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Effect of storage time on physicochemical and textural properties of sausages covered with oxidized banana starch film with and without betalains

Efecto del tiempo de almacenamiento sobre las propiedades de textura y físico-químicas de salchichas recubiertas con películas de almidón de plátano oxidado con y sin betalainas

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In this study, two types of oxidized banana starch films (betalains and no betalains) were prepared and used as covering for sausages, then were refrigerated for 20 days at 4°C and 5% relative humidity (RH). The sausages covered were analyzed (covered with films without betalains = F1 and covered with films with betalains = F2) and they were compared with a control (without covering). The sausages were evaluated every 5 days by physicochemical analysis of color, size, weight loss, pH and quantification of betalains in the films. Textural analysis was performed on the sausages. The results indicated that films did not significantly alter the color or moisture loss during storage ($P > 0.05$), while the F2 films maintained the amount of thiobarbituric acid reactive substances in the sausages during storage ($P < 0.05$). Sausages covered with both types of film maintained their texture characteristics for longer compared to the control.

Keywords: color evaluation; betalains content; texture profile analysis; sausages

En el presente estudio se elaboraron dos tipos de películas de almidón oxidado de plátano (con y sin betalaínas) y se utilizaron como cubiertas en salchichas, las cuales se almacenaron por 20 días en condiciones de refrigeración (4°C y 5% HR). Se analizaron tres lotes de salchichas (sin cubierta = control; cubiertas con películas sin betalaínas = F1; cubiertas con películas con betalaínas = F2). Las salchichas se evaluaron cada cinco días mediante análisis fisicoquímicos de color, tamaño, pérdida de peso, pH y cuantificación del nivel de betalaínas en las películas. Se realizó un análisis de textura de las salchichas. Los resultados indicaron que las películas no alteraron significativamente el color o la pérdida de humedad durante el almacenamiento ($P > 0,05$), mientras que las películas F2 mantuvieron la cantidad de substancias reactivas de ácido tiobarbitúrico (TBARS) en las salchichas durante el almacenamiento ($P < 0,05$). Las salchichas cubiertas con ambos tipo de películas conservaron sus atributos de textura por mayor tiempo en comparación con las salchichas control.

Palabras clave: evaluación de color; contenido de betalaínas; análisis de perfil de textura; salchichas

Introduction

The food industry needs new packing materials that are inexpensive, safe for the environment and biodegradable, but that still offer the practical benefits of commercial packing materials based on polyethylene and polystyrene (Peelman et al., 2013). Naturally sourced materials such as starch are an alternative for use as films or coverings for fruits (Pan, Chen, & Lai, 2013) and vegetables (Das, Dutta, & Mahanta, 2013). For meat products, several different biodegradable materials have been proposed as coatings including polysaccharides such as chitosan (Kanatt, Rao, Chawla, & Sharma, 2013), cellulose (Luchansky & Call, 2004), carrageenans (Martins et al., 2012), alginate (Juck, Neetoo, & Chen, 2010), pectins (Kang et al., 2007) and their various formulations (Bierhalz, Da Silva, & Kieckbusch, 2012) as well as proteins including gelatin, corn proteins and collagen (Lungu & Johnson, 2005). One potential source of food packaging and coatings that has been less studied is film from starches. Several researchers have reported that banana starch (*Musa paradisiaca* L.) can be used for film or covering elaboration due to its high amylose content (the starch element responsible for the formation of film) (Pelissari, Andrade-Mahecha, Sobral, & Menegalli, 2013).

The limitations of native starch films, related to their hydrophilic nature, have been documented. For example, its high water content (Mali, Sakanaka, Yamashita, & Grossmann, 2005) and high rate of water vapor transmission (Krochta & Hernández-Izquierdo, 2008) have been studied. However, these disadvantages can be eliminated by chemical modification of the starch (Pérez-Gallardo et al., 2012). Oxidation is a chemical modification that changes the characteristics and functional properties of polymers (De Moura et al., 2011). Researchers have found that oxidized starch has appropriate properties for creating films (Kuakpetoon & Wang, 2001), with mechanical properties superior to those obtained from native starch (Palma-Rodríguez

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et al., 2012; Zamudio-Flores, Gutierrez-Meraz, & Bello-Pérez, 2011; Zamudio-Flores, Vargas-Torres, Pérez-González, Bosquez-Molina, & Bello-Pérez, 2006).

