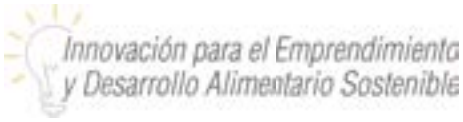

SIAL 2019

6th Simposio Internacional Agroalimentario

Córdoba, Colombia on October 9-11, 2019

Edited by

Marco Túlio Pardini Gontijo and Maryoris Elisa Soto Lopez

Special Issue

CHIRIOTTI
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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "VI SIAL. 6th Simposio Internacional Agroalimentario" organized by the Food Engineering Department (Departamento de Ingeniería de Alimentos) of the Córdoba University (Universidad de Córdoba), held at Montería, Córdoba, Colombia on October 9-11, 2019.
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INTRODUCTION

The sixth Simposio Internacional Agroalimentario (SIAL VI) – 2019 was organized at the Montería city in Colombia, between the 9th and 11th of October of 2019. The aim of the congress was to promote the careers of food engineering of the Universidade de Córdoba approaching important topics in the food science and technology field in the context of emerging economies and/or developing countries.

This international congress had the honor of hold oral presentations from well-known national and international researchers. It is important to mention that VI SIAL had the support of different administrative and technical direction from the Universidad de Córdoba. The symposium also had support from different food companies, such as Café Cordoba, Codelac, Frigosinu s.a., La Bonga del Sinú, Labservis and Agua Cañaveral.

This special edition is the result of the participation of academics, researchers, professionals and students in this international congress. Also, this year's theme "Innovación para el emprendimiento y desarrollo alimentario sostenible", Spanish version for "Innovation for entrepreneurship and sustainable food development". Therefore, this edition highlights researches that show technics developed for the Latin American region such as, technics that allows the improvement of food packaging and food edible films and experiments in the search of regional primary material with higher nutritional content for the development of new food products.

VI SIAL made a precedent of scientific production in the field of food science and technology not only for Colombia but also for other Latin American countries. Thus, SIAL constitute a space for knowledge interchange between academics, researchers, professionals and students in the field of Food Science, Food Engineering and Food Technology.

M.T.P. Gontijo and M.E.S. Lopez

COMPOSITE FILMS AS ALTERNATIVE PACKAGING FOR UV-LIGHT BARRIER INTENDED FOR FOOD PRESERVATION

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ABSTRACT

Food exposure to light result in oxidation. This study aimed to develop films as UV-light barrier using central composite design. Low density polyethylene films were incorporated with tinuvin and iron particles. Physical-mechanical properties (crystallinity, microstructure, mechanical resistance, color properties, thermal stability) and UV-light barrier were evaluated. Iron did no influence film's crystallinity; however, mechanical resistance was affected by iron. Microscopy analyses showed iron agglomeration on films surface. Films presented a maximum decomposition temperature (550.1±8°C). Tinuvin and iron affected L*, a*, opacity and whiteness index. Only tinuvin had a significant effect on UV-light absorbed at 250, 310 and 360 nm wavelengths.

Keywords: food packaging, food preservation, UV light barrier

1. INTRODUCTION

Photo-induced oxidation is considered as one of the major causes of food degradation because it causes alterations in the intrinsic and extrinsic properties of food products in a short period of time, changing nutritional and organoleptic features of foods and reducing their shelf-life (GIBIS and RIEBLINGER, 2011). One of the prune causes of decreased food quality is food product exposition to light, which induces oxidation processes. Food products can be exposed to light on several phases of the food production-chain, such as processing, packaging, distribution retailing and storing, which eventually results in the photo-induced oxidation process (INTAWIWAT *et al*., 2012). Thus, in order to diminish photo-induced oxidation, opaque and nontransparent materials, such as aluminum foil, have been used as a method for reduction of light incidence in food products. Meanwhile the interest and demand for transparent packaging has increased recently in the food industry due to attempts to enhance and differentiate the food product image on the shelves. Moreover, awareness related to environmental contamination has resulted in a reduced use of aluminum and metallized foils (MORTENSEN *et al*., 2004).

On the other hand, food packaging has critical and essential functions required for ensuring food preservation, especially those related to food protection during transporting and storage (YILDIRIM *et al*., 2018). Therefore, innovations related to the development of active food packaging are in constant progress in order to control or minimize external factors that might affect food stability and accelerate food spoilage as a result of chemical reactions, physical changes, or microbial contamination (ESPITIA *et al*., 2012).

In this regard, active packaging with antioxidant properties has emerged to control photo-induced oxidation in food products (SAHRAEE *et al*., 2019). Active packaging can contain food with novel functions, which includes antimicrobial, antioxidant, antiwetting or flavoring properties (FARAONI *et al*., 2008). The development of novel technology, and especially those related to the advancement of the food packaging field is a response to the emerging needs of the food industry and society, which take into consideration factors such as globalization of resources, which means increased distance among producers and consumers, current food regulations, and healthy life-style of consumers (YILDIRIM *et al*., 2018).

According to LEE *et al*. (2008), polyethylene (PE) has a vast market due to its versatility, transparency, high strength to chemical solvents, easy heat-sealing, barrier to moisture and low cost. According to Soares and Hotchkiss (1998), the combination of core technologies for food preservation and active packaging is essential to ensure food safety and increase the shelf-life of the packaged food product. Active packaging is a type of packaging that interacts intentionally with food in order to maintain the organoleptic and nutritional characteristics of the packaged product or even to improve them (RODRÍGUEZ-ROJAS *et al*., 2019).

One of the active compounds that can be incorporated into PE matrix is tinuvin 326. Tinuvin is a benzotriazole used in the packaging industry as UV light absorber. It has low volatility at high temperatures, is resistant to thermal degradation and can be used without significant loss or decomposition during the extrusion of low-density polyethylene (LDPE) films (CIBA, 2002). Moreover, iron particles can be incorporated in food packaging in order to act synergistically with tinuvin and thus improve UV light barrier of films. This study was therefore aimed to develop LDPE films with UV light absorption properties. The LDPE films were incorporated with tinuvin and iron particles and the physical and mechanical properties of developed films were evaluated, including microscopic analysis, mechanical resistance, color properties, thermal stability and potential barrier to UV light. The films were developed using the central composite design

(CCD) and data were analyzed with the statistical approaches of response surface methodology (RSM).

2. MATERIAL AND METHODS

The UV barrier films were prepared using low density polyethylene (LDPE, Braskem S.A., Triunfo, Rio Grande do Sul, Brazil) incorporated with Tinuvin 326 (chemical name: 2-(2'-Hydroxy-3'-tert-butyl-5'-methylphenyl)-5-chlorobenzortriazole; CAS number 3896-11-5) supplied by Ciba - Brazil, and iron powder with particle size of 3 microns (99.67 % iron content) provided by Sigma - Aldrich, São Paulo, Brazil.

2.1. Film Elaboration

The UV barrier films were elaborated as follows: tinuvin 326 and iron powder (at different concentrations) were blended with the LDPE resin according to the CCD (Table 1).

Table 1. Uncoded and coded levels for concentration of tinuvin and iron particles determined by the CCD and incorporated in active food packaging.

Essay	Variables	
	Tinuvin 326 (% w/w)	Iron particles (% w/w)
1	0.07500 (-1)	2.2 (-1)
2	0.07500 (-1)	12.8 (+1)
3	0.42800 (+1)	2.2 (-1)
4	0.42800 (+1)	7.5 (0)
5	0.00189 (-1.41)	7.5 (0)
6	0.50111(+1.41)	7.5 (0)
7	0.25150 (0)	0.0047 (-1.41)
8	0.25150 (0)	15.0 (+1.41)
9	0.25150 (0)	7.5 (0)
10	0.25150 (0)	7.5 (0)
11	0.25150 (0)	7.5 (0)

Each mixture was processed in a twin-screw extruder (Thermo Electron Corporation, Model AX plastic) at 30 rpm of speed and provided with ten heating zones at 165, 170, 170, 175, 175, 175, 175, 180, 180 and 180°C, respectively. After this process, pellets of LDPE incorporated with Tinuvin and iron particles were obtained.
Following this, the UV barrier films were produced in a tubular shape by processing the obtained pellets in a single-screw extruder (Thermo Electron Corp., model HAAKE Polydrive R600/610, Karlsruhe, Germany) at 30 rpm speed and provided with five heating zones at temperatures of 130, 140, 150, 160 and 170°C.

2.2. Experimental Design and Statistical Analysis

The central composite design (CCD) was used to evaluate the combined effects of tinuvin and iron particles on the physical and mechanical properties of developed films. The experiment was planned according to the CCD, constituted by a factorial experiment (2^2),

with the concentration of tinuvin and iron particles being the independent variables. Moreover, the experiment was provided with four treatments in the axial points and three replicates in central point. The whole experiment resulted in 11 treatments (Table 1). The results were analyzed using the statistical approach of response surface methodology using the Statistical Analysis System version 9.1 (SAS Inc., Cary, N.C., U.S.A.).

2.3. Measurement of UV light absorption

The UV light absorption of developed films was determined by the measurement of the light transmitted through the samples. Measurement was done according to methodology described by COLTRO and BURATIN (2004). The percentage of the specular light transmission of the samples was determined using UV-Visible Spectrophotometer (GBC, Model 918, Melbourne, Victoria, Australia). The specular light transmission is the transmittance value obtained when measuring only the light transmitted in the same direction as the incident beam. The spectra were analyzed in the wavelength range of 200-700 nm at 120 nm/min scanning speed. In this measurement, the activity of developed films in the ultra violet region (wavelength below 400 nm), as well as in the visible region (wavelength greater than 400 nm) was monitored. Film samples had dimensions of 4 × 4 cm^2.

2.4. Film Characterization

2.4.1 X-ray diffraction (XRD)

The diffraction patterns of the UV barrier films were taken with the X-ray Diffraction System model X'Pert PRO (PANalytical, Netherland), using an iron filter and Co-Kα radiation (λ = 1.78890 ˚A). The diffraction pattern was obtained at diffraction angles between 10° and 80° (2θ).

2.5. Film thickness

The thicknesses of the samples were determined using a manual micrometer (0.01 mm, Mitutoyo, Suzano, São Paulo, Brazil). The average film thickness was calculated from the measurement of 10 points randomly selected on each film in triplicate. Mean values were used for the calculation of film properties when necessary.

2.6. Mechanical properties

Mechanical properties of the UV barrier films, including tensile strength at break, load at break and elongation at break, were measured according to standard method ASTM D882-02 (ASTM, 2012) using an Instron Universal Testing Machine (Model 3367, Instron Corporation, Norwood, MA, USA). The Instron was equipped with a load cell of 1 kN, with an initial grip separation of 50 mm and 500 mm/min speed. Sample dimension was 25 mm x 100 mm and the test was repeated 10 times for each treatment to confirm the repeatability.

2.7. Microscopic characterization

Morphological analysis of the UV barrier films was directly observed by scanning electron microscope (SEM, Hitachi TM - 3000 Tabletop Microscope, Japan). Also, the surface of developed films was studied using Atomic Force Microscopy (AFM, NT-MDT, Russia).

The AFM images were acquired in an intermittent contact mode at random areas of 100 x 100 μm2. Samples were analyzed in triplicate at room temperature (25°C).

2.8. Thermogravimetric analysis

This analysis was performed in a thermogravimetric analyzer (TGA-1000, Navas Instrument Conway, SC, USA). Samples of each UV absorber film (1 g approx.) were heated to 950°C at a heating rate of 10°C/min under nitrogen atmosphere. Weight losses of samples were measured as a function of temperature.

2.9. Color measurement

The color of the UV barrier films was measured with a colorimeter (COLORQUEST XE HunterLab, Reston, VA, USA). Measurements were done using the CIELAB scale, where each measurement is expressed as L* (indicating the brightness), a* (indicating red in the positive direction and green in the negative direction) and b* (indicating yellow in the positive direction and blue in the negative direction). Calculations were made for D-65 illuminant and 10° observation interval according to ASTM method E308 (ASTM, 2008). Opacity (OP) was calculated using the normalized values of the white background (L* = 93.44; a* = -0.63; b* = 1.21). In addition, the yellowness index (YIE313) and whiteness index (WI E313) were obtained using the Universal software V4.10 according to ASTM method E313-10 (ASTM, 2010). All color measurements were repeated three times.

3. RESULTS AND CONCLUSIONS

3.1. Measurement of UV Light Absorption

The effectiveness of the potential barrier to UV light of packaging material was analyzed considering that the UV light absorption is inversely proportional to transmitted light in the wavelength range varying from 200 to 400 nm. The spectrophotometric analysis showed that UV barrier films at high concentrations of tinuvin presented lower percentage of transmittance in the range of 200 to 400 nm (Fig. 1).
Three main wavelengths, 250 nm (UV-C), 310 nm (UV-B) and 360 nm (UV-A) were selected in order to analyze the results statistically. In all these three main wavelengths the measured absorption of UV light differed among treatments of the developed films with statistical significance and non-significant lack of fit (Table 2). In all three main wavelengths, the linear effect of tinuvin was significant, indicating that increasing concentrations of tinuvin resulted in higher barrier to UV light. In addition, the quadratic effect of tinuvin was significant for UV light absorption when measured at 360 nm. On the other hand, the incorporation of iron particles had no significant effect on UV light absorption. Thus, the analysis of coefficients related to the spectrophotometric analysis at the three main wavelengths showed that the UV barrier films presented low percentage of transmittance in the range of 200 to 400 nm with increasing concentrations of tinuvin.

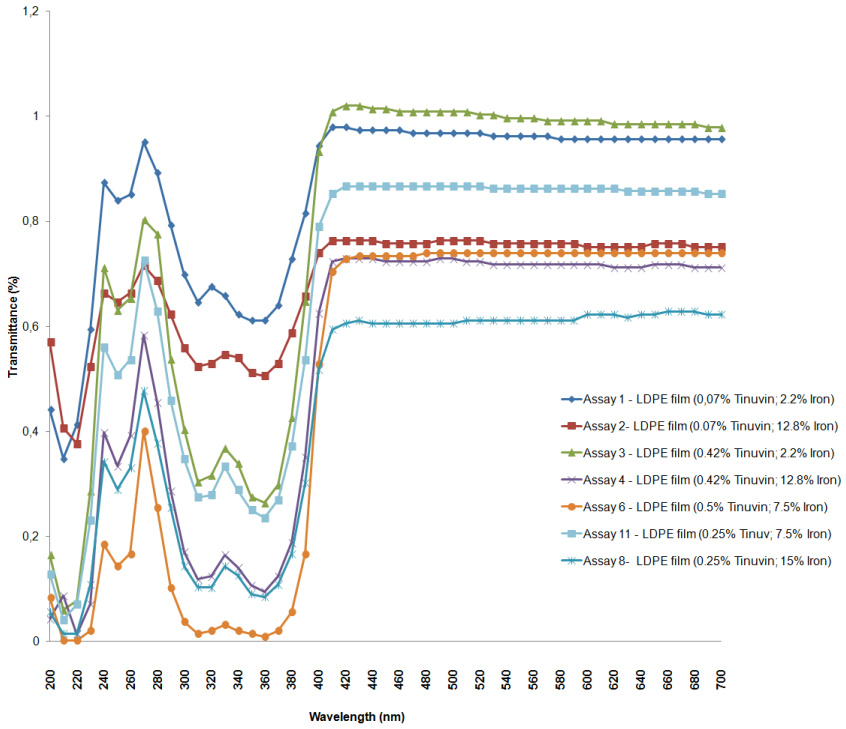
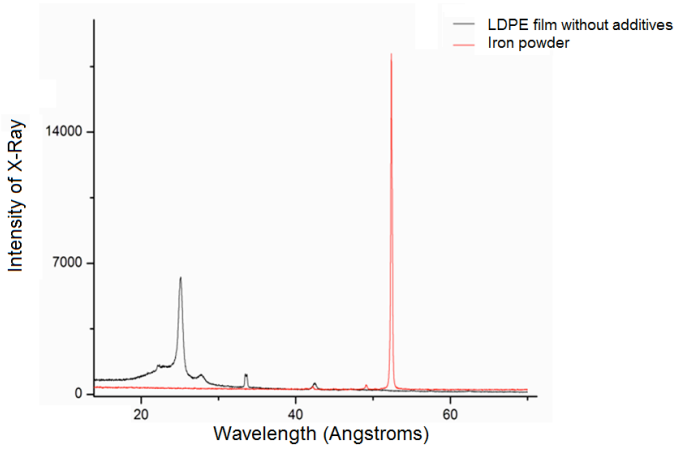


Figure 1. Spectrophotometric analysis of UV light absorption of various developed films.

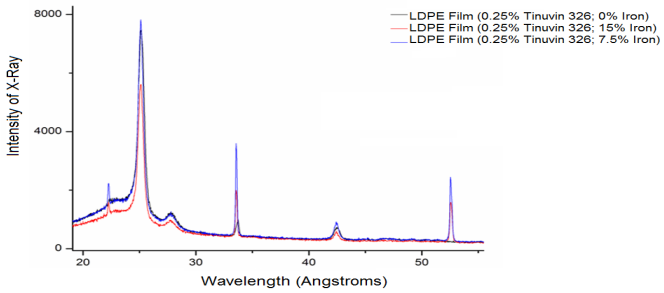
3.2. X-Ray Diffraction (XRD)

Crystallographic structure, chemical configuration and physical features of a material are provided by the XRD technique, in which the X-ray beam reaches the sample and is diffracted in a specific direction. Thus, the diffraction pattern is generated and it is considered a fingerprint of the analyzed sample. This technique is widely used because is nondestructive and the process of sample preparation is relatively simple (LUYKX *et al.*, 2008). The pure iron particles, the control film (without additives) and the UV barrier films in the experiment were analyzed by XRD. The XRD patterns of iron particles and the control film are shown in Fig. 2.a. The sharp peak at 25.112° of 2θ value is assigned to LDPE, while the diffraction peak at 52.363° of 2θ corresponds to iron particles. The presence of iron in the developed films and its crystalline nature was confirmed by XRD analysis (Fig. 2.b). The UV barrier films incorporated with high concentrations of iron particles showed diffraction peak at 2θ equal to 52.363°, as observed in treatment No. 8 (15 % w/w iron) and No. 10 (7.5 % w/w iron), while the treatment No. 7 did not show this characteristic peak, due to low iron concentration in the film (0.01% w/w).
In addition, the characteristic peak of LDPE was observed in the XRD patterns indicating that the crystallinity of the LDPE was not affected by the incorporation of iron. The crystallinity degree and polymer orientation affect directly on physical and morphological structure, and these are considered as major factors in the permeability features of the film. In this way, the behavior of the crystalline regions, which serve as barrier, is significantly different from the behavior of the amorphous regions, where the permeability process takes place.
The incorporation of an oxygen absorber in films of LDPE resulted in decreased crystallinity, up to 20.73 %, in samples with 10 % additive (NOGUEIRA *et al.*, 2005). This probably favored the permeability to gases and water vapor due to the increased space

between the polymeric chains (WANG, 2001). These authors further reported that iron concentration did not affect the crystallinity of the UV barrier films.



a)



b)

Figure 2. XRD patterns of: (a) iron particles and control film (without iron or tinuvin); and (b) UV barrier films with different concentrations of tinuvin and iron particles.

3.3. Thickness and mechanical resistance

The thickness of developed UV barrier films presented no statistical difference among treatments. The developed films presented an average thickness of 0.13 mm, indicating that the incorporated compounds had no effect on this parameter. The mechanical properties of the films measure the material resistance before their rupture. The mechanical performance of the UV barrier films was evaluated by determining the maximum load (N), elongation at maximum load (%), elastic modulus (MPa), and tensile strength at maximum load (MPa). All measured properties differed among treatments and the regression model of tested properties presented statistical significance among developed films, with a non-significant lack of fit (Table 2).

Table 2. Estimated regression coefficients for measured physical-mechanical properties of UV absorber films incorporated with tinuvin and iron particles.

Term		Maximum load (N)	Elongation at maximum load (%)	Elastic modulus (MPa)	Tensile strength at maximum load (MPa)	L*	a*	OP	WI	250 nm (UV-C)	310 nm (UV-B)	360 nm (UV-A)
Mean		***29.78418***	**309.5723**	***74933.1***	***8884.194***	***84.96636***	***-0.72758***	***19.18455***	***57.2603***	***0.4906***	***0.293555***	***0.172253***
Tinuvin		-1.238174	-1.765508	-5545.558	-840.4226	0.6189114	***-0.113825***	-0.206873	0.7553746	***-0.16975***	***-0.21959***	-0.2261
Iron		***-3.205999***	-40.91711	***-10466.6***	***-1770.812***	***-6.292298***	***0.0727124***	***2.5879972***	***-13.39389***	-0.090734	-0.04383	-0.0387
Tinuvin²		0.7753271	2.5020071	3979.814	375.4377	-0.663336	0.025764	0.3693061	-2.071671	0.045177	0.115384	***0.13258***
$Iron^2$		-2.01175	***-37.1685***	-405.9483	-542.3847	0.9883333	-0.025	-0.481667	2.5475	-0.0020476	-0.01154	-0.0091
Tinuvin × Iron		2.7925852	47.716964	7119.698	1337.3354	0.6950083	-0.011736	0.1059686	3.0025213	-0.0020476	-0.01583	-0.0159
Reg.	F^a	6.375988	7.241601	7.032227	9.596485	111.1178	40.25572	79.63881	58.10292	10.64317	19.98182	6.51794
	P^b	0.032497	0.01603	0.026397	0.012767	0.0001	0.0001	0.0001	0.0001	0.009801	0.001554	0.02092
Lack of fit	F^a	1.88709	0.294458	2.859416	1.706253	0.711301	0.511976	0.466179	1.41392	2.431165	6.250935	3.34449
	P^b	0.232769	0.755113	0.126599	0.264242	0.579851	0.777822	0.716495	0.327724	0.163244	0.02817	0.10572

These results indicated that the mechanical strength of LDPE films was affected after the incorporation of iron; however, tinuvin showed no influence on the mechanical properties of the UV barrier films. According to BRODY (2002), one of the biggest challenges of the production of active packaging, when incorporating active compounds to the polymeric matrix is the preservation of mechanical properties of the film. In this work, high concentrations of iron particles resulted in low values of maximum load. WURLITZER (2007) observed similar results after the incorporation of triclosan in polyvinylidene chloride (PVDC) matrix. Probably, these compounds occupied spaces in the polymeric matrix, large enough to create fatigue points which appeared in the polymer structure and resulted in reduced tensile strength.
The elongation at maximum load presented a behavior, in which the value of deformation decreased until a critical concentration of iron particles and beyond this point elongation at maximum load slightly increased. Stiffness of the material is measured by determination of the elastic modulus. In this work, UV barrier films presented a linear reduction of the elastic modulus. The reduction of tensile strength at maximum load of developed films related to increased iron concentration can be explained by the interaction between the resin and iron particles embedded in the film.
The results of mechanical properties were consistent with microscopic observations, which showed agglomerations of iron particles likely to cause stress points, resulting in decreased mechanical resistance of the films.

3.4. Microscopic Characterization

Control film showed homogeneous surface, with the presence of some unmelted polymer resin (Fig. 3.a). On the other hand, tinuvin affected the morphology of UV barrier films and the incorporation of iron particles resulted in heterogeneous surface of developed films, as a result of protrusions promoted by agglomeration of iron particles (Figs. 3.b and 3.c). The AFM presented 3D images of developed films (Figs. 3.d-f). Such images confirmed the results observed by SEM, which showed the formation of unevenness on the surface of the films.

3.5. Thermogravimetric analysis

Dramatic temperature variations may lead to changes in packaging materials, influencing their thermal resistance. The thermal resistance of UV barrier films was studied by the thermogravimetric curve, which shows the mass loss of the sample in relation to the temperature. In general, the developed film showed extensive weight loss (>80%) at temperatures above 550°C (Fig. 4).

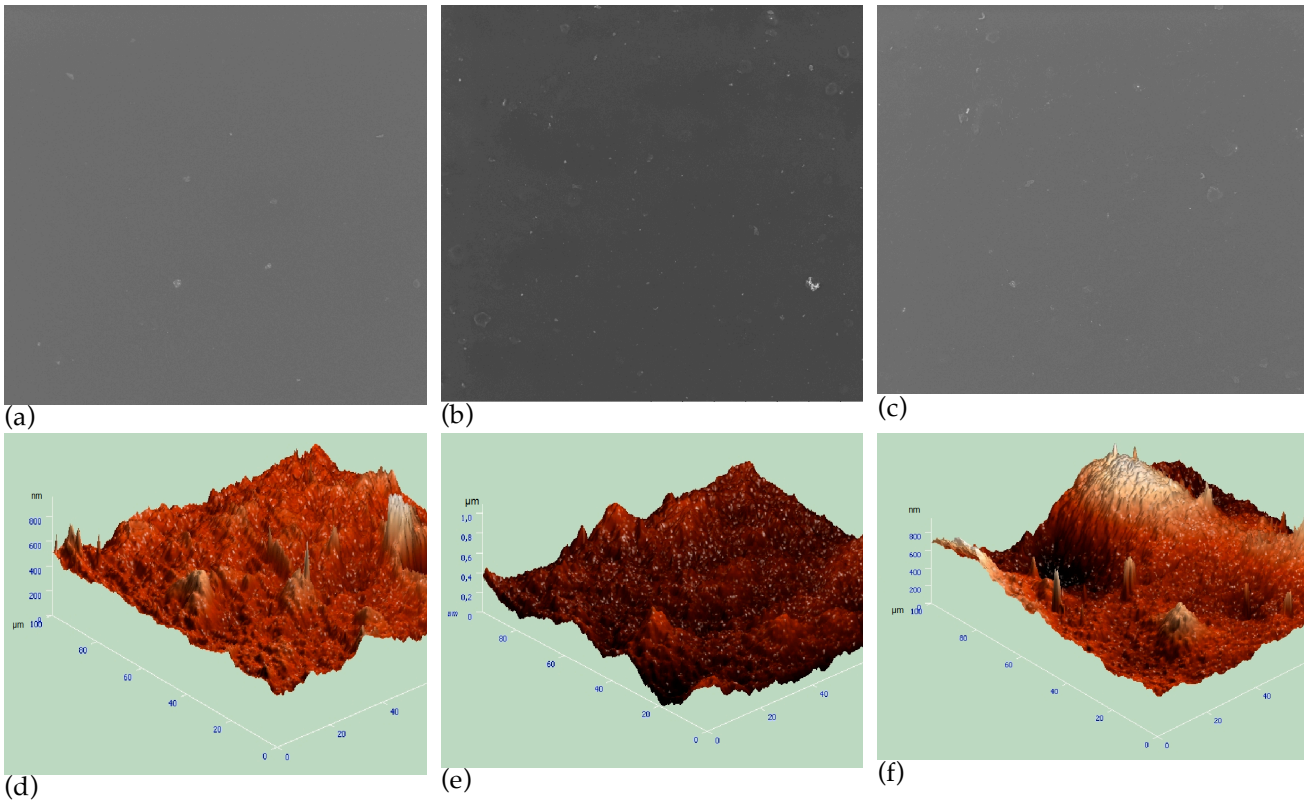


Figure 3. SEM* photomicrographs of control film (a), UV absorber films incorporated with the highest concentration of iron particles (15% w/w) (b) and highest concentration of tinuvin (0.5% w/w), as well as AFM photomicrographs of control film (d), UV absorber films incorporated with the highest concentration of iron particles (15% w/w) (e) and highest concentration of tinuvin (0.5% w/w) (f). *SEM images at 100X magnification.

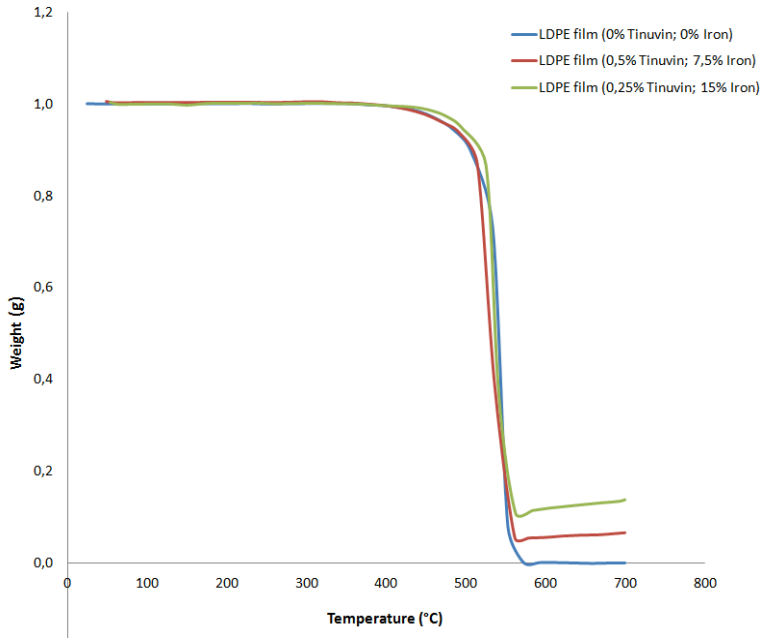


Figure 4. TGA curve of UV barrier films with the maximum concentration of iron particles (15% w/w), highest concentration of tinuvin (0.5% w/w) and control film.

