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Centesimal Evaluation of Two Species of Ora-pro-nobis (*Pereskia aculeata* Miller and *Pereskia grandifolia* Haw) and Application in Extruded Product

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Some vegetables that are rarely used and/or unknown in daily menu, can represent great sources of vital nutrients to maintain the metabolic health of their consumers. Among them is *Pereskia spp* family, commonly known as ora-pro-nobis, "the flesh of the poor". According to RDC No. 54/2012 (BRASIL, 2012), Ora-pro-nobis can be considered a functional food due to its high protein content (approximately 25%), with high digestibility (80%), 3.35g/100g of calcium, 2.42g/100g of potassium, 1.13g/100g of phosphorus, 0.45g/100g of magnesium and 0.98g/100g of sulphur, leaf (140.36 ppm) and stem (88.75 ppm) iron. These data also meet the antioxidant characteristics of the plant. Studies have shown that the extracts, both leaves and stems, have a high content of restive compound to oxygen, given the characteristic of antioxidants. Considering the high nutritional value of this plant, the objective of this study was to verify the bromatological composition of the *Perskia aculeata* Miller and *Pereskia grandifolia* Haw leaves, as well as, evaluate the extrusion of the flour of its dehydrated leaves in proportion of addition of 10% together with corn, in order to evaluate the possibility of its processing. Extrusion tests were performed using 100 grams of sample containing 10% of dehydrated leaf completed with corn grits, obtaining extruded samples of CPA (*Pereskia aculeata* Miller), CPG (*Pereskia Grandfolia* Haw) and 100% corn grits (CP) as the standard for comparison. Samples were pre-stored at humidity of 20% prior to the process, which occurred steadily, without changes in the extruder parameters. Subsequently, the final extruded product was homogenized and dried in a rotary mixing drum at 60°C. Extrusion cooking was performed in duplicate using IMBRA RX50 (INBRAMAQ, Ribeirão Preto, SP, Brazil), with a single thread of 50 mm in diameter and 200 mm in length. The die set used had two 3 mm diameter holes and the parameters were set with the motor amperage at 20A and feed rate of 15g/s with a cut at 120rpm. Sample were evaluated regarding ethereal extract, determined by continuous extractor method Soxhlet type, crude protein, measured by the Kjeldahl method (conversion factor 6.25 (N x 6.25)), ashes were obtained by incineration (550 ºC) in muffle furnace, crude fibres, fibres in neutral detergent and acid detergent, total phenolic compounds (DPPH) and FRAP for antioxidant potential. Results demonstrated the nutritional viability of the two species, *Pereskia aculeata* Miller and *Pereskia grandifolia* Haw, in their bromatological parameters. Inclusion of dehydrated leaves in extruded products demonstrated as viable and the results showed that the final product has satisfactory nutritional characteristics, with substantial increase of protein, iron, fibres and antioxidant capacity in relation to the standard product.

1. Introduction

Some vegetables rarely used in daily food are called unconventional, some of these are used regionally to increase the diversity of meals. Ora-pro-nóbis (*Pereskia aculeata* Miller - PA and *Pereskia grandifolia* Haw - PG), a species of cactus native to the tropics, is considered one of these species used in food (ROSA &

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SOUZA, 2003). Authors have already identified that this plant has a high content of proteins and fibres in its leaves, as well as the absence of toxicity (ALMEIDA-FILHO & CAMBRIA, 1974, BUTTERWORTH & WALLACE, 2005, DAYRELL, 1977). According to RDC No. 54/2012 (BRASIL, 2012), Ora-pro-nobis can be considered a functional food due to its high protein content (approximately 25%) with high digestibility (80%). Conceição, et al., (2014), found in their study values of 3.35g / 100g of calcium, 2.42g / 100g of potassium, 1.13g / 100g of phosphorus, 0.45g / 100g of magnesium and 0.98g / 100g of sulphur, leaf (140.36 ppm) and stem (88.75 ppm) iron (GIRÃO, 1997). Regarding to the presence of antioxidants, Carvalho et al. (2013) evaluated the wound healing property from the mucilage of *Pereskia aculeata* Miller leaves grown on different substrates (sand, rough soil, sand and soil, sand and manure 5:1 and soil and manure 5:1). Concentrations of total phenols obtained from different treatments given with gallic acid equivalents, ranging from 108.2 mg / L to 121.1 mg / L, were found. TURRA et al. (2007) evaluated the antioxidant properties and antimicrobial susceptibility of the crude extract (total alcoholic) and increasing polarity of leaves of *Pereskia grandifolia* Haworth. From the results obtained, the authors concluded that extracts from the leaves of *P. grandifolia* (1000 µg / mL) had relatively low antioxidant properties, varying between 10 and 30%.

