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Effects of the use of raw or cooked chickpeas and the sausage cooking time on the quality of a lamb-meat, olive-oil emulsion-type sausage

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ABSTRACT

Reformulation of cooked sausages using high-protein plant-based food such as chickpea as meat extenders and vegetable oils to replace animal fat can be a suitable approach to promote the consumption of smaller portions of meat. The pre-processing of chickpea and the sausage cooking intensity can potentially affect the quality of reformulated sausages. In this study, an emulsion-type sausage made with lamb meat, chickpea and olive oil was prepared in triplicate following three different formulations containing the same targeted levels of protein (8.9%), lipids (21.5%), and starch (2.9%): control sausage (CON; control, without chickpea), and raw (RCP) and cooked chickpea (CCP) sausages (both with 7% chickpea). Sausages were cooked at 85 °C for two heating times (40 min or 80 min) and were analysed for weight loss, emulsion stability, colour, texture, lipid oxidation and volatile composition. Compared to CON sausages, the use of raw chickpea reduced the elasticity and significantly increased lipid oxidation during the sausage-making process resulting in major changes in the volatile composition. The use of previously cooked chickpea, however, resulted in the sausages having greater cooking loss, hardness and chewiness than CON sausages, while there was no difference in lipid oxidation, and differences in volatile compounds were scarce. The reformulation with cooked chickpea could provide a sausage with more similarity to the CON sausage. The extended heating time of 80 min at 85 °C did not significantly affect the quality traits in either CON or reformulated sausages except for a higher cooking loss.

1. Introduction

Meat and meat products, rich in high-quality protein, vitamins B12, iron, zinc and selenium, are important sources of nutrients in the human diet (Godfray et al., 2018). However, meat production can be associated with large environmental and climate footprints (Eckl, Bierbroek, van't Veer, & Geleijnse, 2021) and, when meat intake levels are high, or meat is consumed on a long-term basis, dietary meat may be associated with adverse health effects, such as colorectal cancer (Godfray et al., 2018; Richi et al., 2015). In a context of increasing rate of demand for meat and meat products and considering the above concerns, reformulation of meat products by partially replacing meat with protein-rich plant foods or replacing animal fat with vegetable oils could be alternatives of choice for the meat industry. These approaches fall under the concept of hybrid meat products (Grasso & Jaworska, 2021) and would aim to promote the consumption of smaller portions of meat (De Boer, Schösler, & Aiking, 2014). Meat products reformulated in this manner must not only be as nutritious as conventional products, but also have sensory characteristics comparable to conventional products to avoid rejection by potential consumers (Hoek et al., 2011; Mateo et al., 2021).

Grain legumes (pulses), in addition to cereals and pseudocereals, oilseeds and mushrooms, as well as protein extracts obtained from them, have been the suggested plant-based ingredients to replace meat in reformulated meat products (Asgar, Fazilah, Huda, Bhat, & Karim, 2017). Pulses have a high nutritional value (Pintado & Delgado-Pando, 2020) and their incorporation into the daily diet has been shown to have advantages and physiological effects in the control and prevention of various diseases prevalent in many countries around the world (Mudryj, Yu, & Aukema, 2014). Among vegetable oils, olive oil has been proposed as a candidate oil to partially or totally replace animal fat in cooked

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emulsion-type sausages because of its beneficial nutritional properties and favourable sensory and functional properties (Shin et al., 2022; Shin, Lee, Lee, Jo, & Choe, 2020).

The effect of the incorporation of pulse flours in meat products on their quality has been studied for several decades and there is currently a renewed interest in research on this topic (Grasso & Jaworska, 2021; Hill, 2022; Pintado & Delgado-Pando, 2020; Verma, Ledward, & Lawrie, 1984a). However, most research has been carried out on fresh meat products, such as burgers, and less on cooked sausages.

The amount of pulse used in experimental reformulations has been up to 50% of the meat batter, although recommended levels have typically been between 5% and 10%.

The use of pulses at limited quantities seems to produce minor sensory differences compared to other meat extenders (Chigwedere, Wanasundara, & Shand, 2021). However, pulses can significantly affect the flavour of meat products if used in high amounts. Pulses in reformulated meat products have been associated with "bean" and "hay-like" off-flavours (Chigwedere et al., 2021), which can have a significant negative effect on consumer acceptance. The colour of meat products can also be affected by the effect of pigments provided by pulses and, in the case of raw pulses, by an oxidative effect on myoglobin (Verma et al., 1984a).

Among the variety of pulses used to reformulate meat products, chickpea (*Cicer arietinum* L) has been one of the most widely used (Mateo et al., 2021). Chickpea has a high nutritional value, being a rich and cheap source of protein, with levels normally above 20%. The protein quality of pulses in terms of essential amino acid ratio is worse than that of meat due to a low content of sulphur amino acids and tryptophan; however, it can be improved when combined with rice or egg (Farooq & Boye, 2011). Chickpea is also a rich source of fibre and a good source of iron, zinc, magnesium, calcium, vitamin E and B9 (Kaur & Prasad, 2021). Moreover, due to the functionality of chickpea components, such as proteins, starch, polyphenols, or carotenoids, this pulse is in demand as a functional ingredient in a variety of food applications (Kaur & Prasad, 2021; Sofi, Muzaffar, Ashraf, & Gupta, 2020). The functional properties of proteins and starch from chickpeas when used as meat extenders can contribute positively to the functional properties of meat proteins, such as water holding capacity (WHC), binding, gelation and emulsification (Li et al., 2020; Vatansever, Tulbek, & Riaz, 2020; Wang et al., 2023).

The processing of pulses prior to use, i.e. grinding, shelling, soaking or cooking, can influence the impact of pulses on the quality of the meat product. Boiling pulses or roasting pulse flours prior to use have the advantage of effectively removing the content of antinutritional factors (ANFs), thus improving their digestibility (Bessada, Barreira, & Oliveira, 2019). Moreover, heating inactivates undesirable enzymes such as oxidative enzymes, i.e. lipoxygenase (LOX; Shariati-Ievari et al., 2016; Shi, Mandal, Singh, & Pratap-Singh, 2020), or hydrolytic enzymes, i.e. α-amylase (Guldiken et al., 2022), thus preventing discoloration, off-flavours, or texture changes in reformulated meat products. On the other hand, heating negatively affects the technological functionality of pulse starch or protein (Aguilera, Esteban, Benitez, Molla, & Martin-Cabrejas, 2009), which might result in poorer binding, gelling, emulsion or stability performances.