Meat products are among the most perishable food products on the commercial market (Cannarsi, Baiano, Marino, Sinigaglia, & Del Nobile, 2005). Their nutritional composition is related to their susceptibility to microbiological and physicochemical degradation (Siripatrawan & Noipha, 2012; Toldrá, 2010). Sausages as processed meat products are used in different and diverse cultures around the world (Savadkoohi, Hoogenkamp, Shamsi, & Farahnaky, 2014). There is scientific evidence that eating certain plant compounds in food (pigments, vitamins, fiber, phenols, etc.) can help reduce the risk of or even slow the progression of various illnesses, including several kinds of cancer and heart disease (Yahia, 2010). Researchers still do not know how exactly these compounds protect the body. Current thinking is that they act as antioxidants that neutralize free radicals, elements that can unleash different pathologies in the body (Bramley et al., 2000; Ferruzzi & Blakeslee, 2007). Betalains are plant compounds often used in the food industry as coloring for fruit juice, but they have not been fully exploited on the industrial level (Delgado-Vargas, Jiménez, & Paredes-López, 2000). Betalains can act as antioxidants, presumably due to their attraction or release of uncoupled electrons (free radicals) in the blood (Ravichandran et al., 2013).

Adding antioxidants and other bioactive compounds to starch films can alter their mechanical properties and change their possible application as a covering material (Mascheroni, Chalier, Gontard, & Gastaldi, 2010) due to potential interactions between bioactive compounds and macromolecules (proteins, lipids and carbohydrates), as was recently reported (Bordenave, Hamaker, & Ferruzzi, 2014). Betalains' antioxidant properties have been thoroughly studied, but their application as a means of improving the shelf life of meat products has not been explored. The goal of this study was to evaluate the effect of storage time on the physicochemical and textural properties of sausages covered with films added with and without betalains.

Materials and methods

Materials

Frankfurt turkey sausages (Dixie Farm) were a gift of BAFAR® (Instalaciones y Maquinaria INMAQ, S.A. DE C.V., Chihuahua, Mexico). The formulation of sausages consisted of 44% turkey meat, 43% water, 11% soybean oil, 1% sodium chloride, 0.4% sodium phosphate, 0.2% red pepper, 0.2% garlic powder, 0.02% ascorbic acid and 0.01% sodium nitrate. In this formulation, nutritional values were less than 25% total carbohydrates, 22% protein and 4% fat. The sausages were removed from their commercial packaging and randomly grouped in three 2-kg lots. Sausages in the first group were covered with film without betalains (F1) and the second group was covered with a film with betalains added (F2). The third group served as a control (sausages not covered with film). Lots were refrigerated (4°C/5% relative humidity (RH)) for 20 days, with researchers performing sampling every 5 days. The films were produced with glycerol (Sigma-Ultra brand, from Toluca, State of México, México) of 99% purity as a dispersant or plasticizer. For the different tests, sausages of each lot (depending of the test) were removed from the refrigerator and they were eliminated after the test. Each lot was evaluated for zero time and different storage times.

Production of the film using oxidized starch

For the film production, researchers used the method proposed by Zamudio-Flores et al. (2006) with some modifications. Two lots of film were produced (one with oxidized banana starch and the other oxidized banana starch with 5.17 mg/g betalains). The oxidized starch and glycerol were mixed with distilled water to make two 2-kg batches. The concentration of glycerol and starch in the solution was 2% and 4% (w/w), respectively. The filmogenic solution (also called the film-forming solution) was heated on a Corning plate (Model PC-620D, New York, NY, USA), rising from 25°C to 95°C. That temperature was maintained for 10 min while being stirred at a uniform rate (250 rpm). Then, the filmogenic solution was cooled to 30°C and an aqueous solution of betalains (extracted from beet juice) was added at a concentration of 0.01 g for every 120 mL of filmogenic solution. The solution was then stirred for another 15 min before being poured into sterile polystyrene Petri dishes (P100). The filmogenic solutions dried at room temperature (20 ± 5°C) for 72 h. Then, the films were removed from the Petri dishes and placed in desiccators with saturated saline solutions of NaBr (RH = 50%) for 48 h. The films were stored for at least 24 h in hermetically sealed bags (Ziploc®, Johnson & Sons, Inc., Racine, WI, USA).

The betalains extract came from fresh beets bought at a local market in Cuauhtemoc, in the State of Chihuahua, Mexico. The beets were washed and cut into 2-cm chunks, placed in a juice extractor (Túrmix, model Ext. hard use, México). The juice was filtered and put in a centrifuge (13,000 × *g*/15 min/4°C). To quantify the betalains, the extract was filtered through a nylon membrane with 0.2-mm pores (Millipore Corp., Bedford, MA, USA) and manually injected (20 mL) into a high-performance liquid chromatography (HPLC, Agilent, model 1200, Tokyo, Japan) system, composed of a degasser, a quaternary pump, a column oven with temperature control and UV-Vis diode array detection. The betalain content in the beet extract was 5.17 mg/g. The chemical nomenclature for the films was as follows: F1 = oxidized banana starch without betalain and F2 = oxidized banana starch with betalains.

