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The Effect of Replacing Water with Tiger Nut Milk (Horchata) Liquid Coproduct on the Physicochemical Properties and Oxidation (Haemopigments and Lipids) of a Cooked Pork Liver Meat Product

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Summary

Tiger nut milk liquid coproduct (TLC) can be used as an ingredient in the food industry because it is a valuable source of natural antioxidants (phenolic compounds). This study analyses the effect of replacing water (50 or 100 %) with different concentrations of TLC in cooked pork liver pâté by measuring the chemical composition, haemopigment and lipid oxidation, physicochemical and sensory characteristics of the obtained product. The pork liver pâtés obtained using this liquid (50 and 100 % of water replacement) had a similar protein and ash content, but the moisture decreased ($p>0.05$) while the fat content increased ($p<0.05$). However, pâtés with added TLC had higher haeme iron content, and showed a lower degree of metmyoglobin formation than the control pâté. Their physicochemical properties (colour, reflectance spectrum, pH and a_w) were not modified ($p>0.05$) by the addition of TLC and their overall acceptance was better. TLC appears to be a valuable alternative for use in the formulation of country-style pork liver pâté (pâté de campagne), while at the same time, reducing waste from tiger nut processing industry, thus increasing its ecoefficiency.

Key words: tiger nut, liquid coproducts, haemopigments, oxidation, country-style pork liver pâté

Introduction

Red meat (beef, veal, ostrich, pork and lamb) contains proteins of high biological value and important micronutrients, among them iron (*1–3*). Iron is known to be an essential nutrient but it is not widely appreciated that iron-deficiency anaemia is the most common nutritional deficiency with a worldwide prevalence of about 30 % (*4*). The continued depletion of iron stores can lead to serious biological impairment. Iron-deficiency anaemia is related to delayed cognitive development and intellectual impairment (*5*). Given the critical role of iron in oxygen transport and storage in muscle, it is not surprising that iron-deficiency anaemia also leads to reduced work capacity (*6*). Iron (Fe) in foods is absorbed as haeme and non-haeme iron, the latter comprising most of the iron intake from a mixed diet. Absorption of both forms can be either enhanced or inhibited by a number of dietary factors. Haeme iron is found in meat (particularly in red meat), poultry and fish, and is better absorbed than non-haeme iron. The absorption of inorganic iron, however, can be enhanced by the addition of haeme iron (meat factor) and vitamin C (*4*). The content of Fe in pork liver tissue (1.66–3.09 g/kg) is higher than in the pork muscle

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tissue (0.1–0.28 g/kg) (*3*), making liver a good nutritional source of iron.

Country-style pork liver meat product, also known as pâté de campagne, is made from pieces of liver (usually from duck, goose or pork), adipose tissue and muscle and is available in terrines, tins, casings or vacuum packs. In liver pâtés in general, lipid oxidation is one of the primary mechanisms associated with quality deterioration (*7*). Indeed, this product is very susceptible to oxidation because it is highly processed, with high fat levels and high iron content (from the liver), but with a low content of natural antioxidants (*7*).

There is a growing demand for natural food products, due to possible negative effects of synthetic food additives on human health and to the increased consumer perception of this problem in recent years (*8*). New references can be found every day in the scientific literature to the beneficial effects of different ingredients and/or bioactive compounds with functional properties, and it is interesting that many of these functional ingredients are obtained from the coproducts of the agro-food industry itself (*9*). The recovery of effluents for different uses is an interesting practice that can contribute to better management of water resources throughout the world. Tiger nuts or 'chufas' (*Cyperus esculentus*) are tubers mainly used to produce 'horchata de chufa' (tiger nut milk), during the course of which high quantities of coproducts (solids and liquids) are produced (*10*). Tiger nut milk liquid coproduct (TLC) can be used as an ingredient for the food industry, because it is a valuable source of natural antioxidants (phenolic compounds) with important antioxidant properties (mainly its reducing power and ability to inhibit lipid peroxidation). Due to its physicochemical properties, it can be used as a substitute for water added to foods. However, due to its high microbial load, it requires pasteurization prior to the addition to foods (*11*).

The purpose of this study is to analyze the effect of substituting water with different concentrations of tiger nut milk liquid coproduct on the chemical composition, haemopigment oxidation, and physicochemical and sensory characteristics of a cooked liver pork product.