Emulsion-type sausages, after stuffing, are heated in ovens or water for pasteurisation, normally at 80 °C to a core temperature near to 72 °C. The heating intensity is a relevant factor for gel structure, strength and cooking yield in sausage making (Barbut, Gordon, & Smith, 1996; Glorieux et al., 2019). In reformulated sausages containing raw pulses, conventional heating (72 °C core temperature) may not be intense enough to obtain a complete gelatinisation of pulse starches, whose gelatinisation temperature ranges between 70 and 90 °C (Farooq & Boye, 2011), which in turn could lead to sub-optimal texture and water retention. Higher than usual heating intensities, i.e. 80–90 °C core temperature, may also be required to achieve a significant inactivation of undesirable pulse bioactive compounds such as lectins or LOX activity, which in short-heating treatments require temperatures between 70 °C and 90 °C for denaturation (He et al., 2014; Shi et al., 2020; Liburdi et al., 2021).

The aim of this research was to determine the effects of the use of chickpea as a partial meat substitute, the pre-treatment of chickpeas (raw vs. cooked) and the intensity of sausage cooking on the quality characteristics of an emulsion-type cooked sausage made with lamb meat and olive oil, namely: yield, texture, oxidative stability and flavour compounds.

2. Materials and methods

2.1. Sausage ingredients

The following ingredients were used in the sausage formulation: leg lean meat (obtained from 4-month-old lambs, which were reared on a local farm under same intensive conditions; legs were purchased two days after slaughter, frozen at −18 °C, stored for up to two months and thawed for two days under refrigeration before use; meat composition ranged 73–75% moisture, 19–21% protein, 3–4% fat), cream-coloured chickpea (*Cicer arietium*, Pedrosillano variety; La Asturiana, Vidanes, León, Spain; containing 41% starch, 18% protein, 14% fibre and 6% fat), 0.4%-acidity refined olive oil, tap water, food-grade potato starch, sodium chloride, polyphosphates (Tai K-7, Pilarica, Paterna, Spain), and powdered black pepper; all purchased from the local market.

2.2. Experiment design

Emulsion-type sausages made with lamb meat, chickpea and olive oil were prepared following three different formulations (three treatments), each with the same target levels of protein, lipid and starch (8.9, 21.5 and 2.9%, respectively): a control sausage (CON; without chickpea) and two sausages with part of the meat replaced by chickpea, one with raw chickpea (RCP) and the other with cooked chickpea (CCP). These sausages were prepared in triplicate (three batches, each prepared on a different day) at the Food Processing Hall of the Faculty of Veterinary Medicine, University of León (León, Spain). The sausages were cooked following two heating times: conventional (72–74 °C core temperature) and prolonged (82–84 °C core temperature). Therefore, the experimental setup consisted of 3 treatments (formulations) x 2 cooking conditions. To evaluate the effects of treatment and cooking condition on sausage quality, the following laboratory analyses were performed the day after cooking: weight losses, pH colour, texture, thiobarbituric reactive substances (TBARS) and volatile compounds. Two sausage pieces were prepared for each combination treatment-heating condition, and the quality analyses were performed in duplicate for each sausage piece the day after cooking. Furthermore, to find out whether reformulation or heating conditions could affect the oxidation stability of the lipids during storage, a portion of each sausage piece was covered with cling film, stored for 12 days under refrigeration (4 °C) and used for an additional TBARS test.

2.3. Sausage preparation and sampling

The formulations for each of the three experimental treatments are shown in Table 1. For the three batches of sausages, each experimental treatment consisted of 2 kg of sausage mix. Each batch was made with leg meat from a different lamb. The main ingredients in CON sausage formulation were meat, oil and starch (44.5, 20 and 2.9%, respectively). The formulation of the sausages with chickpeas, raw (RCP) or cooked (CCP) sausages, was designed to have quantities of protein, lipids and starch equal to those expected in CON sausages. Considering the concentration of starch in chickpeas (41%), the amount of chickpea to be used in the sausage formulation, in order to achieve 2.9% starch (2.9 g starch/100 g sausage batter), was 7% (7 g chickpea per 100 g batter). This amount of chickpea contributed 1.3% protein to the sausage batter,

Table 1
Formulations of the control and reduced meat emulsion-type sausages.

	CON	RCP	CCP
Main ingredients (%)			
Lamb meat	44.5	38	38
Chickpea (untreated)[&]	–	7	7
Olive oil	20	20	20
Water[$]	32.6	35	35
Starch	2.9	–	–
Salts and spices (g/kg)			
Sodium chloride	18	18	18
Phosphates	3	3	3
Powdered black pepper	2	2	2

CON: Control sausage, formulated without chickpea; RCP: sausage formulated with soaked raw chickpeas; CCP: sausage formulated with cooked chickpeas.
[&] The percentage refers to raw chickpea before pre-treatment (soaking or soaking and boiling).
[$] Included in the percentage are the added water and the water absorbed by the chickpeas during soaking and eventually cooking.

which led to a reduction of 6.5 % units in the amount of meat, with 20% protein, in the RCP and CCP sausages (from 44.5% to 38%) – this meant that 15% of the animal protein was replaced by vegetable protein, i.e. CON sausage would contain 8.9% animal protein and chickpea sausages 7.6% animal protein. The formulations of the RCP and CCP sausages complies with the permissible limits for plant-origin ingredients laid down in the Meat Products Quality Standard Regulation (Spain, 2014): no stablished limit for edible oils (quantum satis), <3% plant-origin protein, <10% starch.

