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Effects of the Addition of Pecan Nuts on the Nutritional Properties and Final Quality of Merino Lamb Burgers

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Featured Application: This research contributes to the development of a technological strategy to offer the consumer lamb burgers with pecan nuts as an enhanced product. Extra nutritional value, a healthy fatty acids profile and the use of antioxidants as preservatives can be achieved by incorporating pecan nuts in burgers' formulation.

Abstract: This paper attempts to analyse lamb burgers from meat cuts of lower commercial value to which various amounts of freeze-dried pecan nuts (5%, 10% and 15%) were added to study the influence of the addition of pecans on the quality of the burger. One hundred eight burgers were evaluated by means of physicochemical, sensory and microbiological analyses. The addition of pecan nuts mainly affected the meat's fatty acid profile. Fat content was higher as the amount of pecan nuts was increased, and the monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) levels also increased ($p < 0.001$), whereas the saturated fatty acid content decreased ($p < 0.001$). Pecan nuts also proved able to increase the antioxidant capacity of the product, significantly reducing the oxidation values of lipids ($p < 0.001$) and proteins ($p < 0.05$). In general, no significant differences were identified in the sensory attributes under study. In conclusion, we found that the use of pecan nuts improves the nutritional content of the hamburgers without negatively affecting the technological or sensory properties.

Keywords: healthy; unsaturated fatty acids; antioxidant; nutritive; sensorial analysis

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1. Introduction

Meat and meat products are an important source of protein of high biological value, B vitamins and minerals. However, the consumption of these products currently has adverse connotations. This is mainly due to the associations between their intake (or the intake of some of their components, such as fat, cholesterol, etc.) and the risk they pose to humans in causing some of the main degenerative and chronic diseases (cardiovascular disease, cancer and obesity) [1,2]. For this reason, over the last few years, the meat sector has been taking measures to respond to consumer demand for improvement in these health-related aspects by developing functional meat products [3] with health properties that are more attractive to the consumer [4,5]. Some of the most common practices are a reduction in salts [6], the addition of vegetable fibres [7] and cardio-healthy fats [6,8] or the use of natural antioxidants deriving from plants with bioactive substances [9–11]. Lamb is among the red meats that are considered a risk to consumer health [12]. This has been established as the reason behind the decrease in lamb meat consumption, leading to the need to devise strategies to increase its consumption [8]. The consumption of lamb meat is usually limited to first- or second-category meat cuts, such as the leg or shoulder. Cuts from other categories with lower market value, such as the flank or neck, are usually less accepted in the market. An alternative to increasing the consumption of these meat cuts of lower commercial value could be their use in the processing of meat products such as burgers. Burgers are hugely popular as meat products and they could help diversify the market for this meat product. Furthermore, meat is one of the most perishable foods on the market due to its composition, pH and water activity (aw). In fact, products made from minced meat tend to deteriorate more quickly than meat cuts or fillets [13] due to the high level of handling to which they are subjected and the large area that comes into contact with oxygen, causing rapid lipid oxidation and deterioration by enzymatic and chemical activities [14]. Vacuum packaging or modified atmosphere packaging (MAP) are effective solutions to reduce this damage and are also usually combined with the addition of different ingredients to improve quality and extend shelf life [15]. These ingredients help produce a healthier and more stable final product.

Recently, studies have been carried out on the effects the addition of nuts, such as pecan nuts, can have on meat derivatives. Pecan nuts have a high and beneficial nutritional value provided mainly by their content in bioactive substances [16], such as vitamins, minerals, phytochemicals, fibres and lipids [17,18]. As for their lipid content (50–70%), monounsaturated and polyunsaturated acids are especially important [19].

The objective of our study was to determine the nutritive, physicochemical, sensorial and microbiological parameters of lamb burgers enhanced with different amounts of pecan nuts in their formulation in order to improve consumer and market acceptance of this type of meat, as well as to determine the potential nutritional improvements obtained with this new formula.

2. Materials and Methods

2.1. Freeze-Dried Pecan Nuts as a Natural Ingredient

Pecan nuts (of the "Osage" variety) were harvested at their optimum maturity stage from the CICYTEX Experimental Plant and stored in darkness at 4 °C in open containers until use. A Telstar LyoQuest series freeze dryer was used to achieve complete freeze drying of 900 g peeled and crushed pecan nut portions in 2 days. Vacuum packaging in metal bags at 5 °C ensured no deterioration of bioactive compounds took place until the product was blended into the designated batches of minced meat dough, as defined in the study. Prior to vacuum packaging, a portion of freeze-dried pecan kernels were characterized, showing their potential as natural ingredients for the study (Table S1).

2.2. Lamb Burger Processing

Boneless flanks and legs from "Merino" lambs with the Protected Geographical Indication Cordero de Extremadura CORDEREX trademark were purchased to manufacture a total of 108 lamb burgers. The lambs were slaughtered in a commercial slaughterhouse, and the carcasses were kept refrigerated for 24 h at 2–4 °C. The lamb carcasses were then quartered in a cutting room, and boneless flanks and legs were then trans-ported in a refrigerated vehicle to the CICYTEX meat laboratory. Finally, the lamb meat cuts were individually vacuum-packed and frozen at −20 °C until they could be used in lamb burger manufacturing.

