

Is it possible to produce meatballs made with lamb from animals fed on whole cottonseed without altering the sensory characteristics?

¹*Monego, M. A., ¹Pellegrini, L. G., ²Pellegrini, L. G., ³Roman, S. S., ¹Kubota, E. H., ¹Prestes, R. C. and ¹Mello, R. O.

¹Department of Science and Food Technology, Centre for Rural Sciences, Federal University of Santa Maria – UFSM, Rio Grande do Sul, Brazil

²Federal Institute Farroupilha. CEP 98130-000. Júlio de Castilhos, Rio Grande do Sul, Brazil

³Regional Integrated University of High Uruguay and Missions, Department of Health Sciences, Avenue 7 de Setembro, 1621, 99700-000, Erechim RS, Brazil

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Abstract

The aim of this study was to evaluate the chemical composition and the technological and sensory properties of lamb meatballs made with meat from animals fed with different levels of whole cottonseed (0, 10, 20, 30 and 40%). The meatballs prepared with lamb from animals fed on whole cottonseed presented higher ($P < 0.05$) ash content. All the meatball formulations presented low levels of lipids (2.90%) and cholesterol (74.15 mg/100g), as well as high protein content (18.92%). The difference ($P < 0.05$) observed in the variables of cooking characteristics did not have an effect on the texture profile, which was related to the microscopic observations of the product. In the triangle test for difference, the testers did not perceive difference between the control meatballs (0%) and those with up to 30% of whole cottonseed. However, in the acceptance test, the testers detected sensory alteration starting at a level of 12.5% of whole cottonseed. Thus, it is possible to produce meatballs with meat from lambs fed on up to 12.5% of whole cottonseed in the dry matter of their diet without altering the sensory characteristics of the product.

Keywords

Texture profile analysis
Microscopy
Triangle test
Lamb meat products.

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Introduction

Whole cottonseed is widely produced in Brazil and worldwide. In the period 2015-2016, world production was 21 million tons and the largest producer was India (5.7 million tons) (ICAC, 2016). Brazil occupied fifth place worldwide in terms of production (1.9 million tons). The main producing states are Mato Grosso and Bahia, which are responsible for about 57.4% and 28.8% of national production, respectively (Brasil, 2015).

Whole cottonseed, which is an oilseed, is a by-product of the processing and/or ginning of cotton, both of which are intended to separate the fibres. Whole cottonseed is characterised by high levels of protein, lipids and crude fibre (NRC, 2007); it is considered to be a good protein and energy supplement and it can be used to prepare animal feed because it is a good source of quality protein (EMBRAPA, 2003; Madruga *et al.*, 2008; Paim *et al.*, 2010).

However, the use of whole cottonseed in animal diets is limited because of its possible association with undesirable characteristics in meat. Several studies in the literature have evaluated the effect of whole cottonseed on carcass performance and the

quality of lamb meat (Madruga *et al.*, 2008; Paim *et al.*, 2010; Vieira *et al.*, 2010; Piona *et al.*, 2012; Oliveira *et al.*, 2013; Viana *et al.*, 2014, Rufino Junior *et al.*, 2015; Pellegrini, 2017). These studies have demonstrated the feasibility of using this ingredient in different concentrations (10-40%) in the diet of lambs and for short periods (60 to 90 days) of confinement. However, no studies were found that evaluated the performance of this meat during processing, or the impact on the sensory quality of products. Consequently, this article investigates these issues.

Meat products, such as meatballs, are popular and convenient foods because they are economical, easy to prepare, and also because they contain nutrients that satisfy hunger rapidly, which is in line with current lifestyles (Borba *et al.*, 2013). Brazilian legislation defines meatballs as a processed meat product obtained from the ground meat from one or more species of slaughtered animals. Meatballs are moulded into a rounded form together with additional ingredients and are then subjected to an appropriate technological process; they are sold raw, semi-cooked, fried, cooked or sterilised (Brasil, 2000).

Considering the scarcity of data regarding the

*Corresponding author.
Email: magdaamonego@gmail.com

characteristics of meat products made from meat from lambs fed with whole cottonseed, the present study was designed to evaluate the chemical composition, as well as the technological and sensory properties, of meatballs made with lamb from animals fed with different levels (0, 10, 20, 30 and 40%) of whole cottonseed (WCS).

Materials and Methods

Obtaining the meat

The lamb meat that was used for the experiments was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal Institute of Education, Science and Technology Farroupilha, in the city of Júlio de Castilhos, Rio Grande do Sul, Brazil under Protocol No. 01.0378.2015/001.2015.

The animals that were used were twenty, male, uncastrated, lambs of the Ile de France breed, which had been weaned at 60 days. The animals were finished in confinement in individual, fully covered stalls, with a slatted floor and approximately 2 m² of area. The animals were provided with drinking fountains and feeders.

The lambs were divided into five experimental treatments, with four replicates. Each treatment consisted of the base experimental diet, to which whole cottonseed (*Gossypium hirsutum* L.) was added in proportions of 0, 10, 20, 30 and 40% of WCS in dry matter, respectively. The experimental diet (base) was composed of corn silage (*Zea mays* L.), ground corn (*Zea mays* L.) and soybean meal (*Glycinemax* L.), in a concentrated voluminous ratio (40:60) and mineral mix, as well as an isoproteic in order to meet the nutritional requirements of growing lambs (NRC, 2007).