Color evaluation

Researchers evaluated color on the control sausages and on those with different film formulations. A Minolta CR-300 (Minolta, Co., Ltd., Osaka, Japan) colorimeter, calibrated to standard white, was used. Colorimeter was used with a D65 illuminant and an observation angle of 10°. The film was removed from the sausages and readings were taken at three random points on the sausage surface. The sausage surface was homogeneous without pores or fractures. The readings were in the CIELAB system (L*, a*, b*). Three sausages were analyzed for each lot and three determinations of each sausage were carried out.

Weight determination

Researchers used a Scout Pro SP401 (Ohaus Co., USA) 400 g balance. The films were removed and the sausages were placed on the scale. Weight was measured at 5-day intervals from 0 to 20 days with a 0.01 g of precision. The control sausages received a preliminary measurement (when they were removed from the packaging) and the posterior evaluations. Measurements were taken three times for each group at each measurement period.

Evaluating moisture content

Five sausages were liquefied in an immersion blender (model Robot 180, Taurus S. A. de C.V, Spain). The moisture analysis was performed according to AOAC standard method (952.08). Researchers placed 5 g of the resulting mix in a watch glass, for constant weight, and placed the glass in an oven (1350 GM, VWR Scientific Inc., USA) at 105°C for 24 h. The glass was then removed and cooled in a desiccator (20 ± 3°C) and then weighed. The sample's moisture content was calculated as the difference between the wet weight and dry weight and is expressed as a percentage of moisture. The moisture percentage was used to correct the values of variables that are affected by dehydration.

pH determination

The sausages´ pH was determined using the method recently detailed by Kim et al. (2012). Researchers removed the sausages from their film and liquefied them with an immersion blender Taurus Robot 180 (Taurus, Spain). A 10 g sample was taken, combined with 90 mL of distilled water and measured with a potentiometer (Model HI 221, Hanna Instruments, Mauritania). The pH was checked every 3 days during a 21-day period. All measurements were taken in triplicate.

Measurement of thiobarbituric acid reactive substances levels

Thiobarbituric acid reactive substances (TBARS) were measured following the method proposed by Botsoglou et al. (1994). The sausages were liquefied with an immersion blender (Robot 180, Taurus, Spain), then 1.5 g of the puree was mixed with 20 mL of Millipore water and blended in an Ultra-Turrax (IKA®, T18 Basic) at 3500 × *g* for 10 s. Researchers added 5 mL of trichloroacetic acid 25%, stirring continually (2500 × *g*/ 4°C/ 15 min). The mix was put in a centrifuge (13,000 × *g*/15 min/4°C) and researchers obtained the supernatant liquid (≈3.5 mL), which was mixed with 1.5 mL of an aqueous solution of thiobarbituric acid at 0.6% (w/v). This was incubated at 70°C for 30 min. The reaction's absorbency was recorded at λ = 532 nm using a spectrophotometer Jenway (model 6505, USA). The quantitative data were obtained through a calibration curve plotted with 1, 1, 3, 3-tetraethoxypropane, as an external standard. The results were expressed in milligrams of malondialdehyde per gram of dry sample.

Quantifying betalains in F2 film as a function of time

The films were removed from the sausages, freeze-dried and pulverized in a mortar. The pulverized film was rehydrated with 4 mL of water and left to sit for 10 min. Then, the mix was blended with an Ultra-Turrax (IKA® T18 Basic) at 3500 × *g* for 10 s and placed in a centrifuge (13,000 × *g*/15 min /4°C). Researchers took 2 mL of the supernatant liquid and added 1 mL of ethanol, to separate the fibers. The mix was returned to a centrifuge. The supernatant liquid was filtered through a nylon membrane with a 0.2-mm pore size (Millipore Corp., Bedford, MA, USA) and manually injected (20 mL) into a HPLC system (Agilent, model 1200, Tokyo, Japan), composed of a degasser, a quaternary pump, a column oven with temperature control and UV-Vis diode array detection. The betalains were separated in a Zorbax Eclipse XDB-C18 column and kept at 25°C. Researchers then used a mobile phase isocratic system composed of water and methanol (70:30 w/v). During the mobile phase, the flow was 1 mL/min. The analysis was observed at λ = 538 nm. The betalain quantification was carried out using a calibration curve constructed with three independent series of betalain dilutions as an external standard. The measurement was taken every 5 days for 20 days. The results were expressed in milligrams of betalain per gram of dry sample