The burgers were manufactured in CICYTEX's pilot plant. Prior to producing the lamb burgers, the lamb meat cuts were thawed for 2 days in a refrigeration chamber at 4 °C and minced using an industrial meat mincer with an 8 mm disc (Mainca mod PC-82, Barcelona, Spain). Meat batters were prepared in an industrial vacuum meat mixer (Tologoni mod CATO equipment), and the process consisted of two stages in chilled conditions (4–6 °C). During the first stage, raw meat in a 40:60 (flank:leg) ratio was mixed with salt and white pepper (1.5% and 0.2%, respectively) once in each direction for 4 min. After mixing, the meat dough was subdivided into four equal batches: the "Control" batch (no natural ingredient added); the "Pecan5" batch, which was enriched with 5% freeze-dried pecan nuts (*w*/*w*); the "Pecan10" batch, which was enriched with 10% freeze-dried pecan nuts (*w*/*w*); and the "Pecan15" batch, which was enriched with 15% freeze-dried pecan nuts (*w*/*w*). During the second stage, each batch with its natural ingredient was mixed once in each direction for 4 min.

The burgers, each weighing 95–100 g with a diameter of 10 cm, were formed using a conventional burger maker (Mainca MH/MA, mod MH-100 Spain) and subsequently packaged in an ULMA SMART 300 packaging machine in 150 × 200 × 30 mm (L × W × D) poly-propylene thermosealable trays (PP/PE 1520/30) with 70 µm thick Cryovac Darfresh VSTM100X film for food packaging (7 cm^3/m^2/24 h/bar, 23 °C, RH oxygen permeability), obtaining 112 vacuum skin-packed burgers that were ready to undergo physicochemical, sensory, texture and microbiological analyses (32, 48, 12 and 20, respectively).

2.3. Methods

2.3.1. Nutritional Composition

The proximate composition, including protein content, fat content, moisture and ash content of the burgers, was evaluated according to international procedures [20]. The calorific value was determined using a calorimeter (model 6100 calorimeter Instrument Company; Parr, IL, USA), and the values are expressed as Kcal/g. Total phenol content was determined according to the method of Singleton and Rossi [21] with some modifications and quantified using a gallic acid standard curve in a plate-reading spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Vantaa, Finland). Results are expressed as g of gallic acid (GAE)/kg.

2.3.2. pH and Water-Holding Capacity

The burgers' pH was determined using a digital pH meter (Crison Instruments, S.A., Barcelona, Spain) equipped with a penetration glass probe in accordance with the AOAC method [22]. According to Foegeding and Ramsey's method [23], water-holding capacity (WHC) was quantified as the water released after the exertion of a centrifugal force (3000 rpm during 3 min). The results are expressed as g of water released/100 g meat.

2.3.3. Instrumental Colour

The colour was evaluated using a Minolta colorimeter CR400 (Osaka, Japan) (D65 illuminant and a 10° standard observer). Results are expressed in terms of lightness (L*), redness (a*) and yellowness (b*). The hue angle and chroma values were measured as $\tan^{-1}$ (b*/a*) and (a^{*2} + b^{*2}) 0.5, respectively. The colour was recorded at five random points on each burger, and a result was calculated as the average of the readings.

2.3.4. Cooking Characteristics

The burgers were cooked in an electric double-up to a core temperature of 72 °C. The cooking yield (CY) was calculated according to the equation CY% = 100 × (cooked burger weight/raw burger weight); cooking loss (CL) was estimated using the equation CL% = 100 − CY; diameter reduction (DR) was determined with a digital calliper (MITOTUYO absolute digimatic) by randomly measuring four diameters in the same burger. The measurements of each hamburger were averaged and based on this value. The DR was calculated according to the equation DR% = 100 × (raw diameter − cooked diameter)/raw diameter.

2.3.5. Textural Characteristics of Cooked Burgers

The cooked burgers were tempered to 40 °C in a P-Selecta Incudigit heater before they could be analysed using a TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). The texture characteristics of the burgers were determined using texture profile analysis at 50% deformation (TPA50) and a shear/compression force test using a Kramer Ottawa probe (miniKramer). From cooked burgers, the samples were prepared for TPA50-obtained cubes (1 × 1 × 1 cm) and the shear/compression force test of three burgers from each batch. Finally, 2 × 2 cm samples were obtained. The following parameters were obtained for TPA50: hardness (N/cm^2), springiness (cm), gumminess (N × cm × s^2), chewiness (N × cm × s^2) and cohesiveness (dimensionless). In the shear/compression force test, the samples were weighed before the test to obtain the maximum force per g of sample (N/g).