Feeding was ad libitum, twice a day, at the pre-set times of 7.00 a.m. and 4.00 p.m., with the quantity being adjusted to maintain leftovers at approximately 10% of the total that was offered. The feeding trial of the lambs started after the adaptation period (14 days) and continued until the moment of slaughter. A final live weight of 36 kg was established as the slaughter criterion because the confinement times were different due to the difference in daily weight gain (mean confinement time was 64 days). Prior to being slaughtered, the animals were stunned using a pneumatic gun, followed by bleeding, skinning, evisceration, weighing, washing and cooling. The carcasses were cooled at 2°C for 24 hours. The legs were subsequently collected, packed and frozen in a conventional freezer (Metalfrío, São Paulo, SP, Brasil), at -18°C, until the preparation of the meatballs.

Preparation of meatballs

The meatballs were prepared in accordance with the Technical Regulation of Identity and Quality of Meatballs (Brasil, 2000) and Ordinance No. 1004 (Brasil, 1998). The base formulation (common to all the formulations) was composed of the following: water (8%); corn starch (4%), (Yoki General Mills, São Bernardo do Campo, SP, Brasil); textured soy protein (2%), (Solae, Esteio, RS, Brasil); concentrated soy protein (2%), (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%), (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); smoke powder (0.04%) (ICL, São Paulo, SP, Brasil); and red pepper (0.038%), (Kitano General Mills, São Bernardo do Campo, SP, Brasil). Five formulations of meatballs were developed, in which lamb meat (80.21%) derived from animals fed on the respective diets (0, 10, 20, 30 and 40% of WCS) was added to each formulation, giving rise to five different meatball formulations.

For the preparation of the meatballs, leg of lamb was used, which had been previously thawed under refrigeration (4°C) (Electrolux, Curitiba, PR, Brasil), removing the excess fat. The meat was subsequently ground (Jamar PJ22, Jamar Ltda, São Paulo, SP, Brasil) using discs with a 5 mm hole and taken to a mixer (Jamar MJI 35, Jamar Ltda, São Paulo, SP, Brasil) to add the other ingredients. The mixing process lasted approximately 15 minutes and the temperature of the mixture did not exceed 2°C.

After a homogeneous mixture was obtained, the meatballs were manually moulded in a spherical format with a ± 3 cm diameter (approximately 30 g each), placed in aluminum disposable trays, covered and immediately frozen in a conventional freezer (Metalfrío, São Paulo, SP, Brasil), at -18°C, where they were maintained until analysis.

Physicochemical characterisation

The lamb meat and meatballs were characterised as raw, in triplicate, by the determination of humidity (indirect gravimetric method at 105°C), protein (micro Kjeldahl method), ash (method of incineration in muffle at 550°C), according to the AOAC (2005), and lipids (lipid separation method

with hexane:isopropyl alcohol) according to Hara and Hadin's (1978) methodology. For the meatballs, the carbohydrates were determined by the difference between the other analysed fractions (AOAC, 2005).

The calorific value of the meatballs was calculated by summing the calories provided by carbohydrates, proteins and lipids and by multiplying their values in grams by the Atwater factors 4 kcal, and 4 kcal and 9 kcal, respectively.

Cholesterol was determined in the raw meat and meatballs by the enzymatic method (Saldanha *et al.*, 2004) in duplicate.

To assess the extent of lipid oxidation that occurred in the meatballs, the thiobarbituric acid (TBA) index was used, following the methodology of Raharjo, Sofos, and Schmidt (1992). The analyses were performed in duplicate and the results were expressed as mg of malonaldehyde per kg of sample (MDA mg/kg sample).

The evaluation of pH was performed using a pH meter (pH metro, model DM-23DC São Paulo, Brazil) in accordance with the IAL (2008), with readings performed in triplicate.

The water activity values were determined using Aqualab® (Water Active Meter, version 8, Decagon Devices, Inc., Pullman, Wa, USA) at 25°C, in triplicate.

Histological evaluation

For the histological evaluation, three fragments of each meatball formulation were collected from the centre of the raw samples and they were processed according to the conventional histological technique (Junqueira and Carneiro, 2008). The raw 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 captured with the aid of Motic Images Plus 2.0 software (Motic instruments, INC, Richmond, Canada). The microscopy was performed at the Histology Laboratory of the Pharmacy Department at the Integrated Regional University of Alto Uruguay and the Missions (URI) in Erechim, RS, Brazil.