For the RCP treatment, the chickpeas were previously soaked in tap water for 16 h at room temperature (21 °C) following the recommendations from the provider and for the CCP treatment they were soaked in the same conditions and then traditionally boiled in water for 3 h. Just before the mixing of the ingredients, the meat was minced using a meat grinder (Mainca PM-12; Granollers, Spain) equipped with a 6-mm plate size, and the soaked-raw or soaked-then-cooked chickpeas were grinded with ice (1/1, *w*/w) in a Stephan UMC5 (Saint-Cannat, France) for 8 min at 4 °C and 2400 rpm to form a chickpea paste. A sample of the pulse paste was taken for pH and TBARS analyses. The sausage mix was then prepared in the Stephan UMC5 operating at 4 °C, 2400 rpm and 50.7 × 10^3 Pa pressure in two steps: i) minced meat, ice, NaCl, polyphosphate and either starch (for CON) or the pretreated chickpeas (for RCP or CCP) were mixed for 4 min, and ii) olive oil was added and the mixing continued for another 4 min period. Afterwards, for each sausage treatment, two portions of 70 g of the emulsified batter were sampled for immediate analysis of pH, proximate composition and emulsion stability, and the rest of the batter was stuffed into 34–36 mm-diameter beef casings from small intestine (Navaher, León, Spain) obtaining four similar, c.a., 0.5-kg sausage pieces per treatment.

The sausage pieces were weighed and then cooked suspended in a programmed oven at 85 °C and 95% relative humidity for two different times. Two pieces were cooked for 40 min (conventional heating; 72–74 °C core temperature) and the other two for 80 min (prolonged heating; 82–84 °C core temperature). After cooking, the sausages were cooled in tap water for 10 min, drained at room temperature (21 °C) for 30 min and weighed to calculate the cooking loss. Each (0.5-kg) sausage piece was then cut into three similar portions and each portion was wrapped with polyethylene cling film and cooled in a cold chamber (4 °C). The day after cooking, two portions of sausage were used for analysis. Prior to analysis, each of these two sausage portions was further cut into two parts, one was used to assess water loss by centrifugation, pH, colour and texture, and the other was homogenised using a domestic food processor (DP8108, 1000 W, Moulinex, Alençon, France) and used for analysis of chemical composition, TBARS and volatile compounds. The remaining (third) sausage portion was covered with cling film, stored for 12 days under refrigeration (4 °C) and used for an additional TBARS test.

2.4. Batter composition and technological quality traits

For each batter sample, moisture, fat, protein and ash were determined according to the methods recommended by the AOAC (1999). All these analyses were performed in duplicate. The pH of the chickpea paste and the batters were measured in duplicate using a Basic 20 pH-meter with serial number 047021 (Crison Instruments, Barcelona, Spain) equipped with a penetration electrode (Hach Lange, Düsseldorf, Germany). The pH meter was calibrated daily prior to use with two calibration buffers (pH 7.0 and pH 4.0), and the calibration and measurements of samples were carried out with the buffers and samples at room temperature (20 ± 2 °C). The emulsion stability was determined, in duplicate, from a sample of 25 ± 1 g of raw batter which was placed into a Falcon tube and centrifuged for 1 min at 600 ×*g* using a 2–15 Sigma centrifuge (Osterode am Harz, Germany), following the procedure described by Hughes, Cofrades, and Troy (1997). The filled Falcon tubes were heated in a water bath for 30 min at 70 °C and centrifuged again for 5 min at 1200 ×*g*. The supernatants were poured into pre-weighed crucibles and dried overnight at 100 °C, and the pelleted samples were extracted from the tubes and weighed. In the analysis the dry extracts from the supernatants were considered to be fat. The percentages of fluid released from the batter after cooking and centrifugation and the amount of dry extract in the fluid (fat) was calculated as a percentage of the initial batter.

2.5. Sausage technological quality traits

The day after cooking, using a portion of each cooked sausage, the water retention capacity of the cooked sausage was determined in duplicate following the method described by Hughes et al. (1997) with slight modifications. Two grams of sausage, cut into fine strips, previously weighed using a precision balance (4 decimal places) were placed into 5-ml plastic centrifuge tubes containing a small amount of cotton, c. a. 0.10 g, packed in the bottom. The tubes were then centrifuged at 9000 x*g* for 10 min, at 4 °C, (J2–21 centrifuge, Beckman Coulter, Barcelona, Spain). Afterwards, the strips of sausage were extracted from the tube and weighed to determine the loss of water during centrifugation from the weight difference.

Sausage colour was measured in duplicate on the cut surface of recently-cut 1-cm-thick sausage slices. Three different measurement points for each of two replicates were taken using a CM-700d portable spectrophotometer (Konica Minolta, Osaka, Japan) operating with D65 illuminant D65, SCI mode, 8 mm aperture for measurement and a visual angle of 10°. The values of the lightness (L^*), redness (a^*) and yellowness (b^*) were recorded.

The texture profile analysis was analysed in triplicate on three 1-cm sized cubes each obtained from the inner part of the corresponding 1-cm sausage slices. A texture analyzer TA.XT2i (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5-cm diameter cylindrical probe running with a cross head speed of 1 mm/s and a compression depth of 80% was used. Values for hardness, elasticity and cohesiveness were recorded, and chewiness was calculated as the product of the three values (hardness × cohesiveness × springiness).

2.6. Thiobarbituric acid-reactive substances (TBARS) and headspace volatile composition in sausages

The TBARS of the chickpea paste and the sausages were determined using the procedure described by Nam and Ahn (2003) with slight modification. A 2-g sample was homogenised with 20 mL distilled and deionised water using an IKA T-18 basic Ultra Turrax (Staufen, Germany) working at 9500 rpm for 60 s. The homogenate was filtered through a wire-mesh filter and then 1 mL of homogenate was mixed in a

screw-capped tube with 50 μL of 7.2% butylated hydroxytoluene ethanolic solution and 1 mL of 20 mM thiobarbituric acid/15% trichloroacetic acid. The mixture was vortexed, heated in a water bath at 80 °C for 20 min, cooled in cold water for 10 min, centrifuged at 4000 ×*g* for 20 min and filtered through a 0.45 μm hydrophilic polytetrafluoroethylene 13 mm syringe filter (Membrane Solutions, LLC, Auburn, WA, USA). The absorbance was then read at 531 nm. Quantification was performed on the basis of the absorbance of 1,1,3,3-tetra-ethoxypropane standard solutions.