2.3.6. Fatty Acid Profile

Fatty acid methylated esters (FAMEs) resulting from methylation KOH (85% in MethOH) of the total fat previously extracted were identified and quantified using an Agilent model 6890B gas chromatograph (Agilent, Santa Clara, CA, USA) fitted with a flame ionization detector (FID) and a fused silica column (60 mm length, 0.25 mm inner diameter and 0.25 µm film thickness). The conditions used were as follows: the oven temperature was raised to 220 °C on a ramp temperature, the injector and detector temperatures were 260 °C and 280 °C, respectively, and helium was used as a carrier gas with a constant flow of 1.2 mL min^{-1} and make-up of 25 mL min^{-1}. Injection mode was used with a split ratio of 1:100. Individual FAME identification was carried out on the basis of Sigma standards (Supelco 37 component FAME mix standard, Sigma Aldrich, St. Louis, MO, USA) compared with the retention times obtained. The results are given in g/100 g FAME as an average. Indexes to measure the nutritional quality of the fatty acids were calculated, i.e., PUFA/SFA and the atherogenic (AI) and thrombogenic indexes (TI) [6].

2.3.7. Determination of Antioxidant Compounds

Antioxidant content (α- and γ-tocopherol) was determined as described by Liu, Scheller and Schaeffer's [24], with some modifications by Cayuela, Garrido, Sancho Bañón and Ros [25]. From a saponification solution (KOH 11.5% in EtOH/H2O 55:45), tocopherol extractions were obtained, and both peaks from tocopherol coinciding with the α- and γ-tocopherol standards (0.2–14 µg/mL) were established.

An Agilent Technologies HPLC Series 1100 instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a Kromasil Silica column (5 µm particle size, 150 × 4.6 cm), Guard Column (10 µm) (Symta, Madrid, Spain) and a fluorescence detector (Agilent Technologies Series 1200) was used at a λ excitation of 295 nm and λ emission of 330 nm. The mobile phase was hexane.

Total antioxidant activity was determined following the procedure described by Cano, Hernández-Ruíz, García-Cánovas, Acosta and Arnao [26]. The extract was obtained from 1 g of meat homogenized with 8 mL of phosphate-buffered solution. Then, the extract was centrifugated (4000 rpm; 10 min) and filtered, and to an aliquot of the extract (100 µL), 235 µL of ABTS in phosphate tampon solution was added in order to launch the reaction in a microplate. A plate-reading spectrophotometer (Thermoscientific Multiskan Go) was used for the kinetic development. A standard curve of Trolox in phosphate tampon solution (0–0.25 mg/mL) was used. The results are expressed as mg Trolox/g.

2.3.8. Lipid and Protein Oxidations

Assessment of lipid oxidation was carried out by the 2-thiobarbituric acid (TBA) method [27]. An Agilent Technologies Cary 60 uv-vis spectrophotometer equipment and standard (1,1,1,3-tetraethoxypropane, TEP) curve were used to calculate TBARS values (µg malondialdehyde (MDA)/g sample).

Protein oxidation was determined through the carbonyl groups formed during the incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCL according to Oliver, Ahn, Moerman, Goldstein and Stadman [28]. The above-mentioned spectrophotometer was used at λ-280 nm for protein concentration, and the results are expressed as nmol carbonyls/mg of protein.

2.3.9. Microbial Quality

A total of 16 processed and vacuum-skin-packed lamb burgers were analysed by an external company. For enumeration, 10 g of each sample was placed under sterile conditions and homogenized with 90 mL of sterile peptone water in a stomacher (Stomacher R_ 400 Circulator) for 1 min. Serial decimal dilutions were then made with sterile peptone water, and 1 mL of each sample was spread over each culture medium. Mesophilic aerobic count was determined on plate count agar (PCA) following ISO (2013) [29] and incubated at 31 °C for 72 h; *Enterobacteriaceae* were incubated on VRBG agar at 37 °C for 24 h following ISO (2017) [30]; lactic acid bacteria (BAL) were incubated on MRS agar at 30 °C for 72 h according to ISO 15214 (1998) [31]; *Pseudomonas aeruginosa* were determined on cetrimide agar after incubation at 42 °C for 24 h; *E. coli* was incubated on T.B.X. agar at 44 °C for 18–24 h according to 16649-2/3:2001. The results are expressed as Log10 CFU (colony forming units) g^{-1}. The detection limit of the above techniques was 10 CFU/g. Analysis was performed in duplicate. Finally, *Salmonella* spp. were determined according to ISO (1993) [32]. The results of the analysis prove that these pathogens were present or absent in 25 g of the sample.

2.3.10. Sensory Analysis

Cooked lamb burgers were evaluated by 8 trained individuals selected from the staff members of CICYTEX in a tasting room equipped with private cabins according to Regulation UNE:ISO (2014) [33]. The cabins were individually illuminated with white light and the evaluators were provided with water to help them remove any aftertaste between the samples. Each panellist assessed a meat sample from each of the studied batches in a single session, and a total of 3 sessions were conducted. A random 2×2 cm^2 cut from each treated sample was cooked on an electric double grill to an internal temperature of 72–75 °C and individually encoded with three-digit random numbers to prepare it for tasting and evaluation. Two types of sensory analyses were carried out, i.e., a descriptive analysis and a preference analysis. The following descriptors were evaluated: meat aroma intensity, rancid odour, colour intensity, meat flavour intensity, rancid flavour, juiciness and chewiness. The intensity of each parameter was evaluated on a lineal, non-structured 10 cm scale from 0 (no perception) to 10 (maximum perception). Finally, the panellists were asked to order the packaged raw burgers and cooked burgers according to their preference and purchase intentions.

