Research, Society and Development, v. 9, n. 11, e3469119728. 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9728

Nutritional and rheological evaluation of blends based on ora-pro-nóbis (*Pereskia aculeata Miller*) and wheat flour

Avaliação nutricional e reológica de misturas de farinha de ora-pro-nóbis (*Pereskia aculeata Miller*) e farinha de trigo

Evaluación nutricional y reológica de mezclas de harina de ora-pro-nóbis (*Pereskia aculeata Miller*) y harina de trigo

Received: 11/28/2020 | Reviewed: 11/04/2020 | Accept: 11/12/2020 | Published: 11/17/2020

Micheli Mayara Trentin
ORCID: https://orcid.org/0000-0001-6208-8552
Santa Catarina State University, Brazil
E-mail: mixeli@unochapeco.edu.br
Marlene Bampi
ORCID: https://orcid.org/0000-0002-3030-2099
Department of Food Engeneering and Food Chemistry, Brazil
E-mail: marlene.bampi@udesc.br
Andréia Zilio Dinon
ORCID: https://orcid.org/0000-0002-0676-5050
Department of Food Engeneering and Food Chemistry, Brazil
E-mail: andreia.dinon@udesc.br

Abstract

This work aimed to evaluate the nutritional and rheological quality of ora-pro-nobis flour (OPNF) and its influence on the replacement of wheat flour (WF), through the analysis of physicochemical parameters, determination of phenolic compounds, antioxidant activity and rheological analyzes of wheat flour and mixtures with 10 to 30% OPNF. Flour blends with 10%, 20% and 30% of OPNF in substitution of WF in flour base were evaluated. The OPNF showed higher values of lipids (2.87%), proteins (18.29%), fibers (44.13%), ash (11.19%), minerals (Ca, Mg, K, Na, Fe and Zn), beta-carotene (71.37 μg 100 g^{-1}), lycopene (25.02 μg 100 g^{-1}), total chlorophyll (24.40 μg 100 g^{-1}), ABTS radical-scavenging capacity (15.02 μmol Trolox eq. g^{-1}) and DPPH (IC 50 = 33.30 μmol mL^{-1} extract) in relation to WF. The flour blends with 10%, 20% and 30% of OPNF showed a higher nutritional value and antioxidant activity proportional to the increase on OPNF concentration. The increasing on the concentration of OPNF in flour blends reduced the values of the gluten strength (P), tenacity (W), extensibility (L), water absorption and falling number. Moreover, it increased the values of dough development time, dough stability and water absorption. The results of rheological analyzes indicate that flour blends of OPNF (10%, 20% and 30%) with WF have characteristics suitable for use in biscuits, baked cakes and breads. The data from this study demonstrate the nutritional value and technological potential of OPNF for the development of food products.

Keywords: *Pereskia aculeata*; Physicochemical characterization; Antioxidants; Total phenolics.

Resumo

O objetivo deste trabalho foi avaliar a qualidade nutricional e reológica da farinha de ora-pro-nóbis (OPNF) e sua influência na substituição da farinha de trigo (WF), através da análise de parâmetros físico-químicos, determinação de compostos fenólicos, atividade antioxidante e análises reológicas da farinha de trigo e das misturas com 10 a 30 % de OPNF. Foram avaliadas misturas de farinhas com 10%, 20% e 30% de OPNF em substituição a WF. A OPNF apresentou maiores valores de lipídios (2,87%), proteínas (18,29%), fibras (44,13%), cinzas (11,19%), minerais (Ca, Mg, K, Na, Fe e Zn), betacaroteno (71,37 µg 100 g^{-1}), licopeno (25,02 µg 100 g^{-1}), clorofila total (24,40 µg 100 g^{-1}), capacidade de eliminação de radicais ABTS (15,02 µmol Trolox Eq. g^{-1}) e DPPH (IC 50 = 33,30 extrato µmol mL^{-1}) em relação a WF. As misturas de farinhas com 10%, 20% e 30% de OPNF apresentaram maior valor nutricional e atividade antioxidante proporcional ao aumento da concentração de OPNF. O aumento da concentração de OPNF nas misturas de farinha reduziu os valores de força do glúten (P), tenacidade (W), extensibilidade (L), absorção de água e *falling number*. Além disso, houve aumento nos valores de tempo de desenvolvimento, absorção de água e estabilidade da massa. Os resultados das análises reológicas indicam que as misturas de farinhas de OPNF (10%, 20% e 30%) com WF apresentam características adequadas para uso em biscoitos, bolos e pães. Os dados deste estudo demonstram o valor nutricional e o potencial tecnológico do OPNF para o desenvolvimento de produtos alimentícios.

Palavras-chave: *Pereskia aculeata*; Caracterização físico-química; Antioxidantes; Fenólicos totais.

