection conducted by the Brazilian Ministry of Agriculture, Livestock, and Supply (Frigorífico Angelelli, Piracicaba, Brazil). Salami production was carried out in the Laboratório de Qualidade da Carne e Processamento da ESALQ, Universidade de São Paulo (USP). Italian salami was prepared using pork shoulder (600 g kg^{-1}), beef rib (250 g kg^{-1}), and pork back fat (150 g kg^{-1}). Chilled beef and pork meat were weighed,

Table 1 Levels of salt replacers and NaCl reduction in different treatments ($n = 2$ replicates)

Treatments	Concentration of salt added in g kg^{-1} (m/m%)		
	Sodium chloride (NaCl)	Potassium chloride (KCl)	Calcium chloride ($CaCl_2$)
T1: SC2.5	25.0 (2.5)	0.0	0.0
T2: SC1.0	10.0 (1.0)	0.0	0.0
T3: SL0.25/0.25	10.0 (1.0)	2.5 (0.25)	2.5 (0.25)
T4: SL0.35/0.35	10.0 (1.0)	3.5 (0.35)	3.5 (0.35)
T5: SL0.40/0.40	10.0 (1.0)	4.0 (0.40)	4.0 (0.40)
T6: SL0.45/0.45	10.0 (1.0)	4.5 (0.45)	4.5 (0.45)

SC control salami, *SL* low sodium salami added with NaCl replacers

cut and ground separately using a stainless-steel grinder (HOBART 4B22-2) with caliber of 10, 5, and 8 mmm for the pork, beef, and back fat samples, respectively. Additionally, sodium nitrite (0.15 g kg^{-1}), sodium nitrate (0.15 g kg^{-1}), sodium erythorbate (3.0 g kg^{-1}), condiments (7.0 g kg^{-1}), dextrose (0.70 g kg^{-1}), commercial starter culture (0.25 g kg^{-1}; Bactoferm t-SPX Chr. Hansen) containing *Pediococcus pentosaceus* and *Staphylococcus xylosus* were added to each treatment. After mixing the ingredients, the salts were added according to the experimental design shown in Table 1. The resulting emulsion was added into reconstituted collagen casings (45 mm in diameter). The sausages (400 g) were then fermented for 48 h in a chamber under controlled conditions (23 ± 1 °C, relative humidity, RH, of 85–90%) until a pH between 4.9–5.2 was reached. The ripening stage was conducted under controlled conditions (16–18 °C at 65–75% RH) for 26 days to obtain water activity values between 0.88 and 0.90 for all treatments. Finally, sausages were vacuum packaging and frozen at -25ºC ± 1 °C until analysis.

Experimental design

To collect the physicochemical data, a complete block design was used, considering each process as a block. Each treatment was processed in duplicate ($n = 2$). To generate sensory data, salami samples were presented to consumers in a sequential monadic way, following a Latin Square Design to balance the effects of presentation order of the samples.

Physicochemical analyses

pH level and water activity determinations were analysed both in the raw meat and in the final product (ripened salami). Na^+ levels were analysed in the ripened salami samples.

pH

The pH measurements were carried out using an electrode with a penetration probe coupled to a portable Oakton pH300 pH-meter. Before the analysis of each treatment, the pH meter was calibrated with pH 4 and 7 buffer solutions. The samples were analysed in triplicate.

Water activity (Aw)

The water activity of salami samples (3 mm thickness) was determined by direct measurement using a 4TE Aqualab analyser (Decagon Devices Inc., USA), operating at 25˚C ± 0.3ºC. Aw determinations were carried out at 0 h, after fermentation, and at the final drying stage.

Sodium content

The sodium content was determined by flame photometry, using a Micronal Model B462 (São Paulo, Brazil) flame photometer, following the method recommended by the Association of Official Analytical Chemists (AOAC 2005). The result was then converted through stoichiometric calculation to sodium chloride.

Volatile compounds analyses

Aldehydes were extracted from the headspace of salamis with solid phase micro-extraction (HS-SPME), using a Supelco holder (Bellefonte, PA, USA) and a StableFlex Carboxen/Polidimetilsiloxan 85 µm fibre (CAR/PDMS). In a 40 mL vial lidded with a silicone/Teflon septum, 10 g of ground salami were weighed. The vial was sealed and kept at 30 ºC in a water math for 1 h in order to balance the temperature. After the CAR/PDMS fibre was inserted in the headspace of the vial and kept for 1 h. Separation, identification and quantification of the aldehydes were carried out using a GCMS-QP2010 Plus gas chromatographer coupled to a mass spectrometer detector (Shimadzu, Japan), equipped with a split/splitless injector. Compounds absorbed in the CAR/PDMS fibre were desorbed in the injector for 20 min at 280 ºC in the split mode. Compounds were separated using a RTX-5 MS fused-silica capillary column, 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (Restek US, Bellefonte, PA, USA). The temperature programme of the GC oven was 40 ºC for 1 min, rising until 250 ºC at a rate of 5 ºC/min, and a constant temperature of 250 ºC for 1 min was set. The split ratio was 1:25, gas stream was 1 mL/min, linear velocity at 36 cm/s, with ultra-pure helium as carrier gas. Mass spectra were obtained in the *m/z* range 40 to 450 for each compound. The mass spectrometer was operated in the SCAN mode, a using an ionisation energy of 70 eV. The transfer line and the ion source were maintained at 250 ºC. Hexanal identification was compared with the retention time of a standard hexanal solution, while the other aldehydes were compared with the mass-spectra contained in the WILEY 8 Ed., FFNSC 1.3 library, and by using the Linear Retention Index (LRI). Quantification was based on the total ion chromatogram (TIC) and results were expressed in area units (AU) × 10^5/g (wet basis).

