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Low-fat fresh sausage from rabbit meat: An alternative to traditional rabbit consumption

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ABSTRACT

The study aimed at the development of fresh sausages using rabbit exclusively as raw material. The idea was to offer an innovative product to increase rabbit consumption. Also, to meet currently consumers' requirements, a low-fat version was made. Two final formulations, a control sausage and a low-fat version using konjac gum, were developed through an iterative process and stored in a MAP under refrigeration. Sensory, microbiological and physicochemical analyses were carried out on days 1, 6, 8 and 13 after packaging. The shelf-life of the sausages was determined according to a multivariate criterion. Results showed a significant reduction in fat content and energy value. Sensory analysis showed a decrease in characteristic aroma and flavour and an increase in rancid odour, while hardness and fragility decreased in the low-fat treatment. The shelf-life was 7 days for all treatments, concluding that the multivariate method was a powerful technique as physicochemical, microbiological and sensory criteria were considered.

1. Introduction

The meat of the European rabbit (*Oryctolagus cuniculus*) has a long tradition, especially in the Mediterranean Region, where its consumption dates back to 1100 BCE (Dalle Zotte, 2014). For this reason, rabbit intensive farming for meat obtention pioneered in countries such as France, Italy and Spain, in which rabbit farming became a highly specialized livestock industry (McNitt, Lukefahr, Cheeke, & Patton, 2013). China was the world's largest producer in 2018 (465,733 t/year), followed by the Democratic People's Republic of Korea (150,112 t/year), Egypt (66,944 t/year), Spain (55,824 t/year), France (43,886 t/year) and Italy (23,741 t/year). Europe (21.3%) is the second-largest rabbit meat-producing region behind Asia (75.3%) (FAOSTAT, 2021). However, rabbit meat production is showing a decrease over time with a global decline of 34.7% in the period 2009–2019. In the EU, rabbit meat represents <3% of total meat consumed. There are several reasons for this behaviour: animal welfare, since in many places it is considered a pet, or its price, which is not as competitive as others' meats price (Kallas & Gil, 2012). Nevertheless, it is white meat, easy to cook, tasty and adaptable to all diets, suitable for consumption by children, the elderly and the convalescents, and is even industrialised as deboned meat in the manufacturing of baby foodstuffs (Cury, Martínez, Aguas, & Olivero, 2011).

Rabbit meat has recognised nutritional properties in comparison to other meats such as chicken. It is a lean meat with low-fat content: while rabbit meat fat content is 5.3%, others such as chicken meat have >9%. In addition, the unsaturated fatty acids (56%) predominate compared to saturated fatty acids (BEDCA, Base Española de Composición de Alimentos, 2021). It has also a notable amount of polyunsaturated fatty acids (PUFA) such as linolenic acid (21 mg/100 g), eicosapentaenoic acid (0.15 mg/100 g) and docosahexaenoic acid (0.31 mg/100 g) and has low cholesterol content (47 mg/100 g). These PUFA decrease LDL cholesterol levels and thus decrease cardiovascular risk (Whitney & Rolfes, 2002). They also help brain and vision development in children and brain maintenance in adults (Combes, 2004). Rabbit meat contains also high biological value protein (INTERCUN (Organización Interprofesional de la Carne de Conejo de España), 2011), providing thus all the essential amino acids, especially lysine (2.12 g/100 g) and threonine (2.01 g/100 g). Furthermore, is low in sodium (42 mg/100 g), preventing high blood pressure, and a good source of potassium (430 mg/100 g), phosphorus (228 mg/100 g) and selenium (12 μg/100 g), minerals involved in the regulation of different physiological functions (Dalle Zotte & Szendro, 2011). Rabbit meat is also one of the richest sources of vitamin B12 (8.7 mg/100 g), which prevents nervous system

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pathologies and pernicious anaemia (Stabler & Allen, 2004).

As shown, rabbit meat has outstanding nutritional characteristics. Moreover, there is growing consumer demand for healthy, nutritious and easy-to-prepare products (Brunner, van der Horst, & Siegrist, 2010). Due to the above, it could be interesting to develop novel foodstuffs that meet these requirements as well as being attractive in terms of quality and price. Rabbit meat derivates, in particular fresh sausages, could be a very good option to manage these requirements as it is an important product in Spain and it is in the first position in terms of market share (15.9%) in the group of processed meat products (Mercasa, 2021).

Currently, most of rabbit meat derivatives existing on the market also include other origin fat, such as pork fat. The consequence is the reduction of nutritional properties of rabbit meat. As mentioned, consumers are more conscious of the relationship between health and nutrition. For that reason, they demand products with healthier properties, such as reduced salt or fat content (Pateiro, Domínguez, & Lorenzo, 2017). In this sense, the use of fat substitutes is a trend and, although rabbit fat is among the healthiest, making an alternative product with reduced fat could be a good opportunity to reach as many consumers as possible, according to current market research developed by rabbit companies. Some studies have evaluated the sensory properties of low-fat sausages where fat was replaced with different polysaccharides such as gums and alginates. Most of them concluded that konjac gum is the best option to retain textural properties similar to fat-containing products (Atashkar, Hojjatoleslamy, & Sedaghat Boroujeni, 2018). This gum is used as a fat substitute in meat derivatives because it has thickening properties and forms gels (Salcedo, 2015).

Several studies have been carried out to obtain a rabbit meat derivative. Research carried out by Osburn and Keeton (1994) demonstrated that low-fat fresh pork sausage manufactured with 10 to 20% levels of added konjac flour gel, had substantially less fat and calories while maintaining similar sensory and textural attributes to higher-fat content sausages. Leines, Hernández, Hernández, and Rodríguez (2018) produced a rabbit meat *chorizo*, which was highly accepted by the consumers. Petraci and Cavani (2013) produced rabbit meat-based frankfurters using 7% of pork fat, while Cury et al. (2011) made frankfurters too but used a formulation with a higher pork fat percentage (12%). Other meat products such as ham have been produced using rabbit meat in a similar procedure to the one applied to other meats (Luna, López Fuentes, & Luna Guevara, 2015). As can be seen, these products contain fat or meat from other species, which means a decline in the nutritional quality of rabbit meat.

