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ORIGINAL ARTICLE

Physicochemical, technological and sensory properties of hamburger made with meat from lambs fed on whole cottonseed

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Abstract The physicochemical composition and the technological and sensory properties of hamburgers made with meat from Ile de France lambs fed on different levels (0, 10, 20, 30 and 40%) of whole cottonseed were studied. The addition of whole cottonseed to the lambs' diets decreased the thiobarbituric acid reactive substances in the lamb meat and altered the physicochemical characteristics of the hamburgers, which were characterised by low lipid ($\hat{y} = 4.27$), cholesterol ($\hat{y} = 75.15$) and caloric content ($\hat{y} = 122.04$). The results regarding cooking characteristics were directly related to the microscopic observations regarding the hamburgers; the more cohesive structures exhibited better performance after cooking, with increased cooking yield and moisture retention, and decreased cooking loss. The levels of whole cottonseed did not influence the texture profile, but they negatively affected the acceptability of the hamburgers, since as the levels of cotton seedlings increased, the scores for the sensorial attributes decreased. Thus, a maximum inclusion of 16.7% of whole cottonseed in the dry matter of the diet of lambs is recommended.

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Introduction

There is a growing interest in the consumption of lamb in Brazil, especially in the southern region, and this is provoking a demand for a greater supply of quality products. The quality of carcass, which is highly influenced by the animals' diet, is one of the major factors in relation to the expansion and consolidation of the market for lamb meat (Lima Júnior et al. 2016).

Animal diets involve high production costs and it is appropriate to study alternative ingredients which can provide adequate nutrients at low cost. Brazil has a large level of cotton production and, consequently, there is great availability of low-cost by-products from the textile industry (CONAB 2016). The by-products of cotton production, like cottonseed cake, cottonseed meal and whole cottonseed (WCS), have a high lipid content, which makes it possible to increase the energy density of animal diets without the need to reduce fibre or protein content. There is great potential to reduce the cost of animal diets, especially for ruminants (Paim et al. 2010; Vieira et al. 2010; Costa et al. 2011).

However, one of the limitations of the use of this ingredient in animal diets is related to the possible association of whole cottonseed with undesirable characteristics in the qualitative aspects of the meat, in particular, the sensorial characteristics such as disagreeables flavour and odour (Pellegrini 2017). This is due to the presence of gossypol, which can produce a residual flavour in meats and their derivatives. A study by Viana et al. (2014)

showed that meat from lambs fed with high levels (39% WCS) of whole cottonseed showed no detectable gossypol, demonstrating that whole cottonseed can be used in the diet of ruminants without causing harmful effects to human health.

Other studies have evaluated the effect of whole cottonseed on the quality of lamb meat (Madruga et al. 2008; Vieira et al. 2010; Paim et al. 2010), demonstrating that this practice can performed, for short periods (60–90 days), at levels up to 40% for finishing lambs. However, no studies were found in the literature evaluating the quality of products made with meat from lambs fed on whole cottonseed. Consequently, it is appropriate to investigate the technological behaviour of such meat during processing.

A study by Viana et al. (2013) emphasised the possibility to promote lamb meat as an alternative, in order to structure and add value to the process of sheep farming. One method of expanding the consumption of lamb would be to develop convenience products that satisfy the tastes and needs of consumers. The aim of this study was to evaluate the physicochemical composition, as well as the technological and sensory properties, of hamburgers made with meat from Ile de France lambs fed on different levels of whole cottonseed.

Materials and methods

Obtaining the meat

The lamb meat came from an experiment approved by the Ethics Committee on Animal Use (CEUA) at the Federal Institute of Education, Science and Technology, Farroupilha, Rio Grande do Sul, Brazil. The protocol number was 01.0378.2015/001.2015. Twenty-five male, uncastrated, Ile de France lambs, which were weaned at 60 days, were used. The lambs were distributed into five experimental treatments, with five replicates. Each treatment consisted of the experimental base diet, to which was added whole cottonseed (*Gossypium hirsutum* L.) in proportions of 0, 10, 20, 30 and 40%. The experimental base diet was composed of corn silage (*Zea mays* L.), ground corn (*Z. mays* L.) and soybean meal (*Glycine max* L.), in a voluminous ratio:concentrate (40:60) and mineral mixture. The prepared diets were isoproteic in order to meet the nutritional requirements of growing lambs (NRC 2007). Feeding was ad libitum and the amount consumed was monitored, at the pre-set times of 7:00 a.m. and 4:00 p.m. The feeding trial of the lambs started after the adaptation period (7 days, initial mean weight 19.26 kg, 67 days old) and was extended until the time of slaughter. The slaughter criterion was a final live weight of 36 kg (mean confinement time of 64 days, final mean weight 36.64 kg, 127 days old) (Pellegrini 2017; Pilecco 2016). The animals were slaughtered using a pneumatic gun for desensitisation, which was followed by bleeding, skinning, evisceration, weighing, washing and cooling. The carcasses were cooled to 2 °C for 24 h. The shanks were subsequently collected, packed and frozen in a conventional freezer at − 18 °C, where they were stored for a month, until the moment of production of the hamburgers.