Resumen

El objetivo de este trabajo fue evaluar la calidad nutricional y reológica de la harina de ora-pro-nobis (OPNF) y su influencia en la sustitución de la harina de trigo (WF), a través del análisis de parámetros fisicoquímicos, determinación de compuestos fenólicos, actividad antioxidante y análisis reológicos de harina de trigo y mezclas con 10 a 30 % de OPNF. Se evaluaron mezclas de harinas con 10%, 20% y 30% de OPNF en sustitución de WF en la base de harina. El OPNF mostró mayores valores de lípidos (2,87%), proteínas (18,29%), fibras (44,13%), cenizas (11,19%), minerales (Ca, Mg, K, Na, Fe y Zn), betacaroteno (71,37 µg 100 g^{-1}), licopeno (25,02 µg 100 g^{-1}), clorofila total (24,40 µg 100 g^{-1}), capacidad de captación de radicales ABTS (15,02 µmol Trolox Eq. g^{-1}) y DPPH (IC 50 = 33,30 extracto µmol mL^{-1}) en relación con WF. Las mezclas de harinas con 10%, 20% y 30% de OPNF mostraron un mayor valor nutricional y actividad antioxidante proporcional al aumento de la concentración de OPNF. El aumento de la concentración de OPNF en las mezclas de harinas redujo los valores de fuerza del gluten (P), tenacidad (W), extensibilidad (L), absorción de agua y *falling number*. Además, aumentó los valores de tiempo de desarrollo de la masa, estabilidad de la masa y absorción de agua. Los resultados de los análisis reológicos indican que las mezclas de harina de OPNF (10%, 20% y 30%) con WF tienen características adecuadas para su uso en galletas, pasteles horneados y panes. Los resultados del estudio demuestran el valor nutricional y el potencial tecnológico de OPNF para el desarrollo de los productos alimenticios.

Palabras clave: *Pereskia aculeata*; Caracterización fisicoquímica; Antioxidantes; Fenólicos totales.

1. Introduction

Pereskia aculeata Miller belongs to the Cactaceae family, native from South and Central America, and is widely distributed from California (USA) to Rio Grande do Sul (Brazil). It is an unconventional food plant (UFP) popularly known as ora-pro-nobis, in Brazil. It is characterized as a perennial, rustic, drought-resistant plant that withstands continuous rain and mild frosts, has true leaves, and small white flowers (Agostini-Costa, Pêssoa, Silva, Gomes, & Silva, 2014).

The *Pereskia aculeata* Miller leaves are non-toxic, and they are considered a source of protein, total dietary fiber, mucilage, vitamins and minerals such as calcium, magnesium, manganese, zinc, and iron (Garcia *et al.*, 2019; Martin, Freitas, Sassaki, Evangelista, &

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9728

Sierakowski, 2017). The use of leaves in foods can prevent diseases related to protein deficiencies, and it can reduce the risk of developing obesity, diabetes, cardiovascular diseases, hypertension, osteoporosis and also it shows healing and anti-inflammatory activities (Barbalho *et al.*, 2016; Pinto *et al.*, 2015).

The inclusion of *Pereskia aculeata* Miller leaf flour can contribute with bioactive substances (Agostini-Costa *et al.*, 2014; Martin, Freitas, Sassaki, Evangelista, & Sierakowski, 2017; Barbalho *et al.*, 2016) as a healthy and functional option to replace wheat flour and fats that are the main components in several foods. However, data on the nutritional and technological properties of ora-pro-nobis flour (OPNF) that characterize its application in bakery products are scarce and practically absent in the scientific literature. Therefore, this paper aimed to evaluate the nutritional and rheological quality of OPNF and its influence on the replacement of wheat flour (WF).

2. Materials and Methods

2.1 Samples and preparation of flour mixes

Premium Wheat Flour Type 1 (WF) was purchased from the local market. The OPNF was prepared from *Pereskia aculeata* Miller leaves collected from EPAGRI in Chapecó, Santa Catarina State, Brazil (27°05'25.9"S 52°38'10.4"W). The leaves were selected, washed and sanitized in sodium hypochlorite solution (15 ppm) for 10 min. The leaves were then dried in an oven (Cienlab, Model: CE-205/49) with air circulation at 60 °C for 24 hours. After, they were crushed in a knife mill (MDR302 Cadence, Brazil) and stored in packages (hermetic and metalized), vacuum-sealed and kept refrigerated (5 ºC ± 2) until the moment of use. Three different flour blends were prepared, each one with 10% (OPNF10), 20% (OPNF20), and 30% (OPNF30) of OPNF in replacement of WF.

2.2 Flour analysis

2.2.1 Physicochemical characterization

The moisture content of flours and flour blends was determined by method No. 931.04, lipids content was analyzed by the Soxhlet extraction method No. 30-25.01, total protein by Kjeldahl method No. 950.36 with a conversion factor of 6.25 for OPNF samples and 5.7 for WF and ash samples by method No. 930.22 (AOAC, 2016).

The total dietary fiber (TDF) content was determined according to method No. 985.29 (AOAC, 2000). The mineral contents were determined by atomic absorption spectrophotometry and quantified with reference standards, according to method No. 975.03 (AOAC, 2016). The carbohydrates were determined by difference.

The energy value was calculated according to the AtWater conversion considering the conversion coefficients of 4 kcal g^{-1} for proteins and carbohydrates and 9 kcal g^{-1} for lipids (Association of Official Analytical Chemists, 2016).