Product development mentioned above require knowing product's behaviour during storage and for that reason, these types of foods, are highly perishable from a microbiological point of view and could represent a risk to human health. Therefore, they must have a use-by date, according to Regulations -EC No. 1169/, 2011 and EC No. 2073/, 2005-. Furthermore, other factors that do not represent a health risk but result in a quality decrease should be taken into account when shelf-life is established. In that sense, lipid oxidation due to the high content of unsaturated fatty acids that rabbit fat has should be considered. To extend the shelf-life and delay the rancidity of food, the use of antioxidants is highly recommended. Some compounds with antioxidant capacity are polyphenols, tocopherols, sodium nitrate and ascorbic acid, among others. According to the trend for using non-chemical products, natural antioxidants such as those found in rosemary oil, oregano or thyme could be used (Petraci & Cavani, 2013). It should be noted that rosemary extract [E 392] is authorised as an additive by Regulation No 723, 2013 and has also a bactericide effect.

For the above reasons, developing a sausage-type meat derivative with good sensory characteristics would be a great option to achieve rabbit industries' requirements, based on current consumer trends. Therefore, the main objective of this research was the development and evaluation of low-fat fresh sausages made exclusively with rabbit meat, studying their sensory and physicochemical changes over storage time and establishing the shelf-life of the new products.

2. Materials and methods

2.1. Materials

A total of 53 rabbit carcasses (*O. cuniculus*) aged 2 months and weighing about 1.1 kg each were selected randomly from a slaughterhouse belonging to INCO SL (Valderrobres, Spain). They were transported to the Pilot Plant of the Faculty of Veterinary (Zaragoza, Spain) at 4 °C in polystyrene boxes with flaked ice. After quartering in these facilities, loins, legs and flanks were frozen at −20 °C in 24 batches: twenty of 1 kg and four of 3 kg. Lamb casings were used to stuff the sausages. Spices, salt (NaCl), ascorbic acid [E 300] and Konjac gum [E 425i] were all food grade. Chemicals used in physicochemical and microbiological analyses were analytical grade.

2.2. Production of rabbit sausages

The sausage manufacture was developed following the methodology proposed by Perea-Sanz, Montero, Belloch, and Flores (2019), with a replicate. Overall, 920 sausages of approximately 33 g (Fig. 1), were produced following the same technological procedure and modifying their formulations. The raw material was chopped and minced using a mincer with an 8 mm perforated plate (Gesame, Mod. M-94-32, Spain). The minced meat was introduced into a vacuum mixer (Castellvall, Mod. AVT-50, Spain) and all the ingredients were incorporated. After mixing, the dough was stuffed into lamb casings, which had previously been soaked for 12 h. A sausage filler (Mainca, Mod. EM-30, Spain) was used for this purpose. Finally, the batches corresponding to the final formulations (hereafter treatments 1 & 2 for control and low-fat respectively) were packed in 32 trays (8 for each treatment and replicate) using a packaging machine (Ulma, Mod. Smart-400, Spain). A 70% O_2/30% CO_2 MAP (modified atmosphere packaging) was used (Table 1). Previous research (Luong et al., 2020) has determined that the presence of O_2 maintains oxymyoglobin, which is responsible for the bright red colour; meanwhile, the high CO_2 concentration assured the microbiological stability of the product, being the most adequate MAP. Physicochemical, microbiological, and sensory characterization as well as a shelf-life study were carried out in these two final treatments. All batches and their replicates were produced on different days in a time interval of no >45 days to avoid changes in raw material affecting the final sensory quality of the sausages (Augustyńska-Prejsnar, Ormian, & Sokołowicz, 2018).

2.3. Experimental design development

The procedure used is known as the iterative process and is similar to that carried out by some authors in other fields (Simpson et al., 2017), where the results of one iteration are used as a starting point for the next trial until a product that meets the pre-defined requirements is achieved. According to Fig. 1 five goals were established: sausage-making process feasibility, flavour and colour improvement, texture improvement, fat stability study and final adjustment. This methodology was carried out to find the optimal formulations from a sensory point of view, based on the criteria established by the company INCO SL. For this purpose, a Penalty Analysis (Rothman & Parker, 2009) was used as a tool to identify potential directions for formulation improvement, based on the judgments of 8 expert sensory assessors (ISO 8586:, 2012) belonging to the rabbit's company and their research centre. As mentioned, the first 10 batches with their replicates were produced by modifying the formulations according to the previous Penalty Analysis results. This analysis, consisted, first of an Acceptance Test (Hough, Bratchell, & Wakeling, 1992) using an Unipolar Linear Scale (from 1 –dislike- to 10 –like), followed by a Penalty Analysis using a 5-point JAR (just about right) scale (1 not at all, 2 not enough, 3 just enough, 4 too much and 5 excessive). The samples were chosen according to a Simple Random Sampling (Yates, Moore, & Starnes, 2008), so each sausage of the subset