The volatile composition in the sausage headspace was analysed as previously described by Carballo et al. (2020) with slight modifications. Briefly, volatile compounds were extracted from 20 mL vials containing 3 g of homogenised sausage previously incubated at 45 °C for 20 min, using a 75 μm Carboxen/polydimethylsiloxane 1-cm-coated fused-silica fibre (Supelco, Bellefonte, PA, USA) for 40 min at 45 °C. Afterwards, volatile compounds were desorbed in the injection port of a GC 7890A chromatograph (Agilent System, Zwingen, Switzerland) for 2 min at 260 °C in splitless mode, and separated with a 60 m × 0.25 mm sized, 0.25 mm film thickness DB-5MS column (Agilent System) using helium (1 mL/min) as the carrier gas, with oven programmed at 35 °C (1 min), 35 °C to 50 °C (10 °C/min), 50 °C to 200 °C (4 °C/min), 200 °C to 250 °C (50 °C/min) and 250 °C (11 min). Detection (MS 5975C detector, Agilent System) was carried out in electron impact mode at 70 eV with the spectrometer transfer line and ion source temperatures of 240 °C and 260 °C, respectively, scanning from 40 *m/z* to 350 m/z. Identification was performed by comparing the peak's mass spectrum with those in the NIST/EPA/NIH-98 mass spectral database, and comparing the linear retention indexes calculated from a series of n-alkanes with those reported in the literature. The compound levels were expressed as area units x 10^6.

2.7. Statistical analysis

The general linear model analysis of variance (ANOVA) was performed using SPSS Statistics software (version 26; IBM, Somers, NY, USA). For the sausage batter before cooking, treatment (formulation) was used as a fixed factor. For the sausages, treatment, cooking time and interaction treatment × cooking time were used as fixed factors. In addition, replication consisting of the data from the analysis of two portions of sausages for each treatment-cooking time combination within each production batch was included as a random factor. When the ANOVA proved to be significant ($P < 0.05$) for treatment or the treatment x cooking-time interaction, it was followed by the Tukey test to perform pair-wise comparisons, considering statistical significance for $P < 0.05$.

3. Results and discussion

3.1. Meat batter composition and technological quality

The use of chickpeas in sausage formulation resulted in a sausage batter composition with a lower percentage of moisture. (Table 2). This was expected because the difference in the water added to both RCP and CCP batters and that added to the CON batter (2.4 g of water/100 g batter; Table 1) was lower than the moisture provided from the additional amount of meat in the CON batter (6.5 g of meat/100 g batter; Table 1; contributing 4.8 g of water/100 g batter). The RCP batter contained a higher concentration of the sum starch + fibre than CON formulation. The concentration of starch + fibre in CON batter was consistent with and similar to the amount of starch used, and the significant difference between RCP and CON sausages would be attributed to the fibre content in chickpeas. The intermediate concentration of starch + fibre showed by CCP batter compared to RCP and CON batter could be explained by partial losses in carbohydrates (fibre included) from the chickpeas during cooking (Liu & Serventi, 2020; Ma et al., 2011).

Table 2
Composition and emulsion stability of reformulated emulsion-type sausage batters.

	CON	RCP	CCP	*P*-level
Composition (%) and pH				
Moisture	66.23 ± 0.28[a]	63.41 ± 0.12[c]	64.67 ± 0.24[b]	<0.001
Lipids	18.38 ± 0.26	18.92 ± 0.40	19.45 ± 0.49	0.19
Protein	9.29 ± 0.06	9.31 ± 0.06	9.08 ± 0.02	0.09
Ash	2.87 ± 0.08	3.17 ± 0.13	2.91 ± 0.10	0.09
Starch + fibre[#]	3.23 ± 0.16[b]	5.20 ± 0.42[a]	3.89 ± 0.60[ab]	0.049
pH	6.28 ± 0.03[b]	6.27 ± 0.04[b]	6.35 ± 0.07[a]	0.010
Emulsion stability (%)[&]				
Total fluid losses	0.66 ± 0.09	0.40 ± 0.08	0.76 ± 0.11	0.11
Oil losses	0.62 ± 0.09	0.36 ± 0.07	0.71 ± 0.10	0.10

Results are reported as means (average of triplicate batches and two samples per batch; $n = 6$) ± standard errors of the mean.
CON: Control sausage, formulated without chickpea; RCP: sausage formulated with soaked raw chickpeas; CCP: sausage formulated with cooked chickpeas.
[ab]Means within the same row without a common superscript are significantly different ($P < 0.05$; Tukey post-hoc test).
[#] Obtained by difference: 100 – (% moisture + % fat + % protein + % ash).
[&] Losses due to cooking and centrifuging the batter in Falcon tubes.

The value of pH was slightly but significantly higher in CCP batter, suggesting that cooking of chickpeas increased pH value. Accordingly, we found that the pH values of raw and cooked chickpea pastes were respectively 6.40 ± 0.03 and 6.91 ± 0.03. The increase in pH of chickpeas due to cooking could be at least partially explained by the leaching of soluble acidic compounds in the cooking water, as chickpea cooking water is known to be slightly acidic (Stantiall, Dale, Calizo, & Serventi, 2018).

Water and oil losses in the batters (emulsion stability features) due to cooking and centrifugation were minimal for all the formulations and were not affected by the replacement of meat for raw or cooked chickpeas (Table 2). In contrast, Dzudie, Scher, and Hardy (2002), Thushan-Sanjeewaa, Wanasundara, Pietrasika, and Shanda (2010) and Tahmasebi, Labbafi, Emam-Djomeh, & Yarmand, (2016), all using raw pulse flours, reported that reformulation with pulses at levels of 4% to 11% increased emulsion stability and WHC of cooked sausages. Discrepancies could be attributed to differences in the experimental conditions between this study and those studies that can potentially affect the emulsion stability, such as the type and amount of pulse used or the percentage of meat replacement, the type of meat, fat, water, etc.

3.2. Sausage technological quality traits

The weight loss of the sausages during cooking and the technological quality traits of the cooked sausages after 24 h of refrigerated storage are shown in Table 3. The reformulation showed significant differences in cooking losses, which were mainly due to evaporation of moisture from the sausage surface ($P < 0.001$). In contrast, no significant difference in centrifugation losses was observed ($P = 0.09$). The CCP sausages showed a weight loss of 3 to 7% units higher than CON or RCP sausages. However, the partial substitution of meat by raw chickpea (RCP sausages) showed no effect on cooking losses compared to CON sausages. The evaporation of water (cooking losses) from the sausage, when sausage diameter and cooking conditions are the same, depends on the migration of water inside the sausage and thus on the composition of the sausage and its WHC. Hence, the WHC of the functional components of raw chickpeas, such as protein, starch, fibre, etc., would be comparable to that of the amount of meat replaced.