Cooking characteristics

The meatballs used in the analysis of cooking characteristics, texture profile and sensory analysis 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 analyses of the cooking characteristics were performed in four meatballs from each formulation and the yield was

calculated by the ratio of the weight of cooked meatball to the raw meatball weight, which was expressed as a percentage. The data regarding moisture retention (Eq. 1), fat retention (Eq. 2), shrinkage (Eq. 3) and losses (total, exudate and evaporation) (Eq. 4, Eq. 5 and Eq. 6, respectively) were obtained as described by Gök *et al.* (2011):

$$\text{Moisture retention (\%)} = \frac{\text{weight}_{\text{cooked}} \times \% \text{moisture}_{\text{cooked meatball}}}{\text{weight}_{\text{raw}} \times \% \text{moisture}_{\text{raw meatball}}} \times 100 \quad (1)$$

$$\text{Fat retention (\%)} = \frac{\text{weight}_{\text{cooked}} \times \% \text{fat}_{\text{cooked meatball}}}{\text{weight}_{\text{raw}} \times \% \text{fat}_{\text{raw meatball}}} \times 100 \quad (2)$$

$$\text{Shrinkage (\%)} = \frac{\text{volume}_{\text{raw meatball}} - \text{volume}_{\text{cooked meatball}}}{\text{volume}_{\text{raw meatball}}} \times 100 \quad (3)$$

$$\text{Cooking loss (\%)} = \frac{\text{weight}_{\text{raw}} - \text{weight}_{\text{cooked}}}{\text{weight}_{\text{raw}}} \times 100 \quad (4)$$

$$\text{Exudate loss (\%)} = \frac{\text{weight}_{\text{tray exudate}} - \text{weight}_{\text{tray}}}{\text{cooking loss}} \times 100 \quad (5)$$

$$\text{Evaporative loss (\%)} = \frac{\text{cooking loss} - \text{exudate loss}}{\text{cooking loss}} \times 100 \quad (6)$$

Texture profile analysis

The texture profile analysis was performed using a TA-XT.plus Texture Analyzer, equipped with Texture Expert Exponent Software (Stable Microsystems Ltd., Surrey, England). To perform the analysis, after being cooked according to the cooking procedure described above and cooled to room temperature, the meatball samples were cubed (1 cm³). Four meatballs from each formulation were analysed and 10 cubes were removed from each unit. Each cube was tested under the following conditions: test speed of 5 mm/s; pre-test speed of 1 mm/s; return speed of 5 mm/s; return distance of 20 mm; contact force of 1 g; compression height of 50%; and interval between compressions of 5 s. The texture profile was evaluated based on the characteristics of hardness, cohesiveness, springiness and chewiness (Bourne, 1978).

Microbiological evaluation

In order to evaluate the microbiological quality of the meatballs, the following analyses were performed: *Salmonella* spp., *Clostridium botulinum*, *Staphylococcus aureus* and total coliforms and coliforms at 45°C (*Escherichia coli*), in accordance with Normative Instruction No. 62 (Brasil, 2003) and following the standards recommended by ANVISA (Brasil, 2001). The analyses were performed on the raw meatballs on day one.

Sensory analysis

This study was previously approved by the Research Ethics Committee of the Federal University of Santa Maria under protocol No. 934.222 and Certificate of Presentation for Ethical Assessment, CAAE No. 40246214.3.0000.5346. Sensory evaluation was performed on day 15, after confirmation of the microbiological safety of the products.

The samples offered to the testers were prepared according to the aforementioned cooking procedures. After cooking, the meatballs were wrapped separately in foil and kept at 60°C until serving. The meatballs were served in plastic cups in portions of 30 g with approximately 20 g of traditional tomato sauce (Predilecta®; ingredients: tomato, tomato pulp, salt, starch, sugar, soybean oil, onion, parsley flakes, garlic powder, monosodium glutamate flavour enhancer and potassium sorbate conservative, with an energy value of 22 Kcal per 60 g portion). The meatballs were heated to 60°C.

Based on the criterion that they liked lamb meat, untrained testers were recruited to perform triangle (40 testers) and affective (50 testers) tests (IAL, 2008).

The triangle test was performed in order to detect if the whole cottonseed used in the animals' diets had an effect on the flavour in the products and at what level the testers perceived this differentiated flavor when compared to the control, which was developed with meat from lambs fed on a diet without whole cottonseed. Thus, the triangular test was used to compare each formulation of meatball made with meat from lambs fed with whole cottonseed (10, 20, 30 and 40% of WCS) versus the formulation elaborated with the control formulation (0% WCS).

The interpretation of the results was based on the relationship between the total numbers of judgments versus the number of correct judgments compared to the result of the chi-square table (IAL, 2008). The results of the number of correct judgments greater than or equal to the table value made it possible to conclude that there was a significant difference between the samples at the corresponding probability level (5%). Together with the sensory scoresheet from the triangle test, three coded samples were simultaneously presented to the testers, two identical and one different; and the testers were asked to discriminate the sample(s) that seemed to be different.

The affective test was applied by using a hedonic scale acceptance test, in which the attributes of odour, colour, flavour, texture and appearance were evaluated using a seven-point scale (1 = dislike intensely, 7 = like intensely) and a five-point purchase intent test (1

= would definitely purchase, 5 = would definitely not purchase). For this test, the samples were offered to the testers in a monodic and random fashion, along with the sensory scoresheet of the acceptance test and the purchase intent test (IAL, 2008). From the scores assigned in the acceptance test, the acceptability index (AI) of the product was calculated according to the equation: $AI (\%) = A \times 100/B$, where A = average score obtained for the product and B = maximum score given to the product. In this context, $AI \geq 70\%$ was considered to be a good score (Dutcosky, 2011).