However, despite the many benefits, this food is hardly consumed due to a lack of knowledge caused by the market supply scarcity, considering it as unconventional food (KELLEN et al., 2015).

One way to take better advantage of the benefits of this plant and make this food more accessible to the population, would be to develop a product that contained it and at the same time was already accepted and known by the population. Extruded foods are popularly known and consumed in snacks and cereals Chinellato et al, (2016). The production process is called extrusion, known and of great relevance in the food industry since presents high efficiency and versatility (CARVALHO, R., 2000; MONTEIRO et al, 2016). Snacks have high shelf life stability, requiring no refrigeration or other special storage needs, well-accepted sensory characteristics, which makes this food subject to nutritional improvements (GUERREIRO, L., 2007).

Thus, the aim of this study was to verify the nutritional composition of *Perskia aculeata* Miller and *Pereskia granflolia* Haw leaves, as well as test the possibility of extruding dehydrated leaves (10%) with grits corn (90%), in order to increase nutritional values for the extruded snacks and evaluate the potentiality of its processing.

2. Material and Methods

2.1 Leaves selection and flour production

Leaves of *Perskia aculeata* Miller (PA) and *Pereskia granflora* Haw (PG) were collected at the MUDI seedling nursery of the State University of Maringá between January and May 2018, pick up on harvest after 5 days without rainfall.

The selected leaves were carried to the laboratory, weighed, washed in chlorinated solution and rinsed in running water, allowed to dry in an oven at 105 °C until constant weight.

Dehydrated leaves then were grounded in a knife mill and stored in containers with hermetic sealing and protected from light until analysis.

2.2 Centesimal composition of leaf's flour

The compositions were: lipids using ether extract determined by the Soxhlet type continuous extractor method, crude protein measured by the Kjeldahl method and conversion factor 6.25 (N x 6.25), ashes obtained by incineration (550 ºC) in muffle furnace, crude fibre by neutral detergent fibres and acid detergent, iron analysis by atomic absorption reading.

All mentioned methodologies executed according to AOAC (2005) methods.

2.3 Phenolics compounds and antioxidant activity

For the determination of total phenolic compounds and antioxidant activity a methanolic extraction was performed (1:5). The mixture was left under stirring at 130 rpm and protected from light for 2 hours. After that, each material was vacuum-pump filtered and the obtained liquid was used for the analyses.

2.3.1 Total Phenolic Compounds

Total phenolic compounds were performed according to the methodology described by Singleton & Rossi, (1965). A standard curve with gallic acid was created (r^2 = 0.99) and the results were expressed in µg equivalent gallic acid (EAG) / mg of food.

2.3.2 Antioxidant activity

Iron reducing capability (FRAP) and the sequestering activity of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were performed according to Correa et al. (2017). For the FRAP method, a standard curve was created with Trolox (r^2 = 0.99) and the results were expressed in µM Trolox equivalent (ET) / mg of food. For the sequestering activity of DPPH radical, a standard curve with Trolox radical sequestration efficiency was performed (r^2 = 0.99) and the results were expressed in µM equivalent Trolox (ET) / µg of food.

2.4 Snacks processing

Extrusion cooking was performed according to Dischsen et al (2013) and Oliveira et al (2013) with some modifications in the IMBRA RX50 (INBRAMAQ, Ribeirão Preto, Brazil), equipped with a single screw of 50 mm in diameter and 200 mm in length. The die plate used has two 3 mm diameter holes and the parameters were fixed by motor amperage at 20 A and the cutting speed at 50 rpm. Grits feed rate was 18 $g.s^{-1}$.
The corn grits used in extrusion were humidified by adding 2.5 % water to its total weight and preconditioning for 24 hours at 5 °C.
After extrusion, all snacks were oven dried with forced air circulation at 60 °C for 15 minutes to standardize the moisture in all samples.

2.5 Sample preparation

Three samples were prepared in quantities of 100g each. The samples were conditioned at 20% moisture prior to the process. Each sample preparation details are described in Table 01.