Consumer test

Consumers were recruited within the university community from Universidade de Sao Paulo—Escola Superior de Agricultura "Luiz de Queiroz" in Piracicaba, São Paulo State, Brazil. Thus, 120 academics, researchers, technicians, and postgraduate students were invited to participate in the sensory test, by filling out a recruitment form.

Of the 120 completed forms, 52 regular salami consumers were selected (21 to 60 years old), with the selection criteria being a frequency of salami consumption at least once a week and liking salami to a degree equal to or greater than "like moderately" on the nine-point hedonic scale. For the sensory tests, standard condition of white lighting, air circulation and temperature control were used in the booths. Before analysis, assessors received instructions on how to use the scale and the type of sensory evaluation to be performed. Each consumer evaluated the six samples of salami in a single session receiving monadically a slice of each sample according to the order of presentation of the Latin Square Design, with an interval of 5 min between samples. Consumers were asked to taste the salami sample and to evaluate how much they liked or disliked each sample with respect to texture, flavour, and overall acceptance, using a nine-point hedonic scale (1 = disliked extremely, 5 = neither liked nor disliked, 9 = liked extremely) (Meilgaard, Civille & Carr 2006). Lastly, the consumers evaluated their purchase intention using a five-point structured scale (1 = certainly will not buy, 3 = may or may not buy, and 5 = certainly will buy). This study was registered and approved by the Ethics Committee in Research of ESALQ/USP under protocol nº. 104/2012.

Statistical analysis

The physicochemical measurements and concentration of aldehydes of the experimental treatments were analysed using a one-way ANOVA ($p \leq 0.05$). The acceptance data of the sensory attributes were analysed using a three-way ANOVA ($p \leq 0.05$), considering the effect of the consumer, the order of samples presentation and samples (treatments). To evaluate the differences in physicochemical parameters, concentration of aldehydes and the acceptance of salami samples, paired comparisons of the means were carried out using Tukey's HSD test ($p \leq 0.05$). Pearson correlation analyses ($p \leq 0.05$) were used to quantify relationships between concentration of aldehydes and acceptance scores from six salami formulations. Also, Principal Component Analysis (PCA) of the volatile compounds, NaCl concentration, and consumer acceptance data was performed for discriminating and forming clusters of the salamis based on the similarity of its characteristics. The statistical data analysis was performed using STATISTICA 10.0 software (Statsoft Inc., Tulsa, OK, USA).

Results and discussions

Physicochemical analyses

The fermentation process of salamis was adequate and the pH of 5–5.2 was reached after 48 h, showing that the formation of basic non-protein nitrogen (NPN) compounds was effective (Astiasaran et al. 1990) (Table 2), corroborating previous findings reported in the literature (Cirolini et al. 2010; Vural 1998). Overall, the NaCl substitution with KCl and $CaCl_2$ in the proposed combinations did not inhibit the growth of *Pediococcus pentosaceus* and *Staphylococcus xylosus*. The commercial starter culture is indicated, as noted by the manufacturer, for products with the addition of 2.5% sodium chloride and, it can be proven that addition of the carbohydrates used such dextrose and sucrose were sufficient for the acidification of the sausages. In fact, the acidification process in salami depends on the type of starter culture employed together with the use of a suitable combination of carbohydrates to reduce the fermentation time. The microorganisms used nowadays for the production of salamis are homo-fermentative lactic bacteria for the production of acid and Coagulase negative *Staphylococcus* for colour stability, prevention of lipid oxidation and flavour generation (Drosinos et al. 2005; Sallan, Kaban & Kaya 2022).

Table 2 Physicochemical parameters of salami samples manufactured using different combinations of NaCl, KCl, and $CaCl_2$, (mean ± std, $n = 2$)

Treatments	Physicochemical analysis		
	pH end	Aw End	NaCl (mg/100 g)
SC2.5	$5.2^{a} \pm 0.02$	$0.90^{a} \pm 0.01$	$2442^{a} \pm 14.10$
SC1.0	$5.3^{a} \pm 0.05$	$0.91^{b} \pm 0.01$	$880^{b} \pm 17.51$
SL0.25/0.25	$5.2^{a} \pm 0.06$	$0.91^{b} \pm 0.01$	$968^{b} \pm 1.72$
SL0.35/0.35	$5.2^{a} \pm 0.10$	$0.91^{b} \pm 0.01$	$910^{b} \pm 13.67$
SL0.40/0.40	$5.2^{a} \pm 0.06$	$0.91^{b} \pm 0.01$	$870^{b} \pm 14.01$
SL0.45/0.45	$5.1^{a} \pm 0.01$	$0.91^{b} \pm 0.01$	$834^{b} \pm 35.19$

Means followed by different letters (a, b) in same column differ significantly ($P < 0.05$)

SC control salami, *SL* low sodium salami added with NaCl replacers

The Aw of salamis represents one of the main barriers against the growth of microorganisms and aw values should not exceed 0.92 (Drosinos et al. 2005). Considering the Normative Instruction, nº 22, of 31 July 2000, Brazilian standards, the salami formulations developed herein presented acceptable values of water activity.

Concerning the sodium levels, the substitution of NaCl with other different mixtures of KCl/$CaCl_2$ reduced up to 63% the Na^{+} level. Treatments SL0.25/0.25, SL0.35/0.35, SL0.40/0.40, SL0.45/0.45 and SC1.0, showed NaCl levels between 865–898 mg/100 g, while SC2.5 control treatment (with a value close to the commercial one), had a NaCl level of 2452 mg/100 g. In the Brazilian market, Italian salami contains between 1612 and 2562 mg NaCl/100 g. Therefore, the commercialisation of a

low-sodium product is a good option for industry and consumers. However, a reduction of salt at this level is considered to be critical because of the risk of microbial contamination and hetero-fermentation during processing (Brasil 2000; Chen et al. 2019; Araujo et al. 2021).