Statistical analysis

For the physicochemical analysis of the fresh meat and the meatballs, the statistical analyses were performed using a completely randomised experimental design with five treatments (0, 10, 20, 30 and 40% of WCS) and four repetitions, 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 WCS and j -th repetition; μ = the overall mean of the response variable; α_i = the fixed effect of the i -th level of WCS; ε_{ij} = the random effect associated with observation Y_{ij} , presupposing $\varepsilon_{ij} \sim N(0, \sigma^2)$.

The sensory analysis data were analysed in a randomised block design for storage time (15 days).

The data were subjected to outlier investigation from the studied residues. They were subsequently submitted to univariate analysis of variance (ANOVA) by the GLM procedure. The means were adjusted by the ordinary least squares method with the LSMEANS command and compared by the least significant difference, by 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 regression was adjusted using the RSREG procedure; the r^2 values were expressed in relation to the source treatments (regression + lack of fit). Furthermore, Spearman's partial correlation analysis was performed between the studied variables, with the levels of whole cottonseed being used as covariate.

The statistical analyses were performed using SAS® System for Windows™ version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Physicochemical characterisation of lamb meat

The addition of whole cottonseed to the diets of the lambs changed ($P < 0.05$) the moisture content

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

Variable ¹	Whole cottonseed %					Mean or Equation	Probability value ¹			SEM ²	CV ³
	0	10	20	30	40		WCS	L	Q		
Moisture, %	76.65 ^{ab}	75.17 ^b	74.99 ^b	76.69 ^{ab}	77.42 ^a	(1)	0.0496	0.1308	0.0137	0.32	1.90
Ash, %	1.05	1.14	1.22	1.11	1.19	$\bar{y} = 1.15$	0.3543	0.2187	0.3161	0.03	10.15
Protein, %	18.76	19.90	20.69	18.91	18.35	$\bar{y} = 19.32$	0.1257	0.3953	0.0268	0.33	7.53
Lipids, %	3.32	3.79	3.10	3.29	3.06	$\bar{y} = 3.31$	0.4453	0.2845	0.6379	0.13	17.78
Cholesterol, mg/100g	93.73	85.58	100.02	100.81	92.52	$\bar{y} = 94.20$	0.0681	0.2863	0.3234	1.95	9.02
TBARS, *	0.67 ^a	0.43 ^{bc}	0.39 ^c	0.38 ^c	0.51 ^b	(2)	0.0002	0.0036	0.0001	0.03	26.73
pH	5.68	5.62	5.62	5.58	5.60	$\bar{y} = 5.62$	0.1787	0.0417	0.2765	0.01	1.03
Water activity	0.9875	0.9874	0.9876	0.9877	0.9873	$\bar{y} = 0.9875$	0.9344	0.9006	0.6585	0.01	0.06

Means in the same line, followed by different letters, differ significantly by t-test ($P < 0.05$). (n=3).

*TBARS= thiobarbituric acid reactive substances (mg MDA/Kg).

¹WCS= whole cottonseed levels; L= linear tendency; Q= quadratic tendency.

²SEM= standard error mean.

³CV (%)= coefficient of variation.

⁽¹⁾ $\hat{y}_{\text{moisture}} = 76.47 - 0.15WCS + 0.004WCS^2$ ($r^2 = 0.85$); ⁽²⁾ $\hat{y}_{\text{TBARS}} = 0.66 - 0.03WCS + 0.0006WCS^2$ ($r^2 = 0.97$).

and the TBARS of the meat (Table 1). The moisture levels in the meat were lowest ($P < 0.05$) with the inclusion of 19% of WCS in the diet of the lambs ($\hat{y}_{\text{moisture}} = 76.47 - 0.15WCS + 0.004WCS^2$; ($r^2 = 0.85$)). The TBARS values displayed quadratic behaviour ($P < 0.05$), with a minimum point of 25% of WCS. The decrease in the TBARS values up to 25% of WCS can be explained by the fact that phenolic compounds, tocopherols (Oliveira *et al.*, 2016) and gossypol (Wang *et al.*, 2008; Wang *et al.*, 2009), which are present in whole cottonseed, when deposited in lamb meat (Kim *et al.*, 1996) reduced lipid oxidation (Wang *et al.*, 2008). However, the increase in TBARS values from 25% of 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. This explains the higher TBARS values found for meatballs made with lamb from animals fed with 40% of WCS. Brazilian legislation does not establish a maximum limit of malonaldehyde/kg in meat products. However, data from the literature indicate that values of up to 2.00 mg of MDA /kg of sample are not perceived by consumers (Wood *et al.*, 2004). Thus, the lamb meat used to prepare the meatballs in the present study contained physicochemical conditions that were suitable for processing.

Chemical composition of the meatballs

The meatballs made with lamb from animals fed on diets containing whole cottonseed presented higher ($P < 0.05$) levels of ash content (Table 2). This was because the consumption of whole cottonseed resulted in increased levels of minerals in the diets (Pellegrini, 2017) and, consequently, an increased

deposit of minerals in the meat of the lambs (Paim *et al.*, 2014) and its derivatives. In the present study, the ash content in the meat from lamb fed on whole cottonseed was higher, but not significantly (Table 1).

The results regarding the chemical composition of the meatballs showed that all the formulations were in accordance with the Identity and Quality Standard for Products (Brasil, 2000), which establishes a minimum value of 12% for protein and maximum values of 18% for fat and 10% for total carbohydrates.