Texture profile analysis

Texture profile analysis (TPA) was performed using the double bite-size method using a texture analyzer (TAXT-Plus, Stable Micro Systems, Surrey, UK) at room temperature (20 ± 3°C) as was recently reported by Romero De Ávila, Cambero, Ordóñez, De La Hoz, and Herrero (2014) with slight modifications. The modifications consisted of using a cylindrical probe (P0.25SS, 0.25 in.), and the sausages were maintained at 57% RH (with a NaBr saline solution) at 20 ± 3°C for at least 60 min. Sausages covered with film (those lots with the F1 and F2 films) had their covering removed and were prepared in the same way as the control group. The equipment (with 35 kg load cell) was set up in the following way: pretest speed = 2 mm/s; post-test speed = 5 mm/s; maximum load = 2 kg, crosshead speed of test = 2 mm/s, distance = 8 mm; the auto-trigger feature was set at 5 g. Researchers evaluated variables such as hardness (N), elasticity (mm), cohesiveness (a-dimensional), gumminess (N), chewiness (N × mm) and adhesiveness (N × mm). Measurements were taken using a graphic of force versus time (as control variable). At least eight measurements were taken at every evaluation period (from day 0 to day 20, in 5-day intervals, considering four sausages for each lot evaluated in two points).

Statistical analysis

Researchers used a completely random design. An analysis of variance was used at a 5% significance level (α = 0.05). Results were obtained and normality test was verified using a Sigma-Plot statistical program, version 11.0 (Fox, Shotton, & Ulrich, 1995). When significant differences were found, researchers applied the Tukey range test for the comparison of means (Walpole, Myers, & Myers, 1999).

Results and discussion

Color evaluation

Color evaluations of the sausages (both the control and those covered with film) at different storage times are shown in Table 1. The sausages analyzed were eliminated in each test. Color changes (Factor L*, coordinates a* and b*) in the control sausages can be seen in the evaluation performed on the 5th day (T5), indicating that biochemical changes were occurring during storage. Control sausage showed a decrease in the L* value with the storage time, but those covered presented a slight decrease that was not significantly after 15 days for sausage covered without betalains and 10 days with betalains. The a* value was higher with the storage time in control sausage, a slight increase was found in the sample covered without betalains and no change with the storage time was determined in the sausage covered with betalains. A slight decrease in the b* value during storage of the sausage was found, but those covered with and without betalains did not change after 10 days. The L* value could be the most important because it shows the sausage losing its white tone and becoming darker, which could affect its

Table 1. Effect of storage time on color of sausages covered with films.

Tabla 1. Efecto del tiempo de almacenamiento en el color de salchichas cubiertas con películas.

Storage time (days)	Treatment	L*	a*	b*
T_0	Control	52.11 ± 0.19^{a}	20.41 ± 0.17^{a}	10.80 ± 0.15^{a}
T_5		45.16 ± 0.21^{d}	21.15 ± 0.35^{b}	10.55 ± 0.10^{b}
T_{10}		43.11 ± 1.13^{e}	22.87 ± 0.46^{c}	$10.30 \pm 0.49^{a,b}$
T_{15}		40.15 ± 1.00^{f}	23.10 ± 0.21^{d}	10.08 ± 0.12^{c}
T_{20}		38.17 ± 0.85^{g}	23.05 ± 0.11^{d}	$10.01 \pm 0.08^{c,e}$
T_0	F1	50.15 ± 0.22^{b}	19.95 ± 0.16^{a}	10.19 ± 0.14^{c}
T_5		47.10 ± 0.35^{c}	20.10 ± 0.11^{a}	10.13 ± 0.16^{c}
T_{10}		45.36 ± 0.93^{d}	$20.97 \pm 0.54^{a,b}$	9.15 ± 0.41^{d}
T_{15}		43.40 ± 0.18^{e}	21.08 ± 0.27^{b}	9.10 ± 0.35^{d}
T_{20}		42.17 ± 0.21^{e}	21.85 ± 0.15^{d}	9.05 ± 0.17^{d}
T_0	F2	50.18 ± 0.33^{b}	20.54 ± 0.30^{a}	9.77 ± 0.18^{e}
T_5		48.23 ± 0.80^{c}	20.33 ± 0.27^{a}	$9.45 \pm 0.17^{d,e}$
T_{10}		45.04 ± 0.94^{d}	20.14 ± 0.37^{a}	8.26 ± 0.29^{f}
T_{15}		44.10 ± 0.21^{d}	20.20 ± 0.35^{a}	8.18 ± 0.20^{f}
T_{20}		$43.95 \pm 0.78^{d,e}$	20.35 ± 0.28^{a}	8.08 ± 0.15^{f}

Notes: F1 = film oxidized banana starch without betalain; F2 = film oxidized banana starch with betalain; control was the sausage without film. The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($P < 0.05$).