Preparation of hamburgers

The hamburgers were developed according to the Hamburger Identity and Quality Technical Regulation (Brasil 2000) and Regulation No. 1004 (Brasil 1998). The base formulation was composed of the following: water (3%); textured soy protein (4%) (Solae, Esteio, RS, Brasil); sodium chloride (1.2%) (Diana, São Paulo, SP, Brasil); garlic paste (1%) (Temperalho ind. LTDA, Iacanga, SP, Brasil); monosodium glutamate (0.3%) (Ajinomoto, São Paulo, SP, Brasil); maltodextrin (0.3%) (Nutract, Chapecó, SC, Brasil); sodium tripolyphosphate (0.3%) (Kerry, São Paulo, SP, Brasil); parsley (0.2%) (Kitano, São Bernardo do Campo, SP, Brasil); seasoning (0.2%) (Bremil, Arroio do Meio, RS, Brasil); sodium erythorbate (0.2%) (Nutract, Chapecó, SC, Brasil); sodium lactate (0.01%) (Nutract, Chapecó, SC, Brasil), powdered smoke (0.04%) (ICL, São Paulo, SP, Brasil); sodium nitrite (0.01%) (Nutract, Chapecó, SC, Brasil); cochineal carmine colouring (0.007%) (CHR Hansen, São Paulo, SP, Brasil); and red pepper (0.038%) (Kitano General Mills, São Bernardo do Campo, SP, Brasil). Five hamburger formulations were developed, each of which contained lamb meat (89.195%) from the respective animals' diets (0, 10, 20, 30 and 40% WCS).

For the preparation of the hamburgers, the lamb shank was thawed under refrigeration (4 °C) and the excess fat was removed. The meat was then ground (Jamar PJ22, Jamar Ltda, São Paulo, SP, Brasil) using 5 mm hole discs and taken to a mixer (Jamar MJI 35, Jamar Ltda, São Paulo, SP, Brasil) for the addition of the other ingredients. The temperature of the preparation process did not exceed 2 °C.

After a homogeneous mixture was obtained it was packed in plastic wrappers (10 cm diameter, Spel, Atibaia, SP, Brasil) and immediately frozen in a conventional freezer (Metalfrio, São Paulo, SP, Brasil) at − 18 °C. The following day, the product was sliced (Malpa, São Paulo, SP, Brasil), with an average thickness of 1 cm and average weight of 100 g, the plastic wrapping was removed and the hamburgers were individually wrapped in low-density polyethylene bags and kept at − 18 °C, until the moment of analysis. The analyses of physicochemical characterisation, cooking characteristics, texture profile and histological evaluation were performed in the first 7 days of storage. The sensorial analysis was conducted at 15 and 60 days of storage, after obtaining the results of the microbiological analysis.

Physicochemical characterisation

The lamb meat and the hamburgers were characterised raw, in triplicate, by determining moisture, protein and ashes (AOAC 2005), as well as lipids (Hara and Radin 1978). In the hamburger, carbohydrates were determined by difference (AOAC 2005). The caloric value was calculated by the sum of the calories provided by carbohydrates, proteins and lipids, multiplying their values in grams by the Atwater factors of 4, 4 and 9 kcal, respectively. Cholesterol was determined in duplicate in the crude samples by the enzymatic method (Saldanha et al. 2004). To assess the extent of lipid oxidation that occurred in the hamburgers, the thiobarbituric acid (TBA) index was used, following the methodology of Raharjo et al. (1992). The analyses were performed in duplicate and the results were expressed as mg of malonaldehyde per kg of sample (MDA mg/kg). The evaluation of pH was performed using a pH meter (pH metro, model DM-23DC São Paulo, Brasil) in accordance with the IAL (2008), with readings performed in triplicate. The water activity values were determined using Aqualab® (Decagon Devices, Inc., Pullman, WA, USA) at 25 °C, in triplicate.

Technological properties

For the analysis of cooking characteristics, texture profile and sensory analysis, the hamburgers were cooked in an electric oven (Fischer Grill 44 L, Fischer, Brusque, SC, Brasil) at 180 °C until they reached an internal temperature of 72 °C. The cooking characteristics were determined in quadruplicate. Cooking yield, moisture retention and fat retention (Gök et al. 2011) were all determined. Cooking loss was calculated as the difference in weight between the uncooked and cooked burger divided by the weight of the uncooked burger. Shrinkage was calculated as the difference between the uncooked and cooked burger divided by the uncooked burger, considering thickness and diameter.

The texture profile analysis (TPA) was performed using a TA-XT.plus texture analyser which was equipped with Texture Expert Exponent software (Stable Microsystems Ltd., Surrey, England). After being cooked according to the procedure described above and cooled to room temperature, the hamburger samples were cubed (1 cm^3). Four hamburgers from each formulation were analysed and 10 cubes were removed from each hamburger. Each cube was tested under the following conditions: test velocity (5 mm/s), pre-test velocity (1 mm/s), return velocity (5 mm/s), return distance (20 mm), contact force (1 g), 50% compression height, and interval between compressions (5 s). The TPA was evaluated based on the characteristics of hardness, cohesiveness, flexibility and chewiness (Bourne 1978).

Histological evaluation

For the histological evaluation, three fragments from the centre of raw samples of each hamburger formulation were extracted and processed according to the conventional histological technique (Junqueira and Carneiro 2008). The samples were cut into sections with a thickness of 4 µm and stained with hematoxylin and eosin; three slides of each sample were prepared. The images observed under a microscope (Leica Microscopy Systems, Heerbrugg, Switzerland) were obtained using Motic Images Plus 2.0 software (Motic Instruments, Inc, Richmond, Canada). Microscopy was performed in the Histology Laboratory of the Department of Pharmacy at the Integrated Regional University of Alto Uruguay and Missions (URI), Erechim, RS, Brazil.