Table 01: Description of the snack samples preparation

Sample	Preparation
CM	100% corn grits
CPA	90% corn grits + 10% leaf flour of *Pereskia aculeata* Miller
CPG	90% corn grits + 10% leaf flour of *Perskia grandfolia* Haw

2.6 Expansion ratio

Expansion ratio (ER) was calculated according to Mercier et al. (1998), by the ratio between the average diameter of 10 different expanded products and the diameter of extruder die. Diameter was checked using a Vernier calliper and the ER calculation is represented by Eq. (1).

$$\mathrm{ER} = \frac{\text{sample diameter}}{\text{extruder die diameter}} \quad (1)$$

2.7 Statistical Analysis

Evaluated characteristics results were submitted to correlation analysis and analysis of variance (ANOVA) with subsequent analysis of the means, compared by Tukey test at the significance level of 5% through the software Assistat 7.7.

3. Results and discussion

Results of ash, protein and lipid analyses of the dried leaves of the two families studied and their respective extruded snack are presented in Table 2.

Table 2: Ash, Lipid and Protein content in leaves and extrudates.

Sample	Ashes (%)	Lipids (%)	Protein (%)
PA	45.05±2,57 ^{a}	7.34±1E-04 ^{a}	35.29±0,45 ^{a}
PG	44.65±0,53 ^{a}	6.50±0,04 ^{b}	15.45±0,43 ^{b}
CPA	46.53±0,91 ^{a}	3.86±1E-04 ^{c}	11.73±0,23 ^{c}
CPG	48.85±0,33 ^{a}	3.90±0,01 ^{c}	10.01±0,17 ^{c}
CM	45.23±1,23 ^{a}	3.44±1E-04 ^{d}	8.31±0,002 ^{d}

Equal lower-case letters in the same column do not differ by Tukey test at 5 % probability.

Table 2 presents the results of ashes, lipids and proteins from both extruded snacks and leaves. By then, when comparing the protein values of PA and PG leaf flour, it is possible to observe a statistical difference between them, however, if we compare with previous studies, values are above the ones previously found. In studies with PA, Silva et al. (2005) reported high protein contents of 24.73%, a value below that found in the present study, demonstrating a high protein quality in PA leaves cultivated in the Maringá region. The same behaviour was observed in the study by Rocha et al (2008), who obtained 22.93% protein in the dehydrated flour of the leaves of PA. Lima (2016) in their study on PG leaves and stems, obtained the result of 16.70%

and 7.38% respectively, values very close to that found in the present study. In regard the extruded product protein value, having the same degree of inclusion, the samples do not differ from each other, however, they differ from the control treatment containing only corn in its formulation, where there is an average increase of 32% in the protein content, a satisfactory result since the inclusion was only 10% by total weight of raw material. The results of the ash content found in the present study were also higher than those found in the studies of Silva (2005) and Rocha (2008), 18.07% and lipids (3.64%), respectively. Both showed values higher than those found, showing that the plant can vary in composition according to the region of cultivation. In the cited studies, Silva (2005) and Rocha (2008), were carried out in another region of the country with a different climatic and geographic characteristic, where the essays of this work were made. As for the lipid content, there was a statistically significant difference between the samples, demonstrating that the families have particularities with each other. When correlating with studies such as Magalhães et al. (2011), levels between 1.6% and 5.6% were observed in five different matrices of *Pereskia*, values close to the ones found in cultivars in the Maringá region.

When analysing the ash content of a plant matrix, the amount of minerals contained in the matrix after the burning process of the organic matrix (INSTITUTO ADOLFO LUTZ, 2008) is evaluated. For plant-based products, the average ash value is around 0.4 to 2.1%, however in plants considered unconventional, values are generally higher (KINUPP; BARROS, 2008) and that is the case of the present study.

In all studied parameters we can see that there is a considerable increase in extruded products, where they differ statistically from the standard treatment produced only with the matrix of 100% corn grits. This increase shows that incremented foods have more interesting nutritional characteristics from the dietary point of view since a food to be considered healthy, should contain as much macro and micro nutrients as possible, so that a balanced diet is accomplished.

Table 3: Characterization of the fibre content of the leaves and the extrudates.