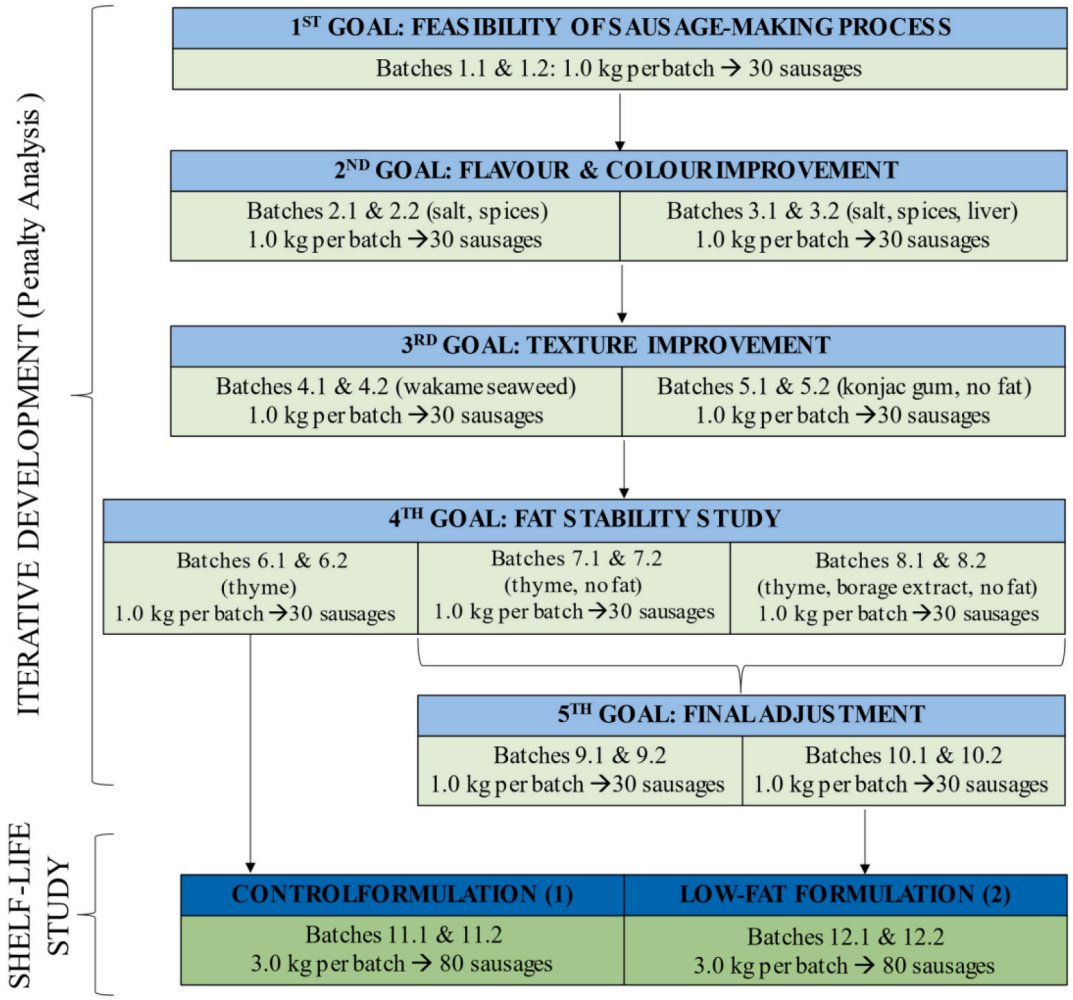

Fig. 1. Experimental design to meet the pre-established goals.
Different letters indicate very significant ** ($P < 0.01$) and highly significant differences *** ($P < 0.001$) among treatments and days for the same sensory attribute. DH: dough homogeneity, SP: spices presence, SF: salty flavour, CF: characteristic flavour, PF: persistent flavour, CA: characteristic aroma, FA: fat aroma, AHA: aromatic herbs aroma, OFF: off flavour, RO: Rancid odour.

Table 1
Final formulations and proximal composition for control (1) and low-fat (2) sausages.

Ingredient	Control (1)	Low-fat (2)
Rabbit meat	91.61	95.97
Rabbit Fat	4.82	0.00
Salt (%)	1.93	1.92
Rosemary (%)	0.48	0.48
Ascorbic acid (%)	0.48	0.48
Thyme (%)	0.39	0.38
Pepper (%)	0.29	0.29
Konjac gum (%)	0.00	0.48
Proximal Composition	Control (1)	Low-fat (2)
Moisture (%)	67.90	66.77
Protein (%)	21.40	19.75
Fat* (%)	5.20[b]	3.70[a]
Energy value* (kcal/100 g)	127.90[b]	112.30[a]

Different letters indicate statistically significant differences * ($P < 0.05$) for the same parameter among treatments.

had an equal probability of being chosen. For this purpose, the 33 sausages of the batch were numbered, and the statistical software XLSTAT, 2016 (Addinsoft©, Paris, France) chose 5 of them. The two ends of each sausage were removed, and the sausage was then divided into two halves to obtain 10 pieces, one for each assessor.

The attributes selected based on the company criteria and in correspondence with the Standard ISO 5492, 2008 were in raw: colour homogeneity, exudation, rancid odour, and typical odour; after cooking: dough homogeneity, rancid odour, typical odour, elasticity, firmness, juiciness, succulence, fibrousness, crumbliness, chewiness, rancidity and typical flavour. A pairwise comparison was performed using Fisher's test with a threshold of 20%. A Spearman correlation was also developed, due to the ordinal nature of the data.

2.4. Proximal composition and quality parameters of final sausages

Moisture determination was carried out using an oven (Selecta, Mod. Ref. 2005167, Spain) at a temperature of 105 °C determining weight losses according to the method AOAC 950.46 (1990). The total protein content of the sausages was determined on a distillation unit (Velp, Mod. UDK 129, Italy) by the Kjeldahl method according to methodology AOAC 2.062 (1984). The final results were expressed as a protein percentage from nitrogen content using the correction factor 6.25. Fat determination was carried out according to the method AOAC 24.005 (1980) in a semi-automatic Soxhlet extractor (Selecta, Mod. DET-GRAS N, Spain). The salt content was established following Mohr's method (AOAC 937.09, 1995). Finally, the energy provided by the sausages

expressed as total kcal per 100 g was calculated using the conversion factor of 4 kcal/g for protein and 9 kcal/g for fat (Merril & Watt, 1955).

The pH was determined with a digital puncture pHmeter (XS Instruments, Mod. PH25, Italy) by inserting the electrode directly into the samples on days 1, 6, 8 & 13 after sausage production. Previously, the equipment was calibrated according to the manufacturer's instructions with buffers of pH 4.01 and 7.00 (XS Instruments). The extent of lipid oxidation was evaluated by the 2-Thiobarbituric Acid Reactive Substances (TBARS) assay. This analysis was determined following Pfalzgraf, Frigg, and Steinhart (1995) methodology on days 1, 6, 8 & 13 after sausage production. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of fresh sausage.