The color parameters were determined using a Minolta colorimeter (Konica-Minolta, chroma-meter CR-400), and the color was expressed by the CIE LAB system, where L* (black = 0 and white = 100), a* (green (-) and red (+)), and b* (blue (-) and yellow (+)). The color variation was determined by the formula $\Delta E *= \sqrt{(\Delta L *^2 + \Delta a *^2 + \Delta b *^2)}$, where ΔE* values < 0.2 until 1.5, indicates imperceptible color difference, 1.5 to 6.0 means an easily distinguishable color, 6 to 12 means a noticeable difference and above 12 means a large color difference (Konica Minolta, 2019).

The carotenoid content was determined on a spectrophotometer (Glod S53 UV-Vis, Ningbo Biocotek) at 450 nm for β-carotene and 470 nm for lycopene (Rodriguez-Amaya, 2001). According to the Nagata and Yamashita methodology, the content of total chlorophyll and cholorophyll a and b were determined (Nagata, & Yamashita, 1992).

The water activity was determined in an Aqualab® Pre (Decagon Devices, USA). The titratable acidity analysis was performed by the method No. 942.15 and the pH determination by method No. 981.12 (AOAC, 2016). The content of total soluble solids (TSS) was determined in a digital refractometer (RTD 95 - Instrutherm, Brazil) and the results were expressed in °Brix.

2.2.2 Determination of phenolic compounds and antioxidant activity

The total phenolic compounds were determined by the Folin-Ciocalteau spectrophotometric method (Swain, & Hills, 1959) and expressed in mg gallic acid equivalent (GAE) per gram of crude extract (mg GAE g^{-1}).

The antioxidant activity was determined by the scavenging of the DPPH radical (Brand-Wiliams, Cuvelier, & Berset, 1995) and the reduction of the ABTS cation (Re *et al.*, 1999). In the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method, the reading was performed on a UV-vis spectrophotometer (Spectrophotometer Femto Cirrus 80 AS) with a wavelength (λ) of 517 nm. Methanol was used as blank. The antioxidant activity value was expressed as IC50, by using a calibration curve with 10 to 60 µM of Trolox. The reduction of the ABTS cation (3-ethylthiabenzoline-6-sulfonate) was determined in a spectrophotometer at 734 nm, with the standard Trolox curve at concentrations of 100 to 2000 µM. The 80% ethanol was used as blank. The results of the ABTS assay were expressed in µmol Trolox eq. g^{-1} (wb). Total antioxidant activity was expressed in %.

The content of total flavonoids in the extracts was determined by spectrophotometer at 510 nm, based on the analytical curve of catechin at a concentration of 0.01 to 0.25 mg mL^{-1} (Zhishen, Mengcheng, & Jianming, 1999). The values obtained were expressed as the concentration of catechin equivalents (CE) in mg per g of extract (mg CE g^{-1}).

2.2.3 Rheological analyzes of wheat flour and flour blends with 10-30% OPNF

The samples WF, OPNF and their flour blends (OPNF10, OPNF20, and OPNF30) were evaluated by alveography analysis (Chopin, model NG), according to method No. 54-30 (AACC, 1999), using 250 g of sample. The parameters evaluated were gluten strength, tenacity and extensibility. The gluten content was determined in Yucebas equipment by methods 38-10 and 38-12A (AACC, 2000). A total of 10 g of the sample was washed with 2% sodium chloride solution to remove starch and soluble proteins. Then, it was centrifuged to obtain and determine wet gluten (wb). After drying, the dry gluten (db) was determined and the sample index was calculated.

The falling number was determined according to the method 56-81B (AACC, 2000). The farinograph analysis were performed according to method 54-21 (AACC, 1995), in a farinograph (Yucebas flour testing device), during 20 min of analysis. The water-solubility index (WSI) and the water-absorption index (WAI) were determined according to the methodology described by Anderson, Conway, Pfeifer and Griffin Junior (1969).

2.2.4 Statistical analysis

The results obtained were statistically evaluated by the Tukey Test, through the analysis of variance (ANOVA), using the Statistic® software to detect the significant difference between the samples ($p < 0.05$), at 95% confidence. All analyzes were performed

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9728

in triplicate, according to the standards of the Association of Official Analytical Chemist (AOAC, 2000; AOAC, 2016).

3. Results and Discussion

3.1 Physicochemical characterization of flours

The OPNF showed lower values of moisture (10.27 mg 100 g^{-1}) in comparison to WF (13.21 mg 100 g^{-1}). However, the samples OPNF10, OPNF20, and OPNF30 did not show a significant reduction ($p < 0.05$) in moisture concerning to WF (Table 1).

Table 1 – Physicochemical characterization of wheat flour, ora-pro-nobis flour and flour blends (n = 3).