Material and Methods

Tiger nut milk liquid coproduct preparation

The raw material was obtained from a local 'horchata' producer, a member of the Association of Horchata Producers of the Community of Valencia, which oversees the production of horchata (or tiger nut milk) with Protected Designation of Origin (PDO) 'Chufa de Valencia'. Tiger nut milk liquid coproduct (TLC) was obtained using the method described by Sánchez-Zapata *et al.* (*11*). The liquid coproduct was separated from the solid coproduct by filtering and manual pressing through a filter (d=0.2 mm, Venex, Ref. V1137, Barcelona, Spain) and stored frozen at -23 °C. Before use in the experiment, it was thawed at 4 °C and pasteurized at 80 °C for 3 min. The TLC was frozen again because it was used in the country-style pâté formula as ice.

Processing of country-style pork liver pâté

Country-style pork liver pâté was manufactured according to a traditional formula (the percentages of non-meat ingredients below are related to the total meat content): 25 % pork liver, 75 % pork back fat, and in g/kg: water (ice) 1.5, whole egg 0.8, sodium chloride 0.18, sodium caseinate 0.15, sodium tripolyphosphate 0.02, sodium ascorbate 0.005, sodium nitrite 0.0015, white pepper 0.02, nutmeg 0.003, laurel 0.003, thyme 0.003 and garlic powder 0.003. This original mixture was used as control sample. Two other batches were prepared according to this formula, but water was substituted with 50 and 100 % TLC (in each batch). Three independent replicates of each batch were prepared.

The products were prepared in the IPOA pilot plant research facilities of Miguel Hernández University (Alicante, Spain) following normal industrial practices. Frozen raw material of animal origin, except the pork back fat, was transferred to the cutter (Tecator 1094 Homogenizer, Tecator AB, Höganäs, Sweden) with the sodium chloride to extract salt-soluble proteins. After chopping, the other ingredients and additives were added. Then, pork back fat, previously divided into 10-cm cubes, was added. This original mixture was split into 3 batches, to two of which TLC was added in different amounts (50 and 100 % of the total water added) and control batch without TLC.

The mixtures were dispensed into aluminium containers and cooked in a convection oven (Balay Activa 505, BSH Electrodomésticos España SA, Pamplona, Spain). The pâtés were kept in the oven until the geometric centre, which corresponds to the thickest part of the product, reached 72 °C. When the endpoint temperature was reached, the pâtés were immediately chilled. After reaching room temperature, the pâtés were stored at 4 °C until analysis (24 h later). Processing was repeated three times with each formulation.

Proximate analysis

The moisture, ash, protein and fat content were determined by AOAC methods (*12*). Moisture (g per 100 g) was determined by drying 3 g of sample at 100 °C to constant mass. Ashing was performed at 500 °C for 5 h (g of ash per 100 g of sample). Protein (g of protein per 100 g of sample) was analyzed according to the Kjeldahl method. Factor 6.25 was used for conversion of nitrogen to crude protein. Fat (g of fat per 100 g of sample) was calculated by mass loss after a 6-cycle extraction with petroleum ether in a Soxhlet apparatus.

Physicochemical analyses

pH

The pH was measured with a pH meter (pH/Ion 510, Eutech Instruments Pte Ltd., Singapore) in a suspension obtained by blending 15 g of sample with 150 mL of deionized water for 2 min.

Water activity

Water activity (a_w) was measured at 25 °C using a Novasina TH-500 hygrometer (Novasina, Axair Ltd., Pfäffikon, Switzerland).

Colour determination

Colour was evaluated using a spectrophotometer (CM-2600D, Minolta Camera Co., Osaka, Japan) with illuminant D_{65}, 10° observer angle, diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for measurement. Colour was described with coordinates: lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*). From these coordinates, hue (h_{ab}) and chroma (C^*_{ab}) were calculated as follows:

$$h_{ab}=\tan^{-1}b^*/a^* \qquad /1/$$

$$C^*_{ab}=(a^{*2}+b^{*2})^{1/2} \qquad /2/$$

The reflectance spectra at every 10 nm between 360 and 740 nm were also obtained. Nine replicate measurements were taken for each sample, following the guidelines for colour measurements of the American Meat Science Association (*13*).