Microbiological evaluation

The microbiological analyses were performed according to Normative Instruction No. 62 (Brasil 2003) following the standards established by ANVISA (Brasil 2001)—(*Salmonella* spp., *Clostridium* sulfite reducing, coagulase positive *Staphylococcus* and total coliforms and coliforms at 45 °C) on the 1st and 45th days of storage to ensure the microbiological quality of the products for sensory analysis, which was performed subsequently.

Sensory analysis

This study was previously approved by the Research Ethics Committee of the Federal University of Santa Maria (UFSM) under protocol No. 936.005 and Certificate of Presentation for Ethical Assessment No. 40264114.4.0000.5346. The sensorial analysis were performed in the Sensory Analysis Laboratory, of the UFSM, in a standardised room, with individualised booths and normal white lighting, at 25 °C.

The sensory analysis tests were performed by untrained testers (who confirmed that they liked lamb meat). They were female and male, aged from 18 to 50, and were recruited from staff, students and general public at the UFSM. The number of testers for each test was chosen as recommended by the Adolfo Lutz Institute (IAL 2008). A total of 187 different testers participated in the sensory analysis, of which 107 participated in the acceptance test (day 15 = 55 testers; day 60 = 52 testers); 55% of these testers were female and 45% were male. Eighty testers participated in the triangular test (day 15 = 40; day 60 = 40), of which 60% were female and 45% were male.

After confirming the microbiological safety of the products, sensorial analysis was performed on days 15 and 60 of storage at − 18 °C: testing on day 15 was to verify the acceptance of the hamburgers and testing on day 60 was to verify the effects of storage. The samples were prepared according to the cooking procedure previously described. After cooking, the burgers were cut into uniform pieces (1 cm^3 cubes), wrapped individually in aluminium foil and kept at 60 °C until ready to serve.

The hedonic test was applied through an acceptance test that evaluated odour, colour, flavour, texture and appearance, using an hedonic seven-point scale (1 = dislike intensely, 7 = like intensely) and purchase intent by using a five-point scale (1 = woud definitely not purchase, 5 = would definitely purchase). The samples were offered to the testers in a monodic and random manner, together with the sensory score sheet (IAL 2008); each taster evaluated the five hamburger formulations. From the scores assigned in the acceptance test, the acceptability index (AI) of the product was calculated according to the following equation: $AI\ (\%) = A \times 100/B$, where A = average score obtained for the product and B = maximum score given to the product (Dutcosky 2011).

The triangular test was applied to detect if the whole cottonseed used in the animals' diets provided a flavour in the developed products and at what level the tester perceived this flavour when compared to the control (0% WCS); each tester tasted the treatments: 0% WCS versus 10% WCS; 0% WCS versus 20% WCS; 0% WCS versus 30% WCS and 0% WCS versus 40% WCS. The interpretation of the results was based on the relationship between the total number of judgements versus the number of correct judgements compared to the result of the Chi square table (IAL 2008). The results of the number of correct judgements that were equal to or greater than the table value made it possible to conclude that there was a significant difference between the samples at the corresponding probability level. Three coded samples were presented simultaneously, two identical and one different, together with the sensory score sheet of the triangular test; the tester was asked to identify the sample that seemed different to them.

During each evaluation, was provided along with samples water for mouth rinsing and cracker as palate moderator.

Experimental design and statistical analysis

Five replicates for each lamb meat experiment and four replicates for each hamburger experiment were performed. For each sample, the analyses were carried out in duplicate (cholesterol and TBARS determination); triplicate (physicochemical, pH, water activity, histological evaluation); quadruplicate (cooking characteristics) and in ten replicates (texture profile analysis).

For the physicochemical analyses of the raw lamb meat and hamburgers, the statistical analyses were carried out in a completely randomised, experimental design with five treatments (0, 10, 20, 30 and 40% WCS in the diet) and a different number of repetitions (five for the lamb meat and four for the hamburgers) according to the following statistical model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} = the value observed at the i-th level of whole cottonseed and the j-th repetition; μ = the average mean of the response variable; α_i = the fixed effect of the ith level of whole cottonseed; ε_{ij} = the random effect associated with the Y_{ij} observation, assuming $\varepsilon_{ij} \overset{iid}{\sim} N(0, \sigma^2)$.

The sensory analysis data were analysed using a randomised block design for each storage time (15 and 60 days).

The data were subjected to outlier investigation from the studentised residuals. They were subsequently submitted to univariate analysis of variance (ANOVA) by the GLM procedure: their averages were adjusted by the ordinary least squares method using the LSMEANS command and compared by the least significant difference (*t* test).

The linear and quadratic trends were tested by means of the contrasts from the coefficients for interpolation of the orthogonal polynomials. In addition, the polynomial and multiple regression were adjusted using the RSREG procedure, and the r^2 values were expressed in relation to the source treatments (regression + lack of fit). Furthermore, Pearson and Spearman correlation analyses were performed between the studied variables.

The statistical analyses were performed using SAS® System for Windows™ software, version 9.4 (SAS Institute 2002).

Results and discussion

Physicochemical characterisation of lamb meat

The addition of whole cottonseed to the lambs' diets altered ($P < 0.05$) the ash content and the thiobarbituric acid reactive substances (TBARS) in the meat (Table 1). The ash content reduced ($P < 0.05$) with the addition of whole cottonseed, although there were no scientific findings that justified this behaviour. In contrast, data from the literature emphasise an increase in ash content in line with increased whole cottonseed in the diet (Paim et al. 2014; Pellegrini 2017).