Analysis	WF	OPNF	OPNF10	OPNF20	OPNF30	RDI	
						Men	Women
Moisture (g 100 g^{-1})	13.21±0.08^{a}	10.27±0.49^{b}	12.90±0.04^{a}	12.61±0.09^{a}	12.31±0.14^{a}	nd	nd
Lipids (g 100 g^{-1})	0.36±0.01^{e}	2.87±0.15^{a}	0.55±0.05^{d}	0.85±0.02^{c}	1.12±0.02^{b}	nd	nd
Protein (g 100 g^{-1})	10.21±1.20^{c}	18.29±0.60^{a}	10.97±0.81^{bc}	11.81±0.76^{bc}	12.62±0.72^{b}	0,8	0,8
Fiber (g 100 g^{-1})	2.00±0.02^{e}	44.13±0.15^{a}	6.21±0.17^{d}	10.42±0.15^{c}	14.64±0.13^{b}	38	25
Ash (g 100 g^{-1})	0.60±0.05^{c}	11.19±1.02^{a}	1.66±0.11^{bc}	2.72±0.21^{bc}	3.78±0.31^{b}	nd	nd
Carbohydrates (g 100 g^{-1})	76.00±0.02^{a}	23.73±0.10^{e}	59.15±0.98^{b}	55.09±0.90^{c}	51.08±0.87^{d}	130	130
Calories (kcal)	348.08±0.04^{a}	193.91±0.09^{e}	285.48±0.44^{b}	275.31±0.69^{c}	264.92±0.79^{d}	nd	nd
Aw	0.35±0.02^{a}	0.22±0.01^{b}	0.33±0.02^{a}	0.32±0.01^{a}	0.30±0.01^{a}	nd	nd
Titratable acidity (g 100 g^{-1})	4.05±0.72^{a}	2.85±0.62^{b}	3.38±0.73^{a}	2.96±0.74^{b}	2.74±0.36^{b}	nd	nd
pH	5.63±0.13^{a}	6.01±0.03^{a}	5.71±0.19^{a}	5.76±0.19^{a}	5.79±0.17^{a}	nd	nd
Total soluble solids (°Brix)	0.55±0.05^{e}	2.60±0.00^{a}	0.75±0.05^{d}	0.96±0.04^{c}	1.16±0.04^{b}	nd	nd
Calcium (mg 100 g^{-1})	18.00±0.09^{e}	3535.05±142.56^{a}	362.96±11.64^{d}	707.86±23.26^{c}	1052.8±34.90^{b}	1000	1000
Magnesium (mg 100 g^{-1})	nd	3263.34±209.87^{a}	326.33±14.84^{d}	652.66±29.68^{c}	979.00±44.52^{b}	420	320
Potassium (mg 100 g^{-1})	nd	1800.7±583.1^{a}	180.07±4.13^{d}	360.14±8.26^{c}	540.21±12.39^{b}	4700	4700
Sodium (mg 100 g^{-1})	nd	51.27±3.60^{a}	5.29±0.29^{d}	10.58±0.58^{c}	15.87±0.87^{b}	1500	1500
Iron (mg 100 g^{-1})	4.20±0.05^{d}	84.41±12.42^{a}	12.21±0.87^{cd}	20.23±1.75^{bc}	28.25±2.63^{b}	8	18
Zinc (mg 100 g^{-1})	nd	12.22±1.14^{a}	1.22±0.08^{d}	2.44±0.16^{c}	3.66±0.24^{b}	11	8
Color:							
L*	92.58±0.24^{a}	28.93±0.15^{e}	56.91±0.75^{b}	47.45±0.99^{c}	45.69±0.50^{d}	nd	nd
a*	-5.61±0.01^{d}	-4.42±0.02^{c}	-4.12±0.04^{b}	-4.03±0.05^{ab}	-4.13±0.05^{a}	nd	nd
b*	10.48±0.001^{b}	15.86±0.09^{a}	9.91±0.11^{c}	10.74±0.25^{b}	11.73±0.13^{b}	nd	nd
ΔE	-	11.15±0.08^{b}	8.83±0.16^{c}	9.48±0.51^{c}	10.01±0.10^{bc}	nd	nd

* Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). * ndt: means not determined. OPNF: 100% ora-pro-nobis flour, WF: 100% wheat flour, OPNF10: 10% ora-pro-nobis flour with 90% of wheat flour, OPNF20: 20% ora-pro-nobis flour with 80% of wheat flour, OPNF30: 30% ora-pro-nobis flour with 70% of wheat flour.
Source: Authors.

All flour samples analyzed were following Brazilian legislation, which establishes the maximum moisture limit of 15 mg 100 g^{-1}. According to Fernandes, Pereira, Germani and Oiano-Neto (2008), the flours used in the manufacture of bread, cakes, doughs and cookies must have a moisture content close to 13%, as higher values may form lumps and it can also favor the development of microorganisms, decreasing their stability and shelf life. This indicates that the flours and flour blends used in the present study have adequate moisture resulting in storage stability.

The OPNF had a higher content of lipids and proteins compared to WF (Table 1). Consequently, the samples OPNF10, OPNF20, and OPNF30 showed an increase in the content of lipids and proteins, proportional to the concentration of OPNF. The total dietary fibers values of OPNF (44.1 g 100 g^{-1}) were higher than Takeiti, Antonio, Motta, Collares-Queiroz and Park (2009) who found 9.8 g 100 g^{-1} in ora-pro-nobis leaves (db). The OPNF and flour blends with 10-30% substitutions can be considered rich in fibers because they have more than 25% of this nutrient in 100 g (ANVISA, 2012). Daily fiber intake is beneficial as it improves serum lipid levels, the immune system, blood glucose in patients with diabetes mellitus (DM), and reduces body weight and blood pressure (Weickert, & Pfeiffer, 2018).