2.3.11. Statistical Analysis

A statistical analysis was performed using a SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) package. The Shapiro–Wilk test was used to verify the normality and homogeneity of variances of all the variables. A one-way ANOVA test was performed in order to assess the effects of the addition of pecan nuts to the different formulations (“Control”, “Pecan5”, “Pecan10” and “Pecan15”). Mean and standard error of mean (SEM) were reported. The differences between treatment means were determined using a Tukey’s HSD test. Significance was accepted at $p < 0.05$.

The relationships among the quality parameters to check the overall effects on burgers (the control and the one with pecans) were analysed using principal component analysis (PCA). For the purposes of modelling, Bartlett’s test of sphericity was performed, and the data matrix was found to be suitable for factorization. The Kaiser–Meyer–Olkin (KMO) test was performed in order to measure sampling adequacy for each variable in the model, and only variables with over KMO 0.6 were selected. Subsequently, the overall KMO of the performed PCA was 0.747.

3. Results and Discussion

3.1. Effects of the Various Portions of Added Pecan Nuts on Nutritional Composition

The results of the nutritional composition of Merino lamb burgers enhanced with different amounts of pecan nuts in their formulation are shown in Table 1. The percentage of pecans added significantly affected the proximate analysis of lamb burgers (Table 1). Although protein content was similar ($p \geq 0.05$), burgers containing pecan nuts had significantly higher ($p < 0.001$) fat content and lower ($p < 0.001$) moisture values than the control burgers. As expected, the fat content of lamb burgers increased as the pecan content increased and, in contrast, the moisture content decreased. These results are consistent with other findings relating to healthier frankfurters with walnuts added [34] and burger formulations, since pecan nuts are rich in fats with a high proportion of unsaturated/saturated fatty acids [35]. Similar results were observed by Reyes-Padilla et al. [36], who found an increase in fat content in low-fat bologna-type meat due to the addition of pecan nuts in their formulation.

Table 1. Nutritional composition of Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecan			SEM	*p*-Value
		5%	10%	15%		
Protein content	16.74	17.46	17.63	16.63	0.20	0.178
Fat content	13.98 c	14.94 bc	15.71 b	18.27 a	0.34	0.000
Moisture	62.25 a	59.10 ab	57.08 bc	53.43 c	0.74	0.000
Ash content	4.34 a	3.24 b	3.61 b	3.29 b	0.10	0.000
Total phenol content	0.82 c	1.17 b	1.49 a	1.54 a	0.06	0.000
Calories (kcal/g DM)	14.66 a	13.98 ab	13.76 bc	13.28 c	0.16	0.002

a, b, c: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means; SEM: standard error of the mean; total phenol content is expressed as g of gallic acid (GAE)/kg DM.

Protein content in all cases varied between 16.63 and 17.63 g/100 g of product, with no differences ($p \geq 0.05$) among batches. These values are slightly lower than the values typically found in lamb meat [37,38]. Reyes-Padilla et al. [36] and Jiménez Colmenero et al. [39] obtained similar results on protein content ($p < 0.05$) due to the addition of walnuts in low-fat bologna-type meat and restructured beef steaks, respectively, since they did not find variations in protein content, although the reported protein values are slightly higher than ours.

The addition of pecan nuts to burgers gradually increased ($p < 0.001$) the total phenol content with respect to the control burgers. Reyes-Padilla et al. [36] also reported an increase in phenol content in low-fat bologna-type meat enhanced with pecan nuts in their formulation. Pecan nuts are a rich source of polyphenols [40]. The phenolic compounds from walnuts and pecans could be beneficial because they scavenge free radicals (superoxide and hydrogen peroxide), and therefore have anti-inflammatory properties and prevent lipid and protein peroxidation [41].

The lamb burgers with pecan nuts proved to have a lower caloric value than the control burgers ($p < 0.01$). The decrease in caloric value was directly proportional to the increase in the amount of pecan nuts in the formulation.

3.2. *Effects of Proportion of Pecan Nuts on pH, Water-Holding Capacity and Instrumental Colour*

The addition of pecan nuts caused a significant increase in the pH ($p < 0.001$) and WHC ($p < 0.05$) of lamb burgers (Table 2). Ayo, Carballo, Solas and Jiménez-Colmenero [42] reported an increase in the pH values of meat batters with the addition of walnuts. However, Cofrades, Ayo, Serrano, Carballo and Jiménez-Colmenero [43] reported that increasing the amount of walnuts used while keeping the meat protein content constant did not have a significant effect on the pH of the meat batters. Likewise, Ayo et al. [34] found that the incorporation of walnuts did not have an impact on the pH of the frankfurters.