Table 1 Physicochemical characterisation of meat from lambs fed on different levels of whole cottonseed

Variable	Whole cottonseed (%)					Mean or equation	Probability value[1]			SEM[2]	CV[3]
	0	10	20	30	40		WCS	L	Q		
Moisture (%)	75.3	74.4	74.6	75.2	73.6	$\bar{y} = 74.51$	0.3863	0.2912	0.7092	0.29	1.82
Ashes (%)	1.38[a]	1.14[ab]	1.03[b]	1.08[b]	1.00[b]	(1)	0.0448	0.0043	0.0992	0.04	15.2
Protein (%)	20.5	20.4	20.5	20.1	21.6	$\bar{y} = 20.5$	0.0928	0.1429	0.0735	0.17	3.80
Lipids (%)	2.79	4.13	3.90	3.60	3.78	$\bar{y} = 3.76$	0.4330	0.3452	0.1716	0.18	22.3
Cholesterol (mg/100 g)	98.9	88.2	79.7	81.6	85.9	$\bar{y} = 85.2$	0.0921	0.0406	0.0242	1.96	10.8
TBARS* (mg MDA/kg)	1.23[a]	0.73[b]	0.60[bc]	0.46[c]	0.52[bc]	(2)	0.0006	0.0001	0.0052	0.06	41.9
pH	5.68	5.78	5.80	5.77	5.83	$\bar{y} = 5.78$	0.1611	0.0292	0.3762	0.02	1.31
Water activity	0.9934	0.9918	0.9918	0.9910	0.9910	$\bar{y} = 0.9916$	0.1001	0.0091	0.2625	0.0002	0.12

Means in the same row, followed by different letters, differ ($P < 0.05$) by *t* test

**TBARS* thiobarbituric acid reactive substances

[1]*WCS* whole cottonseed levels, *L* linear tendency, *Q* quadratic tendency

[2]*SEM* standard error mean

[3]CV (%) = coefficient of variation

$^{(1)}\hat{y}_{ashes} = 1.24 - 0.01\ WCS\ (r^2 = 0.64)$; $^{(2)}\hat{y}_{TBARS} = 1.18 - 0.05\ WCS + 0.007\ WCS^2\ (r^2 = 0.96)$

The TBARS had quadratic behavior ($P < 0.05$) with minimum point at 31% WCS. The decrease of TBARS up to 31% WCS can be explained by reduction of the lipid oxidation (Wang et al. 2008) induced by the possible deposition of whole cottonseed constituents in the lamb meat (Kim et al. 1996) such as phenolic compounds, tocopherol (Oliveira et al. 2016) and gossypol (Wang et al. 2008, 2009). However, the increase in TBARS values from 31% WCS can be explained by the increase in the level of whole cottonseed in the diet of the lambs; there was an increase in the deposition of unsaturated fatty acids (Pellegrini 2017), which are more susceptible to lipid oxidation. Brazilian legislation does not establish a maximum limit of malonaldehyde/kg in meat products; however, data from the literature indicate that values up to 2.00 mg of MDA/kg of sample are not perceived by consumers (Wood et al. 2004).

Madruga et al. (2008) evaluated the effect of including whole cottonseed (0, 20, 30 and 40%) in the diet of Santa Inês lambs on the chemical composition of the meat. They found similar results to those obtained in the present study and found no significant difference ($P > 0.05$) for all the analysed variables (moisture, ash, proteins, lipids and cholesterol), which was attributed to the short confinement period (70 days). Thus, the results of the physicochemical analyses in the present study demonstrated that the meat of lambs fed with different levels of whole cottonseed presented satisfactory characteristics and was considered suitable for the production of hamburgers.

Chemical composition of the hamburgers

The moisture levels in the hamburgers increased ($P < 0.05$) in line with increased levels of whole cottonseed (Table 2), which was reflected in lower protein content ($r = -0.89$, $P < 0.001$), ash content ($r = -0.95$, $P < 0.001$) and caloric value ($r = -0.88$, $P < 0.0001$). The lipids (minimum point = 18.8% WCS) and carbohydrates (maximum point = 20.1% WCS) demonstrated quadratic behaviour. It is believed that these results may have been associated with the heterogeneity of the products because only the ash content was reduced in the meat (Table 1).

All the formulations of the hamburgers made with lamb from animals fed with whole cottonseed were low in fat ($\hat{y} = 4.27$) and low in caloric value ($\hat{y} = 122.04$) when compared to other commercial meat products. It should be noted that the hamburgers prepared in this study did not contain added fat. Furthermore, the cut of meat that was used (lamb shank) contained low levels of fat (3.76 g/100 g, Table 1). Similar results were found by Santos Júnior et al. (2009) when they evaluated oatmeal-enriched lamb meat hamburger, in which the lipid values were 4.3–8.4/100 g and the caloric values were 122.55–153.68 kcal/100 g. Likewise, Linares et al. (2012) studied the effect of the type of meat (shank compared with shank + neck + breast) on the nutritional composition of hamburgers made with meat from Spanish Manchego lamb and found that the hamburgers only made with shank had lower fat content and lower caloric value than those made

Table 2 Proximate composition of hamburgers made with meat from lambs fed on different levels of whole cottonseed