Sensorial analysis

Twenty experienced panellists were recruited from the staff and students of the Miguel Hernández University, Orihuela, Alicante, Spain. Protocols for sensory analysis were approved by the local Ethics Committee for Clinical Research (ECCR Vega Baja Hospital, Orihuela, Alicante). Panellists were chosen on the basis of previous experience in consuming traditional pork liver pâté. Furthermore, a preparatory session was held prior to testing, so that each panel was able to thoroughly discuss and clarify each attribute of the pâté to be evaluated. Testing begun after the panellists had agreed on the descriptors. A quantitative descriptive analysis was carried out (*14*). All sensory work was carried out in the sensory laboratory at the University, which fulfils the requirements of international standards (*15*). During evaluation the panellists sat in private booths under TL 5 fluorescent light (Philips-Ibérica, Madrid, Spain), with an intensity of approx. 350 lux. Rectangular pieces approx. 1.5×2 cm were cut from the centre of each pâté sample, and were served at room temperature (*16*). Each panellist evaluated three replicates of all the formulas; the order of sample presentation was random for each panellist. Spring water was provided between samples to cleanse the palate.

The attributes measured in the pâté samples and their descriptors were as follows: external evaluation: colour intensity (from extremely light to extremely dark) and colour homogeneity (from completely homogeneous to heterogeneous), for odour: from imperceptible to extremely intense, for texture: hardness (from soft to extremely hard) and juiciness (from extremely dry to extremely moist), for taste: fatness (from imperceptible to extremely intense) and flavour (from imperceptible to extremely intense). At the end of the test, the panellists were asked to give a score for overall product liking from 1 to 9.

Determination of oxidative parameters of country-style pork liver pâté

Lipid oxidation

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method following the recommendations of Buege and Aust (*17*). TBA reactive substances (TBARS) values were calculated from a standard curve of malondialdehyde (MA) and expressed as mg of MA per kg of sample.

Haemopigment oxidation

Myoglobin (Mb) and metmyoglobin (MMb) were determined according to Krzywicki (*18*). A mass of 5 g of minced product was used to determine the MMb mass fraction in each sample. Myoglobin was extracted with cold 0.04 M phosphate buffer, pH=6.8, with a sample to buffer ratio of 1:10. Samples were homogenized for 15 s with an ULTRA-TURRAX® homogenizer T20 Standard (IKA, Staufen, Germany) at 13 500 rpm. The homogenates were then centrifuged for 30 min at 5000 rpm. The absorbance of the filtered supernatant was read at 525, 572 and 730 nm. Mb was determined using the Krzywicki's formula (*18*):

$$w(\text{Mb})/(\text{g/kg})=(A_{525\ \text{nm}}-A_{730\ \text{nm}})\cdot 2.303\cdot \cdot 5\ (\text{dilution factor}) \qquad /3/$$

and percentage of MMb was determining using the formula of Krzywicki (*18*):

$$w(\text{MMb})/\%=1.395-((A_{572\ \text{nm}}-A_{730\ \text{nm}})/(A_{525\ \text{nm}}-A_{730\ \text{nm}}))\cdot 100 \qquad /4/$$

Samples were kept on ice at all times during the assay.

The total iron mass fraction was determined in wet-ashed samples using the ferrozine assay (*19*). Haeme iron was determined using the method of Hornsey (*20*). Total pigments, as acid haematin, were calculated using the formula:

$$w(\text{total pigment})/(\text{mg/kg})=A_{640\ \text{nm}}\cdot 680 \qquad /5/$$

Haeme iron was calculated as follows (*21*):

$$w(\text{haeme iron})/(\text{mg/kg})=w(\text{total pigment})\cdot \cdot 8.82/100 \qquad /6/$$

Determination of microbial counts of country-style pork liver pâté

Serial dilutions of samples were prepared in sterile peptone water for most microbial determinations, and in the De Man, Rogosa, Sharpe (MRS) broth for lactic acid bacteria counts. Total viable counts (TVC) were determined by plating the diluted samples on TVC 3M Petrifilm™ (3M, Maplewood, MN, USA) plates followed by incubation at 35 °C for 48 h, lactic acid bacteria (MRS broth) on TVC 3M Petrifilm™ plates incubated at 37 °C for 48 h under anaerobic conditions, enterobacteria on 3M Petrifilm™ plates incubated at 37 °C for 24 h, and moulds and yeasts on Rose Bengal chloramphenicol agar incubated at 28 °C for 5 days. The results were expressed as log CFU/g.