Moreover, the increase in the pH values in burgers enhanced with pecan nuts may be related to the increase in WHC, since an increase in the pH causes an increase in the WHC of the meat due to changes in the electrical charges of the muscle proteins [44]. In addition, the formulation of the burgers affected the colour coordinates (Table 2). Pecan nuts increased the lightness and CIE b* values ($p < 0.001$) of raw burgers. According to Perez Alvarez et al. [45], this could be due to the fact that the fat and oil in the pecan nuts provide a shine to the hamburgers. As for the CIE b* values, their increase may be due to the colour of the nut itself—usually yellowish—which would cause a progressive change in colour as the amount of pecan nuts increases in the hamburger. These results are in line with the results obtained by Serrano Agulló [46] in his study on the production of potentially functional restructured beef through the addition of walnuts. In contrast, CIE a* values decrease ($p < 0.001$) with the addition of pecan nuts in the formulation. According to Florowski et al. [47], this could be due to the fact that the incorporation of different amounts of walnuts, with their yellow colour, reduces the red tone of the burger meat. As with other types of enriched burgers, it is important to take into account the modifications in the colour of the burgers, given the importance that colour has for consumers [4].

Table 2. pH, water-holding capacity and colour properties of raw Merino Lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecans			SEM	*p*-Value
		5%	10%	15%		
pH	6.06 c	6.28 b	6.33 ab	6.37 a	0.02	0.000
WHC (%)	14.85 b	15.99 ab	19.31 a	17.97 ab	0.56	0.014
Colour parameters						
L*	48.13 c	50.38 bc	52.71 ab	54.96 a	0.65	0.000
a*	15.50 a	13.15 b	10.50 d	11.73 c	0.37	0.000
b*	10.81 c	12.50 b	13.05 ab	14.28 a	0.28	0.000
C*	18.96 a	18.18 a	16.75 b	18.48 a	0.23	0.001
H°	34.85 c	43.54 b	51.18 a	50.60 a	1.28	0.000

a, b, c, d: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means. SEM: standard error of the mean; WHC: water-holding capacity.

3.3. Effects of the Pecan Nut Levels on the Cooking Properties

The cooking properties of cooked lamb burgers are presented in Table 3. The addition of pecan nuts only significantly increased the cooking loss when the walnut content reached 2%. Ayo et al. [34] also found no differences in cooking losses in frankfurters when walnuts were added, and according to the authors, this behaviour was probably related to the low moisture of frankfurters, which was similar to the moisture content of the burgers produced in this study. However, Romero Oré [48] reported in his study an increase in cooking losses when walnuts were added to restructured llama meat, which could be associated with the loss of moisture in the product [49]. No significant differences in diameter reduction due to the addition of pecan nuts were reported ($p \geq 0.05$).

Table 3. Cooking yield of Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecans			SEM	*p*-Value
		5%	10%	15%		
% Cooking loss	24.84 b	28.19 a	25.00 b	24.72 b	0.53	0.023
% Diameter reduction	16.358	18.552	16.709	16.078	1.16	0.883

a, b: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means.

3.4. Effects of the Pecan Nut Levels on Textural Properties

The differences found in the textural parameters of the lamb burgers are shown in Table 4. In general, a decrease in Kramer shear force and hardness values was identified due to the addition of different amounts of pecan nuts in their formulation, although this decrease was not significant ($p \geq 0.05$). Ayo et al. [42] have indicated that nuts do not affect textural properties and that softer textures are obtained by increasing nut content and therefore decreasing meat content in different meat products. In meat products made from minced meat, such as hamburgers, texture will depend directly on the protein's capacity to create gels or the emulsifying capacity of the non-meat ingredients added [49]. For this reason, the decreasing trend in hardness values can be attributed to the reduced presence of myofibrillar proteins and a diluting effect of non-meat ingredients (pecan nuts) on meat protein systems as well as the poorer gelling properties of pecan nut globular proteins at the processing temperature (72 °C) [34].

Table 4. Instrumental textural properties of cooked Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecans			SEM	p-Value
		5%	10%	15%		
Shear/Compression force						
Hardness (N)	38.29	36.93	32.96	33.29	7.34	0.387
TPA 50						
Hardness(N/cm^2)	19.20	13.91	14.05	14.89	4.54	0.055
Springiness (cm)	0.85 a	0.81 ab	0.81 ab	0.75 b	0.07	0.022
Cohesiveness	0.66 a	0.69 a	0.62 a	0.53 b	0.08	0.000
Gumminess (N cm s^2)	12.64 a	9.56 ab	8.77 ab	8.20 b	3.32	0.027
Chewiness (N cm s^2)	10.74 a	7.90 ab	7.07 b	6.33 b	2.99	0.011

a, b: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means. TPA: texture profile analysis.