The thermal degradation of the films occurred in a single step, being initiated in the control film (LDPE) at 419°C and completed at 571°C. The thermal decomposition of developed films initiated at a lower temperature (370°C) and their thermal decomposition process was completed at 580°C.
The maximum temperature of films decomposition was calculated from the derivative of the thermogravimetric curve (data not shown). These results revealed that the average maximum temperature for decomposition of control film was 552±3°C while that for the UV barrier films was 550.1±8°C.

3.6. Color Measurement

The color of packaging material is an important factor for the acceptance of food products (BOURTOOM and CHINNAN, 2008). In active food packaging, the active compounds bind to the polymeric matrix, resulting in changes in the natural film color (Rhim *et al*., 2000). Tinuvin and iron particles affected significantly the colorimetric parameters L*, a*, OP and WI (Table 2), while the YI and b* parameters were not influenced by them. The colorimetric parameters L* was influenced by the linear effect of iron particles ($p<0.05$), while the addition of tinuvin had no effect on this parameter. The linear regression coefficient for the concentration of iron particles was negative, which indicated that higher the concentration of the compound incorporated into the polymeric matrix resulted in low values of lightness in the films.
The colorimetric parameter a* was affected by the linear effect of tinuvin and iron particles. Linear effect of iron particles was positive, indicating that higher concentrations of iron results in higher values of a*, a pronounced tendency to redness, while linear effect of tinuvin was negative, indicating that higher concentrations of tinuvin results in lower values of this parameter. The colorimetric parameter OP was affected only by the linear effect of iron, indicating that higher concentrations of iron particles result in more opaque films. This is in agreement with the results of the WI, which showed that higher concentrations of iron particles result in lower values of this colorimetric.
Finally, the incorporation of tinuvin and iron particles in low-density polyethylene allowed the development of new active packaging materials for food preservation. Results obtained from mechanical and physical characterizations of the developed films by incorporating tinuvin and iron showed the influence of these particles in the film performance. Furthermore, the incorporation of tinuvin resulted in significant absorption of UV light, while iron particles had no significant effect. Thus, this work leads to conclude that UV barrier films may be developed with the incorporation of tinuvin to control the oxidation process induced by light in food products.

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EVALUATION OF FIRMNESS AND RELATIONSHIP WITH COLOR COORDINATES CIELAB OF THE PAPAYA (*CARICA PAPAYA* L.) TAINUNG F1 CULTIVATED IN THE HIGH SINÚ (CÓRDOBA - COLOMBIA)

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ABSTRACT

The relationship between firmness and CIELAB color coordinates (a *, b *, L *, C *), of the Tainung F1 papaya, grown in the department of Córdoba was determined. The maximum penetration effort for five degrees of maturity was calculated and statistical regression models were adjusted. An inversely proportional relationship between the firmness of the shell and the coordinates a *, b *, C * and L * was presented. The coefficient of determination R^2 of the model obtained between firmness of the shell and color coordinates resulted in 93.4%. In conclusion all the related parameters in the model were significant.

Keywords: color, correlation, quality, texture, tonality, tropical fruits

1. INTRODUCTION

According to ZAPATA *et al*. (2010), the firmness is the resistance of a material to the deformation or penetration and each material is characterized by a deformation curve in response to variable forces applied on its surface. The relationship between degree of maturity and physicochemical properties has been studied for various tropical fruits through multiple regression models as reported TORRES *et al*. (2013). The measurement of elastic properties of fruits and vegetables can be estimated through a simple penetration test coupled to a texturomete, Thus obtaining the force vs distance curves, necessary for the calculation of mechanical parameters such as force or penetration effort (CIRO *et al*., 2005). The aim of this research was to determine the color coordinates in the peel and pulp, a*, b*, L*, and the expressions *C, h° and coloration index IC, of the papaya Tainung F1 cultivated in the high Sinú (Córdoba- Colombia) to observe their statistical correlation. It is very useful to have measurements that correlate firmness with simple tests such as color determination. The result of the present investigation allowed obtaining a correlation between the firmness and the CIELAB coordinates with a coefficient of determination of 93.4%

2. MATERIAL AND METHODS

2.1. Collection and classification of papaya fruits Tainung F1

The analyses were carried out in the laboratory of the group of research processes and agroindustry of vegetables (GIPAVE) of the University of Cordoba-Colombia, Berástegui. The coordinates of color and firmness of the papaya fruits were determined in five degrees of maturity based on the external color as seen in Fig. 1. The fruits used were obtained from the Producers Association of Papaya of high Sinú (APPALSI). The determinations were performed with fruits harvested and maintained at an average temperature of 27.4°C. Ten fruits were taken for each degree of maturity, for the measurements.

Figure 1. Degree of maturity in Tainung F1 papaya cultivated by the association APPALSI in the municipalities of Valencia and Tierralta - Córdoba.

2.2. Determination of the CIELAB color coordinates in the peel and pulp of the papaya Tainung F1

A Colorflex colorimeter EZ 45 (HunterLab®) was used to measure the color. From the reflection spectra it was obtained the color coordinates of Hunter L* a* b*. Where L* is a brightness indicator, a* represents chromaticity (green (-) to red (+)) and b* represents blue

(-) chromaticity to yellow (+) as well as the color coordinates derived *C, h° and the IC staining index.

2.3. Determination of the firmness of Tainung F1 papaya fruits in peel and pulp

A cylindrical stainless steel probe of 2 mm diameter was used coupled to a texturometer TA.XTPlus Stable Micro Systems® in the whole fruit to calculate the "maximum penetration effort" in five degrees of maturity (external coloration), with a deformation rate of 5 cm / min at room temperature (27°C).

2.4. Experimental design

The analysis of the results obtained in the mechanical characterization was adjusted to an experimental design based on the recommendations of the studies carried out by SANTAMARÍA *et al*. (2015). Completely randomized with 5 repetitions for each degree of maturity. The analysis of the data was performed using the procedure of analysis of variance and the comparison of means with the Tukey test ($p \leq 0.05$). Likewise, multiple regression statistical models were fitted to observe the relationships between firmness and to observe the relationships between the firmness and the color coordinates (CIELAB) measured. For the statistical analysis of all the variables were used, the statistical packages STATGRAPHICS Centurion XVI® and the IBM SPSS Statistics 9.5®

3. RESULTS AND DISCUSSION

3.1. Firmness of peel and firmness of pulp

The initial firmness of the Tainung F1 papaya fruits, harvested at degree maturity 1, was 58.65±0.11 N in the peel. The Fig. 2 shows the decrease in firmness of fruits preserved at room temperature (27.4°C), being found differences between the firmness of the papayas harvested green (degree of maturity 1) and papayas in a state of consumer maturity (degree of maturity 4 and 5).

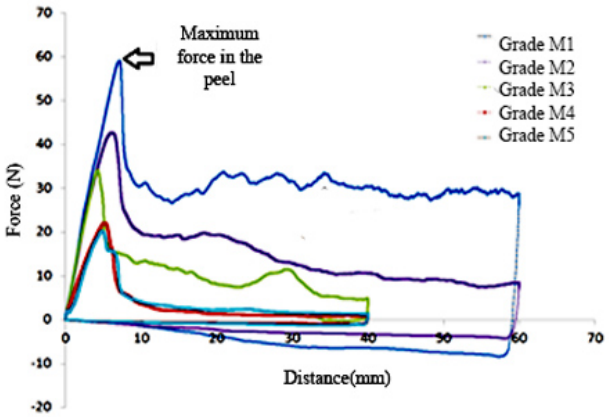

Figure 2. Firmness (N) of papaya Tainung F1 fruit with different degrees of maturity.

The Table 1 shows the values of firmness in the peel and pulp for the five degrees of maturity evaluated.

Table 1. Firmness (N) in papaya Tainung F1 fruits during the ripening process.

Firmness (N)	Degree of maturity				
	1	2	3	4	5
Peel	58,65±0,11a	41,84±0,08b	33,34±0,10c	21,45±0,03d	19,7±0,09E
Pulp	32,6±0,10a	19,83±0,05b	10,42±0,11c	2,93±0,07d	2,63±0,10e

For each variable, the means with the same letter in each row are statistically the same (Tukey $p \leq 0.05$).

3.2. Results obtained for color coordinates

In Tables 2 and 3, it is noted the color coordinate data of the Cielab model measured in pulp and peel respectively for the Tainung F1 papaya.

3.3. Correlation between the firmness and the coordinates of the Cielab color model

The values obtained for the firmness in the peel and pulp of the payaya Tainung F1 were correlated with the results of the color coordinates a*, b*, L*, C* and h°. An inverse proportional relationship between the firmness of the peel and the coordinates a*, b*, C* and L* was presented and a relation directly proportional to the pitch angle h° (Table 4). The firmness of the pulp has an inverse relationship with a* and C*. The regression model obtained is represented by equation 1:

$$\text{Firmness Peel} = 126{,}965 + 2{,}97498 \bullet a - 3{,}82312 \bullet b + 0{,}656391 \bullet C + 0{,}402449 \bullet L \qquad (1)$$

The R^2 determination coefficient resulted in 93.4%, all parameters were significant. The Durbin-Watson statistical test used to determine the correlation of the model data by analyzing the residuals yielded a value of 2.1029 ($p=0.8538$), this value represents the independence of the residues at one level of 95% confidence.

Table 2. Data obtained for the Cielab color model measured in the papaya Tainung F1 pulp.

Degree of maturity	a*		b*		L*	
	Mean	Desvest	Mean	Desvest	Mean	Desvest
1	19,957	0,26	30,361	0,43	66,4544	0,72
2	24,828	0,38	28,832	0,29	53,891	0,69
3	33,373	0,37	34,907	0,19	52,127	0,37
4	31,742	0,88	39,012	0,15	52,01	0,59
5	34,605	1,23	38,614	1,24	51,416	1,35
Degree of maturity	**C***		**h°**		**IC**	
	Mean	**Desvest**	**Mean**	**Desvest**	**Mean**	**Desvest**
1	36,1614	0,21	56,68	0,60	9,7549	0,36
2	37,4472	0,33	49,26	0,66	15,3112	0,37
3	48,2945	0,33	46,28	0,39	18,3474	0,41
4	49,5417	0,63	50,86	1,01	14,0520	0,91
5	51,8602	0,43	48,13	1,03	17,4773	0,97

Consequently with the data obtained, the decrease in firmness is very remarkable in wide range during maturity stages 4 and 5, representing a storage time at 27.4°C for 6 to 9 days. The Tukey test ($p \leq 0.05$) shows that there are significant differences between firmness in the shell and pulp for all degrees of maturity. The loss of firmness is favored by the action of enzymes such as hydrolases, caused by the rate of respiration and the production of ethylene, which cause the degradation of pectic and hemicellulosic substances (THUMDEE *et al*., 2007). KOJIMA *et al*. (1994) indicate that the decrease in resistance to maturation is attributed to the loss of elasticity and viscosity of the pulp due to the conversion of starch to sugars. As the degree of maturity progresses, the appearance of the yellow color also plays an important role as an indicator of the β-galactosidases that influence the detriment of the cell wall, as well as the action of the glucanases and endoxylanases that have effect on the same time in the loss of firmness.
The decrease in firmness in the pulp occurs due to the reduction of cell adhesion as a result of dissolution of the polysaccharides in the cell wall and half of the lamellae, although the mechanism of point action is not clear (BRUMMELL and HARPSTER, 2001; FABI *et al*., 2014). Another explanation of the loss of firmness is associated with the degradation of the cortical parenchyma that conforms the cell wall by the enzymatic degradation that occurs (PÉREZ and KROCHTA, 2000). In other studies of firmness, values of 70 N in the degree of maturity 1 to values of 10 N in the grade of maturity 5 have been obtained for Papaya variety Maradol (KRONGYUT *et al*., 2011).

Table 3. Data obtained for the Cielab color model measured in the papaya Tainung F1 peel.

Degree of maturity	a*		b*		L*	
	Mean	Desvest	Mean	Desvest	Mean	Desvest
1	-8,6560	0,24	18,3100	0,13	34,6430	0,26
2	-9,4200	0,13	23,3940	0,22	39,2590	0,14
3	-2,2220	0,12	33,7800	0,30	48,9200	0,12
4	7,8940	0,16	46,8520	0,20	46,7440	0,30
5	17,7180	0,12	58,8150	0,22	60,2820	0,15
Degree of maturity	**C***		**h°**		**IC**	
	Mean	**Desvest**	**Mean**	**Desvest**	**Mean**	**Desvest**
1	20,2530	0,12	115,3	0,69	-13,6463	0,44
2	25,2194	0,17	111,9	0,44	-10,2567	0,26
3	33,8530	0,29	93,8	0,22	-1,3446	0,08
4	47,5124	0,22	80,4	0,15	3,6045	0,04
5	61,4258	0,23	73,2	0,11	4,9973	0,03

For the Golden variety papaya, BRON and JACOMINO (2006) report values of firmness lower than 20 N in grades 4 and 5 of maturity, similar to the values of firmness presented by the Tainung F1 papaya in the present study for the degrees of maturity 4 and 5.

Table 4. Pearson correlations for the firmness variables in peel and values of the Cielab coordinates in papaya Tainung F1 fruits.

	Firmness peel	a*	b*	C*	h°
Firmness	1				
a*	-0,86••	1			
b*	-0,93••	0,99••	1		
C*	-0,92••	0,99••	0,998••	1	
h°	0,95••	-0,96••	-0,985••	-0,9728••	1
L*	-0,87••	0,91••	0,932••	0,930••	-0,9133••
	Firmness pulp	**a***	**b***	**C***	**h°**
Firmness	1				
a*	-0,77¨••	1			
b*	0,02ns	0,49••	1		
C*	-0,51••	0,91••	0,81••	1	
h°	0,90••	-0,85••	0,04••	-0,55••	1
L*	0,91••	-0,82••	0,07••	-0,52••	0,99••

•, •• Significant and highly significant differences ($p < 0.05$), ns: no differences

4. CONCLUSIONS

An inverse proportional relation between the firmness of the peel and the coordinates a*, b*, C* and L* was presented and a relation directly proportional to the pitch angle h°. The firmness of the pulp has an inverse relationship with a* and C*. The correlation between firmness and color coordinates was significant only for the firmness of the peel and the coordinates a*, b*, C*, L*. The obtained model presented a coefficient of determination of 93.4%. This prediction constitutes an acceptable tool for the monitoring of the mechanical resistance of the fruit to fast and standardized measurements, such as Cielab coordinates.

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BIOCONSERVATION OF HAMBURGUESA DE RES MEAT USING NISINA *LACTOCOCCUS LACTIS* TO INHIBIT THE GROWTH OF *ESCHERICHIA COLI*

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ABSTRACT

The effect of the use of nisin at different concentrations on the physicochemical and microbiological stability of beef burguers was evaluated. The simples were evaluated, pH, microbiological count and sensory atributes. BTC 1235, Humidity (AOAC 930.10), Ashes (AOAC942.05), Protein (AOAC984.18) and Fat (Soxhlet). A Completely randomized desing was used, where ther treatments used were milligrams of nisin in relation to one A completely randomized design was used, where the treatments used were milligrams of nisin in relation to a kilo of hamburger. 21 samples of 110g were taken and a control sample without addition of nisin, the series of burgers that had *Escherichia coli* absent, after being treated with nisin were subjected to sensory analysis. nisin-treated hamburgers (T1) showed better control of the growth of polluting bacteria, keeping the pH of the meat below the value of 5.5 throughout the cooling period and presented the highest sensory acceptance of burgers. According to the results found and due to the antibacterial properties of nisin, this helps to improve and extend the lifetime of meat per-product (Burger meat), is a reliable alternative to obtain a product with adequate microbial stability and high sensory acceptance.

Keywords: bioconservation, *Escherichia coli*, meat burger, Lactocucus lactis, nisina

1. INTRODUCTION

The quality of food has been a concern for man since the beginning of history, But only until the nineteenth century did it become apparent that the alteration of food or its microbiological contamination is directly related to the transmission of certain diseases. In recent years, the industrial sector of processed meat products has a great expansion and acceptance in the consumer market, because, with these, the useful life span increases. The overall trend for this type of product is the use of natural preservatives, which have the greatest potential in the meat industry are bacteriocins. (CASTILLO *et al*., 2001) substances produced by *Lactococcus lactis*, which have received great attention among natural preservatives, alter the structure and functionality of the cytoplasmic membrane through pore formation, causing the release of essential compounds for the cell. (GARRIGA *et al*.,2 001) The antimicrobial activity of bacteria represents a great potential for the food industry as they can be used as biological preservatives, and have the advantage of being peptides that are inactivated by digestive tract proteases and do not form dangerous secondary compounds (FAO/OMS.2004). Nisine is produced by *Lactococcus lactis* subsp. Lactis and is the only commercially available bacterium recognized as safe substance (generally considered safe - GRAS), having been approved for use in food in the United States (European Parliament and of the Council. European Directive 95/2) and in the European Unión (AYMERICH *et al*., 2008). When the meat is ground a larger area of it is exposed to harmful bacteria, such as those that can be contained in animal food, pathogenic bacteria such as Salmonella, *Escherichia coli* , Campylobacter yeyuni, Listeria monocytogenes and Staphylococcus aureus, which diminish the useful life of the product and generate the suffering of diseases transmitted by food (ETA). New food conservation treatments have been developed, including the use of biopreservatives, vacuum storage and under modified atmosphere. FERNÁNDEZ *et al*. (1999), carried out a study of the incorporation of bacteria in plastic surfaces, thus maintaining the activity of the bacteria and inhibiting the bacteria of the meat, For this the Bacteriocin goes directly to the plastic material which has several advantages: the agent would not be a direct additive to the product and the plastic material is biodegradable thus helping the environment. Cooked meat products are not sterile products; however, it is possible to produce them with a minimum of microbial load up to packaging, limiting their development in marketing, through refrigeration (FERNÁNDEZ-ESCARTIN, 2009). Typical examples of this group are sausages and burgers. Control of pathogenic microorganisms (such as *L. monocytogenes* and *Escherichia coli*) is feasible by the addition of commercial preservatives of natural origin, commonly used by industry, in which products based on nisin and organic acids stand out (CEPEDA-MÁRQUEZ *et al*., 2009). Un published data). The aim of this study was to measure the action of nisin to prevent the growth of *Escherichia coli* in the meat burger.

2. MATERIAL AND METHODS

2.1. Microbiological determination of the samples

Meat and fat were subjected to a milling process. Subsequently, the condiments were added in the form of brine held in 4°C, continuously mixing for 8 min. After this period the mixture was ground again, the final mixture was obtained (2310 g), was divided into 7 portions of approximately 330 g, on a digital scale. The manufacture of hamburger meat was carried out at laboratory scale at the facilities of the food laboratory of the University of Cartagena. The process involved the manufacture of 7 batches of hamburger meat, identified as A, B, C, D, E, F, G and a control sample. A completely randomized design

was used, where the treatments used were milligrams of nisin in relation to a kilo of hamburger: F1. 0.01 mg/kg, F2. 0.012 mg/kg, F3. 0.015 mg/kg and a control without nisin in addition. Ninin was mixed by spraying. The samples (batches of hamburger meat) were subjected to analysis, by means of a count of *Escherichia coli* , a procedure that will determine the degree of contamination of the meat mixture, prior to the addition of nisin (T°) (Table 1).

Table 1. Serial concentration of nisin of hamburgers.

FORMULACIONES stradas adosa e ingredientes		
Lot	**Serie**	**Ninin Concentration (g)**
A	AI	0,01%
	AII	0,012%
	AIII	0,015%
B	BI	0,01%
	BII	0,012%
	BIII	0,015%
C	CI	0,01%
	CII	0,012%
	CIII	0,015%
D	DI	0,01%
	DII	0,012%
	DIII	0,015%
E	EI	0,01%
	EII	0,012%
	EIII	0,015%
F	FI	0,01%
	FII	0,012%
	FIII	0,015%
G	GI	0,01%
	GII	0,012%
	GIII	0,015%
Control	Control 1	-----
	Control 2	
	Control 3	

2.2. physicochemical determination of samples

Carried out in accordance with Colombian legislation NTC 1325, Humidity (A0AC 930.10), Ashes (A0AC 942.05), Protein (AOAC 984.18) and Fat (Soxhlet). 2.3 Statistical analysis In this research the variables corresponding to the microbial growth of *Escherichia coli* in meat burgers, supported by two indicators, were analyzed, the first based on the absence or presence of *Escherichia coli* before and after the bioconservation process; and the second for the formation units of the colony (UFC) allowed for this product. The second variable corresponded to the sensory assessment of meat burgers, based on the acceptance of the product by the panellists and the degree of acceptability was statistically measured in order to emit a food attribute; In this case the taste.

3. RESULTS

Proximal analysis determines the nutritional components that are part of the food diet. Table 2 shows the results of the analysis carried out on the different samples with addition of nisin.

Table 2. Result of the proximal analysis of beef burger.

SAMPLE	PROTEIN	GREASE	HUMIDITY	ASHES
HAMBURGER WITH ADDITION OF 0.01% OF NISINA	13.297	3.684	2.75	1.54
HAMBURGER WITH ADDITION OF 0.012% OF NISINA	13.297	3.684	2.75	1.54
HAMBURGER WITH ADDITION OF 0.015% OF NISINA	13.297	3.684	2.75	1.54

Table 3 shows the results obtained from samples of beef burgers, showing that only four batches were found within the limits permitted by INVIMA (National Food and Pharmaceutical Surveillance Institute).

Table 3. Result of microbiological analysis of meat burger without nisin addition.

ANALISIS	MUESTRAS	RESULTADOS	VALORES DE REFERENCIA	VALORES DE REFERENCIA
Escherichia coli	Serie A	>1.100 NMP/g	120-1.100 NMP/g	PRESENCIA
	Serie B	>1.100 NMP/g	120-1.100 NMP/g	PRESENCIA
	Serie C	290 NMP/g	120-1.100 NMP/g	AUSENCIA
	Serie D	290 NMP/g	120-1.100 NMP/g	AUSENCIA
	Serie E	>1.100 NMP/g	120-1.100 NMP/g	PRESENCIA
	Serie F	290 NMP/g	120-1.100 NMP/g	AUSENCIA
	Serie G	>1.100 NMP/g	120-1.100 NMP/g	PRESENCIA

After obtaining the results of the analysis of *Escheria Coli*, those samples that presented "absence" of these microorganisms, will be intentionally added strains of this pathogen (Table 4). These strains were provided by the Microbiology Laboratory of the University of San Buenaventura Cartagena. Ninin was added by spraying. The burgers were packed in Ziploc bags, to be refrigerated at 8ºC for 15 days, simulating the average time of the burgers' commercial stay in the markets. After the storage time (15 days), at a temperature of 5ºC-8ºC, these samples were immediately subjected to microbiological analysis (T1).
The series of samples that showed the absence of *E. Coli*, after being treated with nisin, were subjected to sensory analysis, which allowed the evaluation of a certain attribute of the final product, such as taste. To do this, a population of twenty volunteer panelists will be taken to show the "flavor level" of the cooked samples, using a 6-point hedonic scale. The panel of tasters was composed of students, teachers and administrative staff of the University of Cartagena.

Table 4. Results of microbiological analysis of meat burger with nisin addition.

ANALISIS	MUESTRAS		RESULTADOS	VALORES DE REFERENCIA
Det. Escherichia coli	T^1	C1	560 NMP/g	120-1.100 NMP/g
		D1	290 NMP/g	120-1.100 NMP/g
		F1	420NMP/g	120-1.100 NMP/g
	T^2	CII	490 NMP/g	120-1.100 NMP/g
		DII	290 NMP/g	120-1.100 NMP/g
		FII	480 NMP/g	120-1.100 NMP/g
	T^3	CIII	480 NMP/g	120-1.100 NMP/g
		DIII	290 NMP/g	120-1.100 NMP/g
		FIII	400 NMP/g	120-1.100 NMP/g

Figure 1. Sensory evaluation of beef burgers with T1, T2, T3.

Fig. 1 presents the results of the sensory evaluation to the Beef burgers, prepared with T1 treatment presented the highest sensory acceptance, obtaining a 97% acceptance, followed respectively by samples T2 with 75% and finally the sample with treatment T3 with 55%. The addition of nisin in the beef burger showed to be an effective treatment to inhibit the growth of *Escherichia coli* by decreasing the microbial load, so it can be used as an antimicrobial agent, improving the microbiological quality. The series of burgers that showed *Escherichia coli* absence, after being treated with nisin, were subjected to sensory analysis. the burgers treated with nisin showed better control of the growth of contaminating bacteria, keeping the pH of the meat below 5.5 throughout the cooling period.

4. CONCLUSION AND DISCUSSION

The concentration of nisin of 0,05 mg/kg presented the best microbiological protection to the meat product with effective action on *Escherichia coli* , the preservative effect of the meat in the studied treatments was verified, measuring the pH of the hamburgers during storage and it was observed that they remained below 5.5. The addition of nisine to beef burgers did not cause alteration in the sensory acceptance of meat. According to the

results found and due to the antibacterial properties of nisin, this aid to improve and extend the shelf life of the meat subproduct (Beef burger), is a reliable alternative in obtaining a product with adequate microbial stability and high sensory acceptance.
The use of bacteriocins as natural antimicrobials is of great interest in the food industry, mainly because of its wide antimicrobial aspect, offering the possibility of replacing synthetic preservatives giving way to new advances in new technological processes in food biopreservation.

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THE PHYSICOCHEMICAL AND SPECTROSCOPIC CHARACTERIZATIONS OF GULUPA (*PASSIFLORA EDULIS SIMS*), OR PURPLE PASSION FRUIT, OF PAMPLONA, COLOMBIA

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ABSTRACT

This study determines the Physicochemical and Spectroscopic characterizations of Gulupa in its three states of maturation. The physicochemical characteristics, such as pH, soluble solids, and maturation indices increased as the fruit ripened, while the titrated acidity decreased. The presence of sugars, as indicated by FTIR Infrared Spectroscopy, in the region of the fruit situated between 1400 and 900 cm^{-1} found an augmentation of sugars as it matured. The data regarding the physiochemical characteristics was analyzed using Variance Analysis, ANOVA, with a significance factor of $p<0.05$.

Keywords: FTIR, Gulupa, chemical profile, maturation

1. INTRODUCTION

Today, physical, chemical, and biological methods are in a constant state of change and innovation, looking for faster, more precise and economical methods of analytically determining the physicochemical and sensorial characteristics of fruits (PEIRIS et al., Spectroscopy (FTIR), which is being evaluated and used for industrial purposes to measure the quality of whole, or intact, fruits and vegetables. FTIR is an alternative to carbohydrate analysis in food samples and produces information about the three principle carbohydrates (glucose, sucrose, and fructose). The sugar content of fruits is important to establish a "maturity grade", and as the potential as a principle material in the making of juices, nectars, jellies, and preserves. The Gulupa, *Passiflora edulis sims*, originated in Latin America, specifically Brazil, and was introduced in Colombia in the 1950s. Being the country with the larger wealth of species of this family, it has survived and fared well in both the wild and domestic settings. Its uses range from health and personal hygiene to ornamentation, and it is a highly sought-after product for fruit producers due to its extremely rapid rate of production in comparison to other products and its market demand both for fresh consumption and industrial processing.
As mentioned previously, this study focused on the spectroscopic and physicochemical characterizations of the Gulupa, or Purple Passion Fruit, originating from the surrounding areas of Pamplona, Colombia.

2. MATERIAL AND METHODS

2.1. Selection and preparation of Gulupa samples

2.1.1 Material Vegetal

Fruit was selected that fit exportation-quality criteria. It was collected in three different stages of maturation according to the color of the peel, or outer shell (green, painted, and ripe) and at two different times of the year (April and August), originating from the areas in and around Pamplona, Colombia (Fig. 1).

2.2. Physicochemical characterization

The collection of samples conforms to the parameters established by NTC 756 in 1977.

2.2.1 Caliber

The caliber of the fruit is determined by measuring the equatorial and longitudinal diameter of the entire fruit with a calibrator, or Vernier, or Dial Caliper in millimeters.

2.2.2 Total Soluble Acidity

Measured in triplicate for each sample, we took 10 grams of fruit, soaked and homogenized it in distilled water. We took an aliquot of 5 milliliters and titrated it with Sodium Hydroxide (NaOH) 0.1N. until the pH reached 8. The titulable was expressed grams of citric acid (0.064 factor of citric acid) for every 100 grams of product.

2.2.3 pH

To determine the pH, we used a calibrated HANNA Instruments HI 9 91001.

2.2.4 Total Soluble Solids

We measured this in triplicate implementing an ATAGO refractometer and expressed this in Degrees Brix.

2.2.5 The Maturity Index

This was determined by dividing the total soluble solids (in ºBrix) and the total tituable acidity (in % of citric acid).

