International Food Research Journal 22(1): 318-323 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Emulsified cooked sausages enriched with flour from *ora-pro-nobis* leaves (*Pereskia aculeata* Miller)

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Article history

Received: 13 March 2014
Received in revised form: 16 June 2014
Accepted:17 June 2014

Keywords

Emulsified meat products
Unconventional food plants
Nutritional quality

Abstract

The ora-pro-nobis (*Pereskia aculeata* Miller) is a plant of tropical origin, belonging to the family of Cactaceae. It is a perennial climbing plant, with too many thorns on its branches, fleshy leaves and presence of mucilage. Once its leaves have high protein content, it is so-called "meat of the poor". Furthermore, the leaves are also rich in fiber and minerals, especially iron, calcium, magnesium and manganese. In this study, a flour from ora-pro-nobis leaves was produced and used in sausage formulations at concentrations of 1 and 2%. The flour was characterized for its chemical composition, color and pH. The flour from ora-pro-nobis leaves presented a high content of fiber (11.13%±0.05) and protein (40.68%±1.61), thus it can be considered as an excellent source to nutritionally enhance the products in which it is added. The sausages were evaluated by both physicochemical (chemical composition, color and texture) and sensory analyses. Although it has caused important changes in color and texture of the sausages, these changes have not depreciated the sensory acceptance. Therefore, it can be concluded that the flour from ora-pro-nobis leaves can be used at a level of 2% to increase the nutritional quality of sausages.

Introduction

Consumers are increasingly giving more importance to aspects that improve their quality of life. Diet is not the only factor that affects the well-being and health, but it is one of the most important. The goal is to have a balanced diet with healthy foods and sensory pleasing (Campagnol *et al*., 2011).

A healthy diet should contain a variety of foods, with appropriate types and quantities for the stages of the life course. Fruits and vegetables are rich in vitamins, minerals and fiber and should be present in the daily meals. It is recommended to consume daily at least 400 g of fruit and vegetables and increase consumption of fiber-rich foods (WHO, 2013). The main basis for this recommendation is because these foods have nutrients with significant effects on the general health of individuals and, more specifically, in the prevention of chronic non-communicable diseases such as obesity, type 2 diabetes, cardiovascular disease and certain cancers. Epidemiologic data have shown that individuals with a high fruit and vegetables intake are at a lower risk of several types of cancers, cardiovascular diseases (CVD), and stroke (Riboli and Norat, 2003) than people with a low fruit and vegetables intake. The low intake of fruits and vegetables is responsible for about 19 % cases of gastrointestinal cancer and 31% cases of ischemic heart disease, which has caused death of 2.7 million people per year worldwide (WHO, 2013).

The low consumption of fruits and vegetables by the world's population may be related to the price of the food, not easily accessible to everyone. Unconventional food plants arise as a cheaper and easily accessible solution. These plants are known as "lawn" or "scrub" and as these names suggest, are found in vacant, yards and garden soils, being easy to grow. However, population in general knows little about them and cannot imagine the high nutritional value of these plants.

Although many of these plants have previously been used as food, today they fell into disuse. Even those people of rural origin no longer know which plants have nutritional potential, and the lack of knowledge about which plants can be used and how to prepare are the main reasons for the lack of use in human food (Kinupp and Barros, 2004).

The ora-pro-nóbis (*Pereskia aculeata* Miller) is a tropical plant, belonging to the family of *Cactaceae* (Takeiti *et al*., 2009). It is a perennial climbing plant, with too many thorny branches, fleshy leaves and presence of mucilage (Mercê *et al.* 2001). Their leaves have high protein content, thus the ora-pro-nóbis is so-called "meat of the poor". Furthermore, their leaves are also rich in fiber and minerals,

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especially iron, calcium, magnesium and manganese (Mercê *et al*. 2001; Takeiti *et al*., 2009). Due to its high nutritional value, this plant is an alternative for enriching and improving the quality of food, and can be used both as a food supplement for human consumption, as in animal feed.