The results regarding proteins were similar to those reported by Linares *et al.* (2012) in hamburger made from lamb meat, when prepared with leg (19.56%) or leg + neck + breast (17.23%), and higher than those found in reduced-fat lamb meat nuggets (14.32%) (Gadekar *et al.*, 2016).

The meatballs made with lamb meat from animals fed with whole cottonseed had a lipid content (2.90%) lower than those reported in the literature for lamb meatballs (10.5%), lamb pâté (23.79%) and lamb nuggets (9.47 to 14.32%) (Kitano and Prestes, 2012; Amaral *et al.*, 2015; Gadekar *et al.*, 2016). These differences were specifically attributed to the type of cut used in the preparation of the product, as well as the addition (or not) of fat to the formulation and the method of preparation (roasted or fried). Pre-fried and fried lamb nuggets had a mean lipid content of 12.75% and 17%, respectively (Medina *et al.*, 2015).

The cholesterol content (74.15 mg/100g) found in the meatball formulations was similar to that found by Madruga *et al.* (2008) in meat from Santa Inês lambs fed with different levels of whole cottonseed. The levels reported in the aforementioned study varied from 80.60 mg/100g in animals fed on the control diet (0% of WCS) to 77.96mg/100g in the animals fed with 40% of WCS, which were considered as

Table 2. Chemical composition of lamb meatballs from animals 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, %	70.50	70.00	70.19	70.05	70.30	$\bar{y} = 70.21$	0.1176	0.4319	0.0456	0.07	0.43
Ash, %	3.39 ^c	3.50 ^a	3.40 ^{bc}	3.48 ^a	3.47 ^{ab}	(1)	0.0210	0.0876	0.5566	0.01	1.79
Protein, %	18.60	19.28	19.12	19.10	18.55	$\bar{y} = 18.92$	0.0578	0.6735	0.0077	0.11	2.42
Lipids, %	3.15	3.00	2.64	2.85	2.88	$\bar{y} = 2.90$	0.5102	0.2981	0.2457	0.09	14.04
Carbohydrates, %	4.37	4.23	5.05	4.53	4.81	$\bar{y} = 4.60$	0.4148	0.2702	0.6775	0.15	14.19
Caloric value, Kcal/100g	120.22	121.04	118.49	120.17	119.32	$\bar{y} = 119.92$	0.7937	0.5562	0.8748	0.60	2.19
Cholesterol, mg/100g	70.71	76.77	74.08	72.42	76.77	$\bar{y} = 74.15$	0.8213	0.5804	0.8859	0.07	0.43

Means in the same line, followed by different letters differ significantly by t-test ($P < 0.05$). (n=3).

¹WCS= whole cottonseed levels; L= linear tendency; Q= quadratic tendency.

²SEM= standard error mean.

³CV (%)= coefficient of variation.

⁽¹⁾ $\hat{y}_{ashes} = 3.42 - 0.001WCS$ ($r^2 = 0.21$).

low (<90 mg/100g). The aforementioned results were similar to those found for ostrich meat (filet, 75.39 mg/100g) and chicken (thigh and leg, 75.94 mg/100g), but were higher than those found for pork (leg, 56.97 mg/100g) and beef (rump, 60.96 mg/100g) (Hautrive *et al.*, 2012).

The formulations of meatballs made with lamb meat from animals fed with whole cottonseed presented a nutritional quality that was compatible with the current trends for meat products with reduced fat and cholesterol levels (Barbut *et al.*, 2016).

Histological evaluation

Histological evaluation using light microscopy has been widely used to evaluate the microstructure of meat products (Vidal *et al.* 2014; Londero *et al.*, 2015; Oliveira *et al.*, 2015; Abdel-Naem *et al.*, 2016; Barbut *et al.*, 2016), making it possible to relate structural properties to results regarding cooking characteristics and texture profile of products.

By using microscopic images, it was possible to observe the distribution of the muscular, adipose and connective tissues, as well as the presence of intracellular edema, rupture of the cells, disorganisation and binding of the tissues. There was a histological difference between the formulations. The control formulation (0% of WCS) presented a wide disruption of the muscular and connective tissues, as well as the presence of clusters of adipose cells and extracellular space (A and B, Fig. 1). Such characteristics reflected the disorganisation of the microscopic structures of this formulation, evidencing the absence of tissue binding.

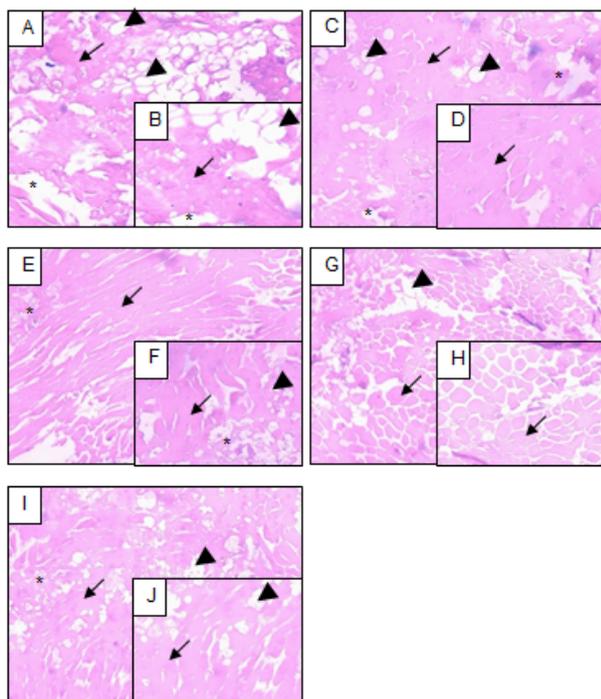
The formulations containing 10% (C and D), 20% (E and F), 30% (G and H) and 40% (I and J, Fig. 1) of WCS had structures that were similar to