The ash content (11.19%) in OPNF was about eighteen times higher than in WF (0.60%) and consequently, flour blends with 10-30% OPNF showed a significant increase in ash content. In OPNF, six mineral elements (Ca, Mg, K, Na, Fe, and Zn) were identified in significant quantities and in WF only two (Ca and Fe) and in smaller quantities, with Fe coming from the mandatory fortification of wheat flour in Brazil. Flour blends with 10-30% OPNF showed an increase in all minerals evaluated proportional to the OPNF concentration (Table 1).

A food is considered a source of vitamins and minerals when it contains at least 15% of the recommended dietary intake (RDI) and rich if it contains at least 30% of the RDI in 100 g of the sample (ANVISA, 2012; Institute of Medicine, 2006). Thus, OPNF can be considered rich in minerals such as Ca, Mg, and Fe, which are essential for human health. Besides, the use of OPNF contributes to the intake of minerals, fibers, and proteins and helps in the prevention and treatment of pathologies.

The lower carbohydrate content determined in OPNF (193.91 kcal 100 g^{-1}) compared to WF (348.08 kcal 100 g^{-1}), indicates the possibility of using OPNF as an ingredient in food products to reduce the intake of carbohydrates in the diet. This caloric difference is due to the higher carbohydrate content present in the WF. The OPNF30 sample (Table 1) can be considered a food with reduced energy value due to a 25% reduction in calories compared to the reference sample WF (ANVISA, 2012).

The observed Aw values (0.22 to 0.35) for samples with OPNF are below 0.8 and do not favor the growth of deteriorating microorganisms. Aw represents the susceptibility to deterioration and to estimate shelf life of food. Dehydrated foods, such as flours, are considered microbiologically more stable and has less risk of developing pathogenic organisms than other products (Jay, Loessner, & Golden, 2005).

The OPNF showed lower values of titratable acidity (2.85 g 100 g^{-1}) and higher pH values (6.01); consequently, it resulted in a decrease in titratable acidity and an increase in pH for samples OPNF10, OPNF20 and OPNF30 compared to WF (Table 1).

The OPNF had a Brix value (2.6 °Brix) approximately five times higher than WF (0.55 °Brix), and the flour blends showed a proportional increase according to the OPNF concentration, this can be attributed to the higher concentration of total sugars in OPNF, resulting from the hydrolysis of fibers and mucilage (Jani, Shah, Prajapati & Jain, 2009).

The WF had the highest values of the color parameter L * (92.58), with a tendency to white, and OPNF had the lowest values (28.93), with a tendency to a darker color. This difference resulted in a significant decrease ($p < 0.05$) in the L * color parameter of the flour blends (OPNF10, OPNF20 and OPNF30) when compared to WF. All samples showed parameter a* negative, which indicates a tendency towards green color. Regarding the color parameter b* positive, which represents the tendency to yellow, the OPNF sample had the highest values (15.86) and the WF (10.48) the lowest and consequently, the flour blends presented intermediate values. The OPNF showed the most significant color difference (ΔE*) about WF. The dark coloration of OPNF can be attributed to carotenoids and chlorophyll in its composition (Silva *et al.*, 2018), while the white color of WF is due to the presence of starch. The OPNF and the flour blends (10-30%) showed a variation of ΔE* between 8.83 and 11.15 (Table 1), which indicates a visible color difference, according to Konica Minolta (2019). This can also be seen in Figure 1.

Figure 1 – Pictures of wheat flour (WF), ora-pro-nobis flour (OPNF) and flour blends (OPNF10, OPNF20 and OPNF30).

WF: 100% wheat flour, OPNF: 100% ora-pro-nobis flour, OPNF10: 10% ora-pro-nobis flour with 90% of wheat flour, OPNF20: 20% ora-pro-nobis flour with 80% of wheat flour, OPNF30: 30% ora-pro-nobis flour with 70% of wheat flour.
Source: Authors.

3.2 Bioactive compounds and antioxidant activity of flours

The contents of β-carotene (71.37 μg g^{-1}) and lycopene (25.02 μg g^{-1}) found in OPNF were higher than the contents of 5.1 μg g^{-1} of β-carotene and 34 μg g^{-1} of lycopene found by Godoy and Rodriguez-Amaya (1998) in tomatoes, whose are recognized as a source of these compounds in foods. There was an increase in the content of β-carotene and lycopene in the OPNF10, OPNF20 and OPNF30 samples proportional to the OPNF concentration (Table 2).

Table 2 – Bioactive compounds and antioxidant activity of wheat flour, ora-pro-nobis flour and flour blends (n = 3).