The tradition of consuming such unconventional plant widely used by previous generations, whose consumption was common and varied, can be resumed if new forms of exploitation are investigated. So far, the addition of ora-pro-nobis in meat products has not been studied. Sausage is an emulsified meat product widely available and accepted by consumers due to its sensory quality, low cost, practicality and convenience. Such characteristics make it a potential product for the use of ora-pro-nobis. This approach is a way to make this meat product healthier, as well as to promote consumption of this type of highly nutritious plant. Based on this, the objective of this study was to produce flour from ora-pro-nobis (*Pereskia aculeata* Miller) leaves and to use it in sausage formulations.

Material and Methods

Manufacturing process of ora-pro-nobis flour

Ora-pro-nobis leaves were purchased in commercial establishments in the city of Uberaba, MG, Brazil. The leaves were rinsed in fresh water and cleaned with a 120 ppm sodium hypochlorite solution for 15 minutes. Subsequently, the leaves were placed in an air-circulating oven (Pardal 60P3) at 60°C for about 24 hours. After drying, the leaves were ground in thermostated knife mill (Marconi MA 345/T) to obtain fine flour. Then, the flour was vacuum-packed and stored at 4°C.

Physicochemical characterization of ora-pro-nobis flour

The moisture content was determined by drying in an oven at 105ºC ± 2ºC; the nitrogen content was determined by the Kjeldahl method and protein content estimated by multiplying the nitrogen content by 5.75; the lipid content was determined by Soxhlet method using petroleum ether; the ash was determined by incineration in a muffle furnace at 550°C; and crude fiber was determined gravimetrically after acidic digestion (AOAC, 2005). The determinations of moisture content, protein, lipids, ash, and crude fiber were performed in triplicate. Color determination was carried out in quintuplicate using Minolta colorimeter CR-400 (Konica Minolta Sensing Inc., Japan) according to CIE $L^*a^*b^*$ system, using spectral reflectance included as a calibration mode, illuminant D65, and observation angle of 10 °. The L^* (lightness), a^* (red intensity), and b^* (yellow color intensity) values were determined. The pH was determined in triplicate by homogenizing the sample with 10 g distilled water (1:10 sample / water). The homogenate was subjected to pH readings (pH meter DM 22, Digimed, São Paulo, Brazil) with time for stable reading after immersion of 5 minutes.

Formulation and manufacturing process of sausages containing ora-pro-nobis flour

Three independent replicates of each treatment were made. Three treatments were carried out to evaluate the effect of different levels of ora-pro-nobis flour on the physicochemical and sensory properties of sausages. The sausages formulations were prepared with pork (35%), beef (50%) and pork back fat (15%). The following ingredients were added in relation to the meat mixture: sodium chloride (2.0%), carrageenan (0.25%), monosodium glutamate (0.15%), sodium nitrite (0.015%), sodium tripolyphosphate (0.2%), sodium erythorbate (0.05%), garlic powder (0.2%), black pepper (0.1%), Jamaica pepper (0.1%) and ice (10%). A control formulation with no addition of flour ora-pro-nobis was prepared, and 1 and 2% ora-pro-nobis flour in relation to the meat mixture were used for both Treatments T1 and T2, respectively. For the preparation of sausages, beef and pork were homogenized with sodium chloride and sodium tripolyphosphate in a cutter (Model KJ20, Jamar, Brazil) to extract the myofibrillar proteins. When the temperature reached 8°C, the other ingredients and pork back fat were slowly added, followed by comminution until complete homogenization. During comminution, the temperature of the batter did not exceed 15°C. The meat mixture was stuffed (Model EJV15, Jamar, Brazil) in cellulose casings (Viskase, Brazil) with 20 mm diameter. The sausages were cooked in water bath in accordance with the following cooking cycle: 60°C for 30 minutes, 70°C for 30 minutes and 80°C until reaching the final core temperature of 72ºC. After cooking, the samples were immediately cooled in an ice bath, the casings were removed and the sausages were vacuum packed (200 Selovac Sealer, Selovac, Brazil), and stored under refrigeration (4°C) until the time of the physicochemical and sensory analysis.