Variables	Whole cottonseed (%)					Equation	Probability value[1]			SEM[2]	CV[3]
	0	10	20	30	40		WCS	L	Q		
Moisture (%)	68.63[b]	68.88[b]	74.55[a]	68.33[b]	75.80[a]	(1)	0.0001	0.0001	0.3051	0.78	4.81
Ashes (%)	3.59[a]	3.47[a]	2.86[b]	3.65[a]	2.89[b]	(2)	0.0004	0.0068	0.8316	0.09	12.64
Protein (%)	19.68[a]	19.53[a]	16.70[b]	18.58[ab]	14.43[c]	(3)	0.0003	0.0001	0.2008	0.51	12.35
Lipids (%)	5.06[a]	3.73[a]	3.18[b]	3.20[b]	6.18[a]	(4)	0.0015	0.2577	0.0001	0.32	33.14
Carbohydrates (%)	3.07[bc]	4.42[ab]	2.71[bc]	6.25[a]	2.19[c]	(5)	0.0030	0.9757	0.0341	0.42	50.40
Caloric value (Kcal/100 g)	136.50[a]	129.32[a]	106.27[b]	128.08[a]	110.01[b]	(6)	0.0002	0.0007	0.1335	3.14	11.17
Cholesterol (mg/100 g)	85.02[a]	80.84[a]	71.69[b]	69.71[b]	68.48[b]	(7)	0.0030	0.0002	0.2342	1.92	11.10

Means in the same row, followed by different letters, differ ($P < 0.05$) by *t* test

[1]*WCS* whole cottonseed levels, *L* linear tendency, *Q* quadratic tendency

[2]*SEM* standard error mean

[3]CV (%) = coefficient of variation

(1) $\hat{y}_{moisture} = 68.58 + 0.13WCS$ ($r^2 = 0.28$); (2) $\hat{y}_{ashes} = 3.53 - 0.01\ WCS$ ($r^2 = 0.25$); (3) $\hat{y}_{protein} = 20.01 - 0.11\ WCS$ ($r^2 = 0.63$); (4) $\hat{y}_{lipids} = 5.23 - 0.24\ WCS + 0.01\ WCS^2$ ($r^2 = 0.91$); (5) $\hat{y}_{carbohydrates} = 2.92 + 0.16\ WCS - 0.004\ WCS^2$ ($r^2 = 0.21$); (6) $\hat{y}_{caloricvalue} = 133.81 - 0.54\ WCS$ ($r^2 = 0.39$); (7) $\hat{y}_{cholesterol} = 83.99 - 0.44\ WCS$ ($r^2 = 0.90$)

with shank + neck + breast (5.16/100 g and 201 kcal/100 g; 10.71/100 g and 246.51 kcal/100 g, respectively).

In the present study, the cholesterol levels decreased ($\hat{y}_{cholesterol} = 83.99 - 0.44\text{WCS}, r^2 = 0.90$) in line with the addition of whole cottonseed and can be considered low (< 90 mg/100 g). The cholesterol values were in accordance with those reported by Madruga et al. (2008) in lamb meat fed with levels of whole cottonseed (80 mg/100 g).

Technological properties

The yield and levels of moisture retention in the hamburgers increased ($P < 0.05$) according to the level of whole cottonseed in the animals' diet (Table 3), which was due to the reduction in cooking loss. No significant difference was observed regarding fat retention and shrinkage.

Cooking loss, which is associated with moisture and fat retention, is important for evaluating the juiciness and mouthfeel of cooked products. The hamburgers made with meat from lamb fed with whole cottonseed levels showed lower values for cooking loss ($\bar{y} = 12.9\%$), as well as high moisture ($\bar{y} = 55.7\%$) and fat ($\bar{y} = 108.5\%$) retention rates. These results can be explained by the ability of the protein matrix to retain lipids, especially in formulations with low lipid content [r ($lipids \times fat\ \ retention$) = − 0.93, $P < 0.001$], which optimises the cooking characteristics of products and guarantees greater succulence for cooked hamburgers. Furthermore, the addition of sodium tripolyphosphate to the formulation may have aided the fixation of water in the product, reducing the cooking loss.

The texture profile results of the hamburgers were not affected ($P > 0.05$) by the levels of whole cottonseed in the lambs' diet (Table 3) and were in agreement with those found in the literature for cooked beefburgers (hardness = 50.8 N; cohesiveness = 0.82; flexibility = 0.71; chewiness = 30.06) (Gutt et al. 2014). Of all the TPA characteristics, hardness is the most important because it best represents the texture characteristic of a product. The hamburgers prepared with lamb meat from animals fed on different levels of whole cottonseed had a soft texture.

Histological evaluation

Microscopy was used to evaluate the relationship between the cooking and textural characteristics of the hamburgers and their microscopic structures. Using photomicrographs it was possible to evaluate the disposition of the muscle fibres, the compact connective tissue, adipose tissue, extracellular matrix and air bubbles in the products. The presence of adipose cells was most noticeable in the hamburger made with meat from lambs fed with 0% WCS (Fig. 1a, b), which was related to the fat content found in this formulation (Table 2). The lamb meat hamburger formulations (Fig. 1c–j) were more homogeneous in relation to the distribution of muscle cells, connective tissue and adipose tissue, exhibiting better structural conditions. These characteristics promote adequate tissue bonding, which provides better technological properties because a more compact and cohesive structure makes it possible to retain more moisture during cooking (Abdel-Naeem and Mohamed 2016). The results for moisture retention and

Table 3 Cooking characteristics and texture profile analyses of hamburgers made with meat from lambs fed on different levels of whole cottonseed