each other, showing well-defined muscle cells, which were associated with well-organised connective tissue and well-distributed adipose tissue cells. Such characteristics guarantee the organisation of muscular tissues with connective and adipose tissues, demonstrating adequate tissue attachment and providing better technological properties for products.

Cooking characteristics and texture profile analysis

The cooking yield of the meatballs was affected ($P < 0.05$) by the levels of whole cottonseed in the animals' diet (Table 3). The worst performance was in relation to the formulation containing 17% of WCS, which was represented by the lowest ($P < 0.05$) cooking yield, lowest ($P < 0.05$) moisture retention and, consequently, the highest ($P < 0.05$) cooking loss and highest ($P < 0.05$) shrinkage.

The cooking characteristics of a product make it possible to verify the influence of the ingredients of a formulation, as well as the steps of the process. With the exception of the lamb meat (from animals fed with 0, 10, 20, 30 and 40% of WCS), all the meatball formulations received the same amount of ingredients. Furthermore, the order of the addition of the ingredients, and the time and temperature of the mixtures, were standardised in order to obtain the solubilisation of the myofibrillar proteins to provide a stable meat emulsion (Gaddekar *et al.*, 2016). Consequently, it was hard to understand the difference found in relation to the variables of cooking characteristics. The retention of moisture and fat in the food matrix have a direct influence on the texture and sensory properties of products because moisture confers juiciness, and fat, in addition to conferring juiciness, promotes flavour, texture, softness and



Detail (A,B) corresponding to lamb meatball with 0% of whole cottonseed (WCS). Note the disruption of muscle cells and connective tissue, showing the absence of ligation between interstitial tissues. Also note a clump of fat cells in some areas. Detail (C,D) corresponding to lamb meatball with 10% of WCS. Note the organisation of muscular tissues with the connective and adipose tissues. Observe the distribution of adipocytes in different tissue areas. Detail (E, F) corresponding to lamb meatball with 20% of WCS. Note the well-defined muscle cells, alongside well-organised connective tissue and well-distributed adipose cells along the tissue. Detail (G,H) corresponding to lamb meatball with 30% of WCS. Observe the small muscle cells with extracellular space in the connective tissue. Detail (I,J) corresponding to lamb meatball with 40% of WCS. Note that the muscle cells and connective tissue are well-defined and grouped together. Observe the well-distributed adipose tissue along the tissue areas. Muscle tissue (arrows); connective tissue (*); adipose cells (arrowheads). Hematoxylin and eosin. 4x and 10x, respectively.

Figure 1. Photomicrographs of lamb meatballs from animals fed on different levels of whole cottonseed.

shine (Costa *et al.*, 2009).

The cohesiveness of the meatballs differed ($P < 0.05$) depending on the level of inclusion of whole cottonseed in the diet of the lambs. Cohesiveness provides the measure of how much the structure of a product supports compression; it is proportional to the strength of the internal bonds that make up the body of the product (the higher the cohesion value the more resistant the product) (Bourne, 2002). The highest value ($P < 0.05$) was found for the control formulation (0% of WCS).

The values for cohesiveness were high and correlated negatively ($r = -0.85$, $P < 0.01$) with the lipid contents of the meatballs. Youssef and Barbut (2011) and Gadekar *et al.* (2016) also reported a decrease in cohesiveness with increased lipid content. However,

the lipid content of meatballs did not differ ($P > 0.05$) in the present study (Table 2).

The texture profile of the meatballs showed low values for hardness, springiness and chewiness (Table 3) when compared to those found by Gutt *et al.* (2014) in a study of the freshness of chopped meat (hardness 50.8 N, cohesiveness 0.82, springiness 0.71, chewiness 30.6), and were therefore considered to be soft and mouthwatering products. The differences between these two studies can be attributed to the different species that were used (lamb and cattle) and possibly the age of the animals.

The differences in relation to cooking characteristics (Table 3) did not reflect a negative effect on the texture profile (Table 3) and the microscopic properties (Fig. 1), between which a certain association was observed. Such behaviour can be attributed to the main constituents of the meatballs (moisture, proteins and lipids), which did not differ ($P > 0.05$) according to the level of whole cottonseed (Table 2).

Microbiological evaluation

Brazilian legislation (Brasil, 2001) recommends 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 *Staphylococcus* coagulase and 3.7 log CFU/g for total coliforms at 45°C. All the formulations of lamb meatballs made with meat from animals fed with whole cottonseed (data not shown) were within these quality control standards established by current legislation and were considered to be safe for consumption.