The lower and higher cooking losses for, respectively, RCP and CCP sausages suggest a heat-induced decrease in WHC of proteins and starch of cooked chickpeas compared to those of raw chickpeas. The addition of native (non-denatured) chickpea protein to myofibrillar protein suspensions increases water retention during thermal gelation of composite

Table 3
Weight loss due to cooking and technological properties of reformulated emulsion-type sausages cooked for conventional (conv) or prolonged (prolong) time.

	Formulations						*P*-level		
	CON-conv	CON-prolong	RCP-conv	RCP-prolong	CCP-conv	CCP-prolong	F	t	F × t
Weight losses (%)									
Cooking	4.26 ± 0.44^{d}	6.84 ± 0.44^{bc}	6.23 ± 0.41^{bcd}	6.67 ± 0.89^{c}	9.08 ± 0.25^{b}	13.39 ± 0.58^{a}	<0.001	<0.001	0.005
Centrifugation	15.7 ± 1.2	14.4 ± 0.7	16.1 ± 0.7	17.8 ± 1.4	19.1 ± 1.9	16.6 ± 1.2	0.092	0.50	0.22
Colour									
*L**	75.9 ± 0.6	76.3 ± 0.6	77.0 ± 0.6	76.8 ± 0.7	77.8 ± 0.6	77.6 ± 0.6	0.055	1.00	0.091
*a**	1.00 ± 0.09^{b}	1.19 ± 0.11^{b}	1.43 ± 0.13^{ab}	1.75 ± 0.13^{a}	1.01 ± 0.11^{b}	1.31 ± 0.11^{ab}	0.005	0.018	0.25
*b**	15.7 ± 0.2^{c}	15.4 ± 0.2^{c}	15.7 ± 0.2^{c}	15.7 ± 0.3^{bc}	16.7 ± 0.2^{ab}	16.8 ± 0.2^{a}	0.002	0.88	0.70
Texture									
Hardness (N)	26.2 ± 0.90^{b}	24.0 ± 0.6^{b}	24.6 ± 1.71^{b}	25.5 ± 1.3^{b}	28.7 ± 1.1^{ab}	31.0 ± 1.2^{a}	0.038	0.39	0.22
Cohesiveness	0.252 ± 0.010^{de}	0.243 ± 0.090^{e}	0.279 ± 0.006^{cd}	0.273 ± 0.032^{bc}	0.317 ± 0.003^{a}	0.299 ± 0.006^{ab}	0.001	0.16	0.14
Elasticity	0.428 ± 0.008^{a}	0.399 ± 0.036^{ab}	0.349 ± 0.024^{b}	0.346 ± 0.015^{b}	0.389 ± 0.050^{ab}	0.365 ± 0.008^{b}	0.011	0.17	0.09
Chewiness (N)	27.4 ± 0.9^{abc}	24.9 ± 0.6^{c}	25.5 ± 1.8^{bc}	25.8 ± 1.3^{bc}	30.7 ± 0.7^{ab}	32.0 ± 1.1^{a}	0.013	0.98	0.85

Results are reported as means (average of triplicate batches and two samples per batch; n = 6) ± standard error of the mean.
CON: Control sausage, formulated without chickpea; RCP: sausage formulated with soaked raw chickpeas; CCP: sausage formulated with cooked chickpeas; conv: conventional cooking time; prolong: prolonged cooking time; F: formulation; t: cooking time.
^{abcd} Means within the same row without a common superscript are significantly different ($P < 0.05$; Tukey post-hoc test).

gels (Li et al., 2021; Wang et al., 2023). On the other hand, protein denaturation in cooked chickpeas results in reduced protein functionality, i.e. reduced ability to interact with water molecules (Aguilera et al., 2009; Ma et al., 2011). Furthermore, in the RCP (and CON) sausages, unlike the CCP sausages, the gelatinisation of the starch took place during the cooking of the sausage which might have also contributed to the higher water retention.

Cooking time exerted a significant effect on cooking losses, finding more losses for the prolonged heating ($P < 0.001$; Table 3). Comparing within each treatment, significant differences in cooking losses were observed between for CON and CCP sausages. In agreement, higher cooking losses are expected as sausage core temperature increases (Glorieux et al., 2019; Shin et al., 2022). Unexpectedly, this effect was not found in RCP sausages. The different pattern observed in these sausages, i.e. the lack of effect of heating time on moisture losses, could be related to the gelatinisation dynamics of raw chickpea starch during the sausage cooking period. Singh, Singh-Sandhu, and Kaur (2004) reported temperature ranges for starch gelatinisation of different chickpea varieties between 62 and 74 °C. Therefore, in RCP sausages the gelatinisation of raw chickpea starch may not have ended with the conventional heating and the ongoing gelatinisation during the extended period of the prolonged heating may have had a slowing effect on moisture migration inside the sausage and this, in turn, may have decreased moisture evaporation.

The colour of the CON sausage, whose values are shown in Table 3, seemed to be considerably different from that of cream-coloured chickpeas, which according to Güzel and Sayar (2012) cream-coloured chickpeas showed L*, a* and b* values of 53–57, 7–8, and 17–23, for L*, a* and b*, respectively. Thus, the reformulation of sausages with chickpea should have an effect on sausage colour, as was the case in our study. However, the specific effects of adding chickpea on the sausage colour are difficult to predict and explain since several complex factors are involved, such as the levels of chickpea and meat pigments in sausage batter, pigment changes during sausage processing, sausage microstructure affecting light scattering, etc. Our results showed that the difference in colour between CON and chickpea-containing sausages depended on the chickpea pre-treatment. Compared to CON sausages, the use of raw chickpea increased a^* and that of cooked chickpea increased b^*. In agreement, Sanjeewaa, Wanasundara, Pietrasika, & Shanda (2010) found the use of raw chickpea flour (5%) in the formulation of a low-fat sausage to increase b^*, whereas a^* and L^* were not affected. Cooking time affected the a^* value ($P = 0.018$); however, this effect could not be evidenced when comparing between heating times within formulations. The effect of heating time on a^* might be related to the higher moisture losses and thus higher protein concentration in the more intensely cooked sausages.