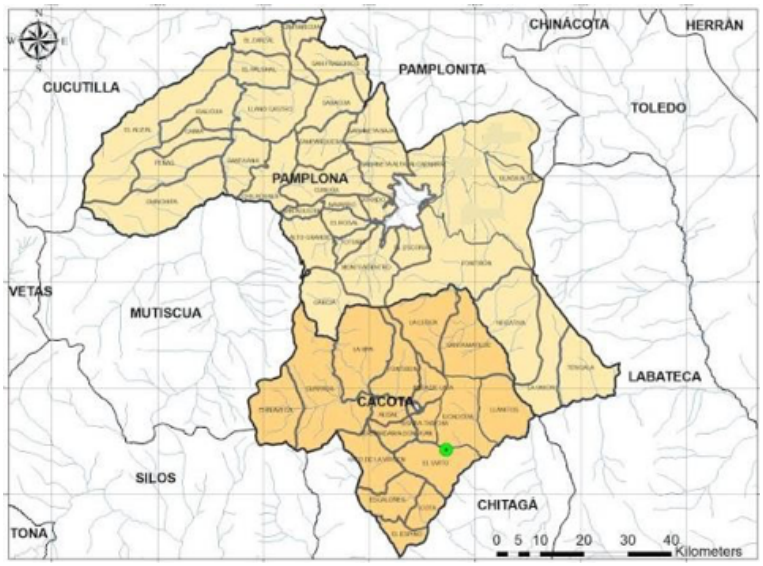

Figure 1. Zone of Study.

2.3. Spectroscopic Characterization

2.3.1 Lyophilization

The Pulp separated from the seed due to filtration and was lyophilized to eliminate the interferences caused by the physical characteristics of the fruit (OLIVEIRA et al., 2014). We used a Labconco Models 74000 Series lyophilized for 8 hours to perform this operation with the fruit.

2.3.2 Infrared Spectrophotometry

The infrared analysis of the lyophilized pulp was performed in a FTIR-ATR Shimadzu IR PRESTIGE-21 with a diamond crystal between 4000 and 600 cm -1, 40 scans and a spectral resolution of 4cm $^{-1}$. We performed this operation in triplicate for each sample. Between each, the cell was cleaned carefully with methanol to avoid interferences and errors between operations.

2.4. Statistical Analysis

We conducted an analysis of variance of a significance factor of 0.05 to determine the differences between the stages of maturation using the Unscramble software.

3. RESULTS AND DISCUSSION

The Gulupa simples were collected on the path of Licaligua which is in the municipality of Cácota, one of the surrounding towns making up the Pamplona region in the Department of Norte de Santander, Colombia, at an altitude of 2,400 msnm. This area has optimal temperatures, ranging being between 15 and 18°C during the day, and from 13 to 16°C at night.
The samples were transported to the Biocalorimetry Lab at the University of Pamplona to undergo the analysis that will be referenced in the following paragraphs.

3.1. Physicochemical characterization

While characterizing the fruits, we found that the pH values increased according to the stage of maturation; while the tituable acidity, measured as a percentage of citric acid, diminished according to the stage of maturation with significantly different values between each stage (Table 1). These data sets coincide with the findings of GUZMÁN and SEGURA (1989), who found that the hydrolysis of starch and other carbohydrates during the maturation process of these fruits and the diminishing of titulable acids can be credited to the consumption of organic acids in the breathing process of the fruits. The soluble solids increased as the Gulupa reached its stage of full maturation. These findings coincide with the findings of FISHER and MARTINEZ in 1999, who found equal manifestations in the uchuva fruit *Physalis peruviana*, or cape gooseberry, which presented a higher sugar content in its physiological maturation stage. This coincides with the findings of the maturation stages of the Gulupa.
OSTERLOH *et al.* (1996), found that the quantity of sugars in the fruit depends on the variey, the leaf a fruit relationship, the climactic conditions during the development and growth of the fruit, the stage of development of the fruit, and the physiological maturation. This also coincides with what was found for the stages of the Gulupa (0.4 and 6). Throughout the maturation process, we observed an ascent in the Maturity Index from 2.77, to 4.22 and 6.70, from the 0, 4, and 6 stages respectively. This behavior of incrementation was compared with what was reported by GALLO (1993) for the Maracuyá, or common passion fruit.
In these climactic fruits, the augmentation of the Maturity Index possibly occurs when they reach their maximum breathing rate and quickly unfold their reserves, organic acids, as a response to the increase of their metabolisms and in consequence, the index is found to increase (HERNÁNDEZ, 2001). OSTERLOH *et al*. (1996) affirmed that the importance of the relationship between SST and ATT to the taste of the fruit and juice, keeping in mind when the fruit has a high sugar content. The level of acids should be sufficiently elevated to satisfy the taste of the consumer.

Table 1. Physicochemical Characterization of Gulupa (Passiflora edulis Sims).

Propiedad	Verde	Pintona	Madura
Total Soluble Solids (°Brix)	12.46±0.05	14.5±0.05	16.5±0.05
pH	2.36±0.06	3.18±0.03	3.90±0.05
Total Soluble Acidity	4.53±0.06	3.41±0.006	2.44±0.01

3.2. Spectroscopic characterization

The spectrum of the pulp of the Gulupa showed the zones of absorption characteristics that correspond specifically to sugars. We identified understood peaks between the 1400 and 900 cm^{-1} assigned to the C-O, C-H and C-C bonds. Characteristic vibration points were identified at 1041, 1063, and 1132 cm^{-1} for glucose, fructose, and sucrose respectively. The incrementation of the carbohydrate content between the maturation stages was 10.5%, 8.6%, and 8% for glucose, fructose, and sucrose respectively. These results are consistent using the degrees Brix of the Gulupa, the data of which was 12.5, 14.5, and 16.5 for each stage of maturation (Fig. 2).

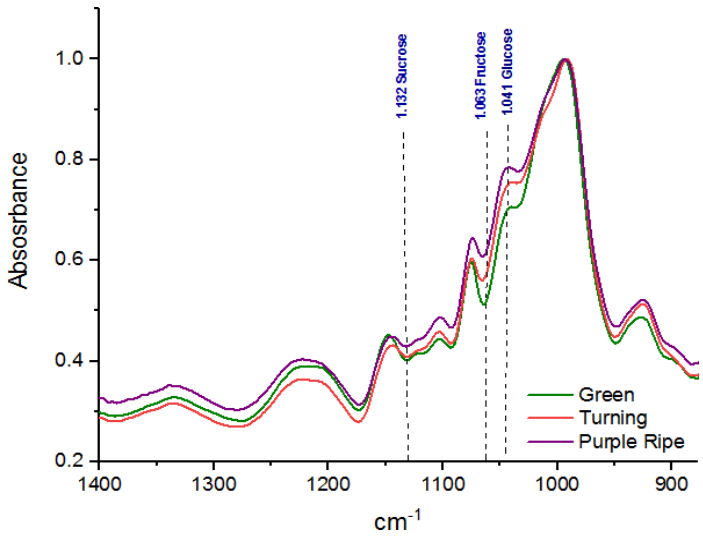

Figure 2. Spectrums of Gulupa in its three stages of maturation.

This finding resembles the study of work with maracuyá, or the common passion fruit, where the sugar characteristics were found to be between 1400 and 900 cm^{-1} (BUREAU *et al*., 2009; RUIZ *et al*., 2008). In the research conducted on the quantification of glucose, fructose, and sucrose in pure juices of the uvilla, which is a name that quantifies a number of small, sphere-shaped plants in Latin America, but of the *Physalis peruviana* family, the pure juices of apple, and the tomate de árbol, or Tamarillo (CRIOLLO *et al*., 2017), distinct characteristic peaks allowed the distinguishing of the least or greatest presence of glucose, fructose, and sucrose, which ranged between 1400 cm^{-1} and 950 cm^{-1}. The bands between 1153 cm^{-1} and 900 cm^{-1} are assigned to the C-O and C-C bonds, while the range of 1400-1199 cm^{-1} are due to the O-C-H, C-C-H and C-O-H bonds, while glucose is found between the wave numbers 1055 and 1138 cm^{-1}. Likewise, in the carbohydrate family appears an extremely high peak between wave numbers 1100 and 1000 cm^{-1}, which changes position in

the function of the specific type of carbohydrate tested (MONDRAGÓN, 2017; STUART, 2004).

4. CONCLUSIONS

Using this non-destructive technique, Spectrofotometry FTIR, for the analysis of sugars, we were provided information rapidly, simply, effectively, at a low cost, and accurately according to the internal and qualitative characteristics of the Gulupa, or purple passion fruit.

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EVALUATION OF TOXIC WASTE OF HEAVY METALS IN BOVES FROM THE MUNICIPALITY OF TUBU NORTH OF SANTANDER, COLOMBIA

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ABSTRACT

The municipality of Tibú, Norte de Santander, depends mainly on oil farms, which produce emissions in the vicinity of the industry, contributing to environmental pollution, affecting animals grazing around them. The objective of this study was to determine the concentration of heavy metals in bovine byproducts, consumed in the municipality of Tibu-Norte de Santander. Sampling was carried out on the sidewalks; Socuavó Norte, J-10, Campo Yuca and P-30, male, adult cattle of the commercial Cebu breed, the quantification of metals Cd, Cu, Mo, Pb and Zn were studied by atomic absorption spectroscopy, using the flame method and the concentrations obtained were processed through the statistical software XLSTAT and its study was based on the analysis of main components (ACP). The results were compared with the European Unions maximum permissible limits, since Colombia does not have a set of rules for this purpose. Itis concluded that the concentrations of heavy metals Cd, Cu, Pb and Zn exceded the limits permitted by the European Union Commission Regulation 488/2012 of 12 May 2014, there fore, the cattle consumed on the Socuavó North sidewalks, J-10, Campo Yuca and P-30 of the municipality of Tibu-Norte de Santander represent a serious danger for the population that consumes it.

Keywords: Cebu, extensive breeding, atomic absorption spectroscopy, heavy metals, bovine byproduct

1. INTRODUCTION

The proliferation of industrial activities and the human presence on the planet itself has facilitated the emission of polluting substances into ecosystems and into the living beings that inhabit them, into the food chain. Consumers are currently subjected to prolonged exposures of toxic substances in very low concentrations in food (CATALÁ *et al*, 1982). This is why the assessment of heavy metals in foods has become important because of their toxicity and their capacity for bioaccumulation in the body causing adverse effects. Heavy metals are chemical elements that possess environmental interest due to their impact on the different environmental compartments, this is associated with the possibility that several of these elements have to bioaccumulate and biomagnify, causing adverse health effects such as cancer, and immunological, reproductive, developmental and neurological problems (FALCÓ *et al*, 2005; SCHECTER *et al*., 2006; MARTÍ-CID *et al*., 2008; SHARIF, *et al*., 2008; WU *et al*., 2009). The main route of exposure of the human population to heavy metals is food, given the diffusion in the environment and accumulation through food chains as they are consumed and accumulated by domestic animals bringing with them public health problems. Within this group of environmental pollutants, mercury, cadmium and lead are noted for their toxicity and distribution. Arsenic and copper are essential elements for animal metabolism, however, both inorganic species of arsenic (arsenite and arsenite) and high concentrations of copper are toxic (MUÑOZ *et al*., 1999). On the other hand, structural changes in the livestock sector, such as increased intensive meat production systems, increased mono specific production, vertical integration, the development of global distribution and the establishment of intensive livestock systems close to urban centres have created an animal interface-people-ecosystems that increase the risk of new diseases infectious or the intensification of those already known (FAO, 2009). At the national and internacional level, a series of standards have been established to ensure the safety of meat products through the production chain, the control of heavy metals in bovine species in order to mitigate the environmental impact, encourage cleaner production and good livestock practices is an important part of this legislation, good manufacturing practices, called "organic agriculture" or "clean agriculture" and contribute to the production of safe foods (CORPOICA, 2007). Within the agricultural and livestock sector, livestock farming in Colombia makes an important economic contribution to the country, to such an extent that it is one of the sectors that most impact the gross domestic product (GDP) with 27% and 4% of the national GDP. Meat production is one of the most representative activities of livestock farming, which means about 12% of the total National Agricultural Production (MADR, 2009) and according to the third national agricultural census, more than 750.000 families live from livestock production (DANE, 2016; MADR, 2016). In Colombia, the meat market is regulated by health entities such as the Colombian Agricultural Institute (ICA), the National Institute for the Surveillance of Medicines and Food (INVIMA) and the Secretaries of Health, as this product is one of the ones with the greatest impact on public health, because its quality and safety is compromised from primary production until it reaches the final consumer. Despite being regulated by these entities, there is no regulation regulating the maximum permitted levels of heavy metals for meat and its byproducts in the country, This means that there is no control over them in Colombia, unlike other countries. In addition, viscera and blood are consumed in some regions of the country and must also be strictly controlled. Therefore, in this investigation the concentration of heavy metals in samples of meat consumed in the municipality of Tibú-Norte de Santander and its potential impact on the health of the local cattle was determined.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

The study was carried out in the municipality of Tibú, Norte de Santander (N. de S.), located in northeastern Colombia, which belongs to the Catatumbo region, characterised by economic activity within the framework of oil exploitation and animal husbandry of the meat type and extensive modality. Sampling was carried out on 20 cattle reared near oil explorations, from the commercial Cebu breed, adult males acquired from the municipalitys slaughter plants. All samples were obtained from cattle that met the legal requirements and the provisions of the guilds and sectors dedicated to livestock in Colombia. 200 grams of samples of liver, muscle, kidney, 100 mL of blood and 2x10 cm of skin were taken, obtained in the slaughter process using always the same anatomical origin of the animal. Liver samples were taken from the square lobe, kidney samples were collected from the right organ, muscle samples were taken from the pillars of the diaphragm. All samples are removed connective tissue, fat and major blood vessels, once cleaned, out of each tissue sample were taken 3 sub samples of approximately 20g that were placed in polypropylene bags duly identified with a code for each animal, which were duly refrigerated and sent to the Quality Control Laboratory of the University of Pamplona.

2.2. Analytical determination of meat, liver, kidney and skin samples

Was carried out according to the standard AOAC 999.10 in the following way; 20g of sample was weighed which was carried muffle at 100º to reach constant weight for two hours, to determine humidity and ash, then the grams obtained from this process were subjected to 550°C for 16 hours to incinerate the samples, once cooled, 3ml of HCl 3N was added and heated for 10 minutes over low heat in order to dissolve the ashes, the obtained solution was filtered and diluted to complete 25ml in volumetric balloon.

2.3. Analytical determination of blood samples

Followed the methodology proposed by ROQUEME *et al*. (2014), in which 30 ml of whole blood were taken from a 50ml porcelain crucible and placed to dry in the stove at 100°C until constant weight was achieved, then the crucibles were deposited in the muffle at 450°C for 16 hours by incinerating the samples, once cooled they were added 2ml of 2N nitric acid and dried in a thermostatic plate. When the acid evaporated, the crucibles were put back into the muffle at 450°C for one hour, 5ml 2N nitric acid and 20ml 0.1N nitric acid were added for the recovery of the ashes, The filter was then made with funnels and filter paper Whatman No. 40, stored in polypropylene containers and placed in refrigeration.
For the quantification of metals by atomic absorption spectrometry in a SHIMADZU AA7000 equipment, with air-acetylene to determine Cd, Cu, Pb and Zn and nitrous-acetylene oxide to determine Mo, performing the respective calibration curves with certified standards for each metal (Table 1).

2.4. Statistical analysis

Of the concentrations obtained were processed through the statistical software XLSTAT and its study was based on the analysis of main components (ACP). Two groups of quantitative variables were used: the first group consists of heavy metals (Cd, Cu, Pb, Mo and Zn) and the second group are bovine byproducts (Meat, liver, skin, kidney and

blood). The extraction of main components was carried out on typified variables to avoid problems derived from scale. The correlation matrix was applied to give equal importance to each and every one of the variables.

Table 1. Calibration curve for the determination of heavy metals

Metal	Equation of the straight	R^2
Cu	A= 0,1183C-0,0125	0,9991
Cd	A= 0,3346+0,0125	0,9976
Pb	A= 123,44-0,0198	0,9988
Zn	A= 1,4435-0,0813	0,9976

3. RESULTS

Table 2 shows the average copper, cadmium, lead and zinc obtained in the bovine products Liver, Muscle, Skin, Kidney and Blood.

Table 2. Metal levels in bovine sub products (mg/Kg). Tibu Norte de Santander.

	Cu mg/kg	**U.E mg/kg**
KIDNEY	1,95-9,99	0,5
BLOOD	0,62-5,61	0,5
LIVER	0,16-3,58	0,5
SKIN	1,08-4,39	0,5
MUSCLE	0,72-1,31	0,5
	Cd mg/kg	**U.E mg/kg**
KIDNEY	1,46-18	1,0
BLOOD	0,92-0,95	0,5
LIVER	1,44-2,10	0,5
SKIN	0,87-2,67	0,5
MUSCLE	0,82-1,16	0,05
	Pb mg/kg	**U.E mg/kg**
KIDNEY	1,12-1,85	0,5
BLOOD	2,22-8,54	0,5
LIVER	0,36-3,12	0,5
SKIN	0,79-1,50	0,5
MUSCLE	1,03-1,94	0,1
	Zn mg/kg	**U.E mg/kg**
BLOOD	39,25-48,40	N. R
SANGRE	25,47-40,54	N. R
LIVER	30,18-63,17	N. R
SKIN	26,38-34,91	N. R
MUSCLE	37,89-50,71	N. R

The ranges obtained in this study were obtained by means of the t-student test with a significance level of 95%. 85% of the liver samples, 70% of the kidney, 60% of the muscle, 75% of the blood and 45% of the skin tested exceeded the values permitted by the European Union regulations.
Heavy metals Cu, Cd, Pb and Zn, evaluated in the bovine products Liver, Muscle, Skin, Kidney and Blood. The graph shows that the highest content of Cu obtained was higher in the bovine sub product Kidney and was found on the Socuavó North sidewalk and in less quantity in the bovine sub product Muscle on the P-30 sidewalk. It is also observed that the highest levels of Cd were found in the blood and in the bovine liver, on the J-10 sidewalk. On the other sidewalks the level of this metal oscillated in a very similar range. Lower levels of cadmium were observed on all sidewalks in the muscle. On the other hand, it is noted that the highest averages obtained for Pb were found on the p-30 sidewalk in the liver bovine sub product, followed by the muscle bovine sub product that had its highest representation on the Socuavó Norte sidewalk. The lowest levels of Zn were obtained in bovine skin on the four sidewalks covered by this study. The p-30, S. Norte and J-10 sidewalk presented the highest concentration of this metal in the Liver (Fig. 2).

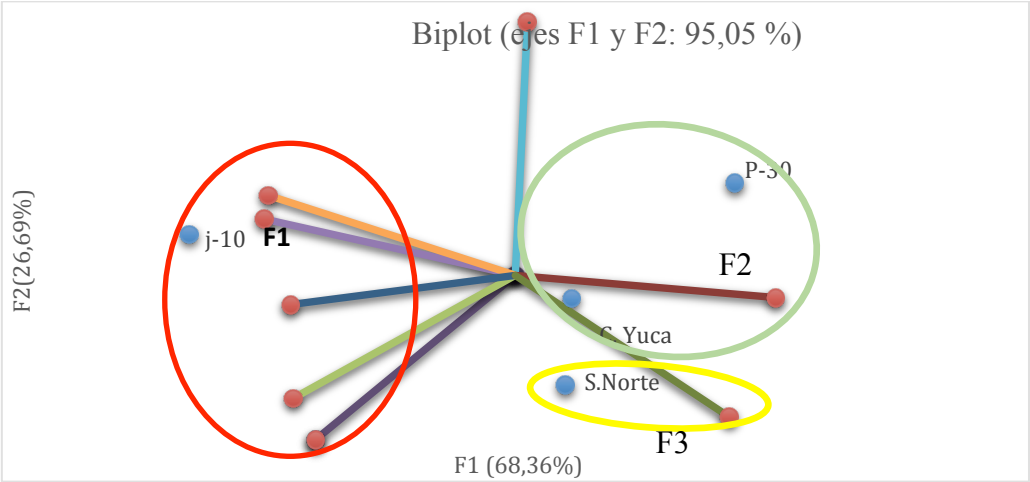

Figure 2. Distribution of Cd, Cu, Pb y Zn concentrations in Campo Yuca, Socuavó Norte, J-10 and P-30 sidewalks in the municipality of Tibú, Norte de Santander.

4. DISCUSSIONS

With the exception of P-3 sidewalk, the levels of cadmium found in the soil of the other study sidewalks were higher than 0,5 mg/Kg, a value established by the European standard, as a permissible limit of that metal in soil. Thus, on the J-10 sidewalk, an average of 0,84-1,102 mg/kg was found for cadmium, followed by the Campo yuca sidewalk (0,921-1,02) and the S. Norte sidewalk (0,682-0,944) (0,5 mg/Kg) and with authors like Brooks (1988), who suggests a limit concentration of Cd in soil of 0,1 to 0,2 mg/kg. As regards Cd levels found in fodder on the same sidewalks, they are above the permissible limits set by the European Standard (Official Journal of the EU, 2014), (Average cassava field between 0,428-0,516 mg/Kg; Socuavó Norte 0,272-0,4408 mg/Kg; vereda P-30 with a range between 0,262-0,435 mg/Kg and vereda 10 - J with an average range of 0,110,110,0000mg; 0/Kg.
When analysing the concentrations found for cadmium in bovine tissues in the study paths, values were found above the normal ranges stipulated by the European Union

(Official Journal of the EU, 2014), so that, in Liver, 85% of the animals, in Kidney 75%, in Blood 65%, in Muscle 60%, and in skin 45% of the animals exceeded the limits allowed by the European standard. A situation which is an indicator of the level of pollution of cadmium in the environment of the surveyed paths and which is consistent with what was reported by the EASA (2009), the Commission which establishes that, in animals, cadmium has a long period of permanence in the liver and kidney tissues, which makes it not easily removed.
The highest accumulation of cadmium was observed at the liver and kidney level, which is in line with what was established by FRIBERG *et al* (1979), who assume that the aforementioned organs are the main ones involved in chronic accumulation of cadmium because they have the capacity of synthesis of metalothionens, to which cadmium is attached.
Moderate to high levels of toxic metals such as Pb and Cd are evident in cattle in the Catatumbo region, especially in muscle, kidney and liver, organs of vital importance in human nutrition, and that having these metals acumulative behavior, that is, they are not degraded or eliminated, but accumulate over the years in the organisms, as the food chain advances, they are causing adverse effects. For this reason, cattle are considered to be a good indicator of environmental health and there is an urgent need to avoid or control cattle grazing in the vicinity of these industries in regions with oil exploitation.

5. CONCLUSIONS

The presence of some heavy metals (Cd, Cu, Mo, Pb and Zn) in the bovine byproducts studied in the municipality of Northern Tibú of Santander exceeded the maximum residue limits laid down in European legislation, it was observed that in most of the studied sidewalks the presence of copper was superior in the kidney and in less amount in the muscle. The highest level of Cd was observed in the Liver and Blood bovine byproducts, obtained on J-10 sidewalk, while the lowest level of Cd was observed in muscle on the four sidewalks under investigation. Cadmium and lead are heavy metals characterized by a slow elimination of the organism, which was consistent with what was found in this investigation, as they were present in most of the matrices analyzed, being more abundant in liver and kidney, which is a warning call due to the dietary habits of the population resident in the Catatumbo region, who consume these two subproduct.
The highest level of lead was found in the bovine byproduct Liver, obtained on the P-30 sidewalk. The highest levels of zinc were found on J-10, P-30 and S. North sidewalks. While on the sidewalk yucca field the level of this metal was found at very low levels. Despite finding levels of heavy metals in bovine byproducts above the European standard, the quality of the meat products offered to the consumer cannot be assured, since there is no regulation in Colombia regulating the permitted levels of metal in these products.
No presence of molybdenum metal was found in bovine production on the sidewalks analysed in this investigation.

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FUNCTIONALITY AND PHYSICOCHEMICAL PROPERTIES OF THE CHERIMOYA FLOUR *(ANNONA CHERIMOLA MILLER)* CV. CUMBE

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ABSTRACT

The objective of the study was to take advantage of the fruits of cherimoya (Annona cherimola Miller), which presented low caliber for fresh marketing and that would allow obtaining a flour with adequate physicochemical and functional properties for the food industry. For this, the fruits of up to 3 days of collecting were manually conditioned to obtain their pulp, which was laminated to 3±1 mm thick and dehydrated in a convective dryer at 50°C and an air velocity of ~3 m / s, until constant weight. Subsequently, it was ground and sieved using an 80 mesh to obtain cherimoya flour (HCh). The results expressed as the mean±SD of at least three independent experiments; where aw values of 0.332±0.025, the humidity of 12.04±1.23%, pH of 5.46±0.05 and °Brix of 20.00±0.01 were obtained. The analysis of the CIELab Color coordinates for the HCh found a luminosity (L*) of 87.9±0.9, a* value of 1.5±0.3 and a b* value of 14.3±2.0, defining a yellowish-white color. The functionality tests yielded values of apparent density (ϱa) of 0.4746±0.019, compacted density (ϱc) of 0.5514±0.0123, cohesiveness by the Hausner Ratio (HR) of 1.1623 and fluidity by Carr index (CI) of 13.94%; values that give "low cohesiveness" and "very good fluidity" to HCh. Likewise, the solubility index (ISA), water absorption index (IAA) and the oil retention capacity (CRA) determined obtained valuables of 27.10±2.55%, 2.6036±0.2466 g H2O/g and 1.0742±0.0930 g oil/g, respectively. In conclusion, cherimoya Flour has physicochemical and functional properties that make possible to use as a

Keywords: *Annona cherimola* Miller, Cherimoya flour, functional properties

1. INTRODUCTION

Cherimoya, scientifically known as *Annona cherimola Miller*, is a fruit that grows in the valleys of the provinces of Lima (Peru). It is a very digestive and nutritious fruit, characterized by its high water content. It has many particular characteristics given to the harmonic combination in its composition of acids and sugars. The last one is product of starch reduction during post-harvest maturation, with glucose (11.75%) and sucrose (9.4%) as predominant sugars (KAWAMATA, 1977); while the main organic acids in its composition are citric and malic acid. This combination results in pulp fruit with pleasant and extremely sweet taste when it reaches its physiological maturity (24 °Brix) that consumption focused on its ripe state.

Although the cherimoya may have limitations in its processing, mainly due to the high phenolic oxidation and its tendency to darkening, the industrial products derived from this pulp are diverse and is very commonly used in the production of ice cream, beverages, and pastry products. Another great limitation is its rapid post-harvest maturity that can generate large losses in the fresh fruit trade, which increases the logistics processes for its conservation and trade due to the application of the cold chain. To which is added the factor of the sale price in the fresh state, which is related to quality criteria such as the selection by calibration, size, weight, etc. All these factors can generate a 60% discard of the national production of Peru (LIRA SEGURA, 2014)

In this sense, in fruits that have a limited shelf life, the development of methods for their conservation is important to avoid losses and add value. Dehydration results in an adequate technique to achieve this objective (VERMA *et al*., 2015) and is commonly used at industrial scale to obtain high-moisture raw material flours, such as cherimoya.

Flour production is an alternative to prolong the shelf life of some food products, as can reduce free water and obtain less water activity; additionally, reduces undesirable physicochemical changes and the growth of microorganisms. Besides, it allows the product to have a better commercial value and adequate handling, reducing the energy costs of storage (CUQ *et al*., 2011). However, when food is dehydrated, an imbalance pressure occurs within the product (BEJAR *et al*., 2011), which can cause pharmacological alterations and affect the quality of fruits; in chemical, physical and functional terms, depending on drying techniques. For example, the color of fruit pulp wastes has been affected after drying at different drying temperatures using a conventional oven (ROSNAH *et al*., 2015), while when using the technique of lyophilization, the resulting changes are minimal.

For this reason, this research studies the physicochemical and functional properties of cherimoya flour (HCh) obtained by conventional drying method, as an alternative to preserve the fruit and contribute to its value chain.

2. MATERIAL AND METHODS

2.1. Vegetal material

Cherimoya fruits (Annona cherimola Miller cv. Cumbe) were collected during the May-August harvest period, from the San Mateo de Otao producing Community of the Province of Huarochirí (Lima, Peru), which was arranged daily following its collection for processing in flour at the Industrial Process Laboratory of the Pontifical Catholic University of Peru (Lima).

2.2. Cherimoya flour production

The cherimoya green fruits were washed with running water and disinfected by immersion in a solution of sodium hypochlorite (100 ppm) for 5 minutes. Subsequently, they were manually stripped with a stainless steel knife and divided into 20 mm slices to facilitate the removal of the seeds. The pulp fractions were suspended in a 0.1% (w/v) sodium bisulfite solution and then laminated with the help of a stainless steel mandolin to obtain slices of 3±1 mm.
The slices were evenly distributed on the surface of the equipment tray (300 mm x 300 mm) and placed in a convective dryer that operated at 50±2ºC and an air velocity of 3 m/s, until a constant weight was reached. Dehydrated pulp was ground in a knife grinder (Bosch, MKM6003, Slovenia), and then screened using meshes of 80 or more mesh (RETSCH, ASTM E11, Germany). The flour contained between the 80-100 meshes, with particle sizes between 150 and 179 μm, was packaged in nylon-PE bags to preserve it until analysis.