Physicochemical analyses of the sausages containing ora-pro-nobis flour

The moisture content was determined by drying in an oven at 105ºC ± 2ºC; the nitrogen content was determined by the Kjeldahl method and protein content estimated by multiplying the nitrogen content by 6.25; lipids content was determined by

Soxhlet method using petroleum ether; and ash was determined by incineration in a muffle furnace at 550°C until constant weight (AOAC, 2005). The determinations of moisture content, protein, lipid and ash were performed in triplicate.

Color determination was carried out using Minolta colorimeter CR-400 (Konica Minolta Sensing Inc., Japan) according to CIE $L^*a^*b^*$ system, using spectral reflectance included as a calibration mode, illuminant D65, and observation angle of 10°. The L^* (lightness), a^* (red intensity), and b^* (yellow color intensity) values were determined in five pieces per treatment, at three different points in the central part for each sample.

The overall color change (ΔE) was determined using the following Equation (Nsonzi and Ramaswamy, 1998). A larger ΔE value denotes greater color change from the control sample.

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$$

To determine the water binding capacity fresh meat batters (22–24 g) were filled in eight containers (diameter: 3.5 cm, height: 5 cm). Containers were sealed and their weight recorded. Containers were then heated in boiling water for 45 min to a uniform temperature (~ 98°C) to induce gel formation in the batter following the procedure suggested by Honikel (1982). Samples were finally cooled and removed from the containers to again record their weight. The water loss percentage, an expression of the water holding capacity of the heated batter, was then determined as:

$$\text{Water loss (\%)} = ((\text{Minitial} - \text{Mend})/\text{Minitial}) \times 100$$

with Minitial being the weight of the sample prior to heating and Mend after heating.

The texture profile measurements were performed using a texture analyzer TA-TX2 (Stable Micro Systems Ltd. England) with a 10 kg load cell. Five pieces were used per treatment. Each sample was cut into 2 cm cylinders and axially compressed in two consecutive cycles of 20% compression with a probe 30 mm in diameter, moving at a constant speed of 1 mm / s. Data collection and construction of TPA curves were performed by Texture Expert program, version 1.11 (Stable Micro Systems Ltd..). The parameters hardness, springiness, cohesiveness and chewiness were determined. Hardness was defined as the peak force during the first compression cycle. Springiness was defined as a ratio of the time between the start of the second area and the second peak, and the time between the start of the first area and the first peak. Cohesiveness was calculated as the ratio between the areas under second and first peak. Chewiness was obtained by multiplying hardness x cohesiveness x springiness.

Table 1. Physicochemical characteristics of ora-pro-nobis flour (*Pereskia aculeata* Miller)

Components	Content (%)
Moisture	9.62±0.04*
Lipids	5.23±0.14
Protein	40.68±1.61
Ash	15.46±0.06
Crude Fiber	11.13±0.05
L*	34.37±0.57
a*	-0.89±0.10
b*	16.47±0.18
pH	6.92±0.01

* Mean ± standard deviation

Consumer study

A sensorial acceptance test was performed using a non-structured nine-point hedonic scale. The color, aroma, flavor, texture, and overall acceptance of the samples were assessed via 100 untrained sausage consumers (Meilgaard *et al.*, 1999), with 53% being female and 47% male, ranging in age from 18- to 50-years old. Consumers were recruited among students, faculty and staff of the Federal Institute of Triangulo Mineiro. The sausages were cooked in water over low heat for 5 minutes, cut into 2 cm long pieces, and served with crackers and water at room temperature. The samples were served to consumers in monadic form following a balanced design as described by Macfie *et al.* (1989). The sensory acceptability index was calculated by dividing the mean score for overall acceptance, by the maximum score of hedonic scale (9.0) and multiplying the result by 100.