Reformulation had a significant effect on all textural characteristics of the sausages (Table 3). Compared to CON sausages, RCP and CCP sausages showed respectively similar or greater hardness and chewiness. Results suggest that the chickpea components involved in the heat-induced gelation in the sausages, such as proteins, mucins, starch and complex carbohydrates, would have compensated (for RCP) or enhanced (for CCP) the bonding strength effect of the replaced meat proteins. Moreover, regardless how was the processing of chickpeas, the replacement of meat by chickpea paste tended to significantly increase the sausage-gel cohesiveness and decrease its elasticity. In previous studies using raw pulse flours as meat extenders in emulsion-type cooked sausages, the effects of pulses on hardness and elasticity have shown partial discrepancy, which might be explained by differences in the experiment conditions. Dzudie et al. (2002), using bean meal at levels up to 10.0% to replace the same amount of beef, thus reducing the moisture level in the sausage with the addition of pulse flour, observed a decrease in hardness. In contrast, Tahmasebi, Labbafi, Emam-Djomeh, and Yarmand (2016) found that replacing beef with pigeon pea flour (up to 16.5%) combined with maize flour increased the toughness of the sausages. Neither study, in contrast to our results, found a significant effect of meat substitution with pulse flour on elasticity. However, as regards to cohesiveness, according to our results, the use of pulse flour increased cohesiveness in the reformulated sausages (Dzudie et al., 2002; Tahmasebi et al., 2016).

Comparing the CCP and RCP sausages separately, the CCP sausages showed higher hardness, cohesiveness, and chewiness than the RCP sausages, despite the expected reduction of protein gelation functionality in the pre-cooked chickpea (Ma et al., 2011). This increasing effect could be related to the higher dry matter and protein contents in CCP sausages resulting from the greater weight (moisture) loss during cooking (Table 2). According to Petridis, Vlazakis, Tzivanos, Derlikis, and Ritsoulis (2010), a higher protein content in frankfurters tends to produce a harder and more cohesive gel network. Regarding cooking time, however, the prolonged heating, although it resulted in significantly higher moisture loss in the CON and CCP sausages, did not increase the texture values in any of the treatments. The protein gelation was probably fully completed with the conventional heating (Barbut et al., 1996), and the excess moisture losses due to prolonged heating were not high enough to significantly change the texture values.

The differences found in the texture profile between the sausages studied would probably have changed their sensory texture perception. The decreasing effect on elasticity found in chickpea reformulated

sausages could be considered negative considering that a high elasticity is a key desired characteristic of frankfurters (Petridis et al., 2010). The relationship between instrumental texture and sensory data in reformulated sausages, however, is not always easy to predict (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2011). Thus, a sensory analysis would be needed to fully understand the effect of the observed changes in texture profile parameters on sausage acceptance. In addition, further research should be conducted to evaluate the combined effect of using chickpeas with texture-enhancing ingredients such as selected proteins or hydrocolloids, thus improving elasticity.

3.3. Lipid oxidation and headspace volatile composition in sausages

Raw pulses seem to produce lipid oxidation when they are mixed with other food ingredients, meat included, to prepare reformulated food products, thus generating secondary lipid oxidation products (Krause et al., 2022; Shariati-Ievari et al., 2016; Verma, Ledward, & Lawrie, 1984b). Accordingly, in our study, the use of raw chickpeas significantly increased TBARS levels in RCP sausage (Table 4). In relation to the lipid oxidation in RCP sausages and in accordance with the findings of Verma et al. (1984b), during mixing we observed that the RCP batter was also discoloured (myoglobin was oxidised) compared to the CON and CCP batters (Supplementary Fig. S1).

The TBARS increase in RCP sausages would have been mainly the result of enzymatic activity by the LOX contained in raw pulses (Karolkowski, Guichard, Briand, & Salles, 2021; Shariati-Ievari et al., 2016; Xu, Zhao, Gu, Rao, & Chen, 2020). The TBARS initially present in the raw chickpea-water (1:1; *w*/w) paste, used in the formulation, would have partially contributed to the TBARS levels of the cooked sausages, since before being mixed with the meat, the TBARS level of the paste was 1.16 ± 0.12 mg malondyaldehyde/kg of paste (mean ± standard deviation; $n = 3$). However, most of the TBARS increase (lipid oxidation) in RCP sausages would have occurred during the mixing, stuffing and initial period of the sausage heating, until the heat was able to inhibit the enzymatic activity. Regarding LOX heat stability, Verma et al. (1984b) observed that heating raw chickpea flour at 60 °C for 1 h inactivated its oxidation effect on meat batters.

In an ad hoc supplementary experiment, we found that neither ascorbic acid (0.5 g/kg) not potassium metabisulphite (0.45 g/kg), two common additives used in the meat industry, were able to significantly reduce neither the discolouration (decrease in a* value) of the RCP batter, nor the lipid oxidation (TBARS levels) in the RCP sausage (Supplementary Table S1). In a previous study, Verma et al. (1984b) reported that an antioxidant containing α-tocopherol at levels of 1% of fat content was able to retard but not prevent the lipid oxidation related to the use of raw chickpea in a sausage, although it did not inhibit discoloration.

The TBARS levels in the sausages within each treatment did not change significantly between conventional and prolonged heating, which indicate that lipoxygenase activity on lipid oxidation was completely inactivated by the conventional heating. No significant

Table 4
Thiobarbituric-acid reactive substances (TBARS) and headspace volatile compounds[&] of the reformulated emulsion-type *sausages* cooked for conventional (conv) or prolonged (prolong) time.