2.5. Microbial characterization of final sausages

Total viable counts at 37 °C (TVC) were carried out according to ISO 4833-1:, 2013. The same standard was followed for psychrotrophic counts (PSY), modifying the incubation temperature to 10 °C. For the enumeration of the *Enterobacteriaceae* family bacteria (ET), the standard ISO 21528-2:, 2017 was followed. These counts were also carried out on the raw material after receipt.

At each of the sampling times (days 1, 6, 8 & 13), 10 g of each fresh sausage were taken and placed in a sterile plastic bag with 90 ml of peptone water (0.1%). After 2 min in a stomacher blender (IUL Instruments, Mod. 1986/470, Spain), appropriate decimal dilutions were poured plated (1 ml) on the following media: Plate Count Agar (PCA) for the total viable count (TVC) and psychrotrophic counts (37 °C for 48 h and 10 °C for 5 days respectively) and Violet Red Bile Glucose Agar (VRBG) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (log cfu/g). In addition, *Salmonella* (ISO 6579-1:, 2017) and *Listeria monocytogenes* (ISO 11290-1:, 2017) investigations were carried out on day 1.

2.6. Sensory analyses of final sausages

A sensory panel from the Meat Science Laboratory of the Faculty of Veterinary at the University of Zaragoza (Spain) constituted by 10 trained sensory assessors according to ISO 8586:, 2012 was employed for the two sensory analyses developed. The assessors had previously demonstrated sensory sensitivity in preliminary tests, received considerable training and they were able to make consistent and repeatable assessments of various commercial sausages samples. This also allowed the assessors to acquaint themselves with the attribute terms and the scoring system. The sessions took place in the test room of the Pilot Plant, which complies with ISO 8589:, 2007. Samples presentation was designed taking into account a complete block design, so each assessor evaluated all the samples to achieve a correct balance. On day 1 after production, two trays were randomly selected. A total of 20 sausages (10 for each treatment) were cooked on an electric grill (Sammic, Mod. GRS-5) until reaching an internal temperature of 72 °C. After discarding the ends, the sausage was divided into 2 pieces (16 g). Each portion was blind-coded with random 3-digit numbers and served to the assessors at a temperature of 60 °C approximately. This procedure was repeated for the second round to assess possible variability between replicates. The panel performance was checked following the guidelines established in section 8 titled "Analysis of Results" of the ISO standard 8556:2012. This procedure was repeated on sampling days 6, 8 & 13 for both sensory analyses carried out.

2.6.1. Quantitative descriptive analysis (QDA)

A QDA (ISO 13299:, 2016) using structured scales anchored at the extremes (1, the attribute is not present – 10, the attribute is very present) for each of the specific sensory attributes highlighted in the penalty analyses was performed, as follows, visual attributes: dough homogeneity, spices presence in the dough; olfactory aspects: sausage characteristic aroma, aromatic herbs aroma, fat aroma, rancid odour; taste attributes: characteristic flavour (rabbit meat), salty flavour, off flavour, and persistent flavour. From the QDA data, a "Product Characterization" analysis was made. Sensory profiles of the treatments were determined by taking into account the means in each product tested as well as the cosine square algorithms to determine those descriptors with the greatest discrimination power in the different treatments and times studied.

2.6.2. Sensory texture profile test of final sausages

A Sensory Texture Profile (Szczesniak, 2002), according to ISO 11036:, 2020, was carried out. A structured scale (0, the attribute is not present – 5, the attribute is very present) was used with different pre-established attributes for the kind of evaluated product: elasticity, juiciness, firmness, fibrousness, gumminess, chewiness, crumbliness, hardness and succulence. The findings were represented by differential semantic graphs for each type of developed sausage.

2.7. Shelf-life study of final sausages

A shelf-life study was carried out taking into account the recommendations established by the standard ISO 16779:, 2015. The methodology "Estimation of failure criteria in multivariate sensory shelf-life" (Giménez, Gagliardi, & Ares, 2017) was developed with some modifications. In this research, sensory attributes were considered, but also other variables such as microbial counts and physicochemical parameters were studied, according to the cited ISO Standard. Sampling points were established on days 1, 6, 8 & 13 to get a test period between 50% and 100% longer than the estimated shelf-life for similar products found at retail. A database was prepared with the results obtained in the sensory characterization, physicochemical analyses, and microbial counts. A Principal Component Analysis (PCA) was carried out on this database to estimate the shelf-life. The factor scores obtained for the first component (F1) on each of the sampling days were used to construct a scatter plot. The cut-off point with the independent variable axis after representing the regression line to the distribution of the different sampling points was considered.

2.8. Statistical study of analytical parameters

All parameters (proximal analysis, microbiological counts and sensory characterization) obtained were synthesized using descriptive statistics. Normal distribution plots were plotted to check the normality of the data and detect outliers. A linear mixed model was carried out to determine differences between treatments 1 & 2. This model included treatments (C & K) and sampling days (1, 6, 8 & 13) as fixed variables and the replicates as a random effect. The interaction between them was also studied. Approximate F-ratio tests for each fixed effect were conducted and the critical value for a statistically important effect was taken at $P < 0.05$. A pairwise comparison between means was carried out using Fisher's multiple comparisons test (LSD). All statistical modelling and presentations were constructed with XLSTAT, 2016 (Addinsoft©, Paris, France). It should be noted that all measurements were conducted in triplicate.