Statistical analysis

Data were evaluated using analysis of variance (ANOVA) and means were compared by Tukey's test, at a significance level of 5% ($p \leq 0.05$), using the statistical package SPSS (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The physicochemical characterization of ora-pro-nobis flour is shown in Table 1.

The ora-pro-nobis flour showed a content of almost twice as higher the protein found in beef, pork and poultry (Honikel, 2009). The high ash content indicates that the flour can be considered an important source of essential minerals, once studies

Table 2. Chemical composition of sausages made with addition of ora-pro-nobis flour (*Pereskia aculeata* Miller)

	Moisture (%)	Lipids (%)	Protein (%)	Ash (%)
Control	55.80±1.52[a]	15.87±0.68[a]	17.75±1.06[b]	3.26±0.14[a]
T1	52.74±2.06[a]	16.07±0.42[a]	19.09±1.08[ab]	3.61±0.08[a]
T2	53.77±1.78[a]	16.83±0.64[a]	20.06±0.94[a]	3.68±0.03[a]

Control: 0% ora pro nobis-flour; T1: 1% ora pro nobis-flour, T2: 2% ora-pro-nobis flour.*Values represent the mean (± standard deviation). Averages followed by the same letter in the same column are not significantly different (p> 0.05) by Tukey's test.

Table 3. Values for instrumental color of sausages made with addition of ora-pro-nobis flour (*Pereskia aculeata* Miller)

	L*	a*	b*	ΔE
Control	49.82±0.42[a]	22.23±0.14[a]	12.84±0.19[c]	-
T1	46.78±0.44[b]	13.68±0.24[b]	13.77±0.31[b]	9,12
T2	46.91±0.41[b]	10.18±1.02[c]	15.33±0.18[a]	12,64

Control: 0% ora pro nobis-flour; T1: 1% ora pro nobis-flour, T2: 2% ora-pro-nobis flour. * Values represent the mean (± standard deviation). Averages followed by the same letter in the same column are not significantly different (p> 0.05) by Tukey's test.

have shown that ora-pro-nobis leaves have a high content of iron, calcium, magnesium and manganese (Wang *et al.* 1996, Mercê *et al*., 2001; Takeiti *et al*., 2009). The flour also presented a high-fiber, nutrient that this is extremely important for health, because its consumption is related to the prevention of various diseases like diabetes, cardiovascular disease, obesity, and some cancers (Jenkins *et al*., 2004; Mann and Cummings, 2009). These results demonstrate that the ora-pro-nobis flour can be considered an excellent way to increase the nutritional value of the products to which it is added. The instrumental color measurements (L^*, a^*, and b^*) indicated that ora-pro-nobis flour have a dark green color. The pH values of ora-pro-nobis flour evidenced that it can be applied in emulsified meat products, since its pH value is far from the isoelectric point of the meat protein (Goli *et al*., 2014).

The chemical composition of sausages made with the addition of now-pro-nobis flour is shown in Table 2. No significant difference was observed in moisture, lipid, and ash contents for all treatments. Once the ora-pro-nobis flour has a high protein content (Table 1), its addition at a level of 2% caused a significant increase in protein content in treatment T2 in comparison to the control, thus improving the nutritional quality of the product.

Table 4. Texture profile and water binding capacity of sausages made with addition of ora-pro-nobis flour (*Pereskia aculeata* Miller)

	Control	T1	T2
Hardness (N)	15.93±0.41[a]	9.68±0.45[b]	9.71±0.36[b]
Cohesiveness	0.60±0.02[b]	0.69±0.02[a]	0.59±0.02[b]
Gumminess (N)	9.56±0.31[a]	6.68±0.33[b]	5.73±0.27[b]
Chewiness (N)	8.32±0.26[a]	6.06±0.28[b]	5.14±0.23[c]
Water loss (%)	5.23±0.34[a]	3.43±0.23[b]	3.72±0.42[b]

Control: 0% ora pro nobis-flour; T1: 1% ora pro nobis-flour, T2: 2% ora-pro-nobis flour. * Values represent the mean (± standard deviation). Averages followed by the same letter in the same line are not significantly different (p> 0.05) by Tukey's test.