	Formulations						*P*-level		
	CON-conv	CON-prolong	RCP-conv	RCP-prolong	CCP-conv	CCP-prolong	F	t	F × t
TBARS	0.60 ± 0.13^{b}	0.43 ± 0.09^{b}	2.58 ± 0.05^{a}	2.17 ± 0.08^{a}	0.57 ± 0.08^{b}	0.45 ± 0.05^{b}	<0.001	0.49	0.095
Aldehydes									
Ethanal	4.4 ± 0.4^{b}	6.2 ± 0.5^{b}	71.0 ± 9.3^{a}	50.0 ± 7.0^{a}	4.8 ± 1.1^{a}	3.4 ± 1.2^{b}	<0.001	0.061	0.005
Methylbutanal	ND	0.8 ± 0.78	3.9 ± 2.5	2.2 ± 1.4	2.9 ± 1.9	4.1 ± 2.7	0.052	0.90	0.401
Pentanal	2.6 ± 1.6^{b}	6.1 ± 2.2^{b}	262.6 ± 17.9^{a}	183.1 ± 17.3^{a}	28.2 ± 8.4^{b}	26.1 ± 9.5^{b}	0.002	0.22	0.69
Hexanal	ND	ND	7693.2 ± 766.2^{a}	5971.1 ± 359.6^{a}	234.3 ± 97.7^{b}	125.1 ± 96.4^{b}	–	0.003	–
Alcohols									
Ethanol	26.0 ± 15.6^{b}	31.1 ± 18.6^{b}	452.6 ± 98.2^{a}	469.9 ± 101.2^{a}	84.2 ± 53.0^{b}	7.3 ± 3.9^{b}	0.003	0.71	0.28
Methylbutanol	ND	ND	41.1 ± 25.6	21.3 ± 8.7	ND	ND	–	0.10	–
Pentanol	ND	ND	60.2 ± 6.3	42.8 ± 3.4	45.6 ± 16.6	ND	–	0.054	–
Hexanol	ND	ND	53.9 ± 21.5	38.4 ± 24.8	ND	ND	–	0.21	–
Hydrocarbons									
Pentane	236.2 ± 15.9^{b}	359.7 ± 18.4^{b}	1471.4 ± 160.9^{a}	1360.8 ± 104.8^{a}	339.2 ± 68.1^{b}	477.9 ± 27.1^{b}	0.012	0.60	0.21
Hexane	6.8 ± 0.8^{b}	11.3 ± 1.5^{ab}	10.3 ± 1.4^{ab}	7.2 ± 1.0^{b}	26.5 ± 9.7^{a}	14.2 ± 1.5^{ab}	0.011	0.027	0.030
Heptane	10.0 ± 1.8^{c}	15.4 ± 2.6^{c}	63.5 ± 7.8^{a}	50.1 ± 3.7^{ab}	18.2 ± 4.0^{c}	40.2 ± 6.8^{b}	0.008	0.071	0.077
Octane	83.7 ± 13.8^{c}	147.1 ± 17.5^{bc}	248.3 ± 6.7^{a}	190.3 ± 12.9^{ab}	120.6 ± 38.3^{bc}	257.4 ± 26.5^{a}	0.007	0.088	0.016
2-Octene	ND	ND	10.6 ± 1.0	8.0 ± 0.9	ND	ND	–	0.20	–
Terpene compounds									
α-Pinene	907.7 ± 41.6^{ab}	909.3 ± 29.2^{ab}	827.0 ± 65.9^{bc}	659.9 ± 23.4^{c}	896.7 ± 21.1^{ab}	1030.1 ± 69.7^{a}	0.004	0.14	0.013
Camphene	73.7 ± 6.6^{abc}	84.2 ± 4.2^{ab}	59.7 ± 7.0^{bc}	43.7 ± 2.6^{c}	93.2 ± 8.3^{a}	96.8 ± 11.9^{a}	0.018	0.94	0.39
β-Pinene	836.2 ± 33.9	657.9 ± 132.9	785.0 ± 58.7	638.7 ± 15.1	753.6 ± 20.8	870.2 ± 51.3	0.50	0.34	0.16
Myrcene	67.4 ± 3.1	71.4 ± 4.4	86.3 ± 6.9	80.4 ± 3.8	71.4 ± 6.0	67.6 ± 8.8	0.16	0.76	0.040
δ-3-Carene	3735.7 ± 149.8	3914.1 ± 136.7	4199.9 ± 102.1	3742.8 ± 77.8	3899.7 ± 64.8	4229.0 ± 175.5	0.19	0.68	0.038
o-Cymene	59.4 ± 3.4	61.2 ± 4.6	58.9 ± 3.0	49.7 ± 1.1	57.3 ± 2.6	67.7 ± 5.8	0.074	0.33	0.089
p-Cymene	223.8 ± 104.3	255.5 ± 17.1	250.7 ± 18.5	230.4 ± 6.6	245.3 ± 5.2	253.2 ± 8.1	0.62	0.48	0.067
Limonene	3118.0 ± 144.3^{bc}	3395.9 ± 149.6^{abc}	3652.0 ± 130.7^{ab}	2961.6 ± 5.8^{c}	3384.3 ± 72.4^{abc}	3764.0 ± 254.9^{a}	0.11	0.86	0.022
β-Caryophyllene	54.2 ± 2.2^{ab}	53.5 ± 3.2^{ab}	59.3 ± 2.6^{a}	54.9 ± 3.0^{b}	35.7 ± 9.6^{b}	50.3 ± 2.6^{ab}	0.056	0.43	0.015

Results are reported as means (average of triplicate batches and two samples per batch; n = 6) ± standard errors of the mean.
CON: Control sausage, formulated without chickpea; RCP: sausage formulated with soaked raw chickpeas; CCP: sausage formulated with cooked chickpeas; conv: conventional cooking time; prolong: prolonged cooking time; F: formulaton; t: cooking time.
ND: not detected.
^{abc}Means within the same row without a common superscript are significantly different ($P < 0.05$; Tukey post-hoc test).
[&] : TBARS expressed as mg of malondialdehyde/kg of sausage and volatile compounds as area units x 10^{6}.

changes in TBARS were observed during refrigerated aerobic storage in any of the sausages (data shown in the supplementary material for brevity; Table S2). The storage-related oxidative stability could be attributed to the protective effect of the olive oil used in the sausages against further lipid oxidation (Pintado et al., 2015; Shin et al., 2022).