Analysis	WF	OPNF	OPNF10	OPNF20	OPNF30
β-carotene (μg 100 g^{-1})	nd	71.37±0.70^a	7.13±0.06^d	14.26±0.13^c	21.39±0.20^b
Lycopene (μg 100 g^{-1})	nd	25.02±0.60^a	2.47±0.04^d	4.94±0.08^c	7.42±0.12^b
Total chlorophyll (μg 100 g^{-1})	nd	24.40±0.04^a	2.43±0.005^d	4.86±0.015^c	7.29±0.015^b
Chlorophyll a (μg 100 g^{-1})	nd	0.80±0.004^a	0.07±0.005^d	0.15±0.01^c	0.23±0.015^b
Chlorophyll b (μg 100 g^{-1})	nd	1.41±0.017^a	0.14±0.00^d	0.28±0.003^c	0.42±.005^b
Total phenolic content (GAE g^{-1})	0.56±0.02^e	70.71±0.43^a	7.6±0.04^d	14.51±0.09^c	21.5±0.14^b
ABTS (μmol Trolox eq. g^{-1})	1.95±0.09^e	15.02±0.03^a	3.24±0.05^d	4.57±0.01^c	5.87±0.02^b
DPPH (IC50)	nd	33.30±0.48^d	93.34±0.01^a	86.67±0.02^b	79.98±0.02^c
Antioxidant activity (%)	nd	22.65±1.08^a	2.25±0.01^d	4.51±0.02^c	6.75±0.04^b

* Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). *nd: means not detected. OPNF: 100% ora-pro-nobis flour, WF: 100% wheat flour, OPNF10: 10% ora-pro-nobis flour with 90% of wheat flour, OPNF20: 20% ora-pro-nobis flour with 80% of wheat flour, OPNF30: 30% ora-pro-nobis flour with 70% of wheat flour.
Source: Authors.

The carotenoids such as β-carotene and lycopene are associated with antioxidant activity and protective effects against various health problems, such as cancer, eye and cardiovascular diseases and it are also useful to the improvement of the immune system (Stahl, & Sies, 2005).

The levels of chlorophyll a, b and total in the flour blends (OPNF10, OPNF20, and OPNF30) showed significant ($p < 0.05$) increase proportional to the increase on OPNF concentration. It has been attributed to chlorophylls the beneficial effects on tissues, organs, and systems about the prevention of coronary heart disease, certain types of cancer, diabetes, and cataracts (Ferruzzi, & Blakeslee, 2007). This demonstrates the importance of using vegetable flours, such as ora-pro-nobis, in human food (Ferruzzi, & Blakeslee, 2007; Hassanbaglou *et al.*, 2012).

The total phenolic content determined in OPNF sample was 125 times greater than the WF sample. The phenolic compounds in plants have reducing properties and antioxidant action (Garcia *et al.*, 2019). Thus, the increase in antioxidant activity and phenolic content in flour blends with 10-30% OPNF was proportional to the OPNF content (Table 2).

The ABTS radical-scavenging capacity value for OPNF sample (15.02 μmol Trolox eq. g^{-1}) and their flour blends were 7 to 2 times higher than the value (1.95 μmol Trolox eq. g^{-1}) found for the WF sample (Table 2). Garcia *et al.* (2019) found values of 40.5 μmol Trolox eq. g^{-1} for dried leaves of ora-pro-nobis, higher than the values of antioxidant capacity for ABTS radical-scavenging observed for the OPNF sample in the present study.

The DPPH radical-scavenging capacity was higher for the OPNF sample compared to WF and increased proportionally for flour blends with 10-30% OPNF. The lower the IC50 value (Table 2), the greater the antioxidant activity observed (Brand-Wiliams, Cuvelier, & Berset, 1995). According to Hassanbaglou *et al.* (2012), the antioxidant capacity is highly associated with the total content of flavonoids and the total phenolic compounds that the plant has in the crude extract. Thus, the highest antioxidant activity was observed for OPNF and OPN30 (Table 2).

3.3 Rheological analysis of flours

The OPNF sample showed higher WAI values compared to the WF sample (Table 3). It can be attributed to the higher protein and fiber content present in OPNF and these compounds can contribute to the water-binding (Ferreira *et al.*, 2015). However, there was no significant difference ($p > 0.05$) in the values found for WSI in the flour samples and all blends (Table 3). The WSI and WAI parameters are important to evaluate the characteristics of the flours for their possible application in food products.

Table 3 – Rheological characteristics of wheat flour, ora-pro-nobis flour and flour blends (n = 3).

Analysis	WF	OPNF	OPNF10	OPNF20	OPNF30
Water Solubility Index (WSI)	1.17±0.12[a]	1.40±0.19[a]	1.19±0.15[a]	1.28±0.17[a]	1.32±0.25[a]
Water Absorption Index (WAI)	2.14±0.20[b]	4.47±0.56[a]	2.54±0.36[b]	2.71±0.30[b]	3.35±0.07[a]
Total gluten (%)	25.16±0.68[a]	ndt	22.64±0.61[b]	20.13±0.55[c]	17.61±0.48[d]
Wet gluten (%)	24.64±0.95[a]	ndt	22.17±0.86[ab]	19.71±0.76[bc]	17.25±0.67[c]
Dry gluten (%)	8.42±0.47[a]	ndt	7.58±0.43[ab]	6.73±0.38[bc]	5.88±0.32[c]
Gluten Index (%)	9.79±0.11[a]	ndt	9.79±0.11[a]	9.79±0.11[a]	9.79±0.11[a]
Gluten strength W (10^{-4}J)	290±1.00[a]	ndt	192±1.00[b]	113±1.00[c]	47±1.00[d]
Tenacity P (mm H_20)	159±1.00[a]	ndt	149±1.00[a]	148±1.00[a]	107±1.00[b]
Extensibility L (mm)	39±1.00[a]	ndt	29±1.00[b]	17±1.00[c]	10±1.00[d]
Tenacity/Extensibility (P/L)	2.15±1.05[c]	ndt	5.14±1.05[b]	8.71±1.00[a]	10.7±1.00[a]
Dough development time (min)	08:00	ndt	08:06	09:12	13:52
Dough stability (min)	08:11	ndt	08:25	08:39	12:24
Water Absorption (%)	59.0[a]	ndt	60.2[a]	62.0[a]	66.8[a]
Falling number (s)	253±2.00[a]	ndt	256±1.00[a]	257±2.00[a]	258±1.00[a]