Variables	Whole cottonseed (%)					Mean or equation	Probability value[1]			SEM[2]	CV[3]
	0	10	20	30	40		WCS	L	Q		
Cooking yield (%)	85.28^{bc}	83.85^{c}	88.33^{ab}	89.80^{a}	88.40^{ab}	(1)	0.0089	0.0031	0.4840	0.67	3.43
Cooking loss (%)	14.74^{ab}	16.13^{a}	11.68^{b}	10.20^{b}	11.60^{b}	(2)	0.0096	0.0033	0.4816	0.67	23.3
Moisture retention (%)	53.05^{b}	54.93^{ab}	55.93^{a}	57.38^{a}	57.25^{a}	(3)	0.0109	0.0008	0.2653	0.49	3.95
Fat retention (%)	75.10	104.05	131.38	145.10	86.83	$\bar{y} = 108.5$	0.0518	0.2475	0.0096	9.05	37.3
Shrinkage (%)	1.34	5.80	3.60	4.34	4.12	$\bar{y} = 3.84$	0.1524	0.2808	0.1585	0.57	66.1
Hardness (N)	51.33	60.05	55.65	60.98	53.40	$\bar{y} = 56.3$	0.2314	0.6357	0.0853	1.57	12.5
Cohesiveness	0.68	0.68	0.70	0.70	0.70	$\bar{y} = 0.69$	0.3719	0.0856	0.6539	0.00	2.15
Springiness	0.98	0.96	1.03	0.97	0.93	$\bar{y} = 0.97$	0.0557	0.2005	0.0494	0.01	5.21
Chewiness	34.05	39.20	39.80	41.03	34.45	$\bar{y} = 37.7$	0.1710	0.7308	0.0211	1.15	13.6

Means in the same row, followed by different letters, differ ($P < 0.05$) by *t* test

[1]*WCS* whole cottonseed levels, *L* linear tendency, *Q* quadratic tendency

[2]*SEM* standard error mean

[3]CV (%) = coefficient of variation

(1)$\hat{y}_{cooking\ yield} = 84.69 + 0.12\ WCS\ (r^2 = 0.61)$; (2)$\hat{y}_{cooking\ loss} = 15.31 - 0.12\ WCS\ (r^2 = 0.61)$; (3)$\hat{y}_{moisture\ retention} = 53.54 + 0.11\ WCS\ (r^2 = 0.91)$

texture profile analysis (Table 3) corroborated with the aforementioned structural conditions in the hamburgers prepared with meat from animals fed with 10, 20, 30 and 40% WCS.

Comparing the results for chemical composition, texture and cooking characteristics with the microscopy results, it was concluded that there was a relationship between the properties and their respective microscopic structures. Although the values for protein (Table 2), total loss (Table 3) and moisture retention (Table 3) of the hamburgers presented a significant difference ($P < 0.05$), the attributes evaluated in the TPA of the product were not affected ($P > 0.05$) and were closely related to the microscopic characteristics observed in the histological evaluation (Fig. 1).

Microbiological evaluation

The hamburgers that were analysed on days 1 and 45 were within the norms of quality control set out in Brazilian legislation (Brasil 2001), which establishes the absence of *Salmonella* in 25 g of sample and a maximum limit of 3.48 log CFU/g for *Clostridium* sulfite reducer, 3.7 log CFU/g for coagulase positive *Staphylococcus*, and 3.7 log CFU/g for coliforms at 45 °C (results not shown). All the formulations were considered safe and suitable for human consumption.

Sensory analysis

At 15 days of storage (Table 4), a difference ($P < 0.05$) was observed only for the attribute of odour. Considering a minimum score of 5 (like moderately) for acceptable odour, the regression equation revealed a maximum limit of the addition of 16.7% WCS ($\hat{y}_{odour} = 5.24 - 0.01\ WCS, r^2 = 0.94$).

At 60 days of storage there was no difference ($P > 0.05$) between the hamburger formulations regarding odour; however, even without the inclusion of whole cottonseed in the animals' diet (0% WCS) odour received an 'indifferent' score (4.5). The scores for all the other attributes (colour, taste, texture, overall impression and purchase intention) decreased ($P < 0.05$) with increased levels of whole cottonseed in the diet, i.e. they worsened. According to the regression equation, these attributes would be acceptable (5 = like moderately) up to the maximum addition limits of 22.3, 29.0, 2.9 and 16.7% WCS for colour, flavour, texture and appearance, respectively. The purchase intention was rated as 'indifferent' for all the hamburger formulations.

All the values found for the acceptance test on both days (15 and 60) of the sensory analysis of hamburgers made with lamb meat from animals fed with different levels of whole cottonseed were higher than those reported by Villalobos-Delgado et al. (2015), who evaluated lamb hamburgers with added hops, which resulted in judgements of 'dislike slightly' or 'indifferent'.