Statistical analysis

All tests were carried out in triplicate (4×3=12 samples). Results are expressed as mean values±standard deviation (S.D.). Analysis of variance (ANOVA) was used to determine significant differences ($p<0.05$) among TLC volume fractions. To assess the differences between the levels of the TLC volume fraction (0, 50 and 100 %), contrasts between mean values (Tukey´s test) were used.

All statistical analyses were performed using the SPSS statistics software package (SPSS v. 16.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Proximate composition of country-style pork liver pâté

Table 1 shows the results obtained for the chemical analysis of the different pâté formulations (0, 50 or 100 % of water replacement with TLC). As can be seen, the moisture content was lower in the samples with added TLC than in the control ($p<0.05$), but no significant differences ($p>0.05$) were found among the formulations with the TLC. This lower moisture content could be due to the fact that the TLC introduced in the formulations instead of water contained dissolved soluble solids, such as carbohydrates (sugars and starch) (*11*). In TLC, carbohydrates represent about 67.44 % of its dry mass. Similar results were determined by Fernández-López *et al.* (*7*) in pâté.

The high fat content ($p<0.05$) of pâtés with TLC (compared to the control without the added TLC) was mainly due to the fat included in the TLC (8.27 %, in dry matter) and the lower moisture content in these products (*11*). The dominant fatty acids in tiger nut milk, and therefore in TLC, are oleic acid (77 %), palmitic acid (11 %) and linoleic acid (9 %) (*22*). The protein and ash contents in the pâtés were not modified ($p>0.05$) by the TLC addition (Table 1).

Oxidative parameters of country-style pork liver pâté

Lipid oxidation was evaluated by determining the levels of TBARS. As regards TBA (Table 1), the replacement of water with TLC (50 and 100 %) did not modify ($p>0.05$) this parameter in the final products with respect to the control. There were no statistically significant ($p>0.05$) differences between the three resulting pâtés. So, the addition of TLC did not result in inhibited lipid peroxidation.

The total iron and haeme iron contents of pâtés are shown in Table 1. The total iron content did not differ significantly ($p>0.05$) between the pâté samples with TLC added, but there were differences ($p<0.05$) between the control and samples with TLC. The total iron content of pâté samples was about 11 mg/kg. Pâté de champagne includes in its formulation muscle (back fat) and pork liver. The content of iron in the muscle and liver is 14.2 and 218 mg/kg, respectively (*3*), so the iron content of the pâté will depend on the iron present in the pork liver and back fat. However, the values are obviously lower in the pâté because it incorporates other ingredients, such as water and some additives that are responsible for decreasing the total iron content.

When meat is cooked, a variable amount of haeme iron is converted to non-haeme iron, depending on the conditions. The ratio of haeme to non-haeme iron is also important because iron within the haeme molecule can be absorbed into enterocyte cells in the wall of the small intestine by a process that is less affected by factors that inhibit the absorption of non-haeme iron, thereby often making it more bioavailable. The bioavailability of haeme iron is also relatively high because most of dietary haeme iron is usually found in meat, and meat in the diet enhances the absorption of all dietary non-haeme iron through the presence of the so-called 'meat factor'. This factor appears to be in the form of the amino acids cysteine and methionine, either alone or within small peptides (*23*). The haeme iron content was lower in control samples than in the samples with added TLC (50 and 100 %): control (5.76±0.94), 50 % TLC (8.38±1.84) and 100 % TLC (8.82±0.42) mg/kg of haeme iron corresponding to 50 % of total iron in pâté control and about 73 % of total iron in pâté with added TLC. The haeme iron content in pork liver pâté decreased with cooking, which agrees with the findings of Tomović *et al.* (*3*). These values indicate that TLC could have antioxidant properties, protecting the iron as haeme iron form and preventing its loss caused by heating. The antioxidant properties of TLC were determined previously by Sánchez-Zapata *et al.* (*11*), who found the total phenolic content expressed

Table 1. Proximate composition and oxidative parameters of country-style pork liver pâté formulated with tiger nut milk liquid coproduct (TLC)