Consumer analysis

The results of the sensory test (Table 3 and Fig. 1), showed that all the salami formulations had a good acceptance by consumers, obtaining averages of acceptance of flavour, texture, and overall acceptance between 6.2 (liked slightly) and 7.5 (liked very much) on the hedonic scale. Likewise, in purchase intention, all the formulations obtained average scores between 3.2 (may or may not buy) and 3.9 (probably will buy). It is important to note that the high control (SC2.5) and SL0.25/0.25 garnered the highest mean hedonic scores for the evaluated attributes. This result showed that the formulation SL 0.25/0.25 did not present significant differences ($P < 0.05$) in flavour, overall acceptance, and purchase intention with the standard commercial products (around 2.5% NaCl).

HRGC-MS analysis of aldehydes produced in salami

Treatment SC2.5 yielded the highest level of hexanal and the lowest level of 3-metilbutanal (Table 4), implying that lipoxidase was more active in this sample. This may be related to the highest NaCl concentration, which is a pro-oxidant agent in meat-based products (Ordónez et al. 1999).

The main stages occurring in lipid degradation in meat products are: (A) breaking of lipids to free fatty acids and triacylglycerides through lipase action and phospholipids by phospholipase action. (B) Oxidation of free fatty acids to peroxides and (C) other reactions that convert peroxides into volatile compounds. However, concerning

Table 3 Flavour acceptance, overall acceptance, texture acceptance, and purchase intent of the different salami treatments manufactured using different combinations of NaCl, KCl, and $CaCl_2$, (mean ± std, $n = 2$)

Attributes	Treatments					
	SC2.5	SC1.0	SL0.25/0.25	SL0.35/0.35	SL0.4/0.4	SL0.45/0.45
Flavour	$7.2^{a} \pm 1.91$	$6.2^{b} \pm 1.88$	$6.8^{ab} \pm 1.76$	$6.4^{ab} \pm 1.53$	$6.5^{ab} \pm 1.81$	$6.2^{b} \pm 1.69$
Texture	$7.5^{a} \pm 1.20$	$6.7^{b} \pm 1.50$	$6.7^{b} \pm 1.50$	$6.3^{b} \pm 1.60$	$6.6^{b} \pm 1.70$	$6.3^{b} \pm 1.60$
Overall acceptance	$7.4^{a} \pm 1.37$	$6.6^{b} \pm 1.46$	$6.9^{ab} \pm 1.47$	$6.4^{b} \pm 1.39$	$6.7^{b} \pm 1.51$	$6.4^{b} \pm 1.50$
Purchase intent	$3.9^{a} \pm 1.14$	$3.2^{b} \pm 1.12$	$3.5^{ab} \pm 1.25$	$3.0^{b} \pm 1.15$	$3.2^{b} \pm 1.23$	$3.1^{b} \pm 1.25$

Means followed by different letters (a, b) in same row differ significantly ($P < 0.05$)

SC control salami, *SL* low sodium salami added with NaCl replacers

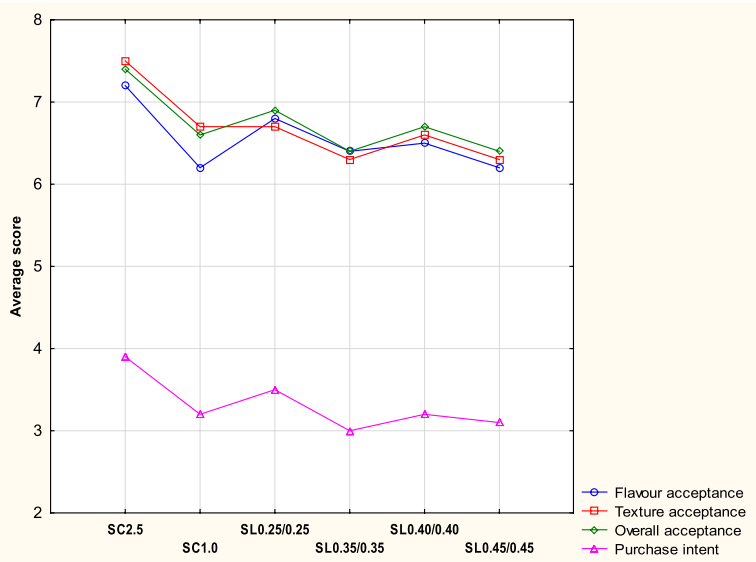


Fig. 1 Average score for flavor acceptance, texture acceptance, overall acceptance, and purchase intent for the six experimental salami formulations

Table 4 Concentration of aldehydes, in area units (AU) × 10^5/g, related to lipolysis and Strecker degradation reactions detected in the salami headspace, (mean ± std, $n = 2$)

Aldehydes	Treatments					
	SC2.5	SC1.0	SL0.25/0.25	SL0.35/0.35	SL0.40/0.40	SL0.45/0.45
Hexanal	$65.24^{a} \pm 5.31$	$23.38^{b} \pm 1.08$	$56.53^{a} \pm 5.09$	$20.58^{b} \pm 2.27$	$9.64^{b} \pm 1.15$	$5.15^{b} \pm 2.07$
2,4-Decadienal	0.58 ± 0.20	0.0	0.0	0.0	0.0	0.0
3-Methylbutanal	$0.36^{b} \pm 0.04$	$0.80^{b} \pm 0.13$	$0.53^{b} \pm 0.06$	$0.87^{b} \pm 0.09$	$2.46^{a} \pm 0.16$	$2.44^{a} \pm 0.25$
Hexanal/3Methylbut	$181.25^{a} \pm 0.50$	$29.74^{c} \pm 6.62$	$109.3^{b} \pm 26.96$	$23.94^{c} \pm 6.02$	$3.96^{c} \pm 0.92$	$2.06^{c} \pm 0.99$

Means followed by different letters (a, b) in same row differ significantly ($P < 0.05$)

SC control salami, *SL* low sodium salami added with NaCl replacers

the aminopeptidases action, NaCl shows an inhibitory effect (Hu et al. 2020; Santos et al. 2015). In fact, this higher NaCl concentration in treatment SC2.5 favoured the highest reduction in the enzyme activities and, consequently, the lowest production of free amino acids. The lowest concentration of the sub-product derived from Strecker degradation, the 3- methylbutanal (Lawrie 2006; Santos et al. 2015, Flores et al. 2018) (Table 4), could be associated to the lowest concentration of free amino acids. The generation of 3-methylbutanal is the result of an epoxyalkenal reaction, originating from the oxidation of 2,4-decadienal, with leucine (del Pugar, Rodan & Ruiz-Carrascal 2013), which is the end product of aminopeptidases action in peptides. The concentration of 3-methylbutanal in the SC2.5 treatment is lower, and this result suggests that there was a low loss of leucine in relation to other treatments.