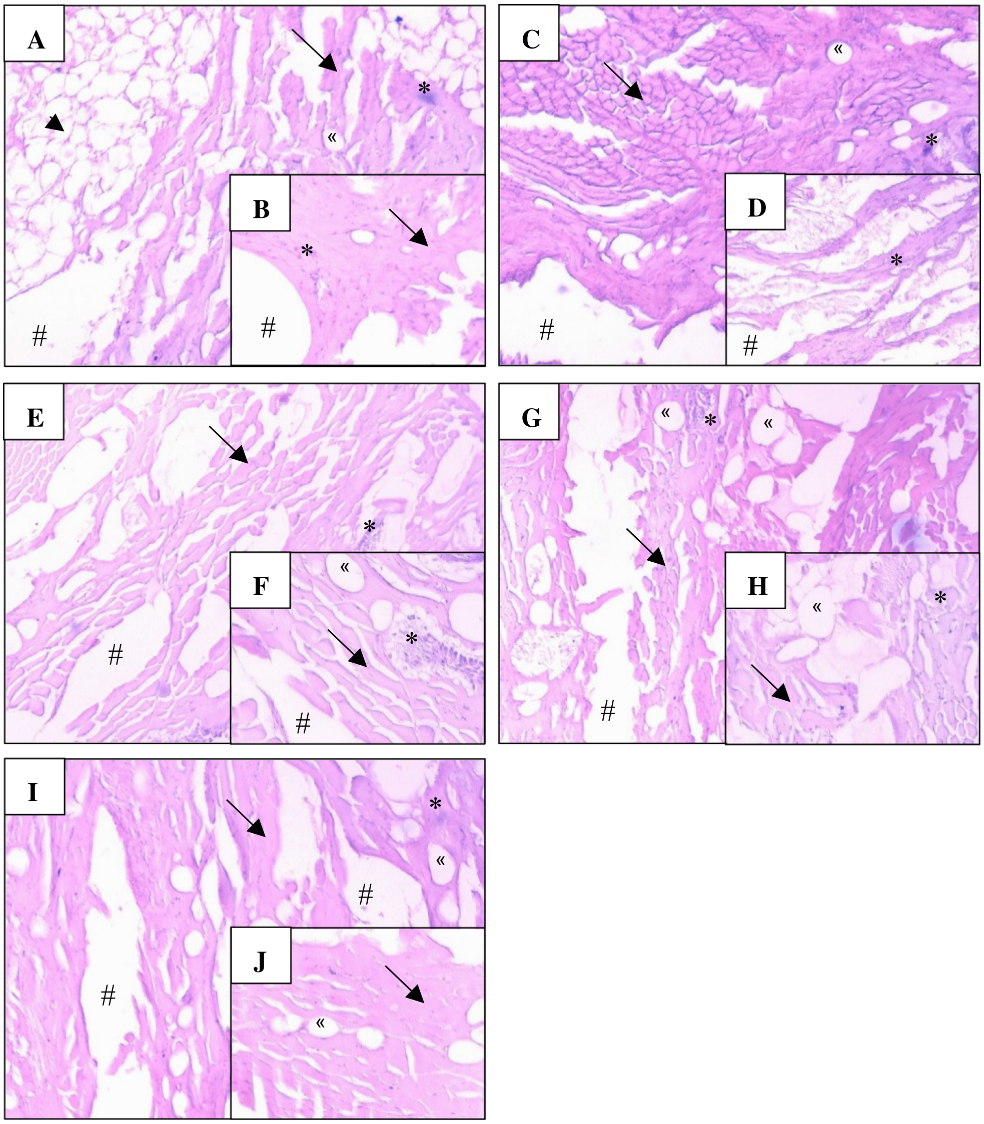

Fig. 1 Photomicrographs of lamb burger from animals fed on different levels of whole cottonseed. **a**, **b** Correspond to lamb burger 0% WCS. Note the disruption of muscle cells between the extracellular matrix, the presence of clusters of adipose cells, connective tissue fibres and large blisters of air bubbles. **c**, **d** Correspond to lamb burger 10% WCS. Note the presence of clusters of small muscle cells, large extracellular matrix in the compact connective tissue and air bubbles. **e**, **f** Corresponds to lamb burger 20% WCS. Note well-organised and better-defined muscle cells, abundant extracellular matrix, interstitial air bubbles of various sizes and clusters of cells in the connective tissue. **g**, **h** Correspond to lamb burger 30% WCS. Observe muscle tissue, connective tissue and air bubbles distributed heterogeneously in the tissue and ample extracellular matrix. **i**, **j** Correspond to lamb burger 40% WCS. Shows the presence of extracellular matrix, well organised muscle tissue and few air bubbles. Muscle tissue (arrows); adipose cells (arrowheads); connective tissue (*); extracelular matrix (#), air bubbles (“). hematoxylin and eosin. 4× and 10×, respectively

Odour and flavour are considered to be the most important attributes in terms of the sensory evaluation of lamb meat and its derivatives (Linares et al. 2012). Therefore, it was interesting to evaluate if the use of whole cottonseed in the diet of the lambs would affect the sensory quality of the hamburgers prepared from this meat. Thus, the acceptability index (AI) was calculated only for odour and flavour because the other attributes had a lesser influence on the acceptability of the product in question. Considering that an acceptability level equal to or greater than 70% is considered acceptable (Dutcosky 2011), at 15 days of storage it was found that the minimum acceptance limit was reached with a maximum inclusion of 29.3% WCS for odour $(\hat{y}_{odour} = 75.79 - 0.20\ WCS, r^2 = 0.94)$ and 27.8% WCS for flavour $(\hat{y}_{flavour} = 73.61 - 0.13\ WCS, r^2 = 0.62)$. However, at 60 days of storage all the formulations were rejected for acceptance in terms of odour $(\bar{y}_{odour} = 66.1)$, while a minimum favourable acceptability was obtained with a maximum inclusion of up to 37.6% WCS in terms of

Table 4 Mean scores of the sensory analysis of hamburgers made with meat from lambs fed on different levels of whole cottonseed, after 15 and 60 days of storage at − 18 °C