However, the addition of pecan nuts showed significant differences in springiness, cohesiveness, gumminess and chewiness between the control sample and the rest of the samples. In each parameter, the values decreased progressively as the amount of pecan nuts in the formulations increased ($p < 0.05$, $p < 0.001$, $p < 0.05$ and $p < 0.05$, respectively). This may be due to the fact that since all the burgers had the same weight (approx. 100 g), as the % of pecans was increased, the meat amount decreased. Similar trends were reported by Rabadán et al. [50] in lamb burgers made with oils and seeds.

3.5. Determination of the Fatty Acid Profile

As expected, the addition of pecan nuts in the burger formulations resulted in significant differences in their fatty acid profiles (Table 5). Palmitic acid and stearic acid values decreased as the amount of pecan nuts in the formulation increased ($p < 0.001$), while in contrast, oleic, linoleic and linolenic acid values increased ($p < 0.001$). The meat of ruminants is generally rich in SFAs [37]. The reduction in major saturated fatty acids resulted in a significantly lower SFA content in burgers with pecans than in the controls ($p < 0.001$). In addition, the MUFA and PUFA levels in burgers with pecans were increased relative to the values obtained for the control samples. ($p < 0.001$). According to the results, this increase in PUFAs was mainly caused by linoleic acid, since its content was more than doubled in the burgers made with pecan nuts compared to the control burgers. Although pecan nuts have a high fat content, they have a fatty acid profile that is beneficial for health due to their high content of unsaturated fatty acids, mainly oleic acid [51,52]. Oleic acid has been shown to have a beneficial effect against cancer and inflammatory diseases [53], and its high content in lamb burgers made with pecan nuts should be considered beneficial for human health. Linoleic acid (C18:2n6) has been found to be the most abundant PUFA in pecan nuts and, additionally, pecan nuts contain a small amount of alpha-linolenic acid (C18:3n3) [52]. Omega-6 linoleic acid and omega-3 alpha-linolenic acid are essential fatty acids that cannot be synthesized endogenously by most animals; therefore, their constant dietary intake is essential.

Besides the evaluation of the fatty acids, the nutritional quality of the fatty acid profile can be evaluated using nutritional quality indexes, such as the PUFA/SFA ratio and the atherogenic and thrombogenic indexes (Table 5).

Considering the improvement in the fatty acid profile of burgers with different concentrations of pecans, these batches showed better nutritional quality indexes than the control. According to nutritional recommendations, the optimal PUFA/SFA ratio is 0.4 [37,54]. In this research, Pecan15 burgers obtained a value of 0.37, which is very close to the optimum value of 0.4 for preventing some coronary disorders, although the rest of the batches obtained values lower than the recommended value. However, other authors [55] noted that the PUFA/SFA index did not consider the effects of MUFAs and took into account that all SFAs were affected in the same way in terms of cholesterol increase. In this sense, other nutritional indexes, such as atherogenic (AI) and thrombogenic indexes (TI), are also used to measure the propensity of a diet or food to influence the likelihood of coronary heart disease. High atherogenicity or thrombogenicity scores are associated with increased risk of ischaemic heart disease, a disorder occurring due to two main reasons: atherosclerosis and thrombosis [54]. Therefore, lower values of these indexes indicate a reduction in the risk of vascular disease. Both indexes decreased as the amount of pecan nuts in the burger formulation increased ($p < 0.001$).

Table 5. Fatty acid profile (g/100 g FAMEs) of raw Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecan			SEM	*p*-Value
		5%	10%	15%		
C16:0	24.41 a	21.04 b	19.07 c	17.17 d	0.49	0.000
C18:0	15.56 a	13.04 b	11.64 c	10.57 d	0.34	0.000
C18:1	45.53 d	49.82 c	52.45 b	54.75 a	0.63	0.000
C18:2 n-6	3.53 d	6.45 c	8.49 b	10.246 a	0.46	0.000
C18:3 n-3	0.41 d	0.58 c	0.68 b	0.76 a	0.03	0.000
SFA	43.75 a	37.25 b	33.47 c	30.06 d	0.93	0.000
MUFA	49.50 d	53.33 c	55.52 b	57.18 a	0.53	0.000
PUFA	3.94 d	7.03 c	9.17 b	11.01 a	0.49	0.000
PUFA/SFA	0.09 d	0.19 c	0.27 b	0.37 a	0.02	0.000
AI	0.73 a	0.55 b	0.46 c	0.38 d	0.02	0.000
TI	1.22 a	0.80 b	0.62 c	0.50 d	0.05	0.000

a, b, c, d: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means. AI: atherogenic index; TI: thrombogenic index.

3.6. Antioxidant Composition and Oxidative Status

Mean values for both α- and γ-tocopherol, total antioxidant activity and oxidative status, are shown in Table 6. α- and γ-tocopherol showed higher values in burgers made with pecan nuts with respect to control samples ($p < 0.05$ and $p < 0.001$ respectively), mainly γ-tocopherol, whose values ranged from 0.16 mg/kg in control burgers to 27.74 mg/kg in burgers made with 10% pecan nuts. These values increased progressively as the amount of pecan nuts increased in the formulation. This is due to the high value of tocopherols (in the form of vitamin E) in nuts, including pecan nuts [41]. Total antioxidant activity also increased gradually in burgers as the amount of pecan nuts in their composition increased ($p < 0.001$). However, in other studies on hamburgers, a trend contrary to that found in the current study was reported [56].