The SC1.0 treatment presented three-fold less hexanal and two-fold more 3-methylbutanal compared to SC2.5 treatment. This discrepancy may be associated to the difference in NaCl concentration between treatments, which decreased the lipid oxidation, hexanal formation and inhibition of aminopeptidase. These data suggest that a higher concentration of amino acids favour Strecker degradation reaction, which is in-line with other authors (Wu et al. 2015).

As the KCl concentration increased, the oxidation process was reduced, and this observation agrees with previous research (Ordónez et al. 1999). When the concentration of KCl + $CaCl_2$ was used in salami, the hexanal concentration and lipid oxidation process were reduced. These salts, under the concentration range assayed in this work, inhibit the action of lipoxidase and enhance the aminopeptidase activity (Armenteros et al. 2009). The enzymes arginine and leucine aminopeptidases are activated more remarkably by KCl and, on the other hand, methionine aminopeptidase and dipeptidyl peptidase I and III are inhibited more significantly by KCl (Armenteros et al. 2009).

SL0.25/0.25 sample presented similar sensory acceptance scores (Table 3) compared to the high control [SC2.5]. In addition, SL0.25/0.25 sample presented the lowest 3-methylbutanal level concentration, the lowest relative leucine loss (del Pugar, Roldan & Ruiz-Carrascal 2013), the lowest relative loss of amino acids and flavour-related peptides, and the highest hexanal concentration.

The ratio of the area units (AU) of hexanal/3-methylbutanal (Table 4) is described (del Pulgar et al. 2013) as an indication of the balance between lipid oxidation reactions and amino acid degradation in fresh meat and processed meat products. In other words, indirect monitoring of loss of amino acids and peptides could be an effective approach to choose the best salami formulation. This ratio might be utilised to guide the use of chloride substitute concentrations in salami to minimise the adverse effects of NaCl reduction on the sensory quality of salami.

Correlation of HRGC-MS analysis of aldehydes and sensory analysis

Many studies have demonstrated the existence of correlations between chemical, instrumental and sensory data of food products (Cafferky et al. 2020; García-González et al. 2008; Pematilleke et al. 2022; Tian et al. 2015). Piao et al. (2019) compared the amount of reducing sugars, sensory characteristics, fatty acid profiles and volatile compounds of *Longissimus thoracis* from Korean cattle, Holsteins and Angus steers. In this study, positive correlations were found between the percentages of acetaldehyde, 3-methylbutanal, 2,3-butanedione and 3-hydroxy2-butanone with taste and overall acceptance ($0.39 \leq r \leq 0.66$, $P < 0.05$), allowing a better understanding of the association of the content of reducing sugars and volatile compounds with the sensory characteristics of beef, information that can help determine the palatability of the meat. Legako et al. (2016) evaluated consumer palatability scores, sensory descriptive attributes, and volatile compounds for beef *Longissimus lumborum* steaks of USDA Prime, Low Choice, and Standard grades. In

this study consumer overall liking was negatively correlated ($P \leq 0.05$) with hexanal ($r = -0.48$), heptanal ($r = -0.53$), octanal ($r = -0.56$), and the sum of aldehydes ($r = -0.49$). Also, positive correlations ($P \leq 0.05$) were found for consumer overall liking with 3-hydroxy-2-butanone ($r = 0.47$) and phenylacetaldehyde ($r = 0.43$). Similarly, flavour liking was positively correlated ($P \leq 0.05$) with 3-hydroxy-2-butanone ($r = 0.41$). These results revealed that variations of headspace compound quantities may be used for predicting beef liking and beef attributes.

Table 5 Pearson correlation coefficients between concentration of aldehydes and consumer acceptance scores from six salami formulations

Aldehydes	Flavour acceptance	Texture acceptance	Overall acceptance
Hexanal	0.89 ($P = .018$)	0.80 ($P = .055$)	0.87 ($P = .025$)
3-Methylbutanal	-0.54 ($P = .264$)	-0.54 ($P = .265$)	-0.53 ($P = .280$)
Hexanal/3Methylbutanal	0.93 ($P = .007$)	0.89 ($P = .019$)	0.93 ($P = .007$)