2.3. Physicochemical properties

2.3.1 Water activity and moisture (%)

The HCh a_w was measured with a water activity measuring device (NOVASINA, LabSwift-aw, SPAIN). While, the moisture content was measured by drying in a forced convection oven (MEMMERT, UN30, Germany) at 105°C until obtaining a constant weight (LI *et al*., 2019).

2.3.2 pH y total soluble solids

The pH of HCh has measured following (SUNTHARALINGAM and RAVINDRAN, 1993) method with some modifications. A suspension of 4% (w/v) HCh was made, at room temperature, stirred for 5 min in a vortex shaker for 30 min. The supernatant was transferred to a beaker for measure pH by a calibrated potentiometer (CRISON, Basic 20, SPAIN). Total soluble solids (TSS) were measured in the same supernatants using a digital refractometer (KRUSS, DR201-95, Germany). The results were expressed as °Brix.

2.3.3 CIELab color

For the measurement of the color of HCh, the CIELab coordinates obtained from a colorimeter (KONICA MINOLTA, CR 400, Japan) were used, using the illuminant D65 and the observer of 10°, following the method reported by (SU *et al*., 2018). The color measurements were expressed in terms of brightness L* from 0 to 100 (degree of lightness) and the parameters a* (degree of redness and greenness) and b* (degree of yellowness and blueness).

2.4. Functional Properties

2.4.1 Apparent density and compacted density

The HCh apparent density (ρ_a) was determined by measuring the weight of the HCh and the corresponding volume. Approximately 1 g of HCh was transferred to a 10 mL graduated cylinder. The apparent density was calculated by dividing the mass of the HCh by the volume occupied in the graduated cylinder. For compacted density (ρ_c), the

graduated specimen was struck at a constant volume with a glass rod. The volume of HCh was measured and used in the mass calculation between volume to obtain the compacted density (JINAPONG *et al*., 2008).

2.4.2 Carr index (fluency) and Hausner ratio (cohesion)

The fluency and cohesion of HCh were expressed in terms of the Carr Index (CI) (Carr, 1965) and Hausner Ratio (HAUSNER, 1967), respectively. Both CI and HR were calculated from the apparent (ρ_a) and compacted (ρ_c) densities of HCh as shown in the following equations:

$$CI = \frac{\rho_C - \rho_a}{\rho_c} x100 \qquad \text{(Ec. 1)}$$

$$R = \frac{\rho_c}{\rho_a} \qquad \text{(Ec. 2)}$$

The fluidity of the powders with IC <15 is classified as "very good"; 15 <CI <20 as "good"; 20 <CI <35 as "regular"; 35 <CI <45 as "bad" and CI> 45 as "very bad" (Carr, 1965). Powders with RH below 1.2 are classified as a "low cohesivity" group; with HR between 1.2 and 1.4 it is considered as "intermediate cohesiveness" and HR of more than 1.4 is considered "high cohesivity" (HAUSNER, 1967).

2.4.3 Water solubity index (WSI) and Water absorption index (WAI)

The water solubility index (WSI) and water absorption index (WAI) of HCh were performed according to RODRÍGUEZ-AMBRIZ *et al*., (2005) with some modifications. 1 g of HCh (P_0) was mixed with 35 mL of distilled water, at room temperature. The mixture was homogenized with a vortex shaker (KGEMMY Industrial Corp, VM300P, TAIWAN), at a maximum level for 5 minutes, then the solution was transferred to a previously weighed 50 mL centrifuge tube; the tube was left at room temperature for 1 hour and centrifuged at 4390 rpm for 20 minutes in a centrifuge (THERMO FISHER, CL10, Germany). The tube was drained at an angle of 45° for 10 minutes in a previously weighed Petri dish. The Petri dish supernatant was dried for 12 h at 105°C until constant weight, (P_1). The WSI was calculated by dividing the dry mass of the supernatant (P_1) by the sample mass of HCh used in the test (P_0), expressed as a percentage. The WAI was calculated as the difference between the centrifuged precipitate mass (P_2) and the HCh sample mass used in the test (P_0) divided by the HCh sample mass used in the test (P_0).

$$WAI = \frac{P_1}{P_0} \qquad \text{(Ec. 3)}$$

$$WSI = \frac{(P_2 - P_0)}{P_0}\ x\ 100 \qquad \text{(Ec. 4)}$$

2.4.4 Oil retention capacity (ORC)

To determine the oil retention capacity (ORC), the RODRÍGUEZ-AMBRIZ *et al*. (2008) method was used, 25 mL of olive oil was mixed with 1 g of HCh (P_0), placed on a vortex shaker (KGEMMY Industrial Corp, VM300P, Taiwan) for 2 minutes and incubated at room temperature for 1 hour. The tube was centrifuged at 4390 rpm for 20 min. The supernatant was decanted and the tube was drained for 10 minutes at a 45° angle. The centrifuged

precipitate was weighed (P_3), and the ORC was calculated as g of oil per g of HCh sample, as follows:

$$ORC = \frac{(P_3 - P_0)}{P_0} \quad \text{(Ec. 5)}$$

2.5. Statistical Analyses

All measurements and analyses were performed in triplicate. The results were expressed as mean±standard deviations, using MS Excel (2016).

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties

The physicochemical properties (water activity, moisture, pH, soluble solids) of HCh are presented in Table 1. Moisture content and water activity of powder products are critical properties that may affect other physical and chemical properties of food. In addition, they are critical factors for shelf life and stability of food. The values obtained for humidity (12.04±1.23) and water activity (0.332±0.03) were within the limits for safe storage. In relation to these, IOMBOR and OLAITAN (2014), LI *et al.* (2019) and SOQUETTA *et al.* (2016) reported a moisture content, lower than HCh, in soursop flour (*Annona muricata*), dry yam and ripe kiwi bagasse, with values of 8.10±0.06%, 6.94±0.13% and 9.18±0.28%, respectively. Regarding the water activity that HCh presented, it was higher than *Marolo Annona crassiflora* flour (0.176±0.00) (CORRÊA *et al.*, 2011) and for ripe kiwi bagasse (0.44) (SOQUETTA *et al.*, 2016). Despite this, HCh can be considered a stable food considering that deterioration reactions occur when water activity is greater than 0.65 for most foods (ETHUR *et al.*, 2010).

The pH results (5.46±0.05) were similar to those reported for green and marolo banana flour with pH values of 5.06 and 5.42, respectively (ALKARKHI *et al.*, 2011) (CORRÊA *et al.*, 2011).

Soluble HCh solids had a value of 20.00±0.01 °Brix, much higher values than those reported for green banana flour that had 0.74±0.09 °Brix (SAVLAK *et al.*, 2016). Similarly, ALKARKHI *et al.* (2011) reported values of 1.22±0.14 °Brix for green banana flour and 4.26±0.24 °Brix for banana flour mature. The response in the °Brix of the HCh is mainly due to the fact that the fruit of the cherimoya at the time of its collection comes to present between 6 and 8 °Brix, reaching 25 °Brix in its senescence (data not shown), with the Dehydration increases to the total solids content, and also the soluble ones (°Brix).

Regarding the color presented by the HCh (Table 2), the value of L* was 87.9±0.90, a brightness value close to white (MATHIAS-RETTIG and AH-HEN, 2014). This value was similar to yam flour that registered an L* of 87.74±0.09 reported by LI *et al.* (2019) and that green banana flour (Musa spp. AAA) obtained by lyophilization and dried with dry air at 55°C that registered luminosity values of 85.00±0.346 and 84.62±1.18, reported by SAVLAK *et al.* (2016) and AHMED *et al.* (2019) respectively. However, ALKARKHI *et al.* (2011) reports a lower luminosity for green and ripe banana flour, with a value of 74.18±4.62 and 70.85±2.53, respectively, by which the flours derived from this banana species were significantly darker. This comparative color advantage is remarkable, given that the HCh had a high content of soluble solids and was dehydrated at 50°C, while that obtained by ALKARKHI *et al.* (2011) had a lower content of soluble solids and was dehydrated at 60°C. Therefore, it is likely that the use of antioxidant has not been effective,

favoring the Maillard reaction and the high enzymatic activity of the polyphenol oxidase present in the banana that could contribute to a certain degree of enzymatic browning (THIPAYARAT, 2007). Enzymatic browning of bananas is a well-known problem, although also in cherimoya, the use of sodium bisulfite as an antioxidant result being effective for this type of Annona. Finally, in terms of color, the parameters a* (red-green axis) and b* (yellow-blue axis) of the HCh presented values of a* of 1.50±0.30, which tends to neutral and b* 14.30±2.00, which tends to yellow, both values added to the L* value, define it with a yellowish-white color.

Table 1. Results of the physicochemical evaluation of cherimoya flour (HCh) and those reported for flours of other fruits.

Fruit	Physicochemical properties			
	a_w	H %	pH	°Brix
Annona cherimola Miller (In this study)	0,332±0,03	12,04±1,23	5,46±0,05	20,00±0,01
Annona crassiflora (Corrêa *et al.,* 2011)	0,176±0,00	4.2±0.87	5,42±0,06	NR
Annona muricata (Iombor and Olaitan, 2014)	NR	8,10±0,06	NR	NR
Musa spp. AAA cv Cavendish verde (Savlak *et al.,* 2016)	0.424±0.015	9.07±0.347	5.665±0.011	0.58±0.035
Musa acuminate L., cv cavendshii verde (Alkarkhi *et al.,* 2011)	NR	NR	5,06±0,52	1,22±0,14
Musa acuminate L., cv cavendshii maduro (Alkarkhi *et al.,* 2011)	NR	NR	5,13±0,29	4,26±0,24

Values are means±standard deviation of at least three repetitions. NR, value not reported.

Table 2. Results of colorimetric parameters of cherimoya flour (HCh) and those reported for flours of other fruits.

Fruit	L*	a*	b*	Chroma	Hue angle
Annona cherimola Miller (In this study)	87,9±0.90	1,50±0,30	14,30±2,00	14,40±2,00	84,20±0,03
Annona crassiflora (Corrêa *et al*., 2011)	71,20±0,17	NR	NR	37.91±0.16	73.60±0.00
Mangifera indica verde (Noor Aziah *et al*., 2012)	72.37±0.20	-3.32±0.05	33.71±0.07	33.88±0.06	95.62±0.09
Musa spp.AAA cv Cavendish verd (Savlak *et al*., 2016)	85.00±0.346	1.83±0.059	11.81±0.125	11.95±0.131	81.18±0.216
Musa spp. AAA cv Mountain Verde *(Ahmed et al., 2019)*	84.62±1.18	1.29±0.03	11.54±0.14	NR	NR
Musa acuminate L., cv cavendshii verde (Alkarkhi *et al*., 2011)	74,18±4,62	2,53±0,78	17,36±0,78	NR	NR
Musa acuminate L., cv cavendshii madur (Alkarkhi *et al*., 2011)	70,85±2,53	3,2±0,80	14,15±2,59	NR	NR

Values are means±standard deviation of at least three repetitions. NR, value not reported.

3.2. Functional properties

The apparent density, the compacted density, the Hausner ratio and Carr index of HCh are recorded in Table 3. GOULA *et al.* (2004) explained that the decrease in these parameters is due to the adhesion of the particles during dehydration and by agglomeration of the product. On the other hand, ABDULLAH and GELDART (1999) assert that free-flowing powders have lower consolidation properties, while a fine and cohesive powder collapses rapidly due to tapping.

The HCh had an apparent density of 0.475±0.019 g·cm^3, values similar to that recorded for other anonaceous flour such as soursop *Annona muricata* (0.421±0.001 g·cm^3) that was reported by IOMBOR and OLAITAN (2014), however it was lower than marolo flour *Annona crassiflora* reported by CORRÊA *et al.* (2011), who reported density values of 1.38±0.09 g·cm^3. This difference may be due to the particle size of the flours, since the study worked with a particle size of 150 and 179 μm, while the other authors do not report the particle size of their respective flours. On the other hand, in flours of other fruits such as green bananas have an apparent density of 0.251±0.00 g·cm^3 when their grain size is less than 212 μm (SAVLAK *et al.*, 2016). This value was much lower than reported by RAYO *et al.* (2015), who reported values of apparent density of 0.515 g·cm^3 for green banana flour of the Nanicão variety.

Regarding the compacted density of the HCh, a value of 0.551±0.012 was found, a value that is between that recorded for green banana flour 0.652 and 0.403 g·cm^3, reported by SAVLAK *et al.* (2016) and RAYO *et al.* (2015). The density value was being important in the development of the products since insoluble or instant foods a higher density is desirable since it facilitates the dispersibility of the product (PADMASHREE, 1987)

The results obtained for the cohesiveness by the Hausner ratio (HR) of 1.1623 and the fluidity by the Carr Index (CI) of 13.94%; they are values that give "low cohesiveness" and "very good fluidity" to HCh; CARR, 1965). The ratio of a good flow capacity with small particle sizes can be explained by a large surface area per unit mass of dust. There is more contact surface area between dust particles available for cohesive forces and frictional forces to resist flow (FITZPATRICK *et al.*, 2004). Therefore, intermolecular forces are strengthened, reducing the ease of dust flow. Other authors such as (SAVLAK *et al.*, 2016) found in green banana flour that, the Carr index changed between 18.3% and 20.95% and the Hausner ratio between 1.22 and 1.27 in the green banana flour, which presented an intermediate fluidity.

Table 3. Apparent and compacted density of cherimoya flour (HCh) and those reported for flours of other fruits.

Fruit	Functional properties			
	ρ_a (g·cm^3)	ρ_c (g·cm^3)	HR	CI, %
Annona cherimola Miller (In this study)	0,475±0,019	0,551±0,012	1,162	13,940
Annona crassiflora (Corrêa *et al.*, 2011)	1,38±0,09	NR	NR	NR
Annona muricata (Iombor and Olaitan, 2014)	0,421±0.001	NR	NR	NR
Mangifera indica verde (Noor Aziah *et al.*, 2012)	0.69±0.01	NR	NR	NR
Musa spp.AAA cv Cavendish verde (Savlak *et al.*, 2016)	0,251±0,00	0.403-0,65	1,22-1,27	18,3-20,95

Values are means±standard deviation of at least three repetitions. NR, value not reported.

On the other hand, the interaction between water and HCh as well as the formation of flour gel, is of great importance in the processing and application of flour. The hydration properties of flour and gel formation are usually affected by the drying method (Fig. 1). For HCh obtained by convective drying at 50 °C, they are presented in Table 4.
The solubility index (CORRÊA *et al*., 2011) (WSI) found for HCh was 27.10±2.55%, relatively high compared to that found for hot air-dried yam flour (11.21%) (LI *et al*., 2019). The authors argue that the yam suffered a thermal effect that destroyed the internal cell structure and starch structure to some extent. Therefore, the low molecular weight amylose was leached, which resulted in a high content of free starch and a greater solubility. Likewise, in comparison to the flour of another anonaceous such as guanabana, it was only slightly higher (20.1±0.06), having in common that both underwent dehydration by hot air at 50°C. In the opposite way, (Corrêa *et al*., 2011) report a higher WSI value for marolo flour (*Annona crassiflora*) 43.87±0.93%. Even the same authors confirm that the dehydration technique significantly influences this parameter, finding a WSI value of 51.87±3.21% when dehydrated by lyophilization (Fig. 1A).
On the other hand, in other fruits of another genus the ISA of the HCh was superior for both green banana flour (7.8±2.55%) (SAVLAK *et al*., 2016) and that of Thai rice flour "Riceberry" (2.03±0.15%) (WIRIYAWATTANA *et al*., 2018).

Table 4. Functional properties of cherimoya flour (HCh) and those reported for flours of other fruits.

Fruit	Functional properties		
	WSI, %	WAI, g H_2O/g	CRA, g aceite/g
Annona cherimola Miller (In this study)	27,10±2,55	2,60±0,25	1,07±0,09
Annona crassiflora (Corrêa *et al*., 2011)	43,87±0,93	6,54±0,20	NR
Annona muricata (Iombor and Olaitan, 2014)	20,1±0,06	0,428±0,002	0,186±0,002
Riceberry sin tratamiento (Wiriyawattana *et al*., 2018)	6.99±0.07	2.03±0.15	NR
Ñame chino (Li *et al*., 2019)	11.21±0.36	4.46±0.17	NR
Mangifera indica verde (Noor Aziah *et al*., 2012)	NR	0.54±0.24	0.20±0.04
Musa acuminate L., cv cavendshii verde (Alkarkhi *et al*., 2011)	NR	5,66±0,17	0,50±0,07
Musa acuminate L., cv cavendshii maduro (Alkarkhi *et al*., 2011)	NR	1,71±0,10	0,82±0,04
Musa spp.AAA cv Cavendish verde (Savlak *et al*., 2016)	0,074±0,00	2,922±0,004	1,804±0,002

Values are means±standard deviation of at least three repetitions. NR, value not reported.

Table 4 shows the water absorption rate and oil retention capacity. The water absorption rate of HCh was 2.60±0.25, this property measures the volume occupied by the starch granule after swelling in excess water (ORTIZ *et al*., 2010). Many researchers report that the rate of water absorption is influenced by the degree of disintegration of native starch granules and is related to the physical state of starch, dietary fiber and proteins in fruit meal (ALKARKHI *et al*., 2011). Comparatively, the WAI of the HCh was significantly lower for the green banana flour reported by (Savlak *et al*., 2016) and (Alkarkhi *et al*., 2011), with a value of 3,702±0.061 and 5.66±0.17 g H2O/g, respectively. The same behavior occurs when compared with the flour of other fruits such as yam (4.46±0.17) and rice berry (2.03±0.15), reported by (LI *et al*., 2019) and (Wiriyawattana *et al*., 2018), respectively. In the case of yam, it is a fruit whose flour has a starch content of 64.23±1.02%, thereby increasing its WAI; while HCh has a starch content of between 28-

37%, being higher when it is dehydrated on the same day of fruit collection (data not shown). Also, with respect to the WAI of other anonas, marolo flour presented a value of 6.54±0.20 g H2O/g (CORRÊA *et al*., 2011), greater than HCh and soursop flour (Fig. 1B); this phenomenon is likely due to the high content of dietary fiber that marolo flour has (18.59%). For (Savlak, Türker, & Yesilkanat, 2016), the result of lower water absorption index usually belong to finer particles, and that is explained by the collapse of the fiber matrix because of the size reduction.

Another functional property of HCh is the oil retention capacity, which had a value of 1.07±0.09. This value was much lower than green banana flour (3,680±0,001) (SAVLAK *et al*., 2016). In this regard, (RODRÍGUEZ-AMBRIZ *et al*., 2005) reported that this property is related to the hydrophilic nature of the starch existing in green banana flour and is mainly due to the physical capture of oil within the starch structure through non-covalent bonds.

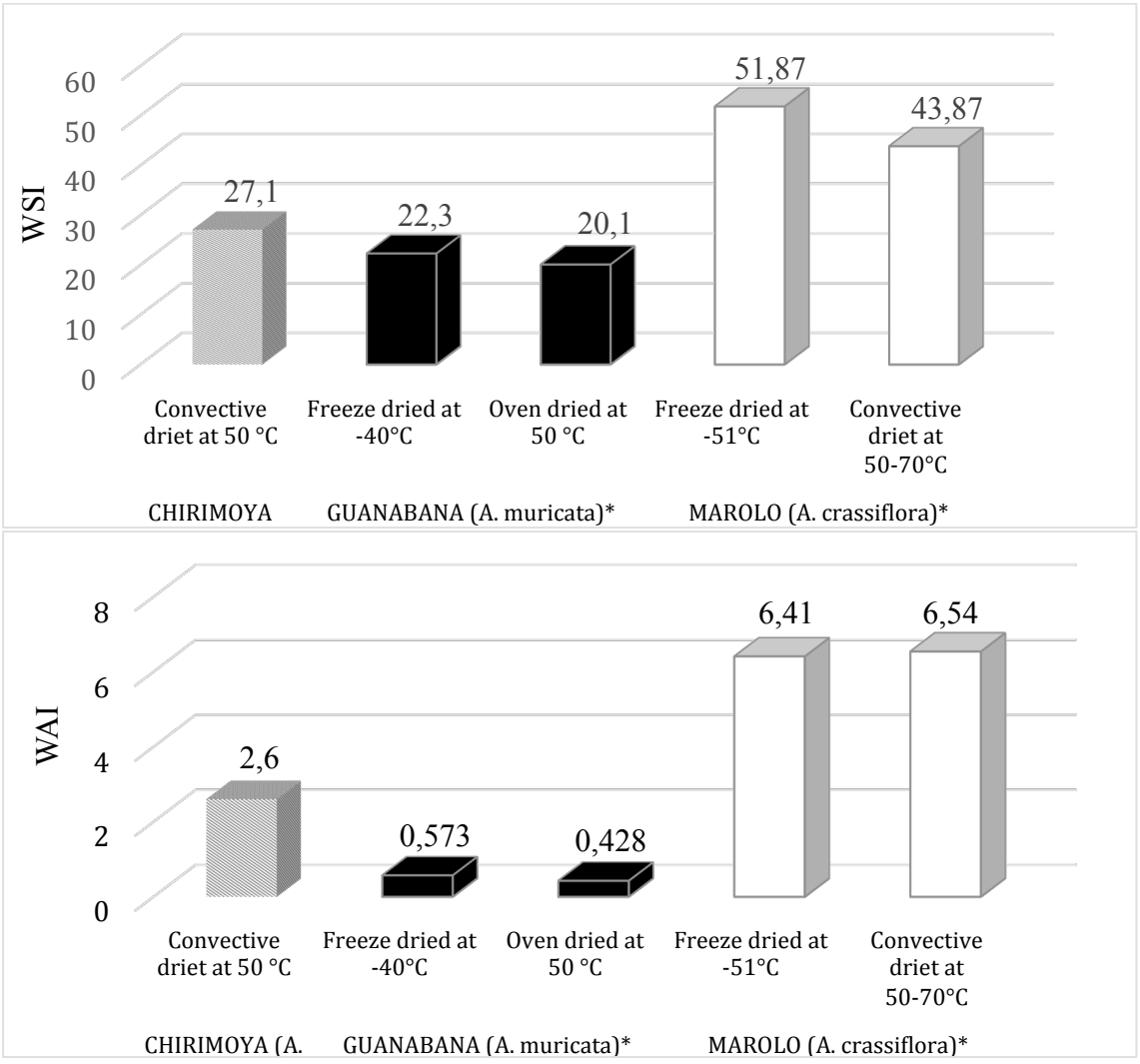

Figure 1. Effects of drying techniques on the functionality of Annonaceae pulp flours (A) WSI: water solubility index, (B) WAI: water absorption index.* Average values of Guanabana and Marolo obtained from (Iombor and Olaitan, 2014) and (Corrêa *et al*., 2011), respectively.

4. CONCLUSIONS

In this study, the functionality and physicochemical properties of cherimoya flour were investigated, and useful information was provided for its commercial applications, to be used thickeners or substitute flour in the food industry, with emphasis on the bakery industry, promoting its industrialization and reinforcing the value chain of the fruit. For further work, it is expected to explore its nutraceutical properties, as it contains a high content of natural antioxidants; as well as, the influence of particle size on rheological properties and their behavior in the design of new products.

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BEHAVIOR OF TOTALS CAROTENOIDS AND COLOR OF A MIXTURE OF PUMPKIN PUREE (*CUCURBITA MOSCHATA*) DURING STORAGE AT 4°C

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ABSTRACT

Cucurbita moschata is a squash rich in carotenoids, which are pigments responsible for providing its yellow or orange color, as well as providing a good nutritional value for being precursors of vitamin A. However, these are susceptible to degradation during food processing. The content of total carotenoids and color (*L**, *a**, *b**, Hue and Chroma) was determined in three lots of a mixture of pumpkin puree with other ingredients such as wheat flour, sugar, pasteurized egg, cinnamon, salt, butter, and additives as potassium sorbate, Tic Gums Ticagel (carrageenan) and ascorbic acid. The lots were stored under refrigeration temperature at 4°C for 3 months. Two samples from each batch were randomly taken weekly for determination of carotenoids and color. The color parameters *L**, *a**, *b**, Hue and Chroma decreased significantly during the storage time, so the mixture became darker, losing red and yellow. Also, the content of total carotenoids in the mixture decreased significantly from 63.30 μg/g on day 0 to 42.07 μg/g on day 70 of storage, indicating that storage conditions influence the changes in color and total content of carotenoids.

Keywords: carotenoid, color, pumpkin, storage

1. INTRODUCTION

Carotenoids represent one of the groups of natural pigments found in fruits and vegetables responsible for giving them the yellow, red or orange color. Carotenoids are also crucial for human health because they act as a precursor to vitamin A, which is an essential nutrient for the normal functioning of the visual system and the immune system, and the growth of body epithelial cells (FAO/WHO, 2002). Carotenoids contribute to the prevention of cardiovascular diseases and some types of cancer (RAO and RAO, 2007).
Pro-vitamin A carotenoids are found in yellow vegetables as the pumpkins. Several authors have reported the presence of these pigments in the pumpkin (*Cucurbita moschata*) (SEO *et al*., 2005, CARVALHO *et al*., 2014, SHI *et al*., 2010, PROVESI *et al*., 2011, de CARVALHO *et al*., 2012, ONWUDE *et al*., 2016). The main carotenoids isolated from pumpkin are β-carotene, α-carotene, and lutein, being the most abundant β-carotene (SEO *et al*., 2005, CARVALHO *et al*., 2014).
The pumpkin is being widely used as a food base ingredient, representing an alternative for the consumption of this vegetable and taking advantage of its contribution of carotenoids. However, the stability of these compounds in foods is essential since their degradation influences the loss of color and the nutritional value of the food. Different factors are associated with their instability, such as exposure to light, heat, acids, and the presence of oxygen or antioxidants (RODRIGUEZ AMAYA, 2001, GONÇALVES *et al*., 2007).
This article aimed to evaluate the changes in the content of total carotenoids and color (L, a, b, Hue and Chroma) in the mixture based on pumpkin puree (*Cucurbita moschata*) during storage at 4°C.

2. MATERIAL AND METHODS

2.1. Raw Material and Ingredients for the mixture

High-methoxyl pectin was used as a gelling agent for the development of edible films. Moreover, glycerol was used as a plasticizing agent, citric acid and ascorbic acid as antioxidants and preservatives. The pulp of papaya (*Carica papaya* L), passion fruit (*Passiflora edulis*) and pineapple (*Ananas comosus*) was used as a polymeric matrix for the development of edible films according to ESPITIA *et al*. (2014a). Additionally, potable water was used for the preparation of the filmogenic solutions. Subsequently, work was done by adding natural species, such as garlic powder, thyme, paprika, and papain. A final test was carried out incorporating flaxseed. The casting method was used for the development of the edible films, which consisted of spreading the filmogenic solution on a sterilized surface and allowing it to dry at room temperature for 24 h.

2.2. Processing of the mixture

The pumpkins were cleaned, washed, and disinfected with chlorinated water (50 ppm sodium hypochlorite-NaClO) at pH 6.0 for 2 minutes. They were cut into pieces (15 cm long by 25 cm wide) and cooked in boiling water for 15 minutes. Then, they were introduced into the Robot Coupe C-80 pulp extractor to get the mash, which was then mixed into a Barco M-20 mixer (Fig. 1b) along with the other ingredients for 10 minutes until a homogenization. Finally, It was added potassium sorbate, ascorbic acid, and stabilizer to the mixture. It was measured and controlled the pH to values less than 4.6 until equilibrium was reached. It was prepared three batches of mixtures of 200 g under

the same conditions (Fig. 1c). Then, they were packed in polypropylene trays and sealed with a film of polyester using polyethylene in the Koch Ultra Source equipment from ILPRA (Fig. 1d). The batches were kept refrigerated at a temperature of 4°C in a hermetic cold room for three months. Two samples from all the batches were randomly taken weekly for determination of total carotenoids and color.

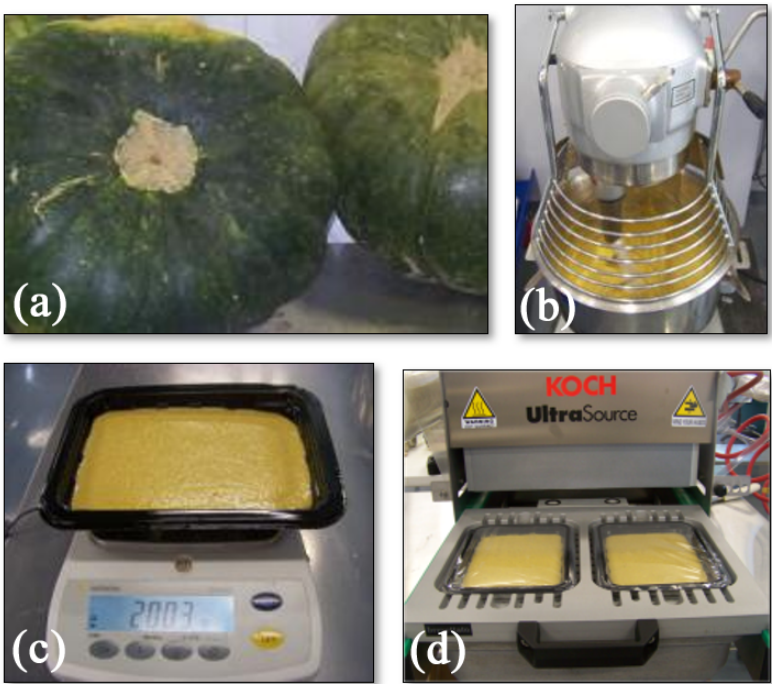

Figure 1. Sample preparation. (a) Pumpkins (*C. moschata*) variety Soler, (b) Barco M-20 mixer (c) batch of pumpkin mixture - 200g, (d) Polypropylene packing - Koch Ultra Source equipment.