3. Results and discussion

3.1. Iterative development process

Fig. 1 shows the procedure that was followed to achieve the final formulations. This process began with a basic formulation (batches 1.1 & 1.2) that included ascorbic acid and rosemary, as this combination, in a 1:1 ratio, presents the greatest antioxidant effect (Perlo et al., 2018). Once the feasibility of the product had been verified, it was decided to carry out two experiments based on that formulation. The first (batches 2.1 & 2.2) included salt, carob gum (E 410) and pepper. The second (batches 3.1 & 3.2) also included rabbit liver. This was discarded due to

the characteristic flavour provided by the liver, while in the first one the texture provided by the carob gum was not sensory well appreciated, although the results in some previous research were satisfactory (Lurueña-Martínez, Vivar-Quintana, & Revilla, 2004). For this reason, starting from batch 2, a third batch (3.1 & 3.2) was developed and carob gum was replaced by wakame seaweed, in an attempt to improve the texture of the product (Cofrades, López-López, Solas, Bravo, & Jiménez-Colmenero, 2008). However, the colour of the product obtained after cooking was not well sensory assessed. Another formulation (batches 5.1 & 5.2) was also produced. In this case, carob gum and fat were replaced by konjac gum, as some studies claim that it is possible to use that as a fat substitute (Jiménez-Colmenero et al., 2012). The gum produced a palate coating sensation, possibly due to a gum excess.

Finally, from batch 4, the sixth batch (6.1 & 6.2) or control treatment (1), was made, including thyme and modifying the spices concentration according to the results of the sensory analysis. On the other hand, from batch 5, batches 7.1 & 7.2 (including thyme) and 8.1 & 8.2 were made. In the latter, borage seed extract was also included according to previous studies in meat products (Bellés, Alonso, Roncalés, & Beltran, 2017) due to its antioxidant activity, although given that no significant differences ($P > 0.05$) were observed between them, it was decided to discard the use of this extract, thus producing batch 9.1 & 9.2, in which the percentage of konjac gum was reduced to 1%. After the sensory analysis, it was considered that this concentration was still high, so it was reduced to 0.5% to obtain the final low-fat formulation (batches 10.1 & 10.2).

3.2. Proximal composition of fresh rabbit sausage

Table 1 shows the proximal composition of the new sausages developed. Concerning fat content, treatment 2 had 1.00% less fat than control, which contained 4,70% fat. This resulted in an energy value reduction of 13.00%, being both statically significant ($P < 0.05$). In all cases, the interaction between fixed effects was tested, and it was not significant, so it was excluded from the model. The replication was not significant ($P > 0.05$) for any of the traits. The proportion of intramuscular fat in rabbit meat increases with age, especially between 11 and 18 weeks (Ramírez, 2004). For this reason, it was not possible to remove more fat during production. However, in contrast to similar products containing fat from other sources, these are dominated by unsaturated fat, which is healthier. Moisture content was higher than those obtained by some authors (Ortega, López-Sobaler, Requejo, & Andrés, 2004) in chicken sausages (55.41%). However, rabbit sausages contained approximately 24.00% less fat than chicken ones (28.10%) and protein content was up to 20.57% in rabbit sausages while in chicken ones it remained at 13.12%. Finally, the salt content was similar in both chicken and rabbit sausages (2,60 - 2,70%). This makes rabbit sausages a healthier product than the variety of sausages currently available on the market.

3.3. Physicochemical quality parameters of fresh rabbit sausage

Regarding pH values obtained in this experiment, the treatments studied showed an increasing trend: from 5.42 ± SE 0.03 and 5.45 ± SE 0.03 for treatments 1 & 2 respectively to 5.70 ± SE 0.02 at the end of the experiment, however, no significant differences ($p > 0.05$) were found. Some authors (Saricaoglu & Turhan, 2019) suggest that typical aerobic bacteria present on the surface of the meat at the early stage of storage, producing and releasing protein metabolites, mainly basic amines (ammonia, primary amines, secondary amines, and tertiary amines) are able to modify the ph in the product. Findings showed that interaction among fixed effects was significant ($P > 0.05$). However, differences among replicates were not observed.

Concerning lipid oxidation (LO), the TBARS index remained relatively constant, not exceeding the value of 0.44 ± SE 0.07 and 0.52 ± SE 0.07 mg MDA/kg for treatments 1 & 2 respectively on day 13. No significant differences were found between treatments ($p > 0.05$) although the interaction between fixed effects was significant. The TBARS values achieved in the sausages would not elicit a sensory response according to a study carried out on chicken meat where a TBARS perception threshold value was set at 0.8 mg MDA/kg for detecting off-flavours and odours (Gallinger, 2015). Regarding the above, Papadima, Arvanitoyannis, Bloukas, and Fournitzis (1999) established a value of 1 mg MDA/kg as a limit for detecting typical rancidity aromas in pork sausages. Our values were also low compared to those of other authors (Moawad, Saleh, Mohamed, & Abdelmaguid, 2020), where values of 1.2 mg MDA/kg were reached in chicken sausages. In contrast, these exceeded the amount of 1.3 mg MDA/kg detected in chicken sausages developed by Deng-Cheng, Ruei Tsz, Yen-Chih, Shyh-Shyan, and Fa-Jui (2009). This finding could be related to the use of a MAP and the incorporation of rosemary and ascorbic acid to extend the shelf-life of rabbit sausages. It should be noted that, as in previous cases, no significant differences ($P > 0.05$) were detected among replicates.

Although significant differences ($P < 0.05$) in these parameters between sampling points were established, these changes were not sufficient to use them as a criterion to determine the use-by date of the product. Due to the above, the need arises for using a broader, more inclusive criterion that takes into account as many variables as possible of different nature (sensory evaluation, microbial counts and physicochemical parameters) to achieve a better power multivariate criterion to establish properly use-by date following the standard "Sensory analysis -Assessment (determination and verification) of the shelf-life of foodstuffs" (ISO 16779:, 2015).