Regarding lipid oxidation, the addition of pecan nuts to the burger formulation had an impact on MDA content ($p < 0.001$). Higher PUFA values in burgers made with pecan nuts increased the risk of oxidation in burgers. However, TBAR values decreased in burgers formulated with pecan nuts. This decrease is explained by the high polyphenol content of pecan nuts [41]. Thus, as the percentage of pecan nuts in the burgers increases, a decrease in lipid oxidation will be reported. Our lipid oxidation values are lower than the values described by De Carvalho et al. [57], who reported values between 7 and 10 mg MDA/kg in lamb burgers reformulated with chia emulsion. The same pattern was found in the protein oxidation values, which were lower in burgers enhanced with pecan nuts with respect to the control samples ($p < 0.05$). This is evidence of the efficacy of the natural antioxidant properties of pecan nuts over other synthetic antioxidants, as reported by some authors [9,58].

Table 6. Antioxidant composition and oxidative status of raw Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecans			SEM	*p*-Value
		5%	10%	15%		
Antioxidant composition (μg g^{-1})						
α-tocopherol	4.21 b	4.97 ab	4.57 ab	5.31 a	0.14	0.019
γ-tocopherol	0.16 d	11.18 b	18.64 b	27.74 a	1.84	0.000
AAT	0.65 c	0.71 c	0.82 b	0.96 a	0.02	0.000
Oxidative status						
Lipid oxidation (μg MDA g^{-1})	1.46 a	0.59 b	0.59 b	0.58 b	0.07	0.000
Protein oxidation (nmol carbonyls mg proteins g^{-1})	2.26 a	1.56 b	1.73 ab	2.13 ab	0.09	0.013

a, b, c, d: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means. α- and γ-tocopherol are expressed as mg/kg DM; AAT: total antioxidant activity.

3.7. Microbial Quality

The mesophilic aerobic count of lamb burgers varied between 5.41 and 5.03 log cfu/g, and there was no significant difference between the control and burgers with pecan nuts (Table 7). Mesophilic aerobic counts reflect the microbial content of raw materials and ingredients, the efficiency of the manufacturing procedure/process and the hygiene conditions of the equipment and utensils, and it is therefore used to monitor the implementation of good manufacturing practices (GMPs). The counts were acceptable according to the microbiological limits established for meat preparations [59–61], although they were higher than the counts found in other studies on lamb meat [62,63].

Table 7. Microbial growth (log10 CFU/g) of raw Merino lamb burgers with different amounts of pecans in their formulation.

	Control	With Pecans			SEM	*p*-Value
		5%	10%	15%		
Mesophilic aerobic bacteria	5.41	5.05	5.09	5.03	0.07	0.188
Enterobacteriaceae	2.38	2.22	2.01	2.22	0.07	0.320
Lactic acid bacteria	2.85 a	2.71 a	2.34 b	2.79 a	0.06	0.000
P. aeruginosa	1.46	1.22	1.52	1.03	0.28	0.941
E. coli	ND	ND	ND	ND	-	-
Salmonella spp.	absence	absence	absence	absence	-	-

a, b: different letters in the same row indicate significant differences during storage according to Tukey's HSD test; values are expressed as means. ND: not detected.

Similarly, no significant differences were observed in the counts of Enterobacteriaceae and *P. aeruginosa* amongst the different batches. However, LAB showed lower counts in burgers made with 10% pecan nuts ($p < 0.001$), although in all formulations, LAB values were far from the limit values considered, as meat spoilage occurs when the LAB concentration reaches 7 log10 CFU g^{-1} [62].

3.8. Sensory Analysis

The effects of the addition of different amounts of pecan nuts on the sensory evaluation of lamb burgers are shown in Figure 1. A sensory evaluation of lamb burgers revealed no significant difference in any sensory attributes between the control and treated burgers, except colour. Variations in the colour of meat products have been associated with the ingredients, the contents of water, fat and the type of package used.

Figure 1. Spider plot of sensory attributes of burgers from Merino lamb with different amounts of pecans in their formulation. (** $p < 0.01$).

The score for colour intensity of the burgers made with pecan nuts was higher ($p < 0.01$) than that of the control burgers. On the other hand, Reyes-Padilla et al. [36] reported that all sensory attributes were affected by the addition of natural ingredients, such as pecan nuts, to low-fat bologna-type meat products. Likewise, Serrano, Cofrades and Jiménez-Colmenero [64] stated that the addition of walnuts had some effects on the sensory quality of restructured steak. Non-significant differences were found in the preference test on cooked burgers ($p \geq 0.05$), which indicated that preferences were similar among the four treatments (Figure 2). However, Lutz, Morales, Sepúlveda and Salviña [65] reported that the use of ingredients rich in antioxidants and omega-3 fatty acids in traditional Chilean dishes improved sensory acceptance among the elderly. In contrast, for raw packaged burgers, 91.67% of the panellists chose the control burgers as their first choice, while 8.33% opted for the hamburgers made with 10% pecans ($p < 0.001$).