Different letters in the same line indicate a significant difference ($p < 0.05$), (Tukey, ANOVA). ndt: not determined. GAE: gallic acid equivalent. CE: catechin equivalent. OPNF: 100% ora-pro-nobis flour; WF: 100% wheat flour; OPNF10: 10% ora-pro-nobis flour and 90% wheat flour; OPNF20: 20% ora-pro-nobis flour and 80% wheat flour, OPNF30: 30% ora-pro-nobis flour and 70% wheat flour.
Source: Authors.

The flour blends with 10-30% OPNF showed a proportional reduction in total, wet and dry gluten compared to the WF sample (Table 3). Gluten is essential for the baked products' structure when it forms a viscoelastic network that retains the gases formed during the dough fermentation process and allows its expansion. The gluten strength is associated with its tolerance for mechanical treatments and also its ability to absorb water. Flour blends with 10% and 20% OPNF had a wet gluten content between 20% and 25% and dry gluten between 7% and 8.5%, which indicates the desirable gluten quality for use in bakery products (Zhang *et al.*, 2010). All the flours analyzed in the present study can be classified as dough weakening for bread making according to Marchylo, Dexter, Clarke, Clarke and Preston (2001), as they have gluten index values below 40.

Flour blends with 10-30% OPNF showed a proportional reduction in gluten strength (W) tenacity (P) and extensibility (L) (Table 3). Gluten strength is used to assess the flour's ability to withstand the mechanical work of the dough deformation. The gluten strength of the sample OPNF10 (192×10^{-4} J) is considered as medium strength, and the OPNF20 (113×10^{-4} J) and the OPNF30 (47×10^{-4} J) samples are considered with weak strength according to Marchylo, Dexter, Clarke, Clarke and Preston (2001), that establishes values about 120×10^{-4} J for conventional, 178×10^{-4} J for medium and 233×10^{-4} J for strong gluten strength.

The P / L results express the dough's resistance to deformation and the dough's ability to extend without breaking it (Borchiani *et al.*, 2011; Sibanda, Ncube, & Ngoromani, 2015). All flour blends with 10-30% OPNF showed P / L values above 1.21, which indicates the potential use for the production of cakes, cookies and breads (Zhang *et al.*, 2010).

The increase in OPNF in flour blends resulted in a proportional increase in dough development time and dough stability (Table 2). According to Sibanda, Ncube and Ngoromani (2015), the flours for baked products should have a dough development time about 14 min and a dough stability about 12 min. Thus, flour blends with 10-30% OPNF showed development time and dough stability, which suggests its use for preparing bakery products.

The water absorption of all samples was greater than 55%. These findings are in agreement with Sibanda, Ncube and Ngoromani (2015), who observed that the addition of sorghum to wheat flour reduces water absorption. Moreover, the water absorption is also important for controlling the dough consistency and moisture, dissolving salts, hydrating the starch, and developing enzymatic activity (Borchiani *et al.*, 2011; Sibanda, Ncube, & Ngoromani, 2015).

The falling number of flour blends with 10-30% OPNF showed no significant difference ($p > 0.05$) compared to WF. The falling number values obtained for WF and for the flour blends with 10-30% OPNF were among 253 s to 258 s. According to Delwiche, Vinyard and Bettge (2015), falling number values less than 300 s often indicate low quality and deleterious effects for bakery products derived from their flour. However, usually, wheat flour suitable for bakery has a falling number between 200 s and 250 s.

It is observed that OPNF presents WSI and WAI superior to WF and flour blends with 10-30% OPNF. This probably occurs due to the higher content of proteins and fibers present in OPNF that increase the interaction and water retention. Flour blends with 10-30% OPNF have a gluten content proportional to the added WF content, increased elasticity and tenacity, and reduced dough stability and dough development time, with less water absorption and falling number. All flour blends 10-30% OPNF showed rheological characteristics that suggest their application to prepare baked products as biscuits, cakes and breads.

Future works can evaluate the use of ora-pro-nobis flour and its mixtures in the production of bakery foods to assess its physicochemical, nutritional and functional characteristics to improve the development of food products.

4. Final Considerations

The OPNF is rich in Ca, Mg, K, Fe, and Zn, and it has the advantage of being reduced in total calories. Moreover, it showed higher protein contents, fiber, lipids, ash, β-carotene, lycopene, and total chlorophyll, compared to WF. The antioxidant potential was also expressive in OPNF due to the presence of phenolic compounds and flavonoids. The rheological analyzes of flour blends with 10-30% OPNF indicate that the addition OPNF in replacement to WF is suitable for use in the manufacture of cakes and biscuits. Therefore, it was possible to verify the nutritional value and rheological potential of blends with OPNF and WF flour as an unconventional raw material that can be applied for the development of products to the food industry.