3.4. Sensory profiles of final sausages

3.4.1. Quantitative descriptive analysis -QDA-

The QDA results for final formulations (1 & 2) are shown in Fig. 2. Firstly, significant differences ($P > 0.05$) were not observed among replicates and the interaction between fixed effects was not significant. Intermediate days were not shown to visualize better the changes that occurred. The sensory profiles remained similar although significant differences ($P < 0.05$) were established for some attributes. In the case of characteristic aroma, characteristic flavour and aromatic herbs aroma,

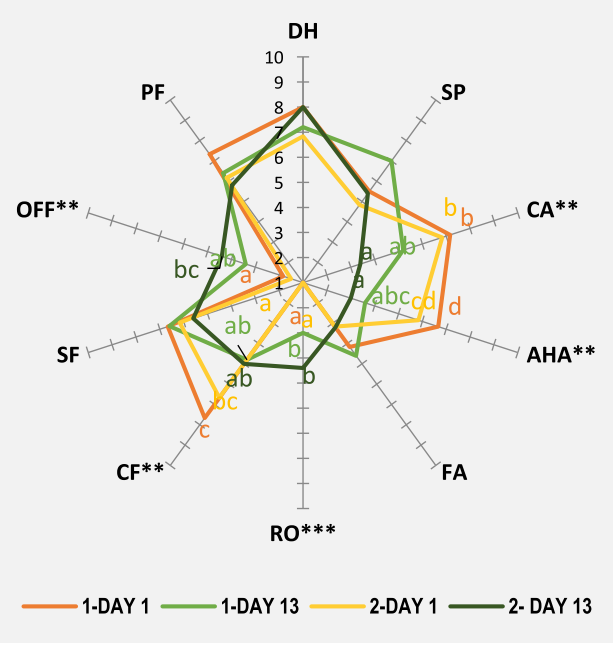

Fig. 2. Specific sensory profiles (spider diagram) for rabbit sausages developed (control −1- and low-fat −2-) packaged in a MAP on days 1 and 13.
*Different letters indicate significant * ($P < 0.05$) and very significant ** ($P < 0.01$) differences for the same descriptor among treatments.

significant differences were found between days, with a decreasing trend. However, these differences were not established between the different treatments, so an effect of the formulation on these attributes could be discarded. The opposite behaviour was perceived for rancid odour and off flavour, whose values increased, establishing two clear clusters in all treatments corresponding to the first and last sampling day. The increase in the value of these attributes could be related to the decrease in the aforementioned attributes, as compounds and aromas generated distort the correct perception of these attributes.

Although significant differences have been established between the days, it is necessary to know the influence of these attributes on the sensory quality. The squared cosines established the discrimination power for each sensory attribute considered. The characteristic flavour was found to have high discriminatory power for treatments 1 and 2 on day 13. In addition, the former also had the fatty aroma and salty flavour as discriminatory attributes. For treatment 2 on day 1, the discriminating attributes were the fat aroma and, again, the characteristic flavour.

3.4.2. Sensory texture profile test

Semantical graphs describing the specific texture profiles in each treatment are shown in Fig. 3. Significant differences ($P < 0.05$) were found among treatments 1 & 2 for hardness and fragility. Sausages containing konjac gum showed lower hardness and fragility than controls. These facts could be related to protein degradation, water liberation and its absorption by konjac gum. It should be noted that water strongly bound to proteins is one of the main characteristics of this type of meat (Ariño, Hernández, & Blasco, 2006). Similar results were found in the study of Atashkar et al. (2018). However, no differences were found between days 1 & 13 for the same treatment or between replicates. The interaction between fixed effects was not significant.

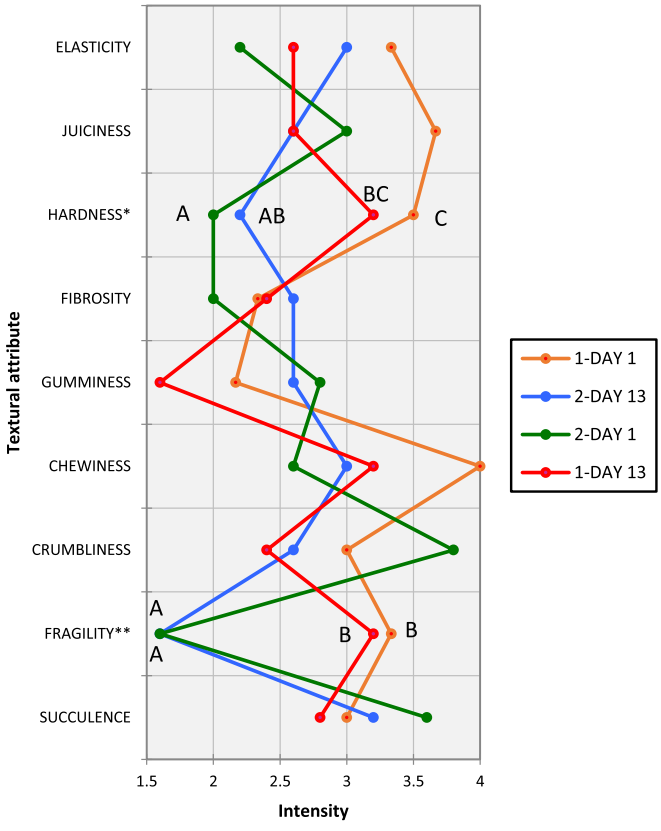

Fig. 3. Differential semantic graph of control (1) and low-fat (2) treatments packaged in a MAP on days 1 & 13.
CF: characteristic flavour, CA: characteristic aroma, AHA: aromatic herbs aroma, OFF: off flavour, RO: Rancid odour, LO: lipid oxidation, ET: *Enterobacteriaceae* counts, PSY: psychrotrophic counts, TVC: total viable counts.