Sample	Crude Fibre (%)	Fibre acid detergent (%)	Fibre neutral detergent (%)
PA	9.30^{a}±0.096	17.558^{a}±0.049	$50.40\ ^{a}$± 0.048
PG	11.09^{b}±0.021	18.02a±0.010	41.69^{a}± 0.323
CPA	2.78^{c}±0.064	2.85^{b}±0.013	$6.85\ ^{b}$± 1.18E-30
CPG	2.20^{c}±0.027	2.66^{b}±0.005	$6.79\ ^{b}$±0.00
CM	0.23^{d}±0.006	0.28^{c}±1.45E-06	$3.46\ ^{c}$±0.0003

Equal lower-case letters in the same column do not differ by Tukey test at 5 % probability.

Table 3 shows the composition in relation to the different types of leaf fibres and extrudates. Related to the fibres, this is a component of the food matrix, which consists of compounds that are not digested by the human body, in some cases, important in food as it aids in intestinal transit, slows digestion and absorption of some compounds present in plants. The study by Silva (2005) showed that *P. grandifolia* and *P. aculeata* can be considered sources of fibre, due to the high content found in their analyses. In addition, the analyses showed a statistical difference between the samples with addition of the leaf flour in comparison to the treatment without addition, proving the increase of this component making the extruded snack richer in dietary beneficial compounds.

For the extrusion test, the addition of 10% leaf flour resulted an average in expansion index of 3.33. HASHIMOTO & GROSSMANN (2003), investigated extrusions with mixtures of starch and cassava flour obtaining expansion indexes varying between 1.6 and 3.2 quoting the influence of the fibre's percentage in the products expansion. These results are lower than those obtained in this work, showing that the chosen percentage of leaf addition in the extrusion process is applicable. It is known that the extrusion process occurs under high temperatures in a short time and is used in the food industry to produce expanded products as well as snacks, breakfast cereals and animal feed, thus any changes in ingredients such as sugar, salt and fibre, can affect the extrusion variables and the product characteristics such as: texture, structure, degree of expansion and sensorial attributes, or even render the whole process unfeasible (MENDONÇA; GORSSMANN; VERBÉ, 2000).

Data showed that the addition of the studied flour is feasible either technically and nutritionally, producing a snack with added value. Snack's iron values were 0.96 ppm for CPA and 1.00 ppm for CPG being higher than the standard treatment CPM (0.30 ppm), thus showing substantial increase.

Table 4: Phenolic compounds and iron from leaves and extrudates

Sample	Iron (ppm)	Total Phenolic Compounds (ug EAG/mg of food)	DPPH (uM ET/ug of food)	FRAP (uM ET/mg of food)
PA	6.355	2.05 ^{a}±0.40	554.17^{a}±20.14	10.12 ^{a}±2.55
PG	8.367	1.86^{a}±0.18	547.81^{a}±7.65	11.47 ^{a}±3.30
CPA	0.958	0.94^{b}±0.09	529.15^{b}±36.26	5.39 ^{ab}±1.01
CPG	0.995	0.93^{b} ±0.08	452.45^{a}±33.58	5.55 ^{ab}±0.35
CPM	0.280	0.17 ^{c}±0.01	115.98 ^{c}±11.51	0.91 ^{b}±0.20

Equal lower-case letters in the same column do not differ by Tukey test at 5 % probability.

In order to evaluate the ability to inhibit oxygen-reactive substances, antioxidant activity through the oxidation inhibition potential using the 2,2-diphenyl-1-picrylidrazyl-64 (DPPH) radical as a reference (BRAND-WILLIANS et al., 1995) and the results are presented in table 4, as well as comparative analyses to FRAP. The present study found values for PA and PG leaves below the references when compared to Santos et. al (2015) which found for PA, 73.4 ± 22.1mg EAGg^{-1} dry leaves and 52.3 ± 3.2mg EAGg^{-1} dry leaves, values found for the analysis of total phenolic compounds. The work conducted by Almeida et al. (2011), the total phenols found in PA and PG were 19.34 and 19.17 mg 100 g^{-1}, respectively, which are lower than the study by Santos et al (2015), showing that these values undergo large variations according to the region and the conditions of cultivation. On the other hand, when the content of iron and phenolic compounds in the added snacks of *Pereskia spp* was evaluated, the expressive increase was evident when compared to the standard, regardless of the species used.

4. Conclusion

As follows, the present work demonstrates that *Pereskia spp* can be used to improve the nutritional characteristics of extruded corn snacks, highlighting a significant increase in iron and fibre content in snacks. It was also evidenced that, as demonstrated in other studies, the varieties and significantly interfere in the centesimal composition of iron and phenolics in the leaves, however in the extruded products these differences do not significantly impact on their nutritional enrichment.

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