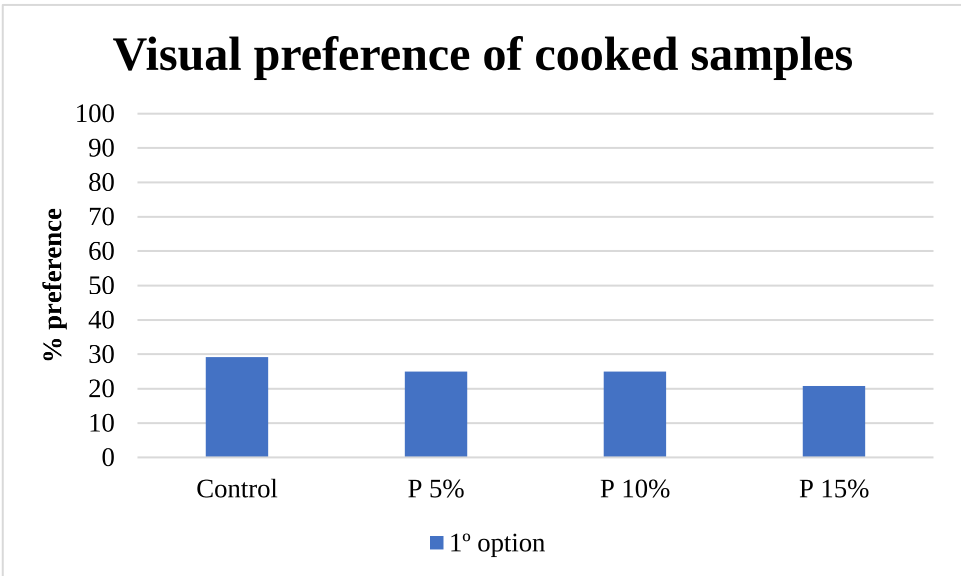

Figure 2. Overall preference for cooked samples.

3.9. Principal Component Analysis

The results of the principal component analysis (PCA) for the different batches of lamb burgers (Control, Pecan5, Pecan10 and Pecan15) are shown in Figure 3. As in most cases, two principal components were sufficient to explain a great proportion of the variation in the original parameters.

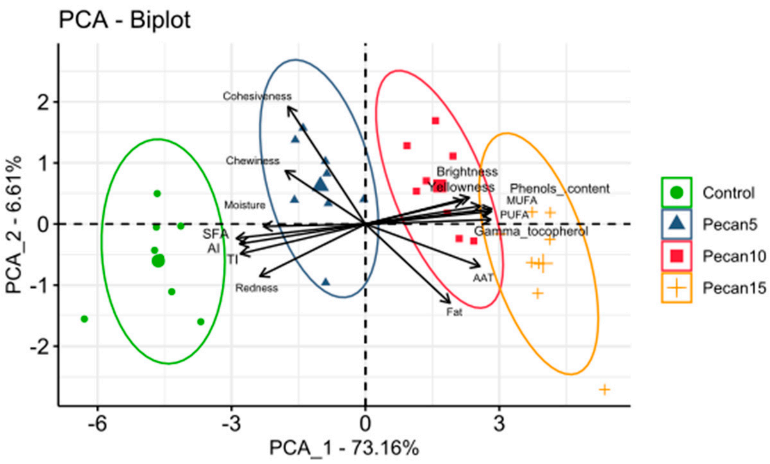

Figure 3. Loading biplot for the first two principal components (PC1 and PC2) of lamb burger with different pecan proportions: Control (without pecan), Pecan5 (with 5% pecan), Pecan10 (with 10% pecan) and Pecan15 (with 15% pecan).

The distribution of the scores for the first two principal components (Figure 3) shows four separate groups of points corresponding to the different treatments and mostly separated by PC1 (73.16%). The samples from Pecan10 and Pecan15 tended to have positive scores in Principal Component 1 (PC1), which could be mainly explained by the positive loadings of MUFAs, PUFAs and gamma tocopherol, whereas the Pecan5 and Control samples were located in the negative part of the PC1 area, since SFAs, AI and TI yielded negative loadings in PC1. Regarding PC2, the variables with high loadings in PC2 (which explained 6.61% of the variance) were cohesiveness, chewiness, redness, AAT and fat.

4. Conclusions

The addition of pecan nuts to the formulation of lamb burgers increased the fat content but improved their fatty acid profile from a health point of view by increasing the MUFA and PUFA content and decreasing the SFA content. In addition to this, the high phenolic content of pecan nuts, which increased in the burgers as the amount of pecan nuts became higher, provided a significant increase in the antioxidant capacity of the product. Thus, the oxidative indexes of lipids and proteins were significantly reduced. In terms of the sensory analysis, no differences were observed in the evaluated attributes and preferences for the cooked burgers, proving that the preference was similar for the different formulations and the control burgers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13116860/s1.

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