Parameter	$\frac{m(\text{TLC})}{V(\text{water})}$/%		
	0	50	100
w(moisture)/(g per 100 g)	(47.74±2.11)^b	(43.07±1.99)^a	(42.66±3.32)^a
w(fat)/(g per 100 g)	(34.40±2.02)^a	(36.99±1.24)^b	(37.41±2.28)^b
w(protein)/(g per 100 g)	(10.74±0.45)^a	(10.96±0.27)^a	(10.32±0.23)^a
w(ash)/(g per 100 g)	(2.34±0.07)^a	(2.42±0.08)^a	(2.17±0.14)^a
w(myoglobin)/(g/kg)	(3.75±0.02)^a	(3.25±0.01)^a	(3.01±0.01)^a
w(metmyoglobin)/%	(66.34±0.12)^b	(64.28±0.09)^a	(64.81±0.04)^a
w(TBA as MA)/(mg of MA per kg)	(0.23±0.04)^a	(0.28±0.05)^a	(0.29±0.07)^a
w(total iron)/(mg/kg)	(11.42±0.37)^a	(11.34±0.38)^a	(11.25±0.29)^a
w(haeme iron)/(mg/kg)	(5.76±0.94)^a	(8.38±1.84)^b	(8.82±0.42)^b

Results are expressed as mean values of three repetitions±S.D. Mean values within a row with different letters are significantly different ($p<0.05$)
TBA=thiobarbituric acid, MA=malondialdehyde

as gallic acid equivalents (GAE) in TLC to be (169.8±10.5) mg/L. Phenolic compounds are presumably present in TLC because tiger nut contains phenolic acids. Parker *et al.* (*24*) identified some monomeric phenols in the tiger nut cell wall, including *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzaldehyde, vanillin, *trans*-*p*-coumaric acid, *trans*-ferulic acid, *cis*-*p*-coumaric acid, *cis*-ferulic acid, and other dimeric phenols. Also, some phenols (*e.g.* organic or inorganic cleaning agents or disinfectants) can be found in the TLC as a result of food processing. Therefore, TLC can be considered a valuable source of natural antioxidants (phenolic compounds) with good antioxidant properties (mainly its reducing power and ability to inhibit lipid peroxidation) (*11*).

The total myoglobin mass fraction of pâtés did not show differences ($p>0.05$) among samples, its values ranging between 3.01 and 3.75 g/kg (on wet basis) (Table 1). However, the percentage of metmyoglobin differed ($p<0.05$) between the control and the samples with added TLC. The control pâté had the highest value (66.34 %) and the pâtés with 50 and 100 % TLC had values of 64.28 and 64.81 %, respectively. It seems that TLC acts as an antioxidant, avoiding the transformation of Mb into MMb.

Physicochemical properties of pâté samples

The physicochemical properties of pâté samples are shown in Table 2. The addition of TLC increased the pH values ($p<0.05$), but did not modify the a_w values. The pH values obtained in this study were lower than those determined in the work of Fernández-López *et al.* (*7*). As regards its colour properties, the addition of 100 % TLC decreased lightness compared with the control ($p<0.05$), but the treatment with 50 % TLC did not cause any differences in this respect ($p>0.05$), although it resulted in a lower L^* value (Table 2). The low L^* values in samples with 100 % TLC could be attributed to the particles dissolved in the TLC, which would have decreased its lightness. The L^* values in this study were higher than those determined by Fernández-López *et al.* (*7*) in ostrich liver pâté since the liver directly affects the L^* values of this type of meat product and the higher the pork liver content, the lower the L^*. The L^* values decreased when TLC was added to the pâté because the solutes contained in the TLC are starchy and may be totally or partially gelatinized. Starch gelatinization allows light penetration into the product and avoids light reflection, so L^* values are lower. This effect was described by Chai and Park (*25*) in surimi gels. They reported that starch significantly reduced lightness of fish protein gels as its concentration increased, regardless of the cooking method. Yoon *et al.* (*26*) and Yang and Park (*27*) also reported that when starch concentration increased, gels became more translucent. They suggested that the decrease in lightness at higher starch concentrations occurs because of the partially swollen starch granules and the water that is consequently absorbed into the starch granules. Another possibility is related to the amount of amylose leaking from the starch granules into the system. When starch granules swell, they allow amylose to leak out into the system (*28*). Because amylose is linked as a linear chain, it has a tendency to align itself in a parallel way, leading to precipitation in a solution or retrogradation in the gel system. The retrogradation of starch normally forms a strong gel and contributes to opacity (*25*).