2.3. Color analysis

The color was measured using the MiniScan in the Hunter Lab color scale (10° observer, Illuminant D-65). L value represents luminosity, with values ranging from 100 for white to 0 for black. Parameter a represents color changes from red to green, with values ranging from 100 (red) to -80 (Green). The parameter b represents color changes from yellow to blue, with values ranging from 70 (yellow) to -80 (blue), (Nielsen, 2017). The equipment was calibrated with standard white and black porcelain plates. Three measurements were taken in each sample. Values of L, a and b were used to calculate Hue (h°) [h°= arctangent (b/a)] and Chroma [C= (a2+b2) 1/2]. Hue, which is an attribute of the color tone, takes values from 0 to 360°. In a plane of color coordinates: 0° (+a) is red, 90° (+b) is yellow, 180° (-a) is green and 270° (-b) is blue. Chroma, which is the measure of the intensity or saturation of the color, takes values ranging from 0 to 60 (TAUB and SINGH, 1997) .

2.4. Carotenoid determination

The determination of the carotenoids was made using the AOAC 941.15 extraction method with acetone and hexane. The absorbance of the solutions was measured with the Thermo Spectronic Genesys TM 8 spectrophotometer at 436 nm using a calibration curve with

standard β-carotene of Sigma, 95% purity. It was determined the concentration of total carotenoids expressed in μg/g of sample.

2.5. Statistical analysis

An analysis of variance (ANOVA) and simple linear regression were used to determine significant differences among samples, batches, and days, with a significance level of 5% using specialized software in experimental design.

3. RESULTS AND DISCUSSION

3.1. Color analysis

Table 1 shows an ANOVA for parameters *L*, a*, *b**, and Hue as a function of the samples, batches, and days, with a significance level of 5%. As can be seen, all the factors and their interactions were significant because of their P-value ($P < 0.05$). However, the sum of squares values (SS) of the factors Day and Lot were the highest indicating that they were the factors that cause the highest effect. The ANOVA for Chrome parameter follows the same pattern.

Table 1. Analysis of variance of parameter *L*, a*, *b** and Hue.

	Parameter *L**					Parameter *a**			
SOURCE OF VARIATION	**DF**	**SS**	**MS**	**P**	**SOURCE OF VARIATION**	**DF**	**SS**	**MS**	**P**
Sample	1	9,44	9,440	0,000	Sample	1	0,636	0,636	0,000
Day	12	1265,18	105,432	0,000	Day	12	29,744	2,479	0,000
Lot	2	187,46	93,730	0,000	Lot	2	682,374	341,187	0,000
Sample*Day	12	20,36	1,696	0,000	Sample*Day	12	2,753	0,229	0,000
Sample*Lot	2	9,54	4,768	0,000	Sample*Lot	2	1,005	0,503	0,000
Day*Lot	24	67,09	2,796	0,000	Day*Lot	24	26,688	1,112	0,000
Sample*Day*Lot	24	16,65	0,694	0,000	Sample*Day*Lot	24	3,498	0,146	0,000
Error	156	2,17	0,014		Error	156	1,154	0,007	
Total	233	1577,89			Total	233	747,852		
	Parameter *b**					**Parameter Hue**			
SOURCE OF VARIATION	**DF**	**SS**	**MS**	**P**	**SOURCE OF VARIATION**	**DF**	**SS**	**MS**	**P**
Sample	1	0,99	0,99	0,000	Sample	1	0,358	0,3582	0,000
Day	12	1401,50	116,79	0,000	Day	12	251,956	209,964	0,000
Lot	2	3470,21	1735,10	0,000	Lot	2	182,533	912,664	0,000
Sample*Day	12	50,18	4,18	0,000	Sample*Day	12	6,975	0,5812	0,000
Sample*Lot	2	25,32	12,66	0,000	Sample*Lot	2	0,524	0,2622	0,000
Day*Lot	24	112,14	4,67	0,000	Day*Lot	24	34,609	14,421	0,000
Sample*Day*Lot	24	68,39	2,85	0,000	Sample*Day*Lot	24	11,183	0,4659	0,000
Error	156	3,90	0,02		Error	156	2,246	0,0144	
Total	233	5132,63			Total	233	490,384		

Fig. 2 shows the main effect plots for all the parameters mentioned before. It can be observed that the Factors Lots and Days has the highest effect validating the information of Table 1. The factor Lot was significant because it refers to different types of Pumpkin. It was an expected result because during the growing of the different types of pumpkin, the speed of nutrient absorption depends on too many environmental factors. For that reason, the Pumpkin has the same internal composition but with different concentrations causing the variation observed in Fig. 2 (Lot zone).

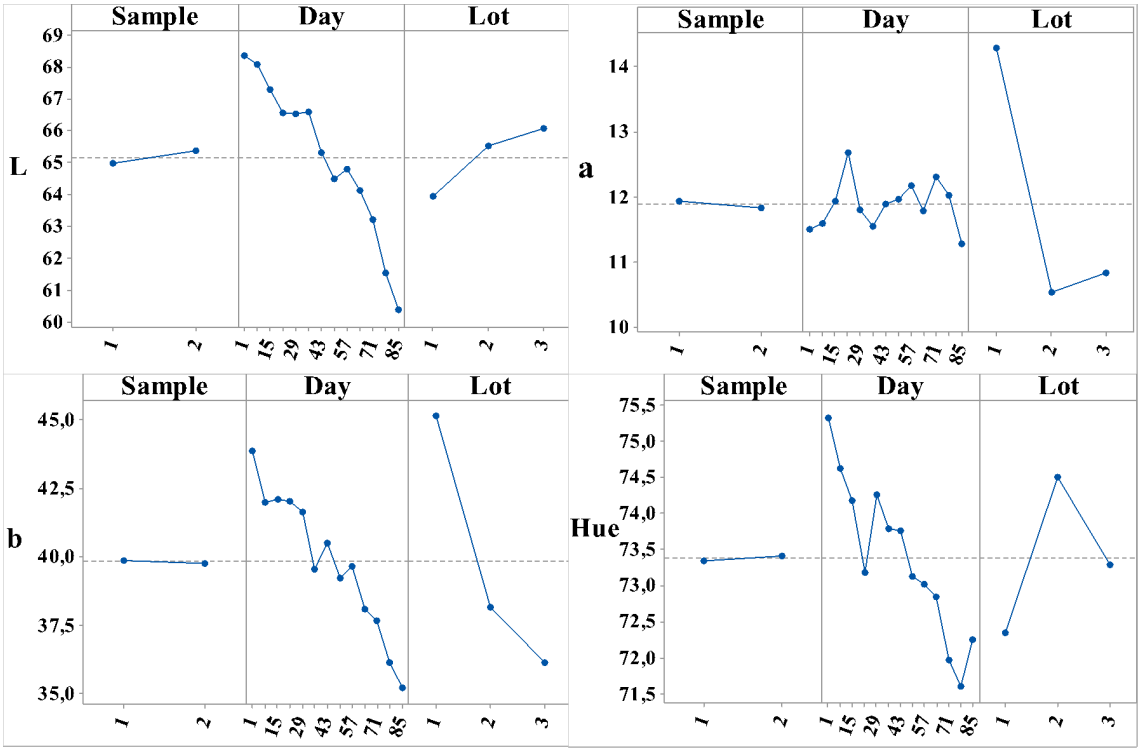

Figure 2. Main effect plot for factors *L*, a*, *b** and Hue.

During the storage of the samples, the color parameters *L*, a*, *b**, Hue, and Chroma in the mixture decreased significantly. The mixture became darker, losing yellow and red color. These same results were observed in pumpkin purees as storage time increased (GLIEMMO *et al*., 2009). During the elaboration and processing of the pumpkin puree, the bioavailability of β-carotene improves because the cellular structure of the plant broke. It makes the pigment more exposed to the reactions of degradation, leading to a decrease in the color of the food during storage.

3.1.1 Analysis of parameter *L*

The values of the parameter *L* represent the darkening of the mixture. The average values found for day 0 correspond to 67.71, 68.57, and 68.67 for lots 1, 2, and 3, respectively. The value of "L" decreases as the time goes (Fig. 3). It happens due to the degradation of pigments, which produce compounds that reduce luminosity (DUTTA *et al*., 2006). On the other hand, the darkening of the mixture may be related to enzymatic reactions that cause brown compounds, as well as non-enzymatic browning (PROVESI *et al*., 2011), due to oxidation reactions of ascorbic acid. During the measurements of the parameter *L* we can

see that the factors days and lots had a significant effect according to the statistical analysis with a p-value lower than 0.05. The samples had no significant effect ($p = 0.364 > 0.05$). The plots for the three lots have the same pattern, a linear trend with negative slope due to the decrease of L^*.

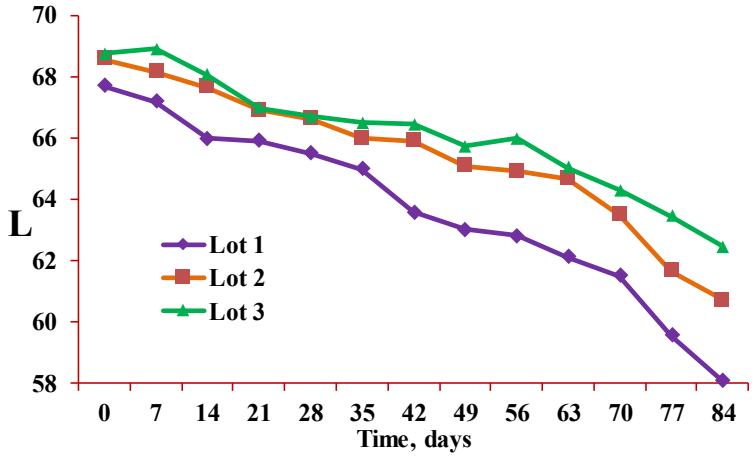

Figure 3. Color parameter "L" of the mixture during storage at 4°C.

3.1.2 Analysis of parameter a^*

The values for the parameter a^* were correlated with red color because they are positive values. Lot 1 had higher values than lots 2 and 3, with initial values of 13.86, 10.16, and 10.51 respectively (Fig. 4). The variation in the colors of the pumpkins used in each batch explains this behavior. These values remained stable during the storage time because it was added ascorbic acid to all the samples. GLIEMMO *et al*. (2009) reported in their study that the addition of ascorbic acid to squash purees packed with polyethylene at a pH of 4.0 protected the red color and increased the loss of yellow color (GLIEMMO *et al*., 2009), as observed in this study.

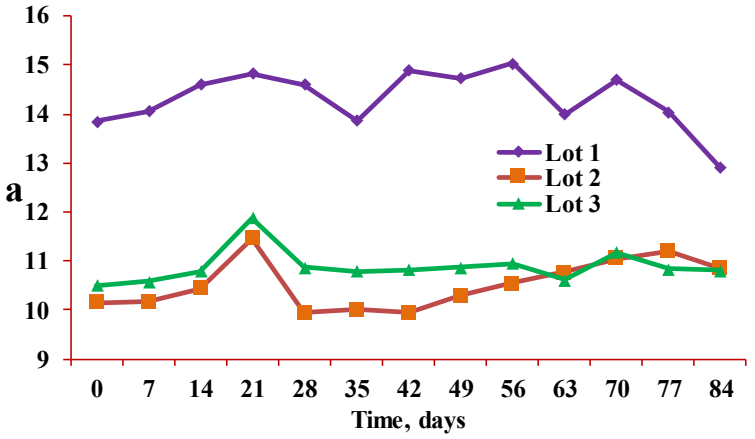

Figure 4. Color parameter a^* of the mixture during storage at 4°C.

3.1.3 Analysis of parameter *b**

The values of parameter *b** remain in the range from 30.0 to 50.0 during the entire storage time (Fig. 5). All of them were positive because of their association with the yellow color. The decrease in the values is low, with a linear trend with the same negative slope that can be detailed visually in Fig. 5, so the mixtures tend to be less yellow through time, maybe to their possible association with brown compounds of enzymatic and non-enzymatic reactions. It was observed that lot 1 presented the highest values, indicating that the pumpkins used for processing were more yellow than those used in the other lots.

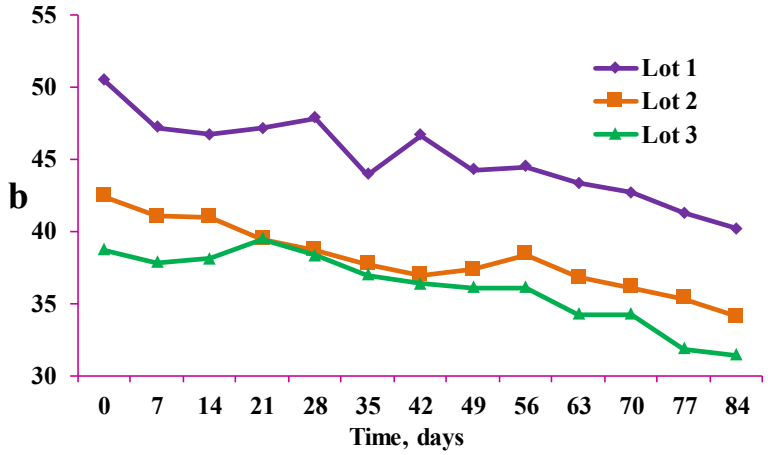

Figure 5. Color parameter *b** of the mixture during storage at 4°C.

3.1.4 Analysis of the Hue parameter

The values of h° decreased as time goes (Fig. 6). There are significant differences in the factors samples, days, and lots ($p < 0.05$). However, the effect of the samples is small compared with the effect of the days and lots. The values of h° for the last day of sampling were 72.19°, 72.32°, and 72.23° for lots 1, 2, and 3, respectively. Despite these values, the mixture is still orange. However, according to the color coordinates, it loses yellow color and retains red color. For all the lots it can be seen the same linear trend with a negative slope.

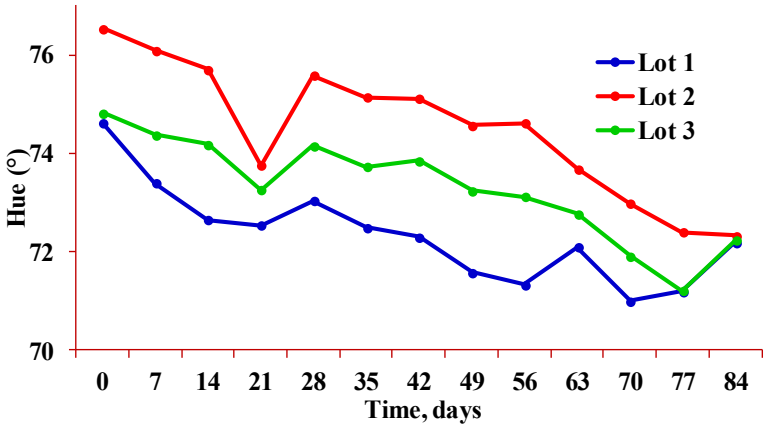

Figure 6. Hue values of the mixture during storage at 4°C.

Previous research found that the color of the pumpkins was consistent with Hue values (Itle and Kabelka, 2009). Low values of Hue correspond to orange color and high values of Hue to yellow color, with an average in the studied pumpkins of 77.5° corresponding to the orange color. Subjectively, the pumpkins used in this investigation corresponded more to a yellow-orange color. Therefore, the mixture will have similar characteristics. The Hue values found for the blend are closer to 90° (yellow) than to 0° (red).

3.1.5 Analysis of the Chroma parameter

In the case of the Chroma parameter, the values decrease through time, as shown in Fig. 7. Significant differences were observed in samples, days, and batches. Applying an ANOVA, there are significant differences in days ($P < 0.05$). The values on day 0 for lots 1, 2, and 3 were 52.35, 43.61, and 40.11 respectively, indicating that there is high intensity in the color of the mixture, which was lost over time. The average values at day 84 were 42.18, 35.77, and 32.96 for lots 1, 2, and 3, respectively. The trend shown in Fig. 7 can is associated with the degradation of carotenoids by oxidation, which helps to attenuate the intense orange color. The same linear pattern was obtained for all the lots.

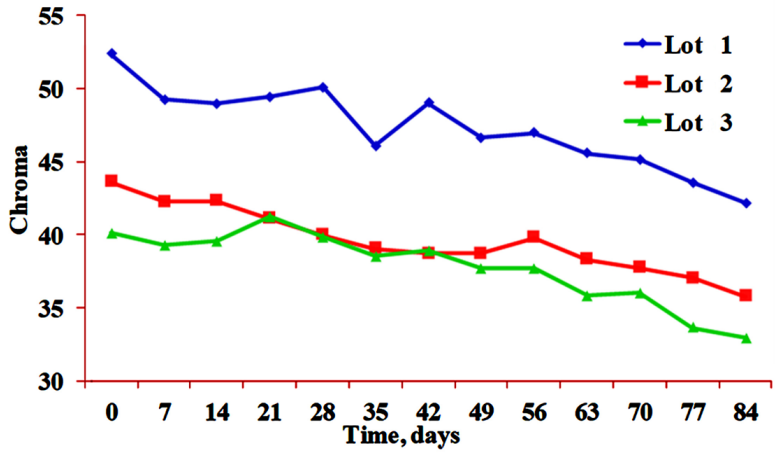

Figure 7. Chroma values of the mixture during storage at 4 °C

3.2. Analysis of carotenoids

The total carotenoid content of the raw pumpkins used for the processing of the mixture was on average, 62.40 µg/g. PANDEY *et al*. (2003) reported values of 23.4 µg/g to 148.5 µg/g in C. *moschata* from India (PANDEY *et al*., 2003). ITLE and KABELKA (2009) reported values in different varieties of C. *moschata* in the range from 1.1 µg/g to 42.3 µg/g in pumpkins grown in Florida, USA (ITLE and KABELKA, 2009). SHI *et al*. (2010) reported 148.8 µg/g of total carotenoids extracted with ethanol (SHI *et al*., 2010). CARVALHO *et al*. (2014) reported values of 236.10 µg/g of C. *moschata* from Brazil (CARVALHO *et al*., 2014). There are qualitative and quantitative differences of carotenoids even within the same species and variety of pumpkins. It happens due to the influence of the factors in the environment in which pumpkins grow as nutrients, soil, climate, amount of sunlight, temperature, the stage of maturation and post- harvest (PROVESI *et al*., 2011, DE CARVALHO *et al*., 2012, ONWUDE *et al*., 2016, RODRIGUEZ AMAYA, 2001).

When comparing the content of total carotenoids in the raw pumpkin to the pumpkin puree for all the lots shown in Table 2, we can see an increase in the values, which indicates that the processing of the pumpkin by cooking produced an increase in the content of carotenoids. Similar results were reported by other authors (CARVALHO *et al*., 2014; AZIZAH *et al*., 2009, DUTTA *et al*., 2009).

Table 2. Content of total carotenoids (μg / g) in raw pumpkin, pumpkin puree and mixtures of day 0.

Lot	Raw pumpkin	Pumpkin Puree	Pumpkin mixture Day 0
1	63.38	72.26	64.72
2	61.71	73.25	62.87
3	62.12	72.84	62.32

Scalding and moderate heat treatments near 100°C for a short time, can increase carotenoid content, being a consequence of better extraction due to cell disruption, moisture loss, and inactivation of the enzymes that oxidize carotene (MacDougall, 2002). Severe heat treatments lead to the degradation of carotenoids by isomerization processes.
The values in the mixture for day 0 were 64.72 μg/g for lot 1, 62.87 μg/g for lot 2 and 62.32 μg/g for lot 3. These values are lower compared to those in the pumpkin puree. However, it must be considered that the mixture has other ingredients, which makes the concentration of carotenoids lower. Also, the acidification process of the mixture influences the isomerization of the carotenoids. During the storage period, a decrease in carotenoid content was observed in the mixture, as shown in Fig. 8. This decrease was gradual until day 63. By day 70, a noticeable decrease was observed with average values of 42.07 μg/g, which were maintained constant until the last day of sampling. Significant effects were found only for lots and days ($p < 0.05$) and their interactions (Table 3).

Table 3. Analysis of variance of the Content of total carotenoids.

SOURCE OF VARIATION	DF	SS	MS	F	P
Sample	1	0.22	0.22	0.83	0.364
Day	12	10915.22	909.6	3397.11	0.000
Lot	2	181.22	90.61	338.41	0.000
Sample*day	12	11.65	0.97	3.63	0.000
Sample*Lot	2	0.03	0.02	0.06	0.940
Day*Lot	24	108.49	4.52	16.88	0.000
Sample*Day*Lot	24	0.55	0.02	0.09	1.000
Error	156	41.77	0.27		
Total	233	11259.16			

The differences that exist among the lots lie in the use of different pumpkins. It can be seen that the carotenoids suffer a degradation process in the mixture through time. The acidification of the mixture plays a crucial role in this process because the carotenoids undergo isomerization reactions due to the presence of acids in the food (RODRIGUEZ AMAYA, 2001).

Regarding the color, the effect of the oxidation and isomerization of the carotenoids causes discoloration in the mixture. That is the reason for the decrease in the values of "L," "Hue," and "Chroma."
Several authors have studied the relationship between the concentration and content of carotenoids with the color of the pumpkin. MURKOVIC *et al*. (2002) studied the content of carotenoids in different varieties of pumpkins and reported that color visualization correlates very well with the carotenoid content (MURKOVIC *et al*., 2002). Varieties with a high content of carotenes showed an orange color, and those with a high content of lutein and low carotene showed a bright yellow color.

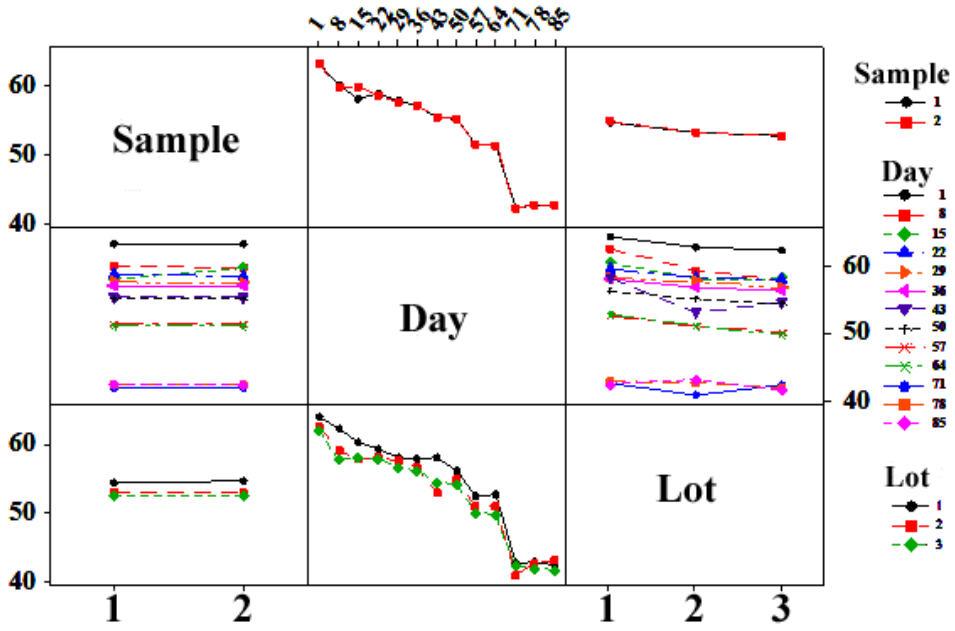

Figure 8. Concentration of carotenoids ($\mu g/g$) in the mixture during storage at 4°C as a function of time, sample and lots.

4. CONCLUSION

A complete study of the behavior of the total carotenoids and color of a mixture of puree pumpkin during storage to 4°c was done. The color parameters L, b, Hue, and Chroma decreased significantly with a linear pattern with a negative slope, so the mixture became darker, losing red and yellow colors. The color parameter was the exception because it remained stable due to the addition of ascorbic acid to the Pumpkin mixture. The values of Hue correspond to orange color, being less orange as the time goes. The Chroma values reflect the loss of the color intensity, which is associated with the degradation of the carotenoid pigments. Pumpkin processing by cooking produced an increase in carotenoid content. The content of carotenoids of the mixture at day 0 decreased with respect to the pumpkin puree (cooked pumpkin). Therefore, the acidification process of the mixture influences the isomerization of the carotenoids. The carotenoid content of the mixture during the storage time decreased considerably as a consequence of various factors that influence its degradation, such as light, oxygen, acidity, and temperature.

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DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILMS BASED ON COLOMBIAN TROPICAL FRUITS

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ABSTRACT

Edible films are thin layer of polymer that can be consumed and used as packaging material for food preservation. Edible films based on tropical fruits (papaya, passion fruit and pineapple) were developed and characterized regarding bromatological, physicochemical, microbiological and sensory properties. Edible films were prepared by casting method. Papaya was chosen as a preferable matrix for the production of films due to its physical integrity. This edible film was added with garlic powder to give aroma. Edible films had a nutritional content similar to the original tropical fruit with positive sensory acceptance.

Keywords: biopolymers, edible film, tropical fruits, food preservation

1. INTRODUCTION

Losses and waste of food constitute a continuous challenge to the food industry as they are considered a global sustainability issue. In this regard, food losses and waste lead to food insecurity, excessive energy consumption, as well as raw material waste. In this regard, food losses and waste have been targeted as one of the Sustainable Development Goals (ONU, 2015, YOKOKAWA *et al*., 2018).

Moreover, food and nutritional security has also been set as one of the main goals by the United Nations in the frame of the Sustainable Development Goals. Food and Nutritional Security has been described as a condition when "all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 1996). The challenge of the food and nutritional security relies on the food supply that meet the criteria of high nutritional value and healthy, safety, affordable and with an extended shelf-life (AUGUSTIN *et al*., 2016). In this regard, previous studies have indicated that the development of innovative food packaging materials has the potential to contribute to the reduction of food losses and waste (WILLIAMS *et al*., 2012), with a positive impact on the food and nutritional security of consumers.

Edible films are innovative food packaging materials, constituted by a thin layer of polymer that can be consumed and be used on food surfaces as packaging materials; edible films can be applied as continuous layers between the different components of the product or used as a cover during its preparation (OTONI *et al*., 2017). Moreover, this kind of packaging are considered an interesting alternative when compared to synthetic since they are characterized by being biodegradable and compostable, being able to carry active compounds to favor food shelf-life extension (BENBETTAÏEB *et al*., 2019). In general, plasticizers and gelling agents are used in their formulation in addition to other components, to provide different biological functions to the developed edible films (ESPITIA *et al*., 2014c).

The development of edible films as packaging materials based on a biological nature has taken greater importance because it contributes to the reduction of the use of synthetic materials, such as plastic bags, which take long time to degrade and generate environmental issues. Thus, the potential application of edible films in the food industry favors the conservation of the environment by reducing pollution and contamination issues. Moreover, the development of edible films as packaging materials constitutes an innovative product for the food market, since as a biodegradable packaging material edible films have the potential to protects and preserves a food product, while can be ingested at the same time promoting other ways of food consumption (Espitia *et al*., 2014b).

Moreover, Colombia has high and varied levels of biodiversity, leading to the production of tropical fruits, which are locally consumed and exported internationally as well. Tropical fruits are currently very interesting in the research field of food science as well as for consumers due to their bioactive compounds and their beneficial effects to human health (LOIZZO *et al*., 2019). Therefore, this research aimed to develop edible films based on tropical fruit from Colombia, such as papaya, passion fruit and pineapple, and characterize the developed films regarding bromatological, physicochemical, microbiological and sensory properties.

2. MATERIAL AND METHODS

2.1. Development of edible films

High-methoxyl pectin was used as a gelling agent for the development of edible films. Moreover, glycerol was used as a plasticizing agent, citric acid and ascorbic acid as antioxidants and preservatives. The pulp of papaya (*Carica papaya* L), passion fruit (*Passiflora edulis*) and pineapple (*Ananas comosus*) was used as a polymeric matrix for the development of edible films according to ESPITIA *et al*. (2014a). Additionally, potable water was used for the preparation of the filmogenic solutions. Subsequently work was done by adding natural species such as garlic powder, thyme, paprika and papain. A final test was carried out incorporating flaxseed. The casting method was used for the development of the edible films, which consisted of spreading the filmogenic solution on a sterilized surface and allowing it to dry at room temperature for 24h.