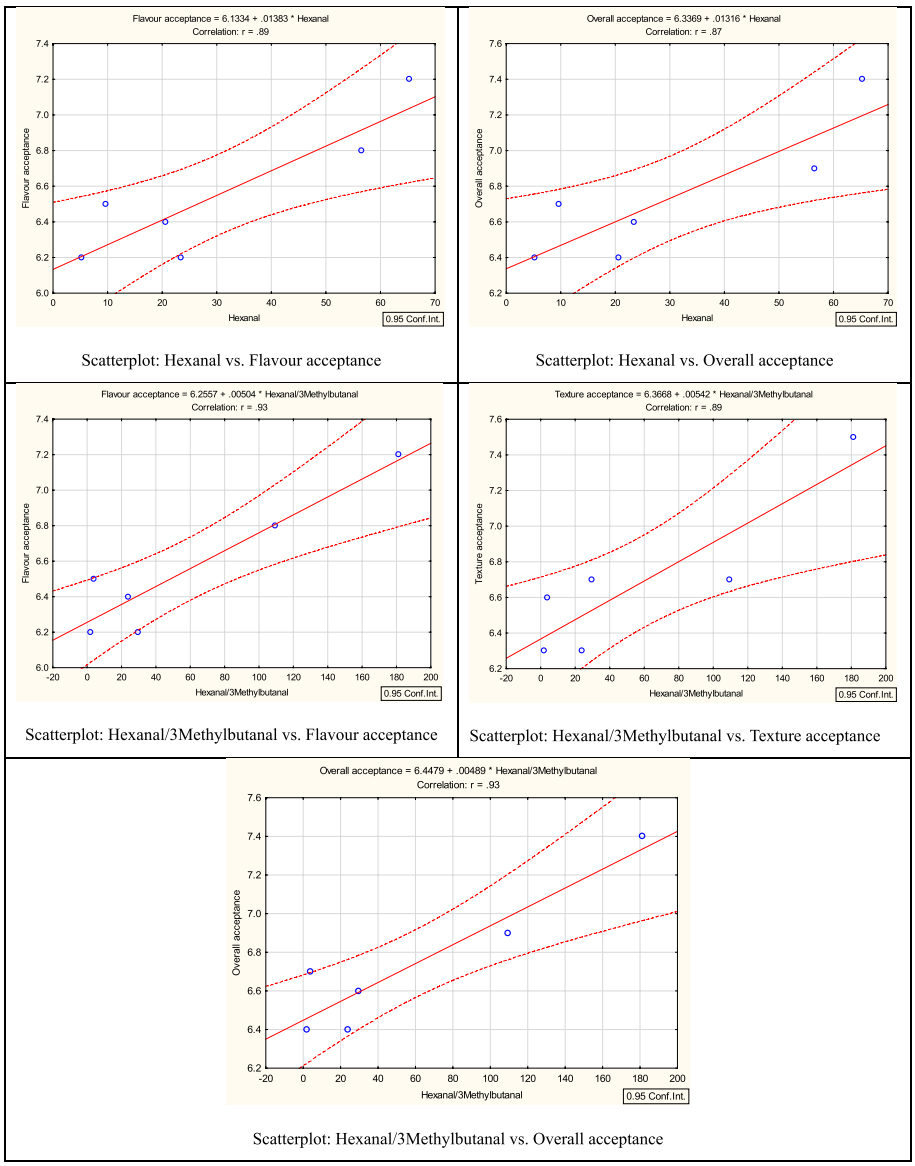


Fig. 2 Scatterplot between the concentration of the aldehydes hexanal and hexanal/3-methylbutanal versus the flavour acceptance, overall acceptance and texture acceptance of salamis

It is hypothesized in this study that the Hexanal/3-methylbutanal ratio can also be utilised as a choice baseline for various meat products. Table 5 and Fig. 2 show a positive correlation ($P \leq 0.05$) between consumer flavour acceptance with hexanal ($r=0.89$) and hexanal/3-methylbutanal ratio ($r=0.93$). Also, consumer texture acceptance was positively correlated with hexanal/3-methylbutanal ratio ($r=0.89$). Likewise, consumer overall acceptance was positively correlated with hexanal ($r=0.87$) and hexanal/3-methylbutanal ratio ($r=0.92$), indicating that an increase in concentration of aldehydes ratio increase the overall acceptance of salamis, information that can be used to predict the level of consumer acceptance of different salami formulations.

PCA

Figure 3 shows the biplot of principal component analysis of the volatile compounds, NaCl concentration, and consumer sensory acceptance data. Two principal components, PC1 and PC2, explained 93.89% of the total variance (81.19.% for PC1, and 12.70% for PC2). The first two principal components showed that there was a separation of treatments into four groups. The treatment T1 (SC2.5) appeared in the 4th quadrant of PC1 and PC2, the treatment T3 (SL0.25/0.25) appeared in the 1st quadrant of PC1 and PC2, the treatments T2 (SC1.0) and T4 (SL0.35/0.35) in the 2nd quadrant, and the treatments T5 (SL0.40/0.40) and T6 (SL0.45/0.45), in the 3rd quadrant. It is apparent that T1 (SC2.5) were more closely