Nota: F1 = película de almidón oxidado de plátano sin betalaína; F2 = película de almidón oxidado de plátano con betalaína; control fue la salchicha sin cobertura. Los valores representan la media y la desviación estándar tres muestras independientes. Diferentes letras en una misma columna indican diferencias significativas ($P < 0,05$).

Table 2. Effect of storage time on weight loss and moisture content of sausages covered with films.

Tabla 2. Efecto del tiempo de almacenamiento en la pérdida de peso y la humedad de salchichas cubiertas con películas.

Storage time (days)	Treatment*	Weight loss (%)	Moisture (%)
T_0	Control	0.00	66.88 ± 0.07^{a}
T_5		22.71 ± 0.90^{a}	58.54 ± 0.25^{b}
T_{10}		42.18 ± 0.73^{c}	44.40 ± 0.44^{d}
T_{15}		50.27 ± 0.41^{d}	35.20 ± 0.75^{g}
T_{20}		55.38 ± 1.20^{e}	29.97 ± 0.44^{i}
T_0	F1	0.00	65.18 ± 0.17^{a}
T_5		25.99 ± 1.07^{b}	55.33 ± 0.25^{c}
T_{10}		43.85 ± 0.73^{c}	41.98 ± 0.76^{f}
T_{15}		51.15 ± 0.73^{d}	33.28 ± 0.56^{h}
T_{20}		55.06 ± 0.25^{e}	28.09 ± 0.21^{j}
T_0	F2	0.00	67.08 ± 0.37^{a}
T_5		25.07 ± 1.15^{b}	55.87 ± 0.52^{c}
T_{10}		42.18 ± 0.95^{c}	43.96 ± 0.50^{f}
T_{15}		49.96 ± 0.73^{d}	34.20 ± 0.45^{h}
T_{20}		53.46 ± 1.15^{e}	27.28 ± 0.06^{k}

Notes: F1 = film oxidized banana starch without betalain; F2 = film oxidized banana starch with betalain; control was the sausage without film. The values represent the mean and standard deviation for three independent samples. Different letters in the same column mean significant differences ($P < 0.05$).

Nota: Los valores representan la media y la desviación estándar tres muestras independientes. Diferentes letras en una misma columna indican diferencias significativas ($P < 0,05$). F1 = película de almidón oxidado de plátano sin betalaína; F2 = película de almidón oxidado de plátano con betalaína; control fue la salchicha sin cobertura.

acceptance among consumers. A falling L* value could be related to lipid oxidation and the production of malondialdehyde, which was higher than the meat's dark color (Park, Rhee, Kim, & Rhee, 1993). Furthermore, some brown coloring could be due to oxidation from light and oxygen, as the light separates nitric oxide from the hemo group of hemoglobin and this, in turn, is oxidized by air (Akesowan, 2008; Gregg, Claus, Hackney, & Marriot, 1993). The starch film reduced oxidation reactions to covered sausages, acting as a barrier to light and oxygen. In general, the sausages covered with the two types of film (with and without betalains) did not show appreciable differences, which indicate that the light and oxygen transmission through the film did not change with the addition of the betalains.

Weight loss and moisture content

In general, all the sausage groups lost weight and moisture, so the differences between the groups were not significant (Table 2). This suggests that during storage, the starch films had a high degree of water loss due to the hydrophilic nature of starch. The weight reduction and humidity were due to water loss (Liu, Tsau, Lin, Jan, & Tan, 2009).

Evaluation of pH

The pH values for control sausages and those with different film formulations can be seen in Figure 1. The covered sausages maintained the same pH value during the evaluation period, with no significant changes in the group with betalains. However, for the control sausages, researchers observed a progressive decrease in pH values (from 6.0 to 4.5) as the evaluation period continued. The starch films (both those with and without betalains) helped maintain the sausages' pH roughly equally until the 18th day of storage. After that, researchers saw a decrease in pH for the group covered with film without betalains. According to Triki, Herrero, Jiménez-Colmenero, and Ruiz-Capillas (2013), the pH changes during storage could be related to microbial growth. Several studies have associated a lower value in pH with an increase in lactic acid, specifically caused