2.2. Bromatological analysis

The moisture content is frequently used as an index of product stability. The moisture content was determined by the desiccation method according to the technique established by A.O.A.C (AOAC, 1990). The determination of ashes serves to represent the mineral content of the food. The gravimetrical method was used at 550 ºC by calcination. The determination of proteins serves to indicate the total nitrogen content of a food. The results of protein content of developed edible films were obtained by the Kjeldahl method according to the technique A.O.A.C 988.05. The determination of fat content was done according to the Soxhlet method. The determination of fiber was carried out through the acid-base hydrolysis method. Finally, the determination of carbohydrates was done by the indirect method through mathematical calculation.

2.3. Physicochemical properties

Thickness measurement was determined using a digital micrometer (Mitutoyo, Japan). The measurements were made in three random points of each film in triplicate. Colorimetric analysis was done using a Konica Minolta colorimeter model CR-20. The colorimetric parameters (L: lightness or darkness; a*: red or green; and b*: yellow or blue) were determined for each sample in triplicate. Water activity of each sample (1 g approx.) was determined by using the Rotronic water activity determinant (model HC2-AW).

2.4. Microbiological analysis

Psychrophiles were determined by plate count method, according to ISO 4832. Aerobic mesophiles were determined by plate count method according to the ISO 4833-1. Mold and yeasts were determined according to the plate count method ISO 21527-1.

2.5. Sensory characterization

Sensory attributes studied were color, aroma and global impression, using a 9-point hedonic scale to indicate the level of acceptance of the sample. The test was done by 50 untrained consumers. In addition, brief survey was applied during the test to characterize the potential consumers.

3. RESULTS AND DISCUSSION

Edible films were prepared based on papaya, pineapple and passion fruit. However, better results were obtained with the papaya pulp, presenting greater physical integrity and better consistency (Fig. 1).

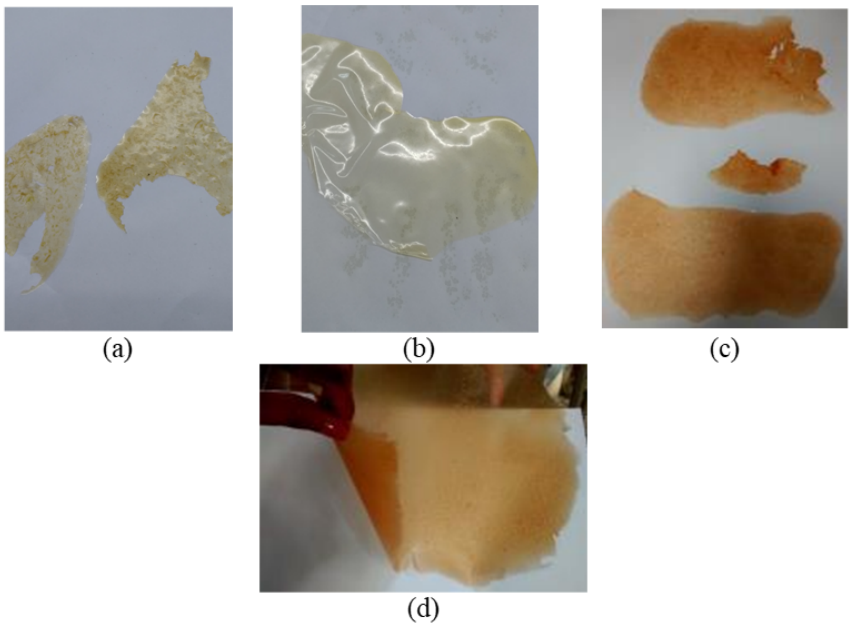

Figure 1. Edible films made from tropical fruits: pineapple (a), passion fruit (b) and papaya (c and d).

Moreover, the incorporation of the species resulted in a saturation of the polymeric matrix and especially the film incorporated with flaxseed favored the indiscriminate growth of molds and yeasts on its surface. From the flavoring species studied, garlic powder allowed to be incorporated (5% and 10% w/w) better in papaya edible film without altering the film physical or microbial integrity.
Similar studies, in which garlic powder has been used as an additive in edible films, have been previously published. In this regard, biodegradable and edible nanocomposites films were incorporated with garlic powder. The results were favorable, since garlic powder provided aroma and flavor to the edible films without altering their physical structure (FAMA *et al*., 2010).

3.1. Bromatological analysis

Developed edible films were characterized by a high percentage of carbohydrates, followed by a high percentage of moisture in their composition (Table 1). These results are probably due to the fact that the main components used in the production of the film are water and papaya. This indicates that the addition of garlic powder did not alter the nutritional characteristics of the developed edible film.

Table 1. Bromatological characterization of edible papaya films.

Treatment	Moisture	Ash	Protein	Fat	Fiber	Carbohydrates
Control	31,26	1,27	0,40	0,20	2,20	64,7
Garlic 5%	31,26	1,26	0,40	0,20	2,20	64,7
Garlic 10%	30,50	1,10	0,45	0,28	2,40	65,3

3.2. Physicochemical properties

The determination of the thickness showed that there is a significant difference between the treatment of edible papaya film incorporated with 10% garlic in relation to the control treatment and edible film incorporated with 5% garlic powder (Table 2).

Table 2. Characterization of the thickness of papaya edible films*.

Treatment	Thickness (mm)
Control	0,108±0,004 [a]
5% Garlic	0,114±0,012 [a]
10% Garlic	0,238±0,036 [b]

*Values followed by the same letter are not significantly different according to 5% probability Tukey test.

The colorimetric analysis allowed to objectively analyze the color of the studied samples. In this regard, the results obtained showed that for all the colorimetric parameters the treatment of edible papaya film incorporated with 10% garlic presented a significant difference in relation to the other two treatments studied (Table 3).

Table 3. Colorimetric characterization of developed edible films†.

Treatment	L*	a*	b*
Control	78,100±0,200 [a]	9,300±0,436 [a]	15,067±0,002 [a]
5% Garlic	78,000±0,985 [a]	9,167±1,002 [a]	18,867±0,001 [a]
10% Garlic	71,900±1,819 [b]	13,500±1,136 [b]	27,000±0,004 [b]

†Values in the same column followed by the same letter are not significantly different according to 5% probability Tukey test.

The determination of water activity helps predict the stability and shelf life of food. The determination of water from the edible films of papaya and incorporated with garlic powder showed that there is a significant difference between the treatment of edible papaya film incorporated with 10% garlic compared to the control film and the edible film incorporated with 5% (Table 4).
In this regard, low values of Aw are favorable for the edible film formation since this process is the result of electrostatic interaction while the Aw is reduced allowing the

gelation of the polymeric matrix (pectin) and consequently the formation of cross-links in the polymeric chains (ESPITIA *et al*., 2014a).

Table 4. Determination of water activity of the developed edible films†.

Treatment	Aw
Control	0,543±0,002 [a]
5% Garlic	0,544±0,001 [a]
10% Garlic	0,565±0,004 [b]

†Values followed by the same letter are not significantly different according to 5% probability Tukey test.

3.3. Microbiological analysis

The results showed a low count of colony forming units per grams (cfu/g) of psychrophiles in all samples. For the control treatment, a higher value was obtained in a logarithmic cycle for mesophiles, while low values were obtained for the treatments of 5% and 10% garlic powder, due to the incorporation of this species (Table 5). Garlic contains aliin, which is a sulfur-containing compound that, thanks to the action of the allinase, breaks down into allicin, pyruvic acid and ammonia. Allicin has been shown to have antimicrobial activity on some strains of *Escherichia coli, Staphylococcus aureus* and other pathogens (MARTINS *et al*., 2016). Additionally, all the treatments evaluated had low values for mold and yeast counts.

Table 5. Microbiological characterization of the developed papaya edible films.

Treatment	Psychrophiles	Mesophiles	Mold and yeast
Control	<10 UFC/g	$4.1x10^2$ UFC/g	50 UFC/g
5% Garlic	<10 UFC/g	$2x10^1$UFC/g	<10 UFC/g
10% Garlic	<10 UFC/g	$5x10^1$ UFC/g	20 FC/g

3.4. Sensory characterization

From the 50 untrained consumers who agreed to do the sensory test of developed edible films, 84% people were female and 16% male. In addition, results showed that there was no significant difference regarding the sensory acceptance of papaya edible films (Fig. 3) with or without garlic (5% and 10%).
This means that on average the sensory acceptance scores were framed between the scores of 6 and 7, which corresponds to the sensory perception of "I like it slightly" and "I like it moderately", respectively. These results indicated that the developed films presented a positive average acceptance. Finally, from a brief survey application it was observed that 96% consumers (from 50 untrained consumers) would buy the product if it were in the market, while 4% would not buy it.

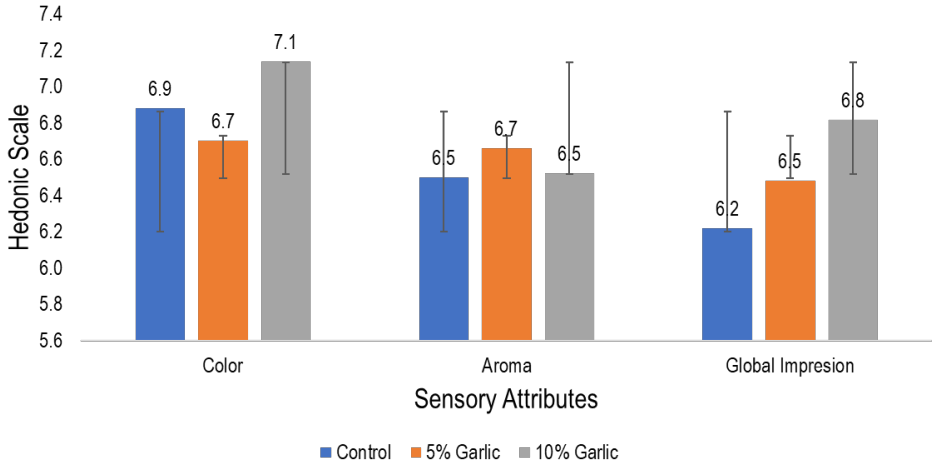

Figure 3. Sensory analysis of papaya edible films.

Finally, developed edible films, especially those based on papaya, despite having a low percentage of proteins, have a high content of carbohydrates, fiber and organic material (fiber), making that polymeric matrix a good material, with potential to be applied in the preservation of food products. Thus, future studies will seek to analyze the potential application of developed edible films in a food matrix. Additionally, it is emphasized that the elaboration of edible films is based on low cost technology. Finally, developed edible films based on papaya proved to have a good sensory acceptance among potential consumers, in such a way, the developed films have the potential to be used as edible food packaging.

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PHYSICOCHEMICAL AND MICROBIOLOGICAL EVALUATION OF FLOUR OBTAINED FROM THE RESIDUAL CAKE OF SACHA INCHI *(PLUKENETIA VOLUBILIS* L.*)* FOR ITS POTENTIAL USE IN THE AGRI-FOOD SECTOR

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ABSTRACT

In the process of extracting Sacha inchi oil (*Plukenetia volubilis* L.), a residual cake is obtained that is considered as waste. From this cake it is possible to obtain a flour. A physicochemical characterization (humidity, ash, protein, fat, fiber, analysis of functional groups by infrared spectrophotometry) and microbiological characterization (mesophilic bacteria, fecal coliforms, molds and yeasts, staphylococci, bacilli, salmonella) of the residual cake and flour of Sacha inchi, with the aim of validating its potential application as a food in the diet of the human being. For the residual cake, protein content values of 46.04%, fiber 3.46%, fat 16.03%, and microbiological analysis were reported. The mesophilic bacteria count was 40 cfu/g and the fecal coliform count was less than 3 mic / g. Likewise, for the flour, protein content values of 54.56%, fiber 4.79%, fat 15.46% were reported. In relation to the microbiological analysis, the mesophilic bacteria count was 80 cfu/g and the fecal coliform count was less than 3 mic/g. For the above, confirmed that the flour obtained from the residual cake, has the appropriate nutritional characteristics to produce products for human consumption.

A food product type brownie, 100% sacha inchi flour was developed, For the management of astringent taste, it was subjected to thermal treatments of 60, 70, 80, 90°C for three hours to the flour and it was found that the astringency disappeared when subjected to 80°C.

Keywords: Sacha inchi, flour, characterization, spectrophotometry, microbiological analysis

1. INTRODUCTION

Sacha inchi (*Plukenetia volubilis* L.) is a plant native from the Peruvian Amazon, which has an oilseed with a high content of fatty acid linolenic (omega 3), linoleic (omega 6) and oleic (omega 9) fatty acids; compared to other plants such as Soy, Peanut, Cotton and Sunflower (BETANCOURTH, 2013). This same seed also contains essential amino acids, those that the body does not synthesize by itself and that are necessary in the diet, and significant amounts of minerals (GUTIÉRREZ, 2011), for that reason there is great interest in its cultivation and industrialization (GÓMEZ, 2005).
From the oil extraction process (INDUCAM, 2017) of the Sacha inchi seed, mainly used in the food and cosmetic industry, a by-product is obtained, the residual cake, which preserves the nutritional qualities of the seed. Currently, it is used as raw material in the production of concentrates (REÁTEGUI, 2010) and in other cases it is classified as waste (MONDRAGÓN, 2009).
Due to the growth in oleochemical agroindustry, it has brought as a consequence, an excess in the generation of residual cake as a byproduct, a fact that would present a virtual problem to the environment and society in which the activity takes place.
Attending to this event and taking into account that from the residual cake a flour can be obtained that would have different organoleptic properties depending on the method used to obtain it, where these properties are important to determine and analyze its potential use as food for human consumption; In this investigation it is desired to carry out a physicochemical and microbiological evaluation of the residual cake and the flour obtained from said cake, in order to know the nutritional characteristics and analyze its value as a potential consumer product in the agro-food industry.

2. MATERIALS AND METHODS

The physical-chemical and microbiological characterization of the residual cake and the flour obtained, was carried out with analyzes of moisture, ash, protein, fat, fiber, functional groups by infrared spectrophotometry and technical standards for microbiological test analysis. Samples evaluated total for each characterization, were 3.
The moisture was determined by gravimetric method (AOAC 1990), by quantifying the loss of mass, drying the sample in a drying oven Brand Memmert, model UM 100 at 105°C. The sample used was 2000 g. To determine the percentage of moisture or water content (WC), Equation (1) was used (SÁNCHEZ-DÍAZ and AGUIRREOLEA, 2000):

$$WC = \frac{S_i - S_d}{S_i} * 100 \qquad \text{(Ec. 1)}$$

Where WC is water content, Si, initial sample mass and Sd, dry sample mass. The samples results were analyzed using descriptive statistics, to verify the accuracy of the data.
The ashes were determined by ash difference (AOAC, 1990). Two samples of 2,000 g each were used in a porcelain crucible. The calcination was carried out in a muffle brand Terrigeno, model L2 at 550°C for 2 hours. The calculation of the ashes percentage is found in equation (2):

$$A = 100 * \frac{m_2 - m_1}{m} * \frac{100}{100 - WC} \qquad \text{(Ec. 2)}$$

Where A is the ashes percentage, m, mass of the sample, m1, mass of the empty crucible, m2, mass of the crucible with ashes and WC, Water Content.

The fat content was determined by acid hydrolysis and Soxhlet (AOAC 1990). A sample of 2 to 5 g was used in a 250 ml Erlenmeyer flask, with 10 ml of Water and 10 ml of HCl (NTC 668). For fat extraction, 50 cm3 of anhydrous petroleum ether was used for 3 hours in a water bath, until the depletion of fat was verified. For the Soxhlet method, the hydrolyzed and dried sample was incorporated into a flask and the fat was extracted with ether for 6 to 8 hours, subsequently removing the solvent in the IKA Brand rotary evaporator, RV 10CS1 model. For the calculation of fat percentage, equation (3) is used:

$$\%\ Few\ Fat = \left(\frac{m_2 - m_1}{m}\right) * 100 \qquad \text{(Ec. 3)}$$

Where m is the mass of the sample, m1, mass of the empty flask and m2, mass of the flask with fat. The values were averaged and expressed with 2 significant digits.
Protein content was determined with the Kjeldahl method (AOAC 1984 and FAO 1986). The sample used was 1 g. The protein calculation is found in equation (5):

$$N\ of\ the\ sample\ (\%) = 100 * \left[\left(\frac{A*B}{C}\right) * 0.014\right] \qquad \text{(Ec. 4)}$$

$$\%Few\ protein = N\ of\ the\ sample\ (\%) * 6.25 \qquad \text{(Ec. 5)}$$

Where A is amount (mL) of hydrochloric acid used in the titration, B, Normality of the acid and C, mass of the sample (g).
In the calculation of the amount of fiber (AOAC 2006) 2 - 3 g of degreased and dried sample was used. Equation (6) shows the operation performed.

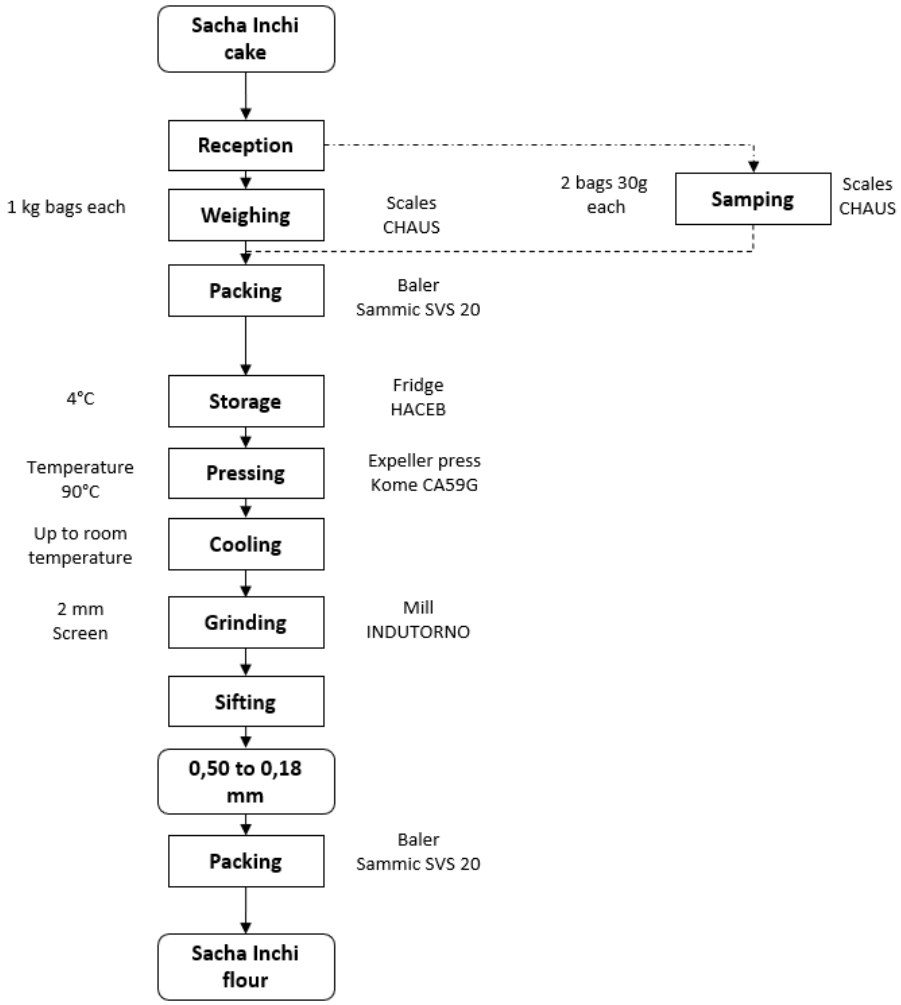
$$\%Few\ Fiber = 100 * \left(\frac{A-B}{C}\right) \qquad \text{(Ec. 6)}$$

Where A is the mass of the crucible with the dry residue (g), B is the mass of the crucible with the ash (g) and C the mass of the sample (g).
All the results of the previous calculations, were analyzed using descriptive statistics, to check the accuracy of the data.
For the determination of functional groups, a solid sample of 100 g mixed with 1g of pure KBr was used. The equipment used was Shimadzu model IR Affinitity-1, with a ratio S/N 30000:1 and a maximum resolution of 0.5 cm^{-1}.
Finally, in the microbiological characterization, the analyzes were performed following the technical standards described in Table 1. The samples were subjected to the stability study in the climatic chamber, BINDER Brand, model KBF 240.
To obtain flour from the cake of Sacha inchi (*Plukenetia volubilis* L.), were prepared: conditioning of raw material, grinding, sieving and packaging.
Fig. 1 shows the flowchart for obtaining flour from the Sacha Inchi cake (*Plukenetia volubilis* L.) from the oil extraction process by pressing in expeller, supplied by a supplier from the city of Medellín.
To obtain the brownie type product, was used flour obtained in the process of the present investigation.

Table 1. Technical standards for analysis and interpretation of microbiological tests and mycotoxins in foods.

Analysis	Method
Mesophilic aerobic count	NTC 4519 (2009)
Total Coliform Count	NTC 4458 (2007)
Faecal Coliform Count	NTC 4458 (2007)
Salmonella Detection	NTC 4574 (2007)
Filamentous fungus count	NTC 4132 (1997)
Yeast count	NTC 4132 (1997)
Staphylococcus aureus coagulase positive count	NTC 4779 (2007)
Bacillus cereus count	NTC 4679 (2006)
Total Aflatoxins	IN-GS-3,404
Deoxinivalenol (DON)	IN-GS-3,404

Figure 1. Flowchart, process of obtaining flour from Sacha inchi.



Source: Authors.

For preparation of the Brownie, it was started by preheating in an oven at 180°C, the butter was mixed with the sugar until a homogeneous cream was left, Sacha Inchi flour and baking powder were added. After having the mixture fully integrated in the container, milk and cocoa were added, finally, blueberries and chia seeds to taste. The already beaten mixture was poured into the aluminum containers, previously greased with butter and taken to the oven, after 30 min of review it was removed from the oven. A pilot test or sensory panel was carried out where a tasting of the product was carried out with which it was intended to give added value to the flour obtained.
A pilot test or sensory panel was carried out where a tasting of the product was carried out with which it was intended to give added value to the flour obtained.

3. RESULTS AND DISCUSSION

The relevance of carrying out this study had its main axis in the insufficient information and studies about the characterization of the flour obtained from the residual cake of Sacha inchi (*Plukenetia volubilis* L.), whose knowledge would allow to develop research on the possible applications that this flour would have in food products. In relation to the above, the following results of the characterization of the residual cake and the flour were obtained:
The average moisture percentage value for the residual cake, was 6.02%+0.17% and for the flour obtained from the cake, 3.56% + 0.32%. The above results are consistent with the regulations for dry products, since they are below 10%, this allows us to conclude that within the raw material there are lower risks of microbiological contamination. On the other hand, the lower moisture value in the flour is rational, taking into account the pressing process that was carried out to obtain it.
The ash content in residual cake was 3.85%+0.07% and into flour was 5.3%+0.25%, comparing them with other research on residual cake, 3.24% (MONDRAGÓN, 2009), 5.5% (JAGERSBERGER, 2013) and 8.72% (PASCUAL and MEJÍA, 2000), it is found that they follow an equivalent behavior where the variation of the values may be due to the contents of the cultivation soils, cultivated genetic load and ability to extract nutrients from the soil. The amount of minerals in food should be approximately 5%, so the values reported in the investigation are in the admissible range.
In the characterization by fat content, the residual cake had a value of 16.03%+4.35% and in the flour was 15.46%. The values obtained for the residual cake differ from other investigations reported with values of 37.33% (MONDRAGÓN, 2009), 19.9% (JAGERSBERGER, 2013) and 8.72% (PASCUAL and MEJÍA, 2000), which may be due to the variety and cultivation region of the Sacha inchi plant, in addition to the times and extraction method used.
The protein content in residual cake was 46.04%, value that is lower compared to other reported data of 59% (Ruiz 2013), 59.13% (PASCUAL and MEJÍA, 2000) and 51.76% (JAGERSBERGER, 2013). On the other hand, the flour obtained from the residual cake, reported a protein content value of 54.56%. It is important that it be a high value, because it is the most important nutrient in the diet, in turn, it allows to control the quality of food that would be acquired or supplied with the use of this flour.
The fiber content values will be obtained after developing the method that simulates the digestion of these polysaccharide compounds. For residual cake a value of 3.46% was reported and for the flour, 4.79%. Compared with other researches 3.16% (MONDRAGÓN, 2009), 4.50% (RUIZ, 2013) and 7.30% (PASCUAL and MEJÍA, 2000). The values of this research have not significant variation, except PASCUAL and MEJÍA. Regular consumption of fiber contributes to the intestinal flora, as well as reducing the

absorption of cholesterol and glucose. The values indicated in the flour indicate an acceptable content for consumption as food. Fig. 2 shows the comparison of results between the residual cake and the flour, where the protein content in the flour stands out with 18% greater than the residual cake.

The determination of functional groups by IR spectrophotometry was performed in order to confirm the nutritional value, taking into account the functional groups of carbohydrates, lipids and proteins. Fig. 3 shows, the infrared spectrum of the residual cake is shown, in which the peaks located at 3305 cm 1 stand out, associated with water molecules, the peaks at 3000, 2920 and 2826 cm^{-1}, correspond to the vibration of Asymmetric stretching of the CH3, CH2 and CH bonds, related to lipids and carbohydrates.

Figure 2. Comparison of residual cake and Sacha inchi flour characterization.

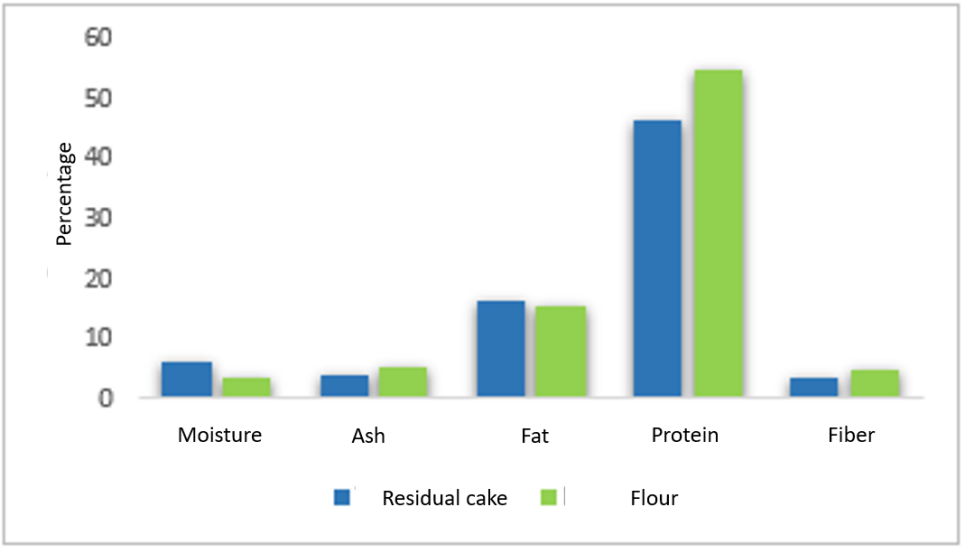


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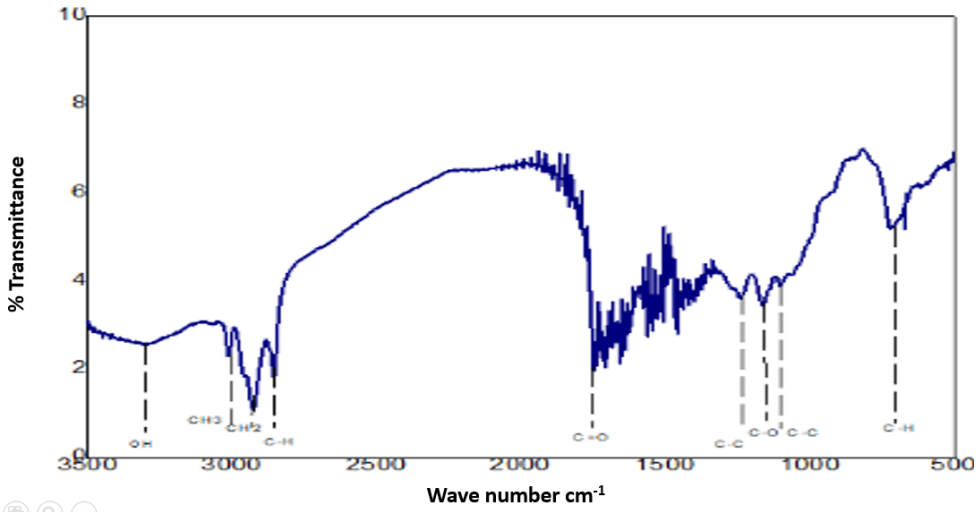
Figure 3. Infrared spectrum results of the residual cake of Sacha inchi.