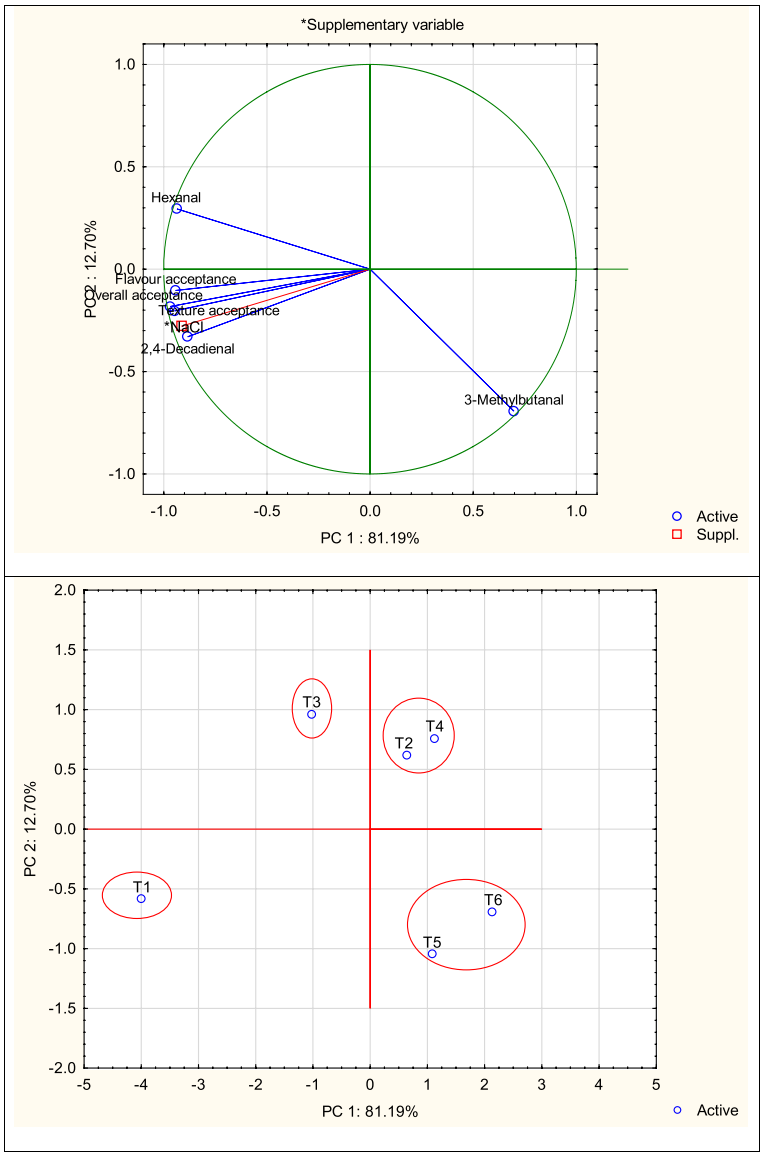


Fig. 3 Plot of principal component analysis of the volatile compounds, NaCl concentration and sensory acceptance

related to presence of hexanal and 2–4 decadienal, NaCl, flavour, texture, and overall acceptance. T2 (SC1.0) and T4 (SL0.35/0.35) were negatively related to 2–4 decadienal, NaCl, flavour, texture, and overall acceptance. T3 (SL0.25/0.25) was associated to hexanal and negatively related to 3-methylbutanal, while T5 (SL0.4/0.4) y T6 (SL0.45/0.45) were closely associated to 3-methylbutanal. PC1 allowed to discriminate treatments T1 and T3 from the others. Both treatments have variables with similar characteristics in terms of hexanal and 3-methylbutanal concentration, overall acceptance, texture and flavor. Still regarding PC1, treatments T2, T4, T5 and T6, in turn, were negatively related to T1. The PCA shows that the increase in the percentage of NaCl replacement in the treatments leads to a departure from the characteristics of the control treatment (T1). PCA confirms that volatile compound data (hexanal and 3-methylbutanal) can be used to discriminate different salami formulations with NaCl substitute salts.

In this study, it was possible to show that the loss of the amino acid leucine leads to a decrease in the acceptance of salami because this amino acid is related to the flavor, leading to a better understanding of the consumers' purchase decision process, who preferred the formulation with the best flavor, reflected in its higher acceptance and purchase intent.

Conclusion

Salami is a highly appreciated and consumed product in Brazil, but with concerns regarding health issues due to the high sodium content that can reach 6% after processing, maturation, and drying, new approaches to make it healthier have been proposed. The use of a mixture of substitute salts for sodium chloride is the focus of this work. Six treatments were formulated, including a control (2.5% NaCl), four with increasing amounts of the salt mixture ($KCl + CaCl_2 + 1\%$ NaCl), and one with 1% NaCl. The formulation of the sodium chloride substitute salts that obtained the highest acceptance in sensory tests was the one with lower amounts of potassium chloride and calcium chloride, each at 0.25% m/m. Among the formulations, this one had the highest concentration of aldehydes (57.06 $AU \times 10^5/g$), which can be represented by the sum of hexanal and 3-methylbutanal (Table 4). Hexanal is a marker of freshness and quality in newly processed products, and 3-methylbutanal is an indicator of amino acid losses related to flavor due to the Strecker degradation. This same formulation was the closest to the control formulation (commercial standard) in terms of hexanal and 3-methylbutanal concentration. The concentration of 3-methylbutanal was the lowest among the formulations (0.53 $AU \times 10^5/g$), suggesting similar quality to the control, as confirmed by higher scores in sensory tests for Flavor, Overall acceptance, and Purchase intent. The higher the value of the hexanal/3-methylbutanal concentration ratio, the lower the loss of amino acids related to flavor. The 0.25/0.25% m/m formulation had the highest value of this ratio among the formulations, with 109.31, being the closest to the control formulation, with a value of 181.25. There is a relationship between the concentration of aldehydes hexanal/3-methylbutanal ratio and the sensory acceptance of salamis, with good accuracy, using SPME/HRGC-MS techniques to quantify volatile compounds, and optimizing the variation in the amount of NaCl and its substitutes ($KCl + CaCl_2$) without conducting parallel sensory studies that are expensive, complex, time-consuming to perform, and demand a high number of participants and specific software for data generation and result analysis.

Acknowledgements

The authors extend sincere thanks to Foundation for Research Support of the State of São Paulo (FAPESP) for the financial support through the Project 2012/07113-2, the Christian Hansen, Ibrac Ingredientes e Aditivos and the Sealed Air, which provided the starter cultures, additives and packaging, respectively. The assistance of Mr. L. C. Rodrigues during the preparation of the Graphical Abstract is highly appreciated.

Authors' contributions

Marcio Aurélio de Almeida: conceptualization, methodology, validation, investigation, data curation, writing original draft, visualization, formal analysis and writing review. Silvina G. Fadda: validation, investigation. Angel S. Vicente: validation, investigation. Nilda D. M. Villanueva: methodology, validation, writing original draft, visualization, writing review and editing. Jair S.S. Pinto: conceptualization, methodology, validation, original draft, formal analysis, writing review and editing. Carmen J. Contreras Castillo: project administration, funding acquisition, resources, supervision, visualization, writing review and editing.

Funding

This project has received funding from the Foundation for Research Support of the State of São Paulo (FAPESP) through the Project 2012/07113–2.