Table 2. Physicochemical properties of country-style pork liver pâté formulated with tiger nut milk liquid coproduct (TLC)

Parameter	$\frac{m(\text{TLC})}{V(\text{water})}$/%		
	0	50	100
pH	(6.02±0.04)^a	(6.07±0.03)^{ab}	(6.14±0.04)^b
a_w	(0.972±0.002)^a	(0.970±0.001)^a	(0.973±0.003)^a
L^*	(57.22±0.96)^b	(56.65±1.14)^b	(55.26±2.73)^a
a^*	(6.37±0.32)^a	(6.14±0.32)^a	(7.75±1.45)^b
b^*	(15.34±0.70)^a	(14.86±0.23)^a	(14.18±0.78)^b
C^*_{ab}	(16.60a±0.77)^a	(16.08±0.29)^a	(16.22±0.86)^a
h_{ab}	(67.46±0.31)^b	(67.57±0.96)^b	(61.40±0.56)^a

Results are expressed as mean values of three repetitions±S.D. Mean values within a row with different letters are significantly different ($p<0.05$)

The addition of 50 % TLC did not modify ($p>0.05$) a^* and b^* values with respect to the control (Table 2). However, samples with 100 % TLC had higher a^* and b^* values ($p<0.05$) than the control and 50 % TLC samples. This indicates that TLC incorporates red and yellow compounds into the pâté, although the CIELAB values for TLC are L^*=54.67, a^*=–1.14 and b^*=9.08 (*11*). Therefore, the effect of TLC on the a^* and b^* values would be due to the increased solute levels in the pâté that remove water from the product (solution effect). C^*_{ab} was not modified by the addition of TLC ($p>0.05$). The control and samples with 50 % TLC showed similar hue values ($p>0.05$), but samples with 100 % TLC had a lower hue value ($p<0.05$).

The reflectance spectra of the different pâtés formulated with TLC are shown in Fig. 1. Reflectance measurements are closely related to what the eye and brain see. Such measurements are a good method for examining the amount and chemical stage of pigments in meat *in situ*. Reflectance measurements are affected by muscle structure, surface moisture, fat content and additives (*29*). The reflectance spectra of all the samples studied were similar up to 400 nm (Fig. 1), and in the last part of the red spectrum (620–740 nm), where there were no statistically significant differences ($p>0.05$) in the reflectance percentages. This means that these wavelengths are isobestic. This plateau could be considered characteristic of cooked pork liver products, regardless of whether TLC is added or not. In the 410–450 nm zone, the TLC samples showed the same shape as the control ($p>0.05$) although the 50 % TLC sample had a higher reflection percentage ($p<0.05$). The addition of TLC had no effect on the shape of the pâté reflectance spectrum, with respect to the control (Fig. 1), indicating that the spectrum shape is not influenced by the addition of TLC, since TLC solutes are incorporated in the meat emulsion. The control and 50 % TLC samples did not differ significantly ($p>0.05$) in the 450–590 nm zone, but the 100 % TLC sample showed a lower percentage of reflection ($p<0.05$). This is because TLC solutes are starchy so they can be totally or partially gelatinized. Starch gelatinization allows light penetration into the product and avoids light reflection (*25*). TLC solutes reduce the amount of avail-

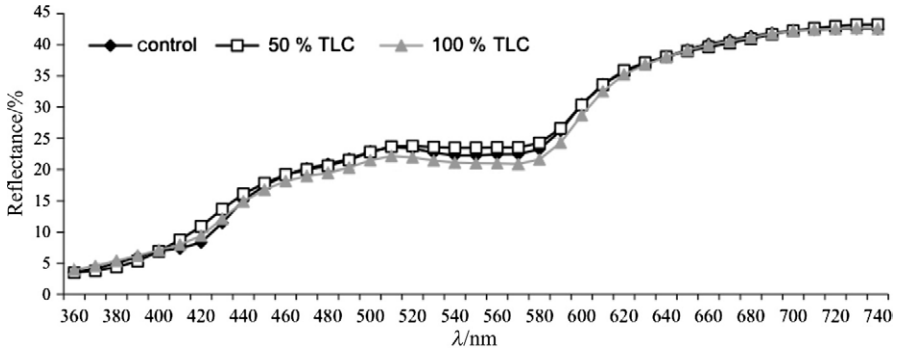

Fig. 1. Reflectance spectra of country-style pork liver pâté formulated with tiger nut milk liquid coproduct (TLC). The added water was replaced with TLC as follows: 0 (control), 50 and 100 %

able surface water, causing a decrease in the reflection spectrum without changing the spectrum shape. This effect can be seen in Fig. 1.