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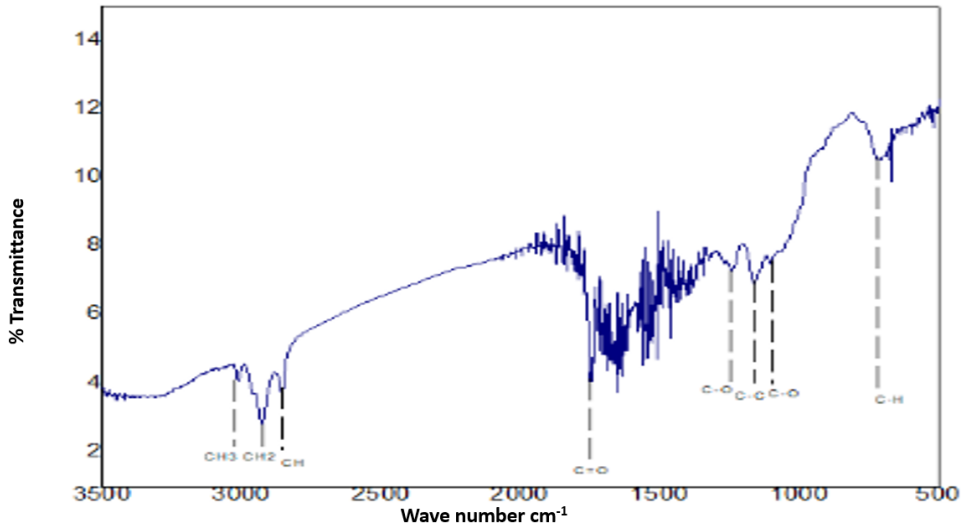
In 1743 cm^{-1} associated with the stretch vibration of the bond C=O related to proteins. Three peaks were observed in the region of 1230, 1170 and 1100 cm^{-1} that are associated with the stretching vibration of the C-O, C-C and C-O bond indicating the presence of carbohydrates. Peak 723 cm^{-1} is associated with the vibration of the C-H bond, indicating the presence of carbohydrates. These results allow to elucidate that the residual cake has the appropriate specifications to be the basis of a food of high nutritional value due to its carbohydrate, protein and lipid content.

Fig. 4 shows the infrared spectrum of Sacha inchi flour, where three peaks were observed at 3000, 2920 and 2826 cm^{-1}, associated with the asymmetric stretching vibration of the CH_3, CH_2 and CH bonds related to lipids and carbohydrates. In 1743 cm^{-1}, associated with the stretching vibration of the C-O bond related to proteins. Three peaks were observed in the region of 1230, 1170 and 1100 cm^{-1} that are associated with the stretching vibration of the C-O, C-C and C-O bond indicating the presence of carbohydrates. The 723 cm^{-1} peak is associated with the rolling vibration of the C-H bond indicating the presence of carbohydrates. As the results obtained with the residual cake, the spectrum confirms that the flour obtained from it, has potential application as food due to its nutritional value represented in the content of carbohydrates, proteins and lipids.

Figure 4. Infrared spectrum results of Sacha inchi flour.



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The results of the microbiological characterization for the residual cake and the flour are shown in Table 2. Among the data reported for the residual cake, 40 $ufc*g^{-1}$ mesophilic bacteria were found, value between the acceptable range. Regarding fecal coliforms, Staphylococcus coagulase, Bacilus cereus and molds and yeasts were found below the normal lower limits accepted.

In the case of the flour, 80 $ufc*g^{-1}$ of mesophilic bacteria were found, like the cake, the values are in the acceptable range, so its potential for agrofood application is confirmed.

The transformation of residual cake to flour began with the milling, later it was taken to dry, four temperatures were handled, which are 60, 70, 80 and 90°C, then sifted in sieves to obtain Sacha Inchi flour.

When the flour was subjected to different temperatures, some organoleptic characteristics such as smell, color and taste were modified. In the case of smell and taste at a higher

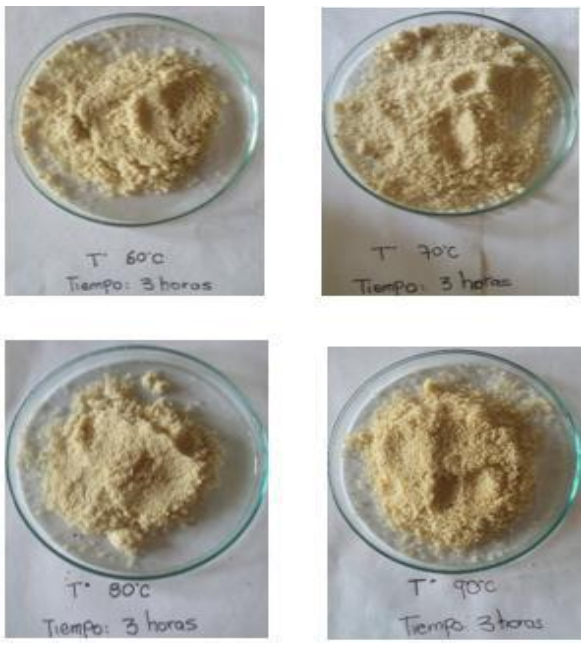
temperature less smell and taste had, being favorable for human consumption. In the case of color, the ratio is directly proportional to higher temperature, greater color, turning the flour darker as evidenced in Fig. 5.

Table 2. Result of microbiological analysis of residual cake and Sacha inchi flour.

Parameter	Result	Lower limit	Upper limit	Unit	Tecnical
Residual Cake					
Mesophilic bacteria	40	Less than 10	150000	ucf/g	Plate count
Fecal coliforms	Less than 3	Less than 3	Less than 3	mic/g	NMP
Staphylococcus aureus Positive coagulase	Less than 100	Less than 100	100	ucf/g	Plate count BP
Bacillus cereus	Less than 100	Less than 100	1000	ucf/g	Plate count Mossel
Molds and yeasts	Less than 10	Less than 10	1500	ucf/g	Plate count YGC
Flour					
Mesophilic bacteria	80	Less than 10	300000	ucf/g	Plate count
Fecal coliforms	Less than 3	Less than 3	menos de 3	mic/g	NMP
Staphylococcus aureus Positive coagulase	Less than 100	Less than 100	2000	ucf/g	Plate count BP
Bacilus cereus	Less than 100	Less than 100	1000	ucf/g	Plate count Mossel
Molds and yeasts	230	Less than 10	100	ucf/g	Plate count YGC
Salmonella	negative	negative	negative	ucf/25g	Salmosyst

Source: Authors.

Figure 5. Flour subjected to four temperature levels to improve organoleptic characteristics. A) 60°C; B) 70°C; C) 80°C and D) 90°C.



Source. Authors

The flour used to make the brownie was the one that was subjected to 80°C for three hours to eliminate undesirable characteristics, such as astringency and smell; as an agrobusiness conservation and flour improvement technique for human consumption.

4. CONCLUSIONS

The results obtained from the physicochemical evaluation, confirm that the residual cake and the flour of Sacha inchi, have a high nutritional value and not-toxic to be used in formulations of nutritional mixtures for food products for human consumption, revealing an added value applicable to This byproduct of the oleochemical industry.
Microbiological tests, both in the residual cake and in the flour, indicated that they are suitable for human consumption, without any risk to health. Especially these microbiological characteristics are maintained because the humidity in both cases remains low, preventing the appearance of saprophytic fungi and pathogens.
The results in the process of heat treatment to the flour for conservation or storage, showed that at a lower temperature (60°C) the color remains stable, but as the temperature in the flour treatment increases, it tends to change, taking a dark tonallity.
The smell has an inversely proportional relationship with the temperature, at a lower temperature it practically does not disappear, but when raised, the smell in the samples changes until it almost disappears at 90°C.
Subjecting the flour to heat treatments, allowed to determine which treatment improved the organoleptic characteristics and recommended a treatment for the handling of the flour at the agroindustrial level.

ACKNOWLEDGMENTS

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BASE LINE TO PROVIDE ADDED VALUE TO FERMENTED HANDCRAFTED RICE BASED PRODUCTS "MASATO"

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ABSTRACT

Fermented artisanal foods increased in popularity and some are considered intangible heritage. "Masato" is a typical drink of Colombia and has lost tradition and even there is ignorance of the product, but it is also the sustenance of many families. There are no reports that correlate natural fermentation, microbiota and the environment with its quality, so we sought to establish a baseline that aims to add value to the product for sustainable development. Samples of "masato" from lots of three volunteer producers who authorized the documentation of processes and the evaluation of microbiological indicators and physicochemical aspects. As a result, it was demonstrated the high prevalence of fermenting microorganisms and others that exceed the criteria allowed for consumption, affecting positively or negatively the quality of the product, this knowledge can be used in training for strategic development and continuous improvement of productivity of microenterprises.

Keywords: natural fermentation, beneficial microorganisms, culinary heritage handcrafted products

1. INTRODUCTION

The popularity of artisanal foods elaborated by generational tradition, together with the culture and customs of their inhabitants, has increased in recent years due to the presence of beneficial microorganisms that could be of interest for the prevention of specific gastrointestinal pathologies or other beneficial effects for health (LÓPEZ, 2010; Dominguez, 2012, VALENCIA *et al*., 2019). Hence the Proinfant-CYTED project to promote cooperation and harmonious development of science and technology in Iberoamerican regions and it was proposed like initiative for promoting strategics on vegetable Functional Foods and at the same time to widen the knowledge on this type of food.
In addition, these traditional foods and culinary knowledge constitute the pillars of the nation's intangible cultural heritage (UNESCO, 2012; MINISTRY OF CULTURE, 2012), therefore the need to document processes and methodologies directly from producers to propose improvements that lead to sustainable developments and add value to their products, especially when many of these products are the livelihoods of families in developing countries.
In this way, the climatic diversity in the different regions has allowed the development of autochthonous native food products (ÁLVAREZ, 2014), elaborated through artisan processes that allow the natural development of the associated microbiota (CHAVES *et al*., 2014; VEGA AND LÓPEZ, 2012). Among some of these fermented foods, those typical of indigenous culture are mainly corn (*Zea mais*) and yucca (*Manihot esculenta*), while those typical of African culture are based on rice (*Oryza sativa*) and banana (*Musa paradisiaca*) (FAO, 2015; ÁLVAREZ, 2014). A typical example in Colombia is the "masato" made from different cereals, whose fermentation and final characteristics depend on the specific preparation of the food, as well as the ingredients with which it is prepared.
The “masato” is a liquid creamy drink, spontaneously fermented for periods ranging from 2 to 5 days for consumption, handmade with uncontrolled processes in the absence of appropriate conservation techniques and are marketed locally. The growing interest in these traditional fermented foods has raised the need for studies focused on the standardization of the manufacturing process (BECERRA, 2014) and physical-chemical and nutritional characteristics (FULA, 2010; BECERRA, 2014). However, only a few studies have reported preliminary microbiological characterization of such raw food materials (LÓPEZ *et al*., 2010).
The development of the natural microbiota depends on many external factors causing bacteria such as lactic acid bacteria (LAB) to be found in this ecosystem, which can modify the fermentable sugars (sucrose, fructose, glucose) present in small quantities in cereals, cost-effectively improving the nutritional value and sensory properties of these foods, and even specific strains of LAB could be found that could have beneficial effects on the health of the consumer (VÄKEVÄINEN *et al*., 2018) special interest has been aroused in those microorganisms that contribute to the intestinal microbial balance of the consumer (some of them called "Probiotics") or those that produce probioactive substances (vitamins, enzymes or antagonistic compounds on specific pathogens (GARCÍA *et al*., 2018; CHAMPAGNE *et al*., 2018).
On the other hand, in these foods there may also be the presence of other microorganisms that may turn the food into a product that is not safe to consume. The presence of all these microorganisms can be correlated with physicochemical analyses that show specific effects of the predominance of some microbial communities. Therefore, this research aimed to establish a baseline to improve the microbiological and physicochemical quality of masatos handmade in Ventaquemada, Boyacá, Colombia (5°21′59″N) as culinary heritage of the country and recovering LAB culture collections like representation of cultivable biodiversity, that will be further characterized for biotechnological traits.

2. MATERIAL AND METHODS

2.1. Collection of "masato" samples

Three artisanal producers of rice masato (P_1, P_2 and P_3) located in the village of Ventaquemada, Boyacá, Colombia (5°21′59″N), who voluntarily accepted to be participants, were selected and signed an informed consent where they accessed the documentation of their processes during the elaboration of three batches of product for each of them and the taking of samples for microbiological and physicochemical analysis. During the elaboration of the batches of "masato", samples were taken at the beginning of fermentation, at an intermediate time of fermentation and at the finished product. The samples were aseptically collected in sterile screw cap bottles, frozen at -18°C±2°C and transported to the laboratory in the shortest possible time for further analysis.

2.2. Microbiological and physicochemical evaluation of "masato" simples

The initial and intermediate samples were evaluated LAB NTC 5034 count from 2002 and moulds and yeasts NTC 5098-2 from 2009, as well as titratable acidity (AOAC method 981.12) expressed in % lactic acid (%p/p) and pH (AOAC method 981.12) and pH curves during fermentation were measured in real time using a device manufactured by the Center for Interfacultative Instrumentation of the University of Antioquia. The electrode was kept in contact with the inoculated mixture for 24 hours and evaluated for 48 - 72 hours.

The finished products were evaluated in addition to the above, the following microbiological criteria: mesophilic counts according to the Colombian technical standard NTC 4519 of 2009, total coliforms and *E. coli* (NTC 4058 of 2018), *Staphylococcus aureus* (NTC 4779 of 2007), *Bacillus cereus* (NTC 4679 of 2006), moulds and yeasts (NTC 5098-2 of 2009) and the test for absence or presence of *Salmonella* spp. (NTC 4574 of 2003). Surface sowing was also performed to count acetic acid bacteria (AAB) using WL agar (Scharlau - Spain) incubating Petri boxes at room temperature for 8 days. And, the LAB count using Man Rogosa Sharpe (MRS- Oxoid-Spain), M17 (Oxoid-Spain) and the yeast agar glucose peptone meat lactose (YGLPB) all supplemented with 100μg/mL of cycloheximide, the BAL boxes were incubated at 30°C for 48 h under anaerobic conditions. (PÉREZ-CATALUÑA *et al*., 2017).

For the microbiological tests, each sample was weighed 10 g and homogenized with 90 mL of 0.1% peptone water. Subsequently, serial dilutions and sowing were made in different culture media. Except for the *Salmonella* spp. test, which had an enrichment process for which 25 g of the feed were weighed in 275 ml of lactose broth. After the incubation period corresponding to each microorganism, the colonies were counted and calculated in Log UFC/ml, for its later analysis.

The physicochemical analyses were: determination of the content of maltose, glucose, fructose, lactic acid, acetic acid, propionic acid and alcohol all by HPLC, for it, the homogenized samples were diluted in 9 mL, filtered and evaluated in a Chromatograph Agilent 1200, Column: HPX87HAminex BIORAD, Column temperature: 35°C, Detector temperature: 35°C, Flow: 0.6ml/min, Mobile Phase: H2SO4 0.008N, Run time: 25 min, Detector: RID, results expressed in g/L. Humidity in the oven (AOAC method 925.45) and rheological behavior of the "masato" in an equipment.

2.3. Statistical analysis of the data

The statistical difference between treatments was determined using the 95% standard deviation limits (LSD). The other response variables were analyzed with the GraphPad Prism 7.0 software using 2-way ANOVA and the Bonferroni test to determine a significant difference between treatments.

3. RESULTS AND DISCUSSION

The average of data obtained from the documentation of the process during its elaboration of the "masato", the standard deviations and the coefficient of variation, for each producer is presented in Table 1.

Table 1. Masato´s Formulations made by producer.

Raw material	Producer 1			Producer 2			Producer 3		
	Quantity (g)	SD	CV	Quantity (g)	SD	CV	Quantity (g)	SD	CV
Water	2667	577	22	3900	173	4	1092	166	15
Rice	500	0	0	500	0	0	527	46	9
Sugar	558	52	9	1167	577	49	250	0	0
Pineapple peel	0	0	0	333	115	0	400	0	0
Bacterial inoculum	0	0	0			0			0
Cinnamon	15	0	0	10	0	0	20	0	0
Clove	6	0	0	3	0	0	0	0	0
Clove water + cinnamon (Rice smoothie)	1500	500	33	0	0	0	1450	87	6
Water to liquefy	3000	1732	58	5667	577	10	503	286	57

The results of the elaborated formulations show a great variation in the content of water, sugar and rice among the same lots of a producer, which indicates the lack of standardization of the process that directly affects the availability of substrate for the microorganisms and variation of the environmental conditions for the growth of the different microbial genera (BECERRA, 2014).

Log average of LAB counts and standard deviations (SD) in samples evaluated during the fermentation process, present at the beginning counts between 7,9 to 8,3 Log CFU/mL, counts that were changing during the process, reaching for P_2 and P_3 the highest values (8,23 and 8,7 Log CFU/mL, respectively), similar values were obtained by Salmerón *et al*. (2015). This behavior is associated with the ability of LABs to tolerate stress conditions such as low pH (BORICHA *et al*., 2019), which is essential for their survival and allows them to metabolize different substrates and be dominant microbiota in vegetable and cereal fermentation processes, similar results were obtained in a rice based fermented chicha (PUERARI *et al*., 2015). The P_1 obtained the lowest LAB Logs with a decrease in the intermediate time, without overcoming the initial number of microorganisms (Fig. 1).

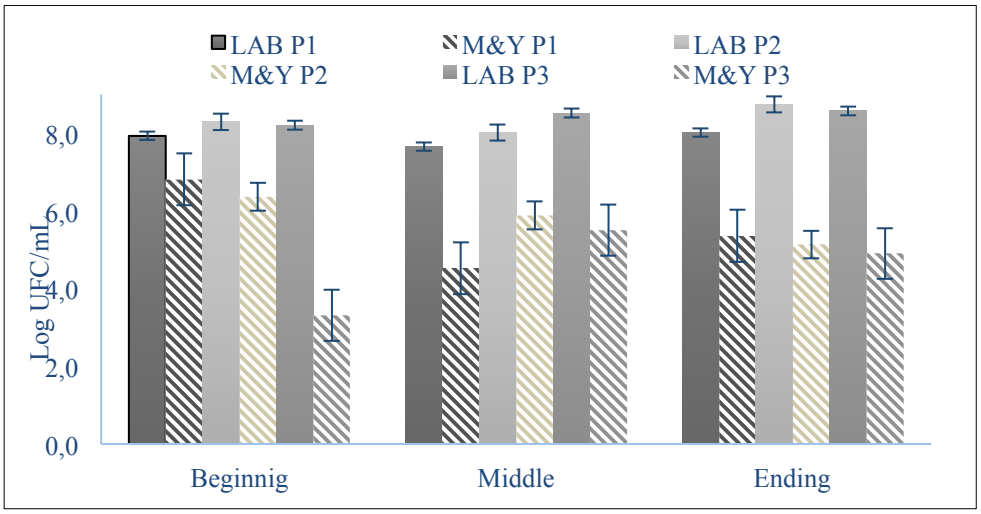

Figure 1. CFU log of lactic acid bacteria and yeasts during the rice masato fermentation process of three different producers.

On the other hand, the diversity and growth dynamics of yeasts reveal that they are important in fermentation, authors such as MENDOZA *et al*. (2017) allude that these microorganisms have been found in several autochthonous fermented products such as Andean Chicha and Colombian Champús (OSORIO *et al*., 2008). In fermented cereals, yeasts make a useful contribution to improving the taste and acceptability of the product. In the case of masato, greater cell concentration is reflected in the initial sample for P1 and P2 with values of 6.8 and 6.36 Log UFC/mL, respectively, which lose viability in the final product, without presenting statistically significant differences in the "masato" of rice for the three producers (5.36 - 5.14 and 4.9 Log CFU/mL). The relationship of LAB and yeasts favor the development of the typical sensory profile, SALMERÓN *et al*. (2014) emphasize that the success of a product is associated with its flavor, which in turn is a critical factor in the acceptance or rejection of a food, for this reason mixtures of yeasts and LAB strains have been proposed, in order to combine their metabolites according to the tastes of consumers (REHMAN *et al*., 2006).

It was also possible to establish that the pH curves were different, with the P_3 curves presenting lower pH, although at the end of the fermentation process, the pH of P_2 did not show significant differences ($P<0.05$) with the pH of P3 (Fig. 2). It should be noted that the fermentations of P_1 rice "masato" presented a pH>5.54, a value that could favor the growth of other microorganisms that generated greater competition for the BALs present there (Fig. 2).

The CFU/mL Log of the counts of microorganisms indicating quality and safety in the finished products of the different elaborated lots are presented in Table 2, where it is observed that the total mesophiles, the AAB and the LAB presented high and similar counts among the producers (less than one logarithmic unit among them), except for the P_1 AAB. The coefficient of variation between the lots of each producer was high for P1, the variability of these microbiological criteria in the same producer could be related to the cross-contamination and the material of the containers used during the elaboration of the masato (Fig. 3), these variations could directly affect the flavor in the finished products and inhibit or favor the presence of undesirable bacteria by means of the production or degradation of different acids (lactic, acetic, propionic). SALMERÓN *et al*. (2015) refers in his research that the variation in the concentrations of lactic acid and acetic acid can change the flavour attributes of a fermented product.

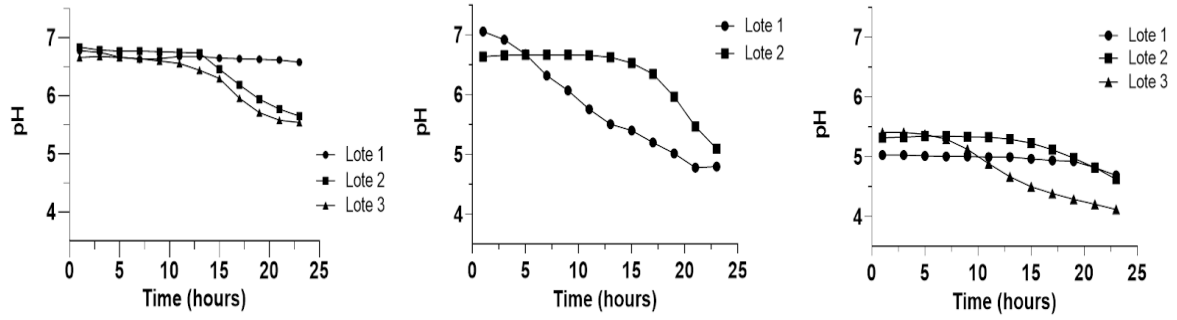

Figure 2. pH during the fermentation process of each producer's batches.

Table 2. Counting of indicator microorganisms in finished product.

INDICATOR	P_1		P_2		P_3	
	X Log CFU/mL	%CV	X Log CFU/mL	%CV	X Log CFU/mL	%CV
Mesophilic	7,8±0,25	3,2	8,7±0,2	2,0	8,5±0,08	0,9
M &Y	5,1±0,71	14,1	5,2±0,2	4,6	3,7±2,32	63,4
TC	4,6±0,15	3,2	4,6±0,6	12,7	1,6±0,87	52,9
E. coli	3,4±2,08	61,2	1,0	0,0	1,0	0,0
S. aureus	2,4±0,32	13,5	4,5±1,1	24,6	1,6±1,00	63,6
B. cereus	1,0	0,0	1,0	0,0	1,0	0,0
AAB	7,6±1,28	16,8	8,3±0,1	1,3	8,4 ±0,10	1,1
LAB	7,9±0,48	6,1	8,7±0,3	3,4	8,6±0,09	1,1

Figure 3. Documented photos during the "masato" production process.

Regarding the prevalence of total coliforms, two of the three producers presented concentrations above 4 Log units and the other, although the count was lower, presented greater variation between the Log units of the batch. In fecal coliforms, only P_1 was above the criteria established for this indicator (3.4±2.08 Log Units), as well as the presence of *Salmonella* spp, in one of the three lots, probably due to deficiencies in hygienic conditions

and the low acidity reached by the lots of this producer. In artisan fermented foods it has been widely reported in the literature values above those allowed for these microbiological criteria (BYAKIKA *et al*., 2019; ILANGO and ANTONY, 2014; NTULI *et al*., 2013; PUERARI *et al*., 2015; VÄKEVÄINEN *et al*., 2018). According to Ilango and Antony (2014), inadequate handling of raw materials and poor hygiene conditions before, during and after the processing of these foods are the main causes.

On the other hand, P_2 showed a growth of 4.5 Log CFU/mL of *Staphylococcus aureus* with a high coefficient of variation between lots, evidencing a lack of good practices. Taking into account the risk that this microorganism represents for human health, because it produces an enterotoxin that causes food poisoning. (CHARLIER *et al*., 2009; RUIZ *et al*., 2017; BYAKIKA *et al*., 2019). *S. aureus* is the third most common cause of confirmed food poisoning in the world, which is why its presence in finished foods is unwanted. In Colombia, the Ministry of Health and Social Protection (2010) has evaluated the risk of this microorganism in non-industrially prepared foods due to the large number of reported outbreaks. However, Villa *et al*., (2012) makes a review highlighting the inhibitory effects of BAL against pathogenic microorganisms such as *S. aureus*, this benefits the industry and public health sectors by increasing the productivity of fermented foods and decreasing the risk of foodborne diseases (TSAS).

Finally, no *Bacillus cereus* counts were evidenced in the evaluated batches of rice "masato". However, authors such as Almeida *et al*. (2007) and Ramos *et al*. (2010) report the presence of *Bacillus cereus* in rice based fermented beverages.

The presence of these indicator microorganisms highlights the importance of implementing good manufacturing practices (GMP) to control the hygiene of the fermentation process by avoiding contamination with unwanted microbiota, without changing the craftsmanship of the process and ensuring that these species do not become dominant and alter the safety, quality and shelf life of the finished product. (RESOLUTION 2674, 2013) This is where LAB plays a crucial role if measures are taken to improve their optimal development. Since, the production of acids and other antimicrobial components during fermentation can promote the safety and stability of the final product by eliminating pathogenic microorganisms. (VÄKEVÄINEN *et al*., 2018; PUERARI *et al*., 2015).

The microbiological counts obtained in the "masato" of rice, allowed to observe the proportion of the evaluated groups of microorganisms and to corroborate the predominance of fermenting microorganisms in the finished product, since, they are in greater quantity LAB, AAB followed by yeasts (Fig. 4); the growth of these microorganisms is favored by the degradation of substrates thanks to the native microbiota that lead to the acidification of the environment and the release of other growth factors such as vitamins and soluble nitrogen compounds, or disadvantaged by the predominance of other different groups that change the characteristics of the finished product.

Thus, the acids produced during fermentation (lactic, acetic and propionic) in the different batches of the producer, present high variability and significant differences between producers, except for the alcohol content (Fig. 5A). These three acids are the most frequent in fermentation processes and are responsible for taste (PUERARI *et al*., 2015). The propionic acid content reflected in P_3 is striking, with no differences with other acids produced in their batches. This acid has a different taste profile than lactic acid (Blandino *et al*. 2003). P_1 presented in the finished products the lowest acid content, data consistent with the pH reached in the batches of this producer. The presence of acid compounds can have inhibitory effects on some bacterial groups, especially those that are demanding and could be affected by pH (VALENCIA *et al*., 2018).

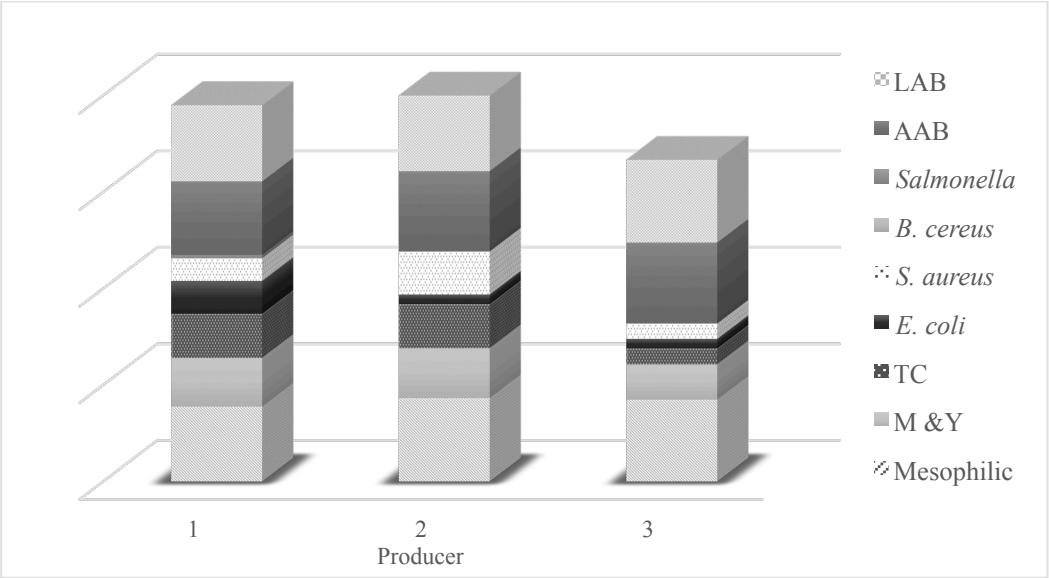

Figure 4. Microbiological predominance in finished product for three producers.