Sensory analysis

A total of 90 testers participated in the sensory analysis; 54% were female and 46% were male. The testers were aged 18-30 (83%), 31-50 (12%) and over 50 (4%).

In the triangle test, 16, 14, 14 and 21 correct judgments were obtained for the meatball formulations containing 10, 20, 30 and 40% of WCS, respectively, when compared to the control (0% of WCS). Considering a significance level of 5%, it would be necessary to have at least 19 correct judgments (IAL, 2008) to deduce that the testers detected a difference between the formulations of meatballs made with meat from animals fed on whole cottonseed (10, 20, 30 and 40% of WCS) compared to the control formulation (0% of WCS). Thus, it can be concluded that the testers detected no difference between the control (0% of WCS) and the formulations made with lamb from animals fed on levels of up to 30% of WCS.

Table 3. Cooking characteristics and texture profile analyses of lamb meatballs made with meat from animals 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		
Cooking yield, %	88.87 ^a	85.28 ^b	86.65 ^b	88.51 ^a	88.64 ^a	(1)	0.0028	0.1698	0.0033	0.40	2.05
Cooking loss, %	11.14 ^b	14.72 ^a	13.35 ^a	11.50 ^b	11.37 ^b	(2)	0.0028	0.1698	0.0033	0.40	14.46
Exudation, %	1.69 ^a	1.64 ^{ab}	0.46 ^c	0.77 ^{bc}	1.77 ^a	(3)	0.0186	0.4617	0.0056	0.17	61.19
Evaporation, %	98.31 ^c	98.36 ^{bc}	99.54 ^a	99.24 ^{ab}	98.23 ^c	(4)	0.0186	0.4617	0.0056	0.17	0.77
Moisture retention, %	57.92 ^{ab}	53.86 ^c	56.02 ^b	58.90 ^a	58.21 ^a	(5)	0.0006	0.0195	0.0104	0.50	3.92
Fat retention, %	84.66	96.49	81.90	75.86	77.71	$\bar{y} = 83.32$	0.6599	0.3097	0.7732	4.45	23.89
Shrinkage, %	15.49 ^{ab}	17.65 ^a	21.61 ^a	16.87 ^a	7.89 ^b	(6)	0.0499	0.0987	0.0114	1.54	43.27
Hardness, N	28.91	27.97	34.33	35.68	34.30	$\bar{y} = 32.24$	0.3110	0.0772	0.6164	1.42	19.75
Cohesiveness	0.62 ^a	0.49 ^b	0.51 ^b	0.52 ^b	0.48 ^b	(7)	0.0001	0.0001	0.0084	0.01	10.52
Springiness	1.04	1.20	1.20	1.22	1.18	$\bar{y} = 1.17$	0.0996	0.0647	0.0503	0.02	9.13
Chewiness	18.46	16.86	21.31	22.50	20.64	$\bar{y} = 19.96$	0.5610	0.2401	0.7019	1.13	25.28

Means in the same line, followed by different letters, differ significantly by t-test ($P < 0.05$). Cooking characteristics (n=4), texture profile analyses (n=10).

¹WCS= whole cottonseed levels; L= linear tendency; Q= quadratic tendency.

²SEM= standard error mean.

³CV (%)= coefficient of variation.

⁽¹⁾ $\hat{y}_{\text{cooking yield}} = 88.16 - 0.2WCS + 0.006WCS^2$ ($r^2 = 0.54$), ⁽²⁾ $\hat{y}_{\text{cooking loss}} = 11.84 + 0.2WCS - 0.006WCS^2$ ($r^2 = 0.53$), ⁽³⁾ $\hat{y}_{\text{exudation}} = 1.88 - 0.11WCS + 0.003WCS^2$ ($r^2 = 0.71$), ⁽⁴⁾ $\hat{y}_{\text{evaporation}} = 98.12 + 0.11WCS - 0.03WCS^2$ ($r^2 = 0.71$), ⁽⁵⁾ $\hat{y}_{\text{moisture retention}} = 56.91 - 0.16WCS + 0.005WCS^2$ ($r^2 = 0.42$),

⁽⁶⁾ $\hat{y}_{\text{shrinkage}} = 14.67 + 0.72WCS - 0.02WCS^2$ ($r^2 = 0.93$), ⁽⁷⁾ $\hat{y}_{\text{cohesiveness}} = 0.59 - 0.07WCS + 0.0001WCS^2$ ($r^2 = 0.68$).

In the affective test, the testers found no difference ($P > 0.05$) between the formulations regarding colour and texture, which received the ratings of ‘indifferent’ and ‘like moderately’, respectively (Table 4). The attributes of odour, flavour and appearance of the meatballs were influenced ($P < 0.05$) by the levels of whole cottonseed, and these attributes obtained a favorable rating (5 = like moderately) in the concentrations up to 34.4, 12.5 and 14.2% of WCS, respectively.

However, the acceptability index calculated for these attributes was higher than 70% (70% odour, 74% flavour, 73% appearance) and the responses for all the attributes in the acceptance test was 5 (like moderately) for all the formulations. In the purchase intent test, the greater frequency was 4 (would probably purchase) up to 30% of WCS and 3 (indifferent) for up to 40% of WCS (data not shown).