3.5. Microbial characterization

Replicates and interactions between fixed effects were not statistically significant for any of the microorganisms studied. TVC counts started at 2.80 ± SE 0.13 and 3.0 ± SE 0.13 log CFU/g and reached 6.18 ± SE 0.09 and 6.26 ± SE 0.09 on day 13 for treatments 1 & 2 respectively. No significant differences were found between samples on any day ($p > 0.05$). This was an acceptable microbial load compared to that obtained by Hernández and Gondret (2006), which ranged between 4 and 5 log CFU/g for rabbit carcasses. The counts obtained were also low compared with Lengkey and Lobo (2016). In that study, sausages were made from rabbit meat with the addition of fat from other origins and TVC counts were higher than 5 log CFU/g. In addition, these counts were also found to be lower than those obtained by Ruiz-Capillas and Jiménez-Colmenero (2010) in air-packaged pork sausages, where 5.27 log CFU/g were reached. Do Amaral, Cardelle-Cobas, and do Nascimento, B. M. S., Monteiro, M. J., Madruga, M. S., and Pintado, M. M. E. (2015) developed a low-fat fresh pork sausage based on chitosan and the TVC doubled those obtained in this study. Álvarez-Astorga, Capita, Alonso-Calleja, Moreno, and García-Fernández (2002) determined the microbial quality of chicken sausages offered for sale, obtaining TVC counts of 7.28 log CFU/g, 4 units higher than those obtained in rabbit sausages. The 4.3 log CFU/g obtained by Deng-Cheng et al. (2009) were higher than those for rabbit sausage. However, more similar behaviour was found by Moawad et al. (2020) in chicken sausages where 4.15 log CFU/g were found on day 0 and 7.14 log CFU/g were reached on day 12.

Enterobacteriaceae counts on day 0 started at 2.00 ± SE 0.13 log CFU/g for both treatments and reached 4.12 ± SE 0.09 and 4.54 ± 0.09 SE on day 13 for treatments 1 & 2 respectively. Statistically significant differences ($p < 0.05$) between treatments could be observed from day 6 onwards, with the highest counts in treatment 2. Replicates and interactions between fixed effects were not statistically significant. These results contrast with those from other authors. In this regard, pork sausages packaged in air did not exceed 3.53 log CFU/g (Ruiz-Capillas & Jiménez-Colmenero, 2010). However, the behaviour of ET in rabbit sausages somewhat resembled that of pork sausages by Do Amaral et al. (2015). The sausages developed by Moawad et al. (2020) had also lower counts than those obtained in rabbit sausage, varying from 2.74 log CFU/g on day 1 to 3.86 log CFU/g on day 12. High ET counts in rabbit sausages could be attributed to the microbial quality of the raw material and to a loss of the cold chain during transport.

Psychrotrophic counts at baseline were similar to those of TVC (3.8 CFU/g). However, growth over time was higher, reaching 7.91 ± SE 0.18 and 9.25 ± SE 0.18 on day 13 for treatments 1 & 2 respectively. Significant differences ($p < 0.05$) among treatments were only observed on this day. Replicates were not significantly different. However, a significant interaction between fixed effects could be observed. The initial counts and their evolution over time were similar to those obtained by Do Amaral et al. (2015) where a chitosan-supplemented pork sausage was developed. The results were also similar to those obtained by Álvarez-Astorga et al. (2002) in chicken sausage, where psychrotrophic counts reached 7.87 log CFU/g. However, in the research carried out by Bostan and Isin Mahan (2011) on beef (90%) and chicken (10%) sausages, PSY counts did not exceed 9 log CFU/g at day 60, which allows observing the effect of the heat treatment applied in this case. Moawad et al. (2020) determined the microbial quality of chicken sausages, whose behaviour was similar to rabbit sausages. However, the final counts are slightly lower in the chicken ones (6.89 log CFU/g). These results contrast with those obtained by Ali, Abdel-Atty, and Helmy (2018) where counts of 6.6 log CFU/g were reached on the sixth day. Finally, investigations carried out for L. *monocytogenes* and *Salmonella* were negative, both at the beginning and at the end of the experiment thus complying with Regulation EC No 2073/, 2005.

Taking as a reference the criteria established by MSSSI (2016) for TVC, all sausages were acceptable for consumption on day 8, as they did not approach 6 log CFU/g. From this point of view, a use-by date of 8 days could be established. However, as a consequence of meat packaging and storage conditions (i.e., storage at low temperature and modified atmosphere or vacuum packaging), mainly psychrotrophic and facultative anaerobic species exhibit fitness to grow in such environments. This may lead to the development of bacteria such as *Enterobacteriaceae* (Zagorec & Champomier-Vergès, 2017). For that reason, ET counts are so high even on day 6. This could have negative consequences in terms of sensory perception, as this group of bacteria is mostly responsible for important spoilage reactions that could lead to consumer rejection.

3.6. Shelf-life study

Fig. 4 shows the biplots for each of the treatments tested. The result was the transformation of the original dependent variables into new dimensions simplifying the data structure and helping data interpretation (Johnson & Wichern, 2007). This shows interrelationships among multiple dependent variables (the descriptors) and objects (the products) (Anderson, 2003; Tabachnik & Fidell, 2006). The first component (F1) of the PCA explained the great majority of the variance of the experimental data (85.41% & 76.74% for treatments 1 & 2 respectively) including physicochemical, microbiological and sensory measurements.

The changes in sensory attributes were highlighted because they showed a good fit with the spoilage development that increased with