The profile of volatile compounds in the headspace of sausages is shown in Table 4. The compounds detected were aldehydes, alcohols, aliphatic straight-chain hydrocarbons and terpenoids. The majority of the aldehydes, alcohols and hydrocarbons, i.e. straight-medium-chain (C5-C8) compounds, would have been formed from lipid breakdown (Resconi, Escudero, & Campo, 2013; Shahidi & Oh, 2020). Terpenoids would have originated from the black pepper (Dosoky, Satyal, Barata, da Silva, & Setzer, 2019; Musenga et al., 2007) used in the formulation.

Formulation had a significant effect on the composition of volatile compounds, with the greatest effect found when comparing the RCP sausages with the other two sausages. RCP sausages showed the highest concentration of most of the lipid-derived compounds detected, i.e. pentanal, hexanal, pentane, octane and octene. This finding, which is consistent with the higher levels of TBARS found in RCP sausages, suggests that lipid oxidation mediated by raw chickpeas during the preparation of RCP sausages was primarily responsible for the formation of these volatile compounds. In agreement, Shariati-Ievari et al. (2016) associated the use of raw chickpea flour in burgers with increased values of medium-chain alcohols, aldehydes and aliphatic hydrocarbons produced through LOX activity. Krause et al. (2022) also reported the formation of a range of lipid-derived volatile compounds attributed to oxidative enzymatic activity when raw pulses were used in reformulated baked cakes.

RCP-sausage headspace also showed the highest levels of ethanal and ethanol. They could have been formed by microbial fermentation during the mixing and filling of the batter and the initial part of the cooking (Krause et al., 2022). These two compounds have also been detected in raw chickpea seeds and flours (Karolkowski et al., 2021; Krause et al., 2022) where, as suggested by Karolkowski et al. (2021), they might originate via amino acid degradation. This pathway could also be a source of methylbutanol and methylbutanal, which, taken together, were more abundant in sausages containing chickpeas than in CON sausages.

The increased lipid-derived volatile compounds, ethanal and ethanol, and methylbutanol in the RCP sausage headspace suggests a more intense flavour in RCP sausages than in the other. Those compounds could probably impart the RCP sausages with a beany flavour. Shariati-Ievari et al. (2016) linked the use of raw chickpea flour (6%) in burgers and the subsequent increase of volatile compounds derived from lipid oxidation to a less desirable aroma, and Krause et al. (2022), in cakes, to the appearance of green-bean flavours. In contrast, Thushan-Sanjeewaa et al. (2010), using flour from raw chickpea (up to 5%) in a low-fat cooked sausage, reported sensory scores of flavour intensity and desirability of chickpea-containing sausages similar to those of the control sausages, and low scores for beany flavour. Further sensory analysis is therefore necessary to determine the possible effect, negative or otherwise, of the use of raw chickpeas (and also cooked chickpeas) on the flavour of the sausages in this study.

Regarding the effect of heating time on the content of aldehydes, alcohol and hydrocarbons in the headspace of the sausages, significant effects were only observed for the levels of hexanal and hexane, with lower values of these compounds being found in the most intensively heated sausages. These effects could be related to increased evaporation of both compounds during cooking due to the longer heating time or, in the case of hexanal, to possible chemical reactions with other components of the sausage (Pignoli, Bou, Rodríguez-Estrada, & Decker, 2009).

Formulation showed effects on the levels of terpenes, presumably from pepper, in the headspace of the sausage. Although all sausages were prepared with the same amount of pepper, lower amounts of the two volatile terpenes that eluted most rapidly in the chromatographic analysis, i.e. α-pinene and camphene, were found for RCP sausages.

This finding could be attributed to an indirect effect of the different concentration of volatile compounds in the sausages (matrix) and in their headspace (in equilibrium with the sausage matrix) between formulations. The higher amounts of aldehydes, alcohols and hydrocarbons in RCP sausages compared to CON and CCP sausages could thus have resulted in a sausage-headspace equilibrium with lower relative proportions of α-pinene and camphene in the headspace. Heating time did not affect the level of terpenes in sausage headspace; however, a significant interaction formulation × time was detected for α-pinene, myrcene, *o*-cymene, limonene and β-caryophyllene. In RCP sausages, their levels, and especially that of limonene and β-caryophyllene, tended to decrease with the longer heating time, while in CON and CCP sausages this tendency was not observed. We have no other reason to explain this observation, apart from a matrix effect, i.e. an increased retention of these volatile compounds by the RCP sausage matrix after prolonged heating.

4. Conclusions

The effects of the reformulation of a lamb meat and olive oil emulsion type sausage using 7% chickpea as a partial replacer of meat and total replacer of potato starch on the sausage quality depended on the pre-treatment of the chickpeas. The performance of raw chickpea on emulsion stability, sausage yield, and sausage hardness and chewiness was comparable to that of the meat and potato starch replaced. However, raw chickpea caused an increased lipid oxidation during the sausage making process, leading to the formation of volatile compounds with a potential effect on off-flavour development. Cooked chickpea reduced cooking yield and, possibly due to the increased moisture loss, led to an increase in sausage hardness, cohesiveness and chewiness. However, cooked chickpea had no effect on the oxidation of the sausage and scarcely affected the volatile compound profile. Both raw and cooked chickpeas reduced the elasticity and slightly changed the colour of the cooked sausages, with raw chickpeas increasing a*, and cooked chickpeas increasing b*. Further research is needed to assess the effects of the volatile compounds associated with raw chickpea use on sausage consumer acceptance or to explore approaches to reduce the oxidation. No apparent advantage was gained by increasing the heating time at 85 °C from 40 min to 80 min. On the contrary, prolonged heating may result in a lower yield of sausages with cooked chickpeas. Reformulation of sausage with cooked chickpea could be recommended. However, additional research is needed to improve the reformulation in order to reduce moisture loss during cooking and to increase the elasticity of the sausage.

CRediT authorship contribution statement

S.A. Kasaiyan: Methodology, Data curation, Investigation. **I. Caro:** Visualization, Writing – review & editing. **D.D. Ramos:** Funding acquisition, Conceptualization, Project administration. **B.K. Salvá:** Conceptualization, Methodology. **A. Carhuallanqui:** Project administration, Investigation. **M. Dehnavi:** Investigation, Methodology. **J. Mateo:** Supervision, Conceptualization, Methodology, Formal analysis, Data curation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2023.109217.

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