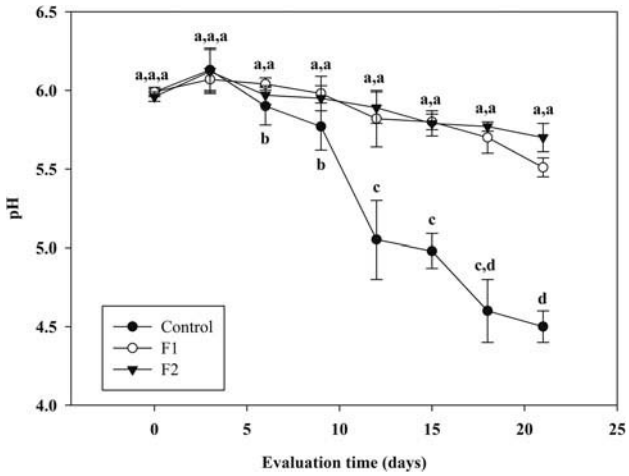

Figure 1. pH evaluation in control sausages and with different films formulations. Mean of five replicates ± standard error bars. Same lowercase letters in the mean values are not significantly different ($P > 0.05$). F1 = sausages with oxidized banana starch films without betalain; F2 = sausages with oxidized banana starch films with betalain.

Figura 1. Evaluación de pH en las salchichas control y con diferentes formulaciones de películas. Media aritmética de cinco repeticiones ± barras error estándar. Letras minúsculas iguales en los valores promedio no son significativamente diferentes ($P > 0,05$). F1 = salchichas cubiertas con películas de almidón oxidado de plátano sin betalaína; F2 = salchichas cubiertas con películas de almidón oxidado de plátano con betalaína.

by the bacteria genus *Lactobacillus*, which is frequently present in fresh meat (Holmer, McKeith, & Killefer, 2008; Salazar, García, & Selgas, 2009). The protection of the sausages with film slowed the development of lactic acid bacteria and was longer lasting in the betalain-enriched film, which could have been acting as an antimicrobial shield (Čanadanović-Brunet et al., 2011; Vulić et al., 2013).

Betalain quantification

The initial betalain content when the films were applied was 5.17 mg/g, and then it shrank by 34.42% during refrigeration (Figure 2). Degradation temperature is around 40°C. Shrinkage reached its peak at 10 days in storage and changed only slightly with additional storage time. This lower value could be due to the following: (1) the betalains reacted with the sausages' free radicals produced by lipid oxidation, (2) the betalains migrated from the film to the sausage and/or (3) betalains were exposed to light and air (Azeredo, 2009). In order for betalains to have a beneficial effect on the human body, it is recommended that the food contain ≈39 µg/g (Kanner, Harel, & Granit, 2001). Even after 10 days of storage, the starch films still have roughly that amount of betalain and can benefit humans if the film is eaten with the sausage. One advantage betalains have over other antioxidants is that they can be absorbed by the body (Butera et al., 2002; Tesoriere, Butera, Allegra, Fazzari, & Livrea, 2005), despite some loss that may occur during the digestive process. Potential betalain absorption varies from 40% to 78% (Tesoriere, Fazzari, Angileri, Gentile, & Livrea, 2008), but once absorbed, the betalains play a protective role in the human body (Lee, Hyun, Li, & Moon, 2001; Sreekanth et al., 2007; Zou et al., 2005). Betalains are reported to boost the immune system (Siriwardhana, Shahidi, & Jeon, 2006), protect the liver (Galati et al., 2005), and shield the brain from harmful elements (Kim et al., 2006).

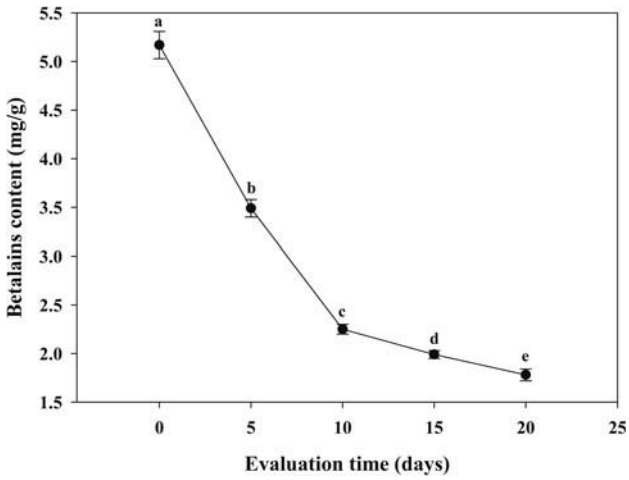

Figure 2. Betalains content (mg/g) in oxidized banana starch films with betalains (F2) during storage time of sausages at 4°C. Mean of five replicates ± standard error bars. Same lowercase letters in the mean values are not significantly different ($P > 0.05$).