Microbial counts of country-style pork liver pâté

Microbial counts of the pâté samples formulated with TLC are shown in Table 3. The presence of enterobacteriaceae, mould or yeast was not detected in any of the samples. Pâté is a cooked product, so the heat treatment prevents mould, yeast and enterobacteriaceae from growing. Similar results in microbial growth were reported by Fernández-Ginés *et al.* (*30*) in vacuum-packed cooked sausages and by Fernández-López *et al.* (*7*) in country-style pork liver pâté and in ostrich pâté. Also, total viable counts were similar ($p>0.05$) in all the studied samples. However, lactic acid bacteria and psychrotrophic bacteria counts were lower ($p<0.05$) in samples with 100 % TLC added, while the control and samples with 50 % TLC did not differ in this respect ($p>0.05$). This suggests that the substitution of water with TLC may have a preservative effect in this product, because it inhibits the growth of lactic acid and psychrotrophic bacteria.

Table 3. Microbial counts of country-style pork liver pâté formulated with tiger nut milk liquid coproduct (TLC)

Microorganism	$\frac{m(\text{TLC})}{V(\text{water})}$/%		
	0	50	100
	N/(log CFU/g)		
total viable counts	(2.62±0.13)^a	(2.83±0.02)^a	(2.60±0.00)^a
lactic acid bacteria	(2.46±0.15)^b	(2.46±0.15)^b	(1.69±0.70)^a
psychrotrophic bacteria	(2.30±0.30)^b	(2.59±0.44)^b	(1.63±0.25)^a
enterobacteriaceae	(0.00±0.00)^a	(0.00±0.00)^a	(0.00±0.00)^a
moulds and yeasts	(0.00±0.00)^a	(0.00±0.00)^a	(0.00±0.00)^a

Results are expressed as mean values of three repetitions±S.D. Mean values within a row with different letters are significantly different ($p<0.05$)

Sensorial properties of pâté samples

As regards the sensorial analysis of the pâté samples, the addition of 50 or 100 % TLC did not cause changes ($p>0.05$) in odour, juiciness, hardness, colour homogeneity or off-flavour in the analysed samples (Fig. 2). Samples with added TLC were perceived as less fatty and their colour was rated as less intense than that of the control samples; however, redness and yellowness measures were higher in samples with 100 % than in 50 % TLC and control, and also the fat content was higher in samples with added TLC.

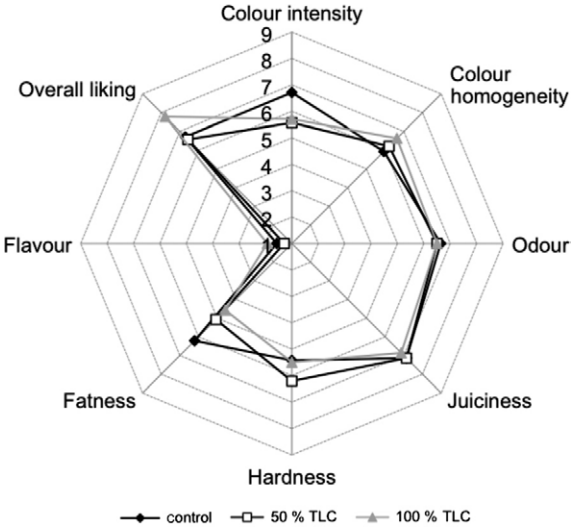

Fig. 2. Sensorial analysis of country-style pork liver pâté formulated with tiger nut milk liquid coproduct (TLC). The added water was replaced with TLC as follows: 0 (control), 50 and 100 %

As regards the overall liking of the pâtés, the panellists scored the samples with 100 % TLC slightly higher ($p<0.05$) than the control and the samples with 50 % TLC.

Conclusion

The results obtained in this study clearly demonstrate that tiger nut milk liquid coproduct can be used as an ingredient in the elaboration of pork liver products (in this case, country-style pork liver pâté). The pork liver pâtés obtained using this liquid coproduct (50 and 100 % of water replacement) had a similar protein and ash content, but moisture mass fraction decreased, and fat content increased slightly. However, pâtés with added TLC had a higher haeme iron content, and showed a

lower degree of metmyoglobin formation than the control pâté. Its physicochemical properties were not modified by the addition of tiger nut milk liquid coproduct and its overall acceptance was greater. The tiger nut milk liquid coproduct used appears to be a valuable alternative for use in the formulation of country-style pork liver pâtés, while at the same time reducing waste from tiger nut processing industry, thus increasing its ecoefficiency.

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