The results for instrumental color are presented in Table 3. The control sample showed a similar instrumental color to that found in commercial sausages (Nurul *et al*., 2010). As expected, the addition of ora-pro-nobis flour influenced significantly the instrumental color parameters, once increasing the level of addition of ora-pro-nobis flour led to a significant decrease in a^* value and a significant increase in b^* value. This means that the product was greener, and more yellow with increasing the concentration of ora-pro-nobis flour. T1 and T2 did not differ in lightness (L^*), but differed significantly from the control, indicating that the modified sausages were also with a darker color. According to Gliemmo *et al*. (2009), the color modifications instrumentally measured can be considered as noticeable visual changes when the ΔE values, which indicated the total color difference (taking the control as reference), are higher than 2. So, in this study the ΔE values confirmed the influence of ora-pro-nobis flour addition on the sausages color.

The water loss and the texture profile of sausages containing ora-pro-nobis flour is shown in Table 4. A decrease of water loss and thus an increase of water binding was found in the sausages containing ora-pro-nobis flour. The higher water binding was probably due to the fiber content of ora-pro-nobis flour (Table 1), because according to Anderson and Berry (2001), the fibers may interact with meat proteins forming a network that prevents water migration from the product to the surface. A significant decrease was observed for the parameters hardness, springiness, and chewiness with addition of 1 and 2% of ora-pro-nobis flour (T1 and T2) as compared to the control.

Table 5. Consumer acceptability of the color, aroma, flavor texture and overall acceptance of sausages made with addition of ora-pro-nobis flour (*Pereskia aculeata* Miller)

	Color	Aroma	Flavor	Texture	Overall acceptance
Control	$7.28 \pm 1.70^{a*}$	7.72 ± 1.36^{a}	7.72 ± 1.51^{a}	7.64 ± 1.37^{a}	7.54 ± 1.50^{a}
T1	7.50 ± 1.08^{a}	7.48 ± 1.28^{a}	7.65 ± 1.14^{a}	7.78 ± 0.88^{a}	7.89 ± 0.90^{a}
T2	7.40 ± 1.54^{a}	7.38 ± 1.40^{a}	7.38 ± 1.43^{a}	7.62 ± 1.14^{a}	7.64 ± 1.17^{a}

Control: 0% ora pro nobis-flour; T1: 1% ora pro nobis-flour, T2: 2% ora-pro-nobis flour. *Values represent the mean (± standard deviation). Averages followed by the same letter in the same column are not significantly different (p> 0.05) by Tukey's test.

The higher water binding may be the probable cause of this phenomenon as shown by Schuh *et al* (2013).

The results of the sensory acceptance of the sausages containing ora-pro-nobis flour are shown in Table 5. No significant difference was observed between the control and T1 and T2 in any of the sensory attributes. This demonstrates that despite the color differences found in the instrumental evaluation (Tables 3 and 4), consumers have approved both formulations containing 1 and 2% ora-pro-nobis flour. The scores ranged from 7.28 to 7.50, 7.38 to 7.72, 7.38 to 7.72, 7.62 to 7.64 and 7.54 to 7.89 for the attributes color, aroma, flavor, texture, and overall acceptance, respectively. These values are in the range between both hedonic terms "liked" and "liked very much". All treatments showed acceptability index close to 85% (p>0.05). These results are very positive, since an index values higher than 70% indicate good sensory acceptance of the product (Meilgaard *et al*., 1999).

Conclusion

Due to the high fiber and protein contents, the ora-pro-nobis flour can be considered as an excellent source to nutritionally enhance the products in which it is added. Although it has caused major changes in the color and texture parameters of the sausages, these changes have not depreciated the sensory acceptance of the product. Therefore, it can be concluded that the flour from ora-pro-nobis leaves can be added to sausage formulations at a concentration of 2% to increase the nutritional quality of the sausages.

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