Figura 2. Contenido de betalaínas (mg/g) en películas de almidón oxidado de plátano con betalaínas (F2) durante el almacenamiento de las salchichas a 4 °C. Media aritmética de cinco repeticiones ± barras error estándar. Letras minúsculas iguales en los valores promedio no son significativamente diferentes ($P > 0{,}05$).

Content of thiobarbituric acid reactive substances

In the control group, TBARS were higher slightly up until the 10th day of storage (Figure 3) and then increased exponentially. TBARS content was higher in sausages covered with a film without betalains during observation at the 20th day, suggesting that, even without betalains, the film succeeded in slowing the growth of these reactive substances. However, the sausages covered with film with betalains showed no increase in TBARS after day 5. This result could be attributed to the antioxidant effects of betalains. Several studies have shown that

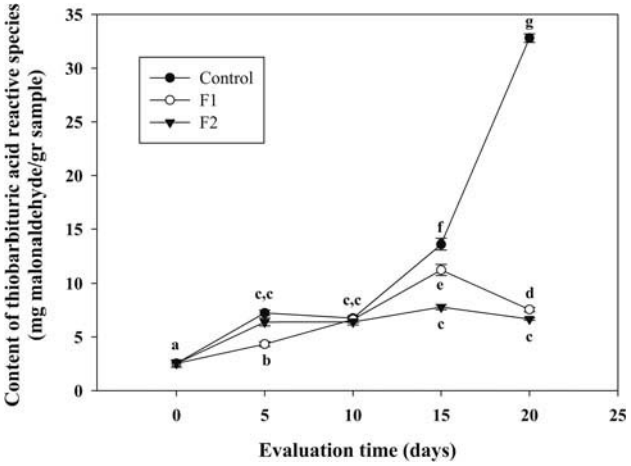

Figure 3. Content of thiobarbituric acid reactive species (mg malonaldehyde/g sample) in control sausages and with different films formulations. Mean of two replicates ± standard error bars. Same lowercase letters in the mean values are not significantly different ($P > 0.05$). F1 = sausages with oxidized banana starch films without betalain; F2 = sausages with oxidized banana starch films with betalain.

Figura 3. Contenido de especies reactivas de ácido tiobarbitúrico (mg malonaldehído/gr muestra) en salchichas control y con diferentes formulaciones de películas. Media aritmética de dos repeticiones ± barra de error estándar. Letras minúsculas iguales en los valores promedio no son significativamente diferentes ($P > 0{,}05$). F1 = salchichas cubiertas con películas de almidón oxidado de plátano sin betalaína; F2 = salchichas cubiertas con películas de almidón oxidado de plátano con betalaína.

Table 3. Texture profile analysis in control, sausages with oxidized banana starch films without betalain (F1) and with betalain (F2) at different storage times.

Tabla 3. Análisis del perfil de textura en salchichas control y con películas de almidón oxidado con betalaína (F1) y sin betalaína (F2) a diferentes tiempos de almacenamiento.