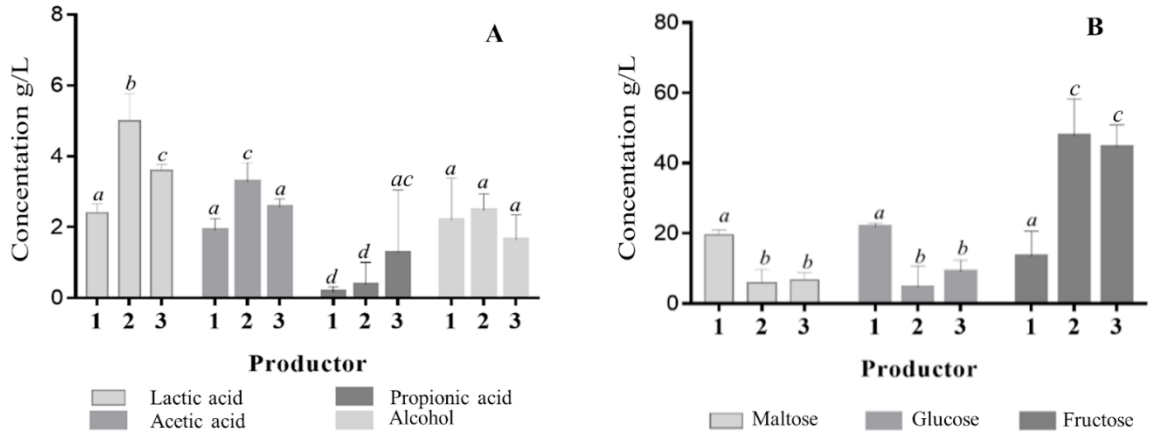

Figure 5. Averages and SD in the finished products of the three masato producers. A: Organic acids (lactic, acetic, propionic) and alcohol. B: Determined sugars. Different letters on the bars show significant difference between treatments (n = 3).

With regard to the quantity of sugars evaluated, the average fructose content of the P2 and P3 lots (47,8 g/L y 44,6 g/L) is striking, which could indicate a low number of microorganisms that degrade fructose, contrary to what is observed in P1 where there are no differences between the average of the quantified sugar content.

In general, the moisture of the finished products did not differ among the "masato" producers (Table 3) despite the differences in the addition of water in the initial formulation, this is probably related to the water retention capacity of starch.

Finally, after the microbiological and physicochemical characterization processes, a total of 80 strains were isolated among the three producers, which met the basic characterization criteria for BAL: Catalase (-), oxidase (-), Gram (+) and hemolysis (-). The highest number of isolates was obtained from P3 with 41%, followed by P2 with 34% and lower amount P1 with 25%

Table 3. Average moisture content of "masato" producer.

"masato" Producer	Moisture (%)	CV (%)
P_1	86,57±1,55	1,8
P_2	85,97±2,11	2,5
P_3	82,53±1,83	2,2

Con n = 3.

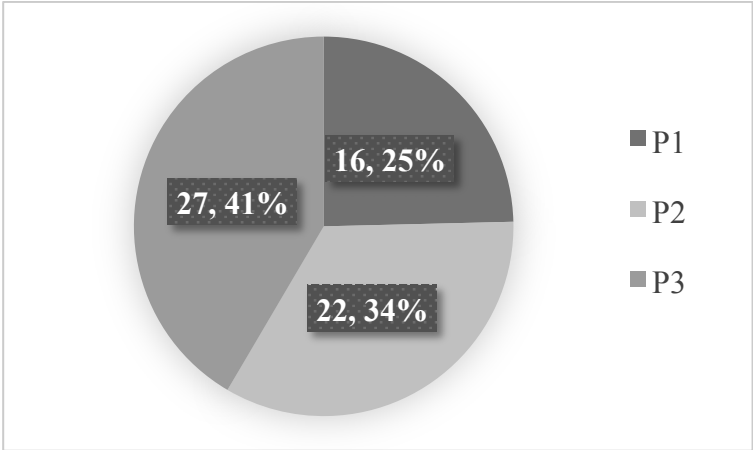

Figure 6. Isolated LAB strains with basic identification characteristics for the three producers.

Thus, the production process of artisan fermented foods, such as "masato", is susceptible to improvement without affecting tradition, since some of these products are made with poorly controlled processes, without adequate technology, these studies provide relevant information, so that the competent authorities can accompany and strengthen these processes, as well as establish specific standards that include adequate criteria for acceptance or rejection ensuring the safety of them. In addition, they provide information that points to the isolation of microorganisms as defined starter cultures that give added value in their contribution to health.

4. CONCLUSIONS

It is exposed that: the variability of the processes of elaboration of the "masato", with the predominance of specific microbial groups that arrive by natural inoculation, and some of these put in risk the sustainability of the producer. In addition, the dependence of this variability on hygienic habits, cross-contamination and production processes to strengthen the number and permanence of beneficial microorganisms for the production of compounds responsible for taste, aroma and preservation of the product. On the other hand, a high number of BAL isolates were achieved, which through the use of molecular techniques may indicate the richness that fermented products of vegetable origin may have.

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EFFECT ON THE TENDERNESS, COLOR AND FUNCTIONAL PROPERTIES OF THE PAPAINA ENZYME IN A CHORIZO PROCESSED WITH COW MEAT OF LOW COMMERCIAL VALUE

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ABSTRACT

Papain is a protease that has been widely used by the meat industry, since hydrolyzing the protein allows to achieve a better tenderness; which allows better use of meat because of its importance due to the contribution of high quality protein, minerals (iron) and vitamins (B12), its use is recommended. The low purchasing power of a large part of the population, allows us to see the possibility of processing meat products using meats of less commercial value, such as jars and meats from muscles with a lot of movement whose hardness is high; One of these products is the economic sausage, typically Spanish product, whose formulation has changed from the conquest to the present day, in each region of the country there are different formulas and according to the NTC 1325 standard there are three types of Premium, Selected and Standard products. The objective of this project was to prepare economic sausage (standard) used meat of low commercial value (cogote), to observe the effect of the application at different concentrations of papain and compare the textural, functional properties and color. The meat was adapted, pre salted, after 24 hours it was ground, mixed with the ingredients and additives, papain concentrations were applied (T_1 0.3; T_2 0.6; T_3 0.9 g/Kg), it was stuffed and portioned, it was scalded (73°C), dried and then cooled, the samples were vacuum packed to perform functional properties analysis (CRA and pH), tenderness by Warner Blatzler method, used a TXT-plus Instrument texturometer, color using a Color Flex EZ colorimeter (Cielab scale,

coordinates *a**, *b** and *L**). The results showed in terms of tenderness significant differences between the control and the other treatments, in turn between the treatments there are significant differences, the T_3 treatment showed the lowest levels of tenderness, as for the color in the coordinate *a** is observed a higher value in T_3, on the other hand with respect to the functional properties, in the pH no significant differences are observed, while with respect to the CRA, if there are significant differences between the control and the treatments. It can be concluded that the enzyme papain does have an effect on the properties of processed sausage at low cost with hard meats, improved tenderness and therefore its chewiness to be used nutritionally, without affecting other quality characteristics.

Keywords: cogote, chorizo, WB, tenderness, color, CRA

1. INTRODUCTION

The chorizo is a raw sausage, of Spanish origin, made with ground pork and fat, with spices and condiments, salt and sodium nitrite, which is stuffed in natural or artificial edible casings (FAO, 2006). It must have a characteristic color and flavor that fundamentally provides the ingredients and spices (MADRID, 2005). Traditionally, one way of preserving meat was by making sausage by fermentation and/or drying. Currently, sausages of different qualities and prices are offered in the market, appreciated for their appearance, texture and flavor. The products obtained do not always have the desired sensory characteristics. The causes of this are the raw material, ingredients and additives as in the manufacturing processes used (ARNAU, 2011). The quality of a sausage depends on the set of product characteristics that determine the degree of acceptability by the consumer. . The tenderness of the meat varies with age, race, sex and the location of the cut in the canal that is where the importance of the use of enzymes in this industry lies; Proteases are a type of technological use, (VIOQUE and MILLÁN, 2001). Studies have shown an increase in the speed of maturation of meat and meat products that have been treated with enzymes such as Carica papaya papain, *Streptomyces griseus pronase* E, *Aspergillus orizae acid aspartyl proteinase*, *Anana comusus bromelain* and *pancreatic lipase* (DIAZ, 1993; FERNÁNDEZ, 1993; GÉLVEZ, 2006). Research such as Sorour and NAFISEH (2016) combined an ultrasonic treatment and application of papain; they found that the application of the enzyme, individually or in combination with ultrasound decreased the shear force of Warner Bratzler

2. MATERIAL AND METHODS

2.1. Sample preparation and storage

The commercial cut of meat was cogote or nape obtained from the slaughter and slaughter of a 4-year-old male Zebu cattle acquired in the public market of Cereté, in the same way the porcine fatty material was acquired. Additive inputs and ingredients (gut, condiments, phosphates, curative salts) were purchased from a marketing company in Medellín (Colombia). On the other hand, Protease (Pure Papain), was supplied by the company Insuquimicos Colombia S.A.S The meat was adapted and presalted with a mixture of common salt and commercial curative salt (150 ppm nitrite) according to NTC 1325, subsequently, the fat meat was ground in a JAVAR number 22 mill, disc No. 8, the ingredients were subsequently calculated according to the sausage formulation shown in Table 1.

This formula was kept constant, on the total mass it was divided into 4 parts corresponding to the control and three treatments, the enzyme was not added to the control, while to the remaining treatments, the enzyme was added as shown in Table 2.

For the addition of the ingredients the recommended order was followed, the mixtures were stored for 2 hours at 4°C and subsequently stuffed using a KRAMER brand manual stuffer, 3 liters capacity, the gut used was a collagen-based corrugated. All treatments were dried and smoked for 30 minutes at a temperature of 70°C, using a chamber for smoked drying. The samples were cooled and stored (24 h/4°C), then vacuum packed for further analysis.

Table 1. Chorizo formulation.

Ingredients	Percentage
Beef	80%
Pig fat	20%
% In relation to the weight basis	
Starch	5%
Ice	10%
Condiments	1%
Cumin	0,3%
Garlic	0,4%
Onion	1%
Liquid smoke	0,1%
Phosphate	0,3%
Enhancer	0,1%

Table 2. Papain concentration.

Treatment	Concentration (g/Kg)
To (control)	0,0
T_1	0,3
T_2	0,6
T_3	0,9

2.2. Sample analysis

To carry out the Tenderness Analysis, the Warne Blatzler shear force was measured on a TXT-plus texturometer of the Mycrom System, the parameters were: compression speed 1 m/s, 30% compression force of 20 gf (PACHECO *et al*., 2011). On the other hand, the color was measured using a Huntelab ColorFlex EZ Spectrophotometer Brand colorimeter and the parameters used in the CIELAB methodology were measured, using L^*: luminosity (black-white), a^* (green-red), b^* (blue-yellow), method proposed by the Commission Internationale de l'Eclairage (CIE); slices millimeters thick were cut to cover the circular area of the colorimeter. Regarding the pH this was determined following the procedure used by GUERRERO and ARTEAGA (1990) using a pH-meter brand OAKTRON pH 700, and for the CRA Water Retention Capacity, the analysis was performed using the compression method, followed the method proposed by GRAU and HAMM (1953). The processing of the statistical data was carried out using the SAS package.

3. RESULTS AND DISCUSSION

Regarding the pH, in Table 3, it can be seen that between the control and the treatments, there are no significant differences (P>0.05). This is possibly due to the regulatory action of phosphates that keep this property stable in scalded sausage products; GONZALEZ *et al*. (2013), they found values below, because the samples corresponded to artisanal sausages handled in environmental conditions, and LEYTON (2017) obtained pH values between 6.09-6.22 in sausages that were subjected to different scalding temperatures. It can be seen

that they do not exceed the limit values of the ICONTEC 1325 standard for processed processed meat products with a minimum value of 5.8. The values obtained for CRA of the treatments, there were significant differences (P <0.05) between treatments T0 and T3, myosin is the main responsible for the CRA (VARNAM and SUTHERLAND, 1998). Papain is a plant enzyme that has a softening effect because it acts on myofibrillar proteins and connective tissues (ASHIE *et al*., 2002), it also affects CRA. T3, having a higher percentage of papain, cannot absorb water in the same way. The Technique Colombian Standard NTC 1325 allows a maximum of 67%.

Table 3. Functional properties.

Treatment	pH	CRA
To (control)	5,486±0,145[a]	51,755±9,884[a]
T1	5,517±0,128[a]	47,116±9,358 [a]
T2	5,489±0,142[a]	49,227±8,934 [a]
T_3	5,503±0,085[a]	39,952±9,970 [b]

The changes of the CRA are presented by the decomposition that occurs in myofibrils, due to the action of this proteolytic enzyme, responsible for water retention.
Table 4 shows the results of WB tenderness, significant differences (P <0.05) are observed between treatments T_0, T_1, T_2 and T_3, demonstrating that the enzyme papain had a softening effect on treatments T_1, T_2 and T_3, so much so that in the T_3 treatment. Other studies such as SOROUR and NAFISEH, (2016), in which they obtained hardness reduction. of cuts of meat exposed to ultrasonic radiation and simultaneous treatment with papain, obtaining average values of 5,098 Kgf higher than those found in this investigation. Hardness influences, among other factors, the length of the sarcomero and myofibrils, so that the greater the state of contraction, the greater the hardness (VAN HOOF 1981). In addition the nature of the connective tissue (NAKAMURA *et al*. 1975). A greater amount of collagen implies greater hardness (TOURAILLE, 1978).

Table 4. Tenderness determined by WB.

Treatment	WB cutting force
To (control)	2,487±0,652[a]
T1	1,229±0,176[bc]
T2	1,642±0,386b
T3	1,152±0,207[c]

Exogenous enzymes such as papain, are used as meat softeners, this is due to its ability to break down mifibrillar proteins and connective tissues (ASHIE *et al*., 2002). Cut resistance values less than or equal to 2.27Kg of pressure means tender meat; values between 2.27-3.63 Kg represents moderately tender meat and more than 5.44 Kg represents extremely hard meat (AMSA, 1995), on the other hand, in investigations carried out by other authors both in chorizo and in similar meat products, it is observed that the data obtained are within similar ranges (ROMERO *et al*., 2016; TIRADO *et al*., 2015; PACHECO *et al*., 2011). Table 5 shows the results of the color of the treated samples. From the analysis of variance (ANOVA) it was obtained as a result that there are no statistically significant differences

(P> 0.05) between the treatments for the parameters evaluated in color, which It indicates that papain had no effect on the color of the sausages. The amount of moisture or fat, which has a direct and marked influence on the luminosity, as these ingredients remained constant, the parameter L* did not vary significantly.

Table 5. Color of sausage samples versus papain application.

Treatment	*L**	*a**	*b**
T_0 (control)	39,807 [a]	8,220 [a]	13,130 [a]
T_1 (0,03%)	42,617 [a]	7,430 [a]	11,045 [a]
T_2 (0,06%)	41,082 [a]	7,767 [a]	11,887 [a]
T_3 (0,09%)	42,940 [a]	10,537 [a]	14,407 [a]

As for the coordinate a* there are no significant differences for a reason similar to the gray scale, by keeping the proportion of meat constant as it provides the myoglobin responsible for the color red. Other researchers such as COBOS *et al.* (2014), in chorizo with fiber they found similar values in parameter L*, but different in a* and b*, possibly the addition of fiber influences these parameters.

4. CONCLUSIONS

The application of the enzyme in a sausage using hard meats of less commercial value reduces the shear or shear force, despite this, an excess in the concentration of papain has a very drastic effect on the texture of the sausage. On the other hand, it can be concluded that the application of papain does not affect the color, mainly attributed to the interaction of myoglobin and nitrites. As for pH, it is not altered by the addition of papain in the different concentrations; while, the Water Retention Capacity (ARC), is reduced as the percentage of papain increases.

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EFFECT ON TEXTURE, FUNCTIONAL PROPERTIES AND COLOR OF COOKED HAM FROM CHIGUIRO AND PORK MEAT (*HYDROCHOERUS ISTHMIUS*)

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ABSTRACT

Since ancient times wild animals have been a source of protein, some aboriginal communities and even settlers still consume these species, the problem that arises is the threat posed by its extinction; One of these species typical of the Sinú region is the chigüiro or poche (Hydrochoerus hydrochaeris), which is very popular for typical dishes due to its flavor. An alternative to mitigate the problem is the production of this species in captivity, so that in addition to protecting the species, it becomes a source of protein and an economic possibility. To give added value this meat can be transformed into meat products, one of them is ham cooked in mold; The objective of this study was to prepare cooked ham with the partial or total replacement of pork as well: control (C) 100% pork, T1 50% pork 50% chigüiro, T2 75% pork 25% chigüiro and T3 100 % chigüiro, to analyze and compare textural, functional properties and color. The meat was adapted, selected, massaged and mixed with the ingredients and additives, and after 24 hours it was molded and scalded at 73°C, until it reached 70°C in the center of the product, cooled and demolished to to obtain the samples, to which TPA texture profile analysis (hardness, chewiness, cohesiveness, elasticity), color and functional properties (CRA and pH) were performed. The results showed significant differences in texture for the different treatments, mainly in hardness; a greater hardness is observed in treatments T2 and T3 in terms of color, similar results were presented mainly in the coordinate of *, there is a greater tendency in treatment T3; on the other hand, there were also significant differences in functional properties, with a higher CRA in chigüiro meat; It is concluded that it is possible to prepare pressed ham of good textural quality using totally or partially chigüiro meat.

Keywords: chigüiro, ham, TPA, color, CRA

1. INTRODUCTION

The species H. isthmius is distributed in the Caribbean region as follows: the Ranchería river valley; in the rivers of the north and western slopes of the Sierra Nevada de Santa Marta; the valleys of the low and middle Magdalena the valley of the Cesar river and the low valleys of the Sinú river (LOPEZ *et al*., 2014). The department of Córdoba is one of the departments with more diversification in its natural resources, standing out in its fauna and flora, as is the case of chigüiro (Hydrochoerus isthmius) (CARRASCAL *et al*., 2011). Wild species have not been exploited as is the case of Chigüiro (*Hydrochoerus isthmius*), also known in this region as Punch, whose meat is similar to that of pork, is rich in proteins, vitamins, and many other beneficial substances for man, so that the consumption of this meat is suitable and appropriate (ARAUJO and GONZÁLEZ, 2017), BUITRAGO and GONZÁLEZ (2007), in their study on the use of chigüiro meat, they applied smoking as a method of conservation, and established that this The method increases the shelf life of the product, it was also shown that the meat of this species has excellent properties for the production of meat products. On the other hand, it was established that captive breeding is possible, it reproduces well in unexploded anegadistic areas. CORZO and ÁVILA (2006), elaborated meat products 100% with chigüiro meat, and they could find that the nutritional content of this meat is higher compared to meats such as pork since its protein content is greater, in the same way, it was found that myoglobin concentration is lower providing a moderate red color. Ham is a product that has different presentations and types of processing, worldwide matured raw ham is known (Serrano in Colombia or Iberian pork in Spain), it is also processed as cooked smoked ham with bone, or boneless ham pressed and cooked (ICONTEC 1325 standard). REGINO (2007), conducted an investigation using rabbit meat (Oryctolagus cuniculus), in the elaboration of two types of smoked ham, thus demonstrating that meats such as rabbit, or in this case chigüiro, are a source of high quality protein, which by its same qualities could improve physicochemical characteristics. The pH is an indicator of acidity in meat products and the values of hams average 5.8 (KOTZEKIDOU and BLOUKAS, 1996). The color is determined by the coordinates "L* luminosity, a* scale of red and b* yellow; By measuring it, you can evaluate the pigmentation changes of a product during processing or storage (LLORET *et al*., 2016); DURAND P. 2002). One of the most valued attributes Texture is one of the main sensory attributes; In cooked ham it is affected by constituents such as connective tissue, moisture and emulsion structure, which modify sensory attributes. On the other hand, acceptance studies have been carried out. In other research work such as that carried out by OROZCO (2005) (cited by REGINO, 2007), he demonstrated the acceptance of consumers of products based on chigüiro. Also in a research work carried out by LÓPEZ *et al* (2014), they processed various sausages and smoked products, among them the chigüiro leg of the Orinoquia. (Hydrochoerus hydrochaeris) with a high degree of acceptance by panelists The production of chigüiro in captivity is practically nil, and the meat of this species is the result of indiscriminate hunting, becoming an exotic dish. The objective of this work was to evaluate the functional properties (CRA and pH), textural and color of a ham cooked with mixtures of chigüiro meat and pork.

2. MATERIAL AND METHODS

2.1. Sample preparation and storage

Chigüiro meat was purchased in the municipality of San Pelayo and pork in the municipality of Cerete, which were transported in cavas at a temperature of 4ºC to the facilities of the pilot plant of the University of Córdoba. The meat was boned, then it was passed to the cleaning that included the degreasing and the removal of all the nerves, ossicles, and cartilage, then the brine was prepared with the formulation shown in Table 1, once the meat was suitable massaged using a BERKEL brand tenderizer, the massaged meat was then mixed with the brine (5°C/20 min) using a JAVAR brand mixer, capacity 15 liters, the binder was incorporated last until a suitable texture was observed. The obtained mass was stored (4°C/24 h), after this time had elapsed, this dough was stuffed using a KRAMER brand manual stuffer, in synthetic casing based on 140 gauge polyamide, and introduced into a press mold for ham, the whole was subjected to scalding (average heating temperature 73°C), until reaching 70°C in the heart of the product, the mold was rethought, subjected to thermal shock, the molds were stored in a refrigeration cellar (5°C/24 h).

Table 1. Brine formula for the different mixtures.

Formulation based on meat (%)	
Water	20
Salt	2
Ham Phosphate	2,1
Sugar	0,05
Enhancer	0,05
Liquid smoke	0,05
Binder	4

The hams were demoulded, and cut in the experimental units, vacuum packed using a JAVAR vacuum chamber, and finally the samples were stored until analysis in refrigeration cellars 1 at 4°C.

2.2. Sample analysis

The samples were determined CRA water retention capacity, pH, color and texture. To determine the water retention capacity CRA, the compression method was used by the procedure followed by the technique described by GRAU and HAMM (1953) modified by BOAKYE and MITTAL (1993). On the other hand, for the color measurements of the L*, a* and b* coordinates, on the Cielab scale (LLORET *et al.*, 2016), were made using a Huntelab ColorFlex EZ Spectrophotometer colorimeter by making three different measurements on the sample. To evaluate the texture, a TXT-plus Instrument texturometer was used, where the texture values of a meat sample of dimensions 2x2x2 were obtained, the adjusted variables were: 40% compression rate, probe drop speed 1 mm/min, trigger force of 7.

And the following textural properties were considered: hardness, cohesiveness, elasticity and chewiness, 6 repetitions were performed for each simple (BARBIERI *et al*., 2016).

3. RESULTS AND DISCUSSION

Table 2 shows the results of the functional properties (pH and ARC), it is observed that there are no significant differences ($p>0.05$) between the control sample and the T1 treatment, while there are significant differences ($p<0.05$) between the control and the treatments T2 and T3, between these two treatments, all the samples reached a pH that is within the established limits.

Table 2. Functional properties of the samples studied.

Treatment	pH	CRA
Tc	6,276±0,05[a]	55,545±0,371 [a]
T_1	6,303±0,01[a]	53,156±0,333[b]
T_2	6,743±0,01[b]	54,776±0,158 [c]
T_3	6,493±0,03[c]	48,153±0,641[d]

There is a tendency to increase the pH by replacing pork with chigüiro, the effect of phosphates as a regulatory solution allowed maintaining pH values. In other research works such as that carried out by ALARCÓN *et al*. (2007) in hams cooked with PSE meat, the pH was between 5.87 and 6.20, well below this work, the levels found favor the production yield. Regarding the water retention capacity, CRA presented a different behavior despite the fact that the values are within the ranges. Table 3 shows that there are significant differences ($p<0.05$) between the control and all treatments, and significant differences were found between treatments T1, T2 and T3. ALARCÓN *et al*. (2007), found a variation of the CRA between 39 and 46, it was observed in this investigation that there is no trend in the relationship between pH and CRA. As for the TPA, there are 3 significant differences in the table ($p<0.05$) for the hardness between the control and the samples; There are also significant differences ($p<0.05$) between the samples, it should be noted that the sample that has less hardness is that of ham made with 100% chigüiro meat and the highest value with pork,

Table 3. Textural properties of pork and chigüiro ham samples.

	Average			
	Control	T_1	T_2	T_3
Hardness (N)	66,40±4,74[a]	47,89±3,58 [b]	36,29±4,41[c]	23,83±2,97[d]
Springiness	0,89±0,12[a]	0,88±0,13[a]	0,90±0,11[b]	0,91±0,03[b]
Cohesiveness	0,79±0,06[a]	0,82±0,014 [b]	0,85±0,01[c]	0,82±0,06[b]
(g)	46,75±2,44 [a]	37,36±3,23 [b]	30,801±4,74	17,840±0,91[d]

It is also seen that the hardness decreases as the percentage of chigüiro meat increases, which was corroborated in another part of the research related to sensory analysis.

Regarding elasticity, the values were very close to each other; however, there were significant differences (p <0.05) between the control and the T3 treatment, and there were no significant differences between the control and the T1 and T2 treatments, the quality of the muscle fiber is possibly a reason for these differences. On the other hand, in the cohesiveness it is observed that there are significant differences (p <0.05) between the control and the treatments T1, T2 and T3, while there are no significant differences (p> 0.05) between the T1 treatments, and T3, the control has a lower cohesiveness value, with T3 being higher, which would mean a more compact slice of ham. Likewise, regarding the chewableness, there is a tendency to decrease between samples with mixed pork and chigüiro meats, and that of 100% chigüiro, significant differences (p <0.05) were found between the control and the treatments, and each other. The relationship between hardness and chewiness is observed. GONZALEZ *et al*. (2009) reported a hardness of 51 N for the first day of storage of a cooked pork ham, the results are attributed to age, sex, type of feeding, KRZYWDZINSKA *et al*. (2016), found hardness values close to 51 N FRONTELA *et al*. (2006) established values for hardness between 8, 68 and 11.3 N in commercial samples of Spanish cooked hams, regarding chewable values are close to 388 g.cm very similar to the T2 treatment, in terms of elasticity, the values found were 1.1, close to those found in this investigation in the cohesiveness these values were lower (0.38 and 0.48). On the other hand KRZYWDZINSKA *et al*. (2016), found cohesivity values in cooked hams of 0.36. The results of TPA of these investigations differ from each other because they depend on raw materials, supplies and processing procedures. Table 4 shows the results obtained when analyzing the color parameters of the samples. It can be observed that in the gray scale there are significant differences (p <0.05) between the control and the treatments, in turn there are no significant differences (p <0.05) between treatments T1 and T2, but if There are significant differences between these and T3.

Table 4. The color in the pork and chigüiro ham samples.

Coordinate	Half			
	Control	T_1	T_2	T_3
*L**	60,48±1,300 [a]	52,15±1,872[b]	53,17±1,215[b]	43,93±4,253 [c]
*a**	9,43±1,119 [a]	11,46±0,764[b]	9,71±1,489[a]	11,86±0,699 [b]
*b**	9,55±0,167 [a]	9,23±1,009[b]	8,64±0,832[c]	9,17±0,453 [a]

A decrease in this parameter is noticeable as the percentage of chigüiro meat increases, one reason may be because in chigüiro meat the fat is darker and its content is greater. It can be observed, in another way, that in the red scale there are significant differences (p<0.05) between the control and treatments T1 and T3 and between T2 and the latter two. In this table it is observed that all samples containing chigüiro meat have a greater pigmentation than the control, since when reacting myoglobin with nitrite and by the action of heat producing nitrosylhemochrome, as chigüiro has a greater mobility than pig Myoglobin content is higher. As for the yellow coordinate, b*, significant differences (p<0.05) were found between the control and the T_1 and T_2 treatments, while there were no significant differences between the control and the T3 treatment, and observed significant differences (p<0.05) between treatments, the reasons for these differences are related to the difference in the type of feeding between a wild animal and a domestic animal. Authors such as GARCIA *et al.* (2000) and HUANG *et al.* (1997) found values for *L** near 55.94, with normal meat and 58.25 with PSE meat, it is observed that as the pH increases the value of *L**, in relation to the coordinate *a**, GARCIA *et al.* (2000), found a value of 9.85 in normal meat,

while WILSON *et al.* (1994), found a value of 11.6. As for the *b** coordinate, the same authors found similar results.

4. CONCLUSIONS

The meat of wild animals such as chigüiro or punch (Hydrochoerus isthmius), despite having functional properties to supply animals such as pig, have good qualities for the production of meat products, some measurable attributes such as hardness, chewiness Red scale color and tech-functional properties are within the quality parameters. Due to the type of food and growth conditions, the differences with pork are notorious, but in the same way, a combination of the two meats makes it possible to obtain cooked ham of good quality. It is recommended to repeat this type of research with chigüiro meat in captivity to adjust certain conditions such as genetics, animal welfare and food, it would be advisable to carry out bromatological studies to evaluate the lipid profile, considering polyunsaturated fatty acids and the poly peptide chain.

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