Attributes[1]	Whole cottonseed (%)					Mean or equation	Probability value[2]			SEM[3]	CV[4]
	0	10	20	30	40		WCS	L	Q		
Day 15											
Odour	5.2[a]	5.1[a]	5.0[ab]	4.8[bc]	4.6[c]	(1)	0.0044	0.0002	0.4928	0.02	12.73
Colour	4.6	4.8	4.6	4.7	4.6	$\bar{y} = 4.7$	0.7838	0.6924	0.5338	0.02	13.37
Flavour	5.0	4.9	4.9	4.9	4.6	$\bar{y} = 4.9$	0.0956	0.0269	0.2661	0.02	15.22
Texture	4.8	4.9	4.5	4.9	4.8	$\bar{y} = 4.8$	0.1132	0.6666	0.4380	0.02	13.77
Appearance	4.8	4.8	4.7	4.7	4.9	$\bar{y} = 4.8$	0.8280	0.8234	0.5131	0.02	12.38
Purchase intent	3.4	3.5	3.4	3.2	3.2	$\bar{y} = 3.4$	0.1481	0.0338	0.2466	0.02	18.49
Day 60											
Odour	4.4	4.6	4.6	4.4	4.5	$\bar{y} = 4.5$	0.3753	0.7034	0.2730	0.02	15.60
Colour	5.5[a]	5.3[a]	5.2[a]	4.4[c]	4.8[b]	(2)	0.0001	0.0001	0.2418	0.02	14.14
Flavour	5.7[a]	5.3[ab]	5.5[a]	4.5[c]	5.0[b]	(3)	0.0001	0.0001	0.2226	0.02	13.12
Texture	4.8[ab]	5.1[a]	5.1[a]	4.7[b]	4.7[b]	(4)	0.0067	0.0528	0.0195	0.02	14.58
Appearance	5.1[ab]	5.1[ab]	5.4[a]	4.3[c]	4.9[b]	(5)	0.0001	0.0002	0.7001	0.02	12.75
Purchase intent	3.7[ab]	3.8[ab]	3.9[a]	3.0[c]	3.5[b]	(6)	0.0001	0.0001	0.8902	0.02	16.17

Means in the same row, followed by different letters, differ significantly by *t* test ($P < 0.05$). Day 15 (n = 55), day 60 (n = 52)

[1]Scores for odour, colour, flavour, texture and appearance (1 = dislike intensely; 2 = dislike a lot; 3 = dislike moderately; 4 = indifferent; 5 = like moderately; 6 = like a lot; 7 = like intensely); scores for purchase intent (1 = would definitely not purchase, 2 = would probably not purchase, 3 = indifferent, 4 = would probably purchase, 5 = would definitely purchase)

[2]*WCS* whole cottonseed levels, *L* linear tendency, *Q* quadratic tendency

[3]*SEM* Standard error mean

[4]CV (%) = coefficient of variation

(1) $\hat{y}_{odour} = 5.24 - 0.01\ WCS\ (r^2 = 0.94)$; (2) $\hat{y}_{colour} = 5.51 - 0.02\ WCS\ (r^2 = 0.66)$; (3) $\hat{y}_{flavour} = 5.63 - 0.02\ WCS\ (r^2 = 0.54)$; (4) $\hat{y}_{texture} = 5.02 - 0.01\ WCS\ (r^2 = 0.25)$; (5) $\hat{y}_{appearance} = 5.22 - 0.01\ WCS\ (r^2 = 0.27)$; (6) $\hat{y}_{purchaseintent} = 3.79 - 0.01\ WCS\ (r^2 = 0.29)$

flavour ($\hat{y}_{flavour} = 80.55 - 0.28\ WCS, r^2 = 0.53$). This demonstrates that the storage time may have influenced the testers in their perception of odour but it did not affect their perception of flavour.

In addition to the affective tests, a discriminatory test was carried out which compared each hamburger formulation prepared with lamb meat from animals fed with whole cottonseed (10, 20, 30 and 40% WCS) and the formulation prepared with lamb from animals with no whole cottonseed in their diet (0% WCS). At 15 days of storage it was found that the testers were able to detect the hamburgers that had been prepared with meat from animals fed on whole cottonseed (Table 5). However, at 60 days of storage, the testers were only able to detect hamburgers made meat from animals fed on whole cottonseed with more than 20% WCS in their diet. Perhaps the natural oxidation of the product during storage may have influenced the sensory perception of the testers.

Table 5 Number of correct judgments in the triangle test of hamburgers made with meat from lambs fed on different levels of whole cottonseed, after 15 and 60 days of storage at − 18 °C

Triangle test	Whole cottonseed (%)			
	10	20	30	40
Day 15	23	19	17	23
Day 60	15	17	21	19

Minimum number of correct judgments to establish significance at various levels of probability: 19 (5%), for day 15 (n = 40) and day 60 (n = 40)

Conclusion

The use of lamb meat from animals fed with whole cottonseed in their diet to prepare hamburgers positively influenced the chemical composition, cooking characteristics (decreased cooking loss, and increased cooking yield and moisture retention) and structural properties (increased homogeneity and cohesion), as well as negatively influencing the sensory characteristics of the product, reducing its acceptability with increasing levels of whole cottonseed.

In order to guarantee the sensory acceptability of such a product (based on this appearance) it is recommended that a maximum level of 16.7% of whole cottonseed must be included in the dry matter diet of lambs.

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