Availability of data and materials

The data and material that support the finding of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participation

The Ethics Committee in Research of University of São Paulo, College of Agriculture Luiz de Queiroz, Piracicaba, Sao Paulo, Brazil, approved the study protocol with reference number 104/2012. The experimental design was conducted in accordance with the force laws and regulations for human.

Consent for publication

Note applicable.

Competing interests

Dr. Carmen J. Contreras Castillo is a member of Editorial Board of *Food Production, Processing and Nutrition* and she was not involved in the journal's review of, or decisions related to this manuscript.

Author details

[1]Departamento de Agroindústria, Alimentos E Nutrição, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo 13418-900, Brazil. [2]Centro de Referencias Para Lactobacilos (CERELA), CONICET, Tucumán, Argentina. [3]Instituto de Agroquímica y Tecnología de Alimentos, CSIC, Paterna, Valencia, Spain. [4]Facultad de Ingeniería Agraria, Universidad Católica Sedes Sapientiae (UCSS), Lima, Peru.

Received: 13 July 2023 Accepted: 13 January 2024
Published online: 03 September 2024

References

Almeida, M. A., Villanueva, N. D. M., Ribeiro, F. A., Fadda, S. G., Pinto, J. S. S., & Contreras Castillo, C. J. (2015). Sensory acceptability of partial replacement of sodium chloride by potassium chloride and calcium chloride in production of salami with low sodium. *Meat Science, 101*, 104–105.

Andrés, A., Barat, J. M., Grau, R., & Fito, P. (2007). Principles of drying and smoking. In: Handbook of fermented meat and poultry. Toldrá, F. (Ed.). Ames: Blackwell.

AOAC. (2005). *Official Methods 920.153. Ash of meat*. Washington: Association of Official Analytical Chemists.

Araujo, D. H. D., Rodrigues, R. T. D., da Costa, M. M., de Miranda, J. O., de Lira-Alencar, N. R. C., Queiroz. M. A. A., de Alencar, M. G., Oliveira, T. P. R., Figueredo, A., Bonfa, H. C., de Carvalho, F. A. L., & Gois, G. C. (2021). Reduction of sodium content in frozen goat sausage using different types of salt. LWT-Food Science and Technology. 135(1). 10.j.lwt.2020.110272.

Armenteros, M., Aristoy, M. C., Barat, J. M., & Toldrá, F. (2009). Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. *Food Chemistry, 117*, 627–633.

Astiasaran, I., Villanueva, R., & Bello, J. (1990). Analysis of proteolysis and protein Insolubility during the manufacture of some varieties of dry sausage. *Meat Science, 28*, 111–117.

BRASIL. Ministério da Agricultura, Pecuária e do Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa nº 22, de 31 de julho de 2000. Aprova o Regulamento Técnico de Identidade de Qualidade de Salames. Diário Oficial da União, Brasília, 03 ago. 2000.

Cafferky, J., Sweeney, T., Allen, P., Sahar, A., Downey, G., Cromie, A. R., & Hamill, R. M. (2020). Investigating the use of visible and near infrared spectroscopy to predict sensory and texture attributes of beef M. longissimus thoracis et lumborum. Meat Sci., 159. https://doi.org/10.1016/j.meatsci.2019.107915.

Campagnol, P. C. B., Santos, B. A., Terra, N. N., & Pollonio, M. A. R. (2012). Lysine, disodium guanylate and disodium inosinate as flavor enhancers in low-sodium fermented sausages. *Meat Science, 91*, 334–338.

Chen, J. X., Hu, Y. Y., Wen, R. X., Liu, Q., Chen, Q., & Kong, B. H. (2019). Effect of NaCl substitutes on the physical, microbial and sensory characteristics of Harbin dry sausage. *Meat Science, 156*(10), 205–213. https://doi.org/10.1016/j.meatsci.2019.05.035.

Cichoski, A. J., Zis, L. C., & Franceschetto, C. (2009). Características físico-químicas e microbiológicas da superfície do salame tipo italiano contendo solução de lactato de potássio. *Food Science and Technology., 29*(3), 546–552.

Cirolini, A., Fries, L. L. M., Terra, N. N., Milani, L. I. G., Urnau, D., dos Santos, B. A., Cervo, G. D., & Rezer, A. P. S. (2010). Fermented italian sausage elaborated with native starter cultures. *Food Science and Technology, 30*(1), 171–179.

Da Silva, S. L., Lorenzo, J. M., Machado, J. M., Manfio, M., Cichoski, A. J., Fries, L. L. M., Morgano, M. A., & Campagnol, P. C. B. (2020). Application of arginine and histidine to improve the technological and sensory properties of low-fat and low-sodium bologna-type sausages produced with high levels of KCl. Meat Science, 159 (1). https://doi.org/10.1016/j.meatsci.2019.107939.

Del Pulgar, J. S., Roldan, M., & Ruiz-Carrascal, J. (2013). Volatile Compounds Profile of Sous-Vide Cooked Pork Cheeks as Affected by Cooking Conditions (Vacuum Packaging, Temperature and Time). *Molecules, 18*, 12538–12547.

Drosinos, E. H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F., & METAXOPOULOS, J. (2005). Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science, 69*(2), 307–317.

Estevez, A., Camacho, C., Correia, T., Barbosa, V., Marques, A., Lourenco, H., Serrano, C., Sapata, M., Duarte, M. P., Pires, C., Gonçalves, A., Nunes, M. L., & Oilveira, H. (2021). Strategies to reduce sodium levels in European seabass sausages. Food and Chemical Toxicology, 153(7). https://doi.org/10.1016/j.fct.2021.112262.

Flores, M. (2018). Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Science, 144*, 53–61.

García-González, D. L., Tena, N., Aparicio-Ruiz, R., & Morales, M. T. (2008). Relationship between sensory attributes and volatile compounds qualifying dry-cured hams. *Meat Science, 80*, 315–325.