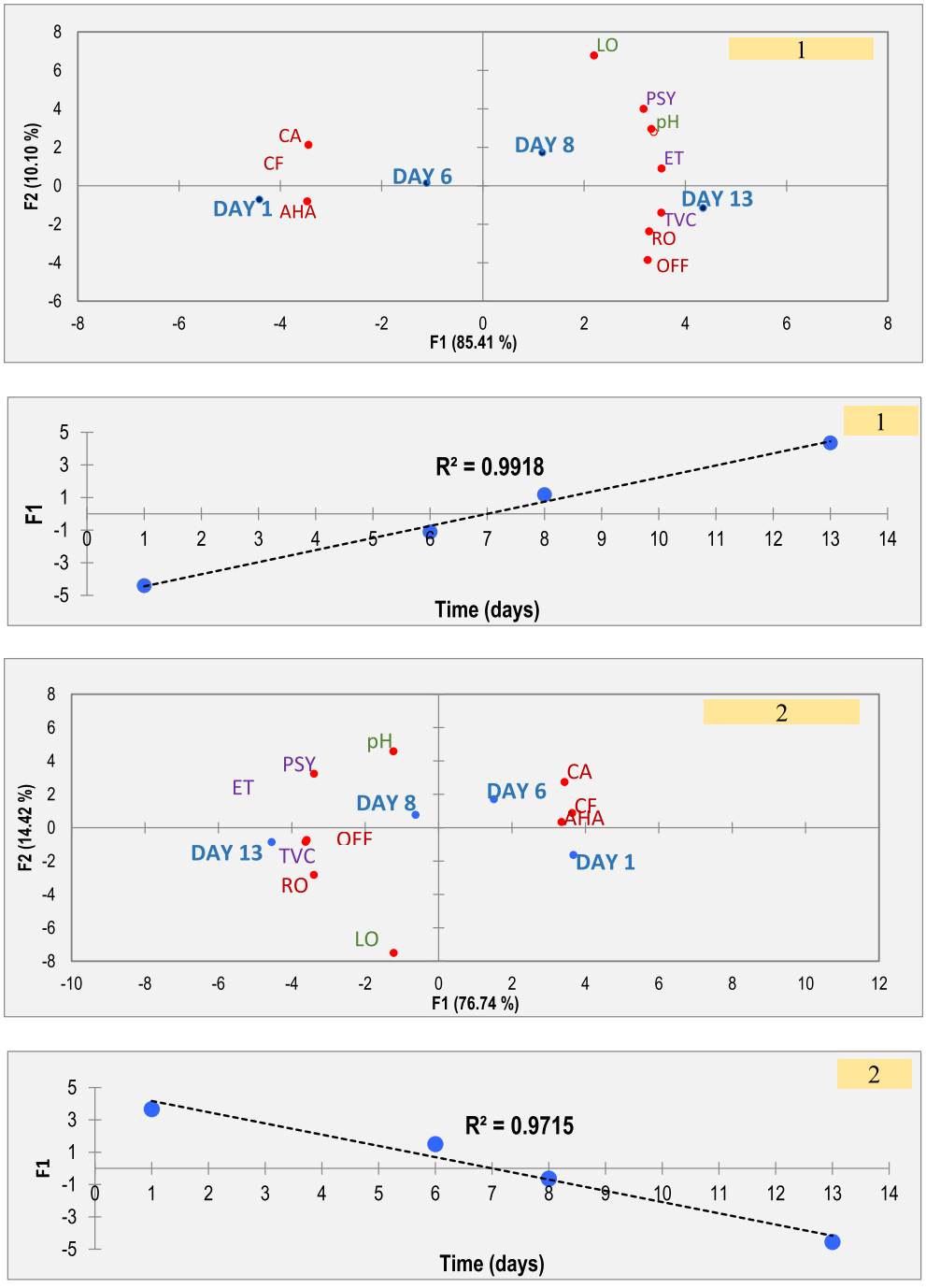

Fig. 4. Principal component analysis (PCA) and their respective representations of the coordinates in the first component for control (1) and low-fat (2) treatments packaged in a MAP.

time so negative correlations with those characteristics that identify a fresh product were found. In particular, treatment 1-A was characterised by the aromatic herb aroma and by the typical meat product flavour. On day 6, the sample started to move away from these descriptors. By day 8, the sample was no longer characterised by these attributes and began to be influenced by lipid oxidation, PSY, and ET. On day 13, other variables such as TVC, rancid odour and off flavour were incorporated.

Treatment 2-A started in a neutral position. On day 6 the sample was associated with the characteristic aroma and flavour as well as the aroma of the aromatic herbs. Again, on day 8 most of the variables responsible for product degradation appeared, especially bacterial growth (PSY & ET). For the last sampling day, lipid oxidation caused the presence of the rancidity odour attribute. TVC, as well as off flavour, were also predominant.

The microbial growth showed typical behaviour of a perishable product and similar to that observed in other similar products, such as chicken sausages (Barbosa et al., 2014). About sensory characterization, two facts occurred (Giménez et al., 2017): an increase in the intensity of sensory defects (off flavour or the rancid odour would be an example of that) and a decrease in the intensity of desirable properties (for instance, characteristic flavour and aromatic herbs aroma).

Shelf-life was 7 days according to the cut-off point criterion (Fig. 4). It was concluded that the addition of konjac gum seemed not to influence the shelf-life of the rabbit sausages. It should be noted that treatment 1 was the only regression line that presented a positive slope as its factor scores increased over time. As seen before, while in treatment 1 the variables that made a greater contribution were those related to microbiology (whose values increased over time), in treatment 2 the variables with a greater contribution were those that decreased over time (i.e. most of the sensory variables). This is the main advantage of the multivariate analysis: all criteria are taken into account at the same time with a global perspective that is closer to reality, and the shelf-life (use-by date) is not only based on microbial criteria as sausages could be safe to eat but maybe they were rejected by consumers due to a loss of sensory quality.

4. Conclusion

The findings of this study corroborated that, after applying an iterative development process to get the best sensory evaluated product, it was possible to replace some of the fat in the product with konjac gum, maintaining proper sensory characteristics and achieving a healthier product with the capacity to increase rabbit meat consumption. Concerning the sensory analysis, over time there was a decrease in the attributes associated with an organoleptically optimal product (characteristic aroma, characteristic flavour and aromatic herbs aroma) and an increase in undesirable attributes associated with poor quality that could produce sensory rejection (off flavour and rancid odour). Konjac gum produced changes mainly in texture, in particular in hardness and fragility. The multivariate method used proved to be a useful tool by considering microbial, sensory and physicochemical parameters at the same time to determine the use-by date, which was 7 days for fresh sausages developed in this study. However, given the minimal variations in the case of pH and the low lipid oxidation values obtained (TBARS), these had no relevant influence on food shelf-life. Future studies could focus on comparing these developments with those made from other meats maintaining the proportions of the ingredients. This would provide insight into consumer preferences for these products.

Author contributions

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All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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