Similar results were reported by Pellegrini (2017), who evaluated the sensory quality of lamb meat from animals fed on whole cottonseed (0, 100, 200, 300 and 400g Kg⁻¹). In the multiple comparison test the testers (8) could detect no difference ($P > 0.05$) between the flavor of meat from lambs fed with or without whole cottonseed. However, the testers rejected ($P < 0.05$) meat from animals fed with 23.5% of whole cottonseed in dry matter in the diet, proving that the addition of whole cottonseed in the animals’ diet increased the sensory rejection of lamb meat.

In general, products made from lamb meat have shown good acceptability, and consumer demand

is increasing. Kitano *et al.* (2012) obtained good acceptance for lamb meatballs, with 94% purchase intent. Amaral *et al.* (2015) reported good acceptance for lamb pâté stored for up to 30 days. Furthermore, lamb nuggets made from different cuts (Medina *et al.*, 2015) and reduced-fat lamb nuggets (control 10% fat, 7.5% fat and 2.5% inulin, 5% fat and 5% inulin) also obtained good sensory acceptance (Gaddekar *et al.*, 2016).

Thus, it can be stated that in the present study, the use of whole cottonseed in the animals’ diet compromised the sensory characteristics of the products. In addition, it was inferred that the flavoring used in the formulation of the meatballs and the tomato sauce used in the sensory evaluation inhibited the sensory detection of off-flavour compounds. However, they were not sufficient to inhibit the aftertaste in the lamb meatballs made with meat from animals fed with whole cottonseed. Therefore, it is evident that the inclusion of whole cottonseed in the lambs’ diet produced an undesirable taste in the meatballs, which was a limiting factor in terms of flavour, but which was accepted up to 12.5% of WCS.

In lamb meat and lamb meat products, off-flavour has been referred to as a “strange” or “strong” flavour (Khan, Jo and Tariq, 2015; Andrade *et al.*, 2016), which can lead to the rejection of lamb products by testers. The development of off-flavour can be associated with the following factors: the physiological condition, castration and stress

Table 4. Mean scores for the sensory analysis of lamb meatballs from animals fed on different levels of whole cottonseed.

Attributes ¹	Whole cottonseed, %					Mean or Equation	Probability value ²			SEM ³	CV ⁴
	0	10	20	30	40		WCS	L	Q		
Odour	5.26 ^a	5.18 ^{ab}	4.92 ^b	5.15 ^{ab}	4.94 ^b	(1)	0.0313	0.0224	0.5342	0.01	9.81
Colour	4.54	4.84	4.74	4.83	4.63	$\bar{y} = 4.71$	0.2236	0.6232	0.0465	0.02	12.97
Flavour	5.13 ^a	5.19 ^a	4.67 ^b	5.10 ^a	4.53 ^b	(2)	0.0005	0.0022	0.5288	0.02	12.61
Texture	5.16	4.94	5.05	5.22	5.05	$\bar{y} = 5.09$	0.4836	0.9050	0.7102	0.01	10.40
Appearance	5.00 ^a	5.20 ^a	4.93 ^a	5.09 ^a	4.61 ^b	(3)	0.0040	0.0128	0.0268	0.01	10.51
Purchase intent	3.60 ^{ab}	3.72 ^a	3.38 ^{bc}	3.60 ^{ab}	3.30 ^c	(4)	0.0253	0.0262	0.4936	0.02	14.75

Means in the same line, followed by different letters, differ significantly by t-test ($P < 0.05$). $n = 50$.

¹Scores 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).

²WCS= whole cottonseed levels; L= linear tendency; Q= quadratic tendency.

³SEM= standard error mean.

⁴CV (%)= coefficient of variation.

⁽¹⁾ $\hat{y}_{\text{odour}} = 5.18 - 0.005WCS$ ($r^2 = 0.61$), ⁽²⁾ $\hat{y}_{\text{flavour}} = 5.15 - 0.01WCS$ ($r^2 = 0.45$), ⁽³⁾ $\hat{y}_{\text{appearance}} = 5.12 - 0.008WCS$ ($r^2 = 0.40$), ⁽⁴⁾ $\hat{y}_{\text{purchase intent}} = 3.66 - 0.007WCS$ ($r^2 = 0.45$).

of animals before slaughter (Monte *et al.*, 2012); age at slaughter, body mass and animal feed (Brito *et al.*, 2016; Erasmus *et al.*, 2016; Jaworska *et al.*, 2016; Pellegrini, 2017); as well as the levels of fat deposition, lipid oxidation and volatile compounds (Vasta and Luciano, 2011; Khan, Jo and Tariq, 2015; Ma *et al.*, 2016; Brito *et al.*, 2017).

Conclusion

The formulations of meatballs made with meat from lambs fed with different levels of whole cottonseed complied with Brazilian legislation in relation to physicochemical and microbiological characteristics. The meatballs presented reduced levels of lipid, cholesterol and calorific content, as well as high protein content. An association was observed between the results of chemical composition, texture profile and the structural properties of the meatballs. The whole cottonseed used in the diet of the lambs influenced the acceptability of the meatballs, mainly affecting odour, flavour and appearance. It is possible to produce meatballs with meat from lambs fed on levels of up to 12.5% of whole cottonseed in the dry matter of the diet without altering the sensory characteristics of the product.

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