Holck, A., Axelsson, L., McLeod, A., Rode, T. M., & Heir, E. (2017). Health and Safety Considerations of Fermented Sausages. J. Food Quality. Volume, UNSP 9753894. https://doi.org/10.1155/2017/9753894.

Hu, Y., Zhang, L., Zhang, H., Wang, Y., Chen, Q., & Kong, B. (2020). Physicochemical properties and flavour profile of fermented dry sausages with a reduction of sodium chloride. *LWT-Food Science and Technology, 124*, 109061. https://doi.org/10.1016/j.lwt.2020.109061.

Kirkyol, M., & Akköse, A. (2022). Investigation of textural and physicochemical changes during the manufacturing process of pastirma, a dry-cured meat product. *Food Science and Technology International, 29*(7), 739–747. https://doi.org/10.1177/10820132221112736.

Lawrie, R. A. (2006). Meat science. (7th ed.) Cambridge: Woodhead Publ.

Legako, J. F., Dinh, T. T. N., Miller, M. F., Adhikari, K., & Brooks, J. C. (2016). Consumer palatability scores, sensory descriptive attributes, and volatile compounds of grilled beef steaks from three USDA Quality Grades. *Meat Science, 112*, 77–85.

Lorenzo, J. M., Bedia, M., & Bñón, S. (2013). Relationship between flavor deterioration and the volatile compound profile of semi-ripened sausage. *Meat Science, 93*, 614–620.

Meilgaard, M., Civille, G. V., Carr, B. T. (2006). Sensory evaluation techniques. (4th ed.). CRC Press: Boca Raton.

Muchaamba, F., Stoffers, H., Blase, R., von Ah, U., & Tasara, T. (2021). Potassium lactate as a strategy for sodium content reduction without compromising salt-associated antimicrobial activity in salami. Foods, 10(1). https://doi.org/10.3390/foods10010114.

Ordónez, J. A., Hierro, E. M., Bruna, J. M., & De La Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition, 39*(4), 329–367.

Pematilleke, N., Kaur, M., Adhikari, B., & Torley, P. J. (2022). Relationship between instrumental and sensory texture profile of beef semitendinosus muscles with different textures. *J. of Texture Studies, 53*(2), 232–241. https://doi.org/10.1111/jtxs.12623.

Piao, M. Y., Lee, H. J., Yong, H. I., Beak, S. H., Kim, H. J., Jo, C., Wiryawan, K. G., & Baik, M. (2019). Comparison of reducing sugar content, sensory traits, and fatty acids and volatile compound profiles of the longissimus thoracis among Korean cattle, Holsteins, and Angus steers. *Asian-Australas J. Anim Sci, 32*(1), 126–136. https://doi.org/10.5713/ajas.18.0065.

Pires, M. A., de Noronha, R. L. F. (2019). Understanding consumer's perception and acceptance of bologna sausages with reduced sodium content and/or omega-3 addition through conjoint analysis and focus group. J. Sensory Studies. 34(6). https://doi.org/10.1111/joss.12495.

Polatoglu, K., Demirci, B., Calis, I., & Baser, K. H. C. (2017). Difference in volatile composition of Chenopodium murale from two different locations of Cyprus. *Records of Natural Products, 11*(1), 88–91.

Raybaudi-Massilia, R. J., Mosqueda-Melgar, J., Rosales-Oballos, Y., Citti de Petricone, R., Frágenas, N. N., Zambrano-Durán, A., Sayago, K., Lara, M., & Urbina, G. (2019). New alternative to reduce sodium chloride in meat products: Sensory and microbiological evaluation. *LWT-Food Science Technology, 108*, 253–260.

Sallan, S., Kaban, G., & Kaya, M. (2022). The effects of nitrite, sodium ascorbate and starter culture on volatile compounds of a semi-dry fermented sausage. LWT-Food Science and Technology, 153(1). https://doi.org/10.1016/j.lwt.2021.112540.

Santos, B. A., Campagnol, P. C. B., Morgano, M. A., & Pollonio, M. A. R. (2014). Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. *Meat Science, 96*, 509–513.

Santos, B. A., Campagnol, P. C. B., Fagundes, M. B., Wagner, R., & Pollonio, M. A. R. (2015). Generation of volatile compounds in Brazilian low-sodium dry fermented sausages containing blends of NaCl, KCl, and $CaCl_2$ during processing and storage. *Food Research International, 74*, 306–314. https://doi.org/10.1016/j.foodres.2015.04.042.

Stahnke, L. (2002). Flavour formation in fermented sausage. In: Research advances in the quality of meat and meat products. Toldrá, F. (Ed.). Kerala: Research Signpost. (pp 193–223).

Tian, H. X., Li, F. H., Qin, L., Yu, H. Y., & Ma, X. (2015). Quality evaluation of beef seasoning using gas chromatography-mass spectrometry and electronic nose: Correlation with sensory attributes and classification according to grade level. *Food Analytical Methods, 8*(6), 1522–1534. https://doi.org/10.1007/s12161-014-0031-4.

Vural, H. (1998). The use of commercial starter cultures in the production of Turkish semi-dry fermented sausages. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung a, Berlin, 207*, 410–412.
World Health Organization [WHO]. (2012). Guideline: Sodium intake for adults and children. https://www.who.int/publications/i/item/9789241504836. Accessed on 23 March 2022.
Wu, H., Zhuang, H., Zhang, Y., Tang, J., Yu, X., Long, M., Wang, J., & Zhang, J. (2015). Influence of partial replacement of NaCl with KCl on profiles of volatile compounds in dry-cured bacon during processing. *Food Chemistry, 172*, 391–399.
Zhao, G. M., Zhou, G. H., Tian, W., Xu, X. L., Wang, Y. L., & Luo, X. (2005). Changes of alanyl aminopeptidase activity and free amino acid contents in biceps femoris during processing of Jinhua ham. *Meat Science, 71*, 612–619.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.