Treatment/evaluation time	Hardness (N)	Elasticity (mm)	Cohesiveness	Gumminess (N)	Chewiness (N × mm)	Adhesiveness (N × mm)
Control						
T_0	7.61 ± 0.30^{a}	1.61 ± 0.07^{a}	0.60 ± 0.04^{c}	4.75 ± 0.18^{a}	1.90 ± 0.60^{b}	−1.01 ± 0.21^{a}
T_5	10.24 ± 0.41^{b}	1.58 ± 0.16^{a}	0.72 ± 0.07^{d}	4.51 ± 0.53^{a}	11.64 ± 1.35^{c}	−0.84 ± 0.11^{b}
T_{10}	15.33 ± 0.38^{c}	1.50 ± 0.23^{a}	0.88 ± 0.10^{e}	8.09 ± 1.58^{b}	20.23 ± 4.11^{d}	−2.66 ± 0.20^{c}
T_{15}	20.15 ± 0.25^{d}	1.63 ± 0.15^{b}	0.90 ± 0.11^{f}	8.15 ± 1.11^{b}	29.56 ± 3.10^{e}	−3.55 ± 0.10^{d}
T_{20}	26.12 ± 0.33^{e}	1.60 ± 0.20^{a,b}	0.97 ± 0.06^{g}	9.27 ± 0.69^{b}	40.54 ± 2.08^{f}	−4.60 ± 0.25^{e}
F1						
T_0	7.90 ± 0.71^{a}	1.32 ± 0.06^{a}	0.41 ± 0.02^{a}	4.87 ± 0.29^{a}	4.28 ± 0.44^{a}	0.90 ± 0.20^{a}
T_5	8.10 ± 0.50^{a}	1.40 ± 0.07^{b}	0.48 ± 0.03^{b}	5.57 ± 0.20^{a,b}	5.44 ± 0.61^{b}	−1.16 ± 0.26^{b}
T_{10}	9.13 ± 0.74^{a,b}	1.25 ± 0.06^{a}	0.54 ± 0.04^{c}	5.79 ± 1.58^{a,b}	6.16 ± 0.81^{c}	−3.47 ± 0.74^{c}
T_{15}	10.25 ± 1.55^{b}	1.13 ± 0.08^{c}	0.56 ± 0.06^{c}	6.80 ± 0.65^{c}	6.50 ± 1.09^{c}	−3.55 ± 0.68^{c}
T_{20}	11.31 ± 0.68^{b}	1.21 ± 0.11^{c}	0.60 ± 0.09^{c}	7.13 ± 0.77^{c}	8.21 ± 0.95^{d}	−4.00 ± 0.35^{d}
F2						
T_0	7.62 ± 0.35^{a}	1.44 ± 0.30^{a}	0.45 ± 0.03^{a}	4.77 ± 0.20^{a}	4.94 ± 0.42^{a}	−1.24 ± 0.13^{a}
T_5	8.91 ± 0.43^{a}	1.35 ± 0.40^{a,b}	0.45 ± 0.03^{a}	5.37 ± 0.29^{a}	5.41 ± 0.60^{a}	−1.07 ± 0.16^{a}
T_{10}	9.80 ± 1.81^{a,b}	1.23 ± 0.15^{b}	0.49 ± 0.03^{a}	5.57 ± 1.86^{a}	5.91 ± 1.80^{a,b}	−1.75 ± 0.37^{a}
T_{15}	10.75 ± 1.05^{b}	1.33 ± 0.15^{b}	0.51 ± 0.09^{a}	6.77 ± 0.50^{b}	7.29 ± 1.12^{b}	−2.10 ± 0.35^{a}
T_{20}	11.21 ± 0.58^{b}	1.38 ± 0.21^{b}	0.52 ± 0.10^{a}	7.20 ± 0.57^{b}	8.04 ± 0.51^{b}	−2.72 ± 0.50^{a}

Notes: The values represent the mean and standard deviation for eight independent samples. Different letters in the same column are significant differences ($P < 0.05$).

Nota: Los valores representan la media y la desviación estándar ocho muestras independientes. Diferentes letras en una misma columna indican diferencias significativas ($P < 0,05$).

betalains have a high antioxidant effect, almost double the effect found in extracts from apples, pears, tomatoes, bananas, white grapes and oranges (Butera et al., 2002; Wang, Cao, & Prior, 1996). According to studies, betalains stabilize the dislocation of uncoupled electrons – either through attraction or through release of electrons – within their aromatic ring.

Texture profile analysis

The control group showed significant differences in variables such as hardness, cohesiveness, chewiness, and adhesiveness during the evaluation period, without changes in gumminess and elasticity during the measurements taken at 10 and 15 days, respectively (T_{10} and T_{15}) (Table 3). Compared to the control, the sausages covered in film with betalains (F2) maintained their hardness, cohesiveness chewiness and adhesiveness for longer time (Table 3). Sausage covered with film showed a higher value in hardness with the storage time without change after 10 days. No difference was found in the hardness of the sausage covered with or without betalains. These results suggest that films could be a way to protect the positive attributes and texture of meat products. Some reports suggest that these changes are due to biochemical processes during the meat's aging more than weigh loss (Jiménez-Colmenero et al., 2010).

Sausages covered with banana starch film without betalains (F1) had significant changes ($P < 0.05$) in gumminess and chewiness up to 15 days (T_{15}), just as was observed in sausages covered with banana starch with betalains (F2). However, researchers did not see significant changes in the later evaluations ($P > 0.05$) in the categories of elasticity, cohesiveness and adhesiveness. The last category, adhesiveness, registered negative values in all the evaluations, dropping in value further as the storage time increased. The decrease was greater in the control sausages and in those with a film without betalains (F1). This decrease is related to the presence of carbohydrates, such as starch, which retain moisture in processed meats (Feng et al., 2013; Grossi, Søltoft-Jensen, Knudsen, Christensen, & Orlien, 2012).

Conclusions

The starch films did not significantly alter the color, pH or loss of moisture during storage. The addition of betalains to the starch film maintained the amount of TBARS in the sausages during storage. Sausages covered with both types of film (with and without betalains) maintained their texture characteristics (hardness, adhesiveness and cohesiveness) for longer time compared to the control (no covering).

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