Conflict of interest statement

The authors declare that they have no conflict of interest in the work reported in this paper.

Acknowledgment

This study was supported by FAPESC 2019TR648 and Michelli M. Trentin was supported by the Fellowship of Postgraduate Monitoring Scholarship Program (PROMOP) from Santa Catarina State University (UDESC), Brazil.

References

Agostini-Costa, T. S., Pêssoa, G. K. A., Silva, D. B., Gomes, I. S., & Silva, J. P. (2014). Carotenoid composition of berries and leaves from a Cactaceae – *Pereskia* sp. *The Journal of Functional Foods, 2*, 178-184. https://doi.org/10.1016/j.jff.2014.09.015

American Association of Cereal Chemists (AACC). (1995). *Approved methods of the AACC* (8th ed.). Saint Paul: AACC.

American Association of Cereal Chemists (AACC). (1999). *Approved methods of the AACC* (8th ed.). Saint Paul: AACC.

American Association of Cereal Chemists (AACC). (2000). *Approved methods of the AACC* (10th ed.). Saint Paul: AACC.

Anderson, R. A., Conway, H. F., Pfeifer, V. F., & Griffin Junior, L. (1969). Gelatinization of Corn Grits by Roll-and Extrusion-Cooking. *Cereal Science Today*, *14*(1), 4-12. https://doi.org/10.1002/star.19700220408

Association of Official Analytical Chemists (AOAC). (2000). *Official Methods of Analysis of Association of Official Analytical Chemists* (17th ed.). Gaithersburg: Association of Official Analytical Chemists.

Association of Official Analytical Chemists (AOAC). (2016). *Official Methods of Analysis of Association of Official Analytical Chemists* (20th ed.). Gaithersburg: Association of Official Analytical Chemists.

Barbalho, S. M., Guiguer, E. L., Marinelli, O. S., Bueno, P. C. S., Pescinini-Salzedas, L. M., Santos, M. C. B., Oshiiwa, M., Mendes, C. G., Menezes, M. L., Nicolau, C. C. T., Otoboni, A. M., & Goulart, R. A. (2016). *Pereskia aculeata* Miller Flour: Metabolic Effects and Composition. *Journal of Medicinal Food, 19*(9), 890-894. https://doi.org/10.1089/jmf.2016.0052

Borchiani, C., Masmoudi, M., Besbes, S., Attia, H., Deroanne, C., & Blecker, C. (2011). Effect of date flesh fiber concentrate addition on dough performance and bread quality. *Journal of Texture Studies, 42*, 300-308. https://doi.org/10.1111/j.1745-4603.2010.00278.x

Brand-Wiliams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brazilian Health Regulatory Agency (ANVISA). (2012). *Resolution – RDC nº 54, November 12nd 2012*. Technical Regulation on Complementary Nutritional Information. Brasília: Brazilian Health Regulatory Agency.

Delwiche, S. R., Vinyard, B. T., & Bettge, A. D. (2015). Repeatability precision of the falling number procedure under standard and modified methodologies. *Cereal Chemistry, 92*(2), 177-184. DOI: 10.1094/CCHEM-07-14-0156-R

Fernandes, A. F., Pereira, J., Germani, R., & Oiano-Neto, J. (2008). Effect of the partial replacement of wheat flour for potato skin flour (*Solanum tuberosum* L.). *Food Science and Technology*, *28*, 56-65. http://dx.doi.org/10.1590/S0101-20612008000500010

Ferreira, M. S. L., Santos, M. C. P., Moro, T. M. A., Basto, G. J., Andrade, R. M. S., & Gonçalves, E. C. B. A. (2015). Formulation and characterization of functional foods based on fruit and vegetable residue flour. *Food Science and Technology*, *52*(2), 822-830. DOI: 10.1007/s13197-013-1061-4

Ferruzzi, M. G., & Blakeslee, J. (2007). Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research, 27*(1), 1-12. https://doi.org/10.1016/j.nutres.2006.12.003

Garcia, J. A. A., Corrêa, R. C. G., Barros, L., Pereira, C., Abreu, R. M. V., Alves, M. J., Calhelha, R. C., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2019). Phytochemical profile and biological activities of 'Ora-pro-nobis' leaves (*Pereskia aculeata Miller*), an underexploited superfood from the Brazilian Atlantic Forest. *Food Chemistry*, *294*(1), 302-308. https://doi.org/10.1016/j.foodchem.2019.05.074

Godoy, H. T., & Rodriguez-Amaya, D. B. (1998). Occurrence of cis isomers of provitamins A in Brazilian vegetables. *Journal of Agricultural and Food Chemistry*, *46*(8), 3081-3086. https://doi.org/10.1021/jf9708655

Hassanbaglou, B., Hamid, A. A., Roheeyati, A. M., Saleh, N. M., Abdulamir, A. B., Khatib, A., & Sabu, M. C. (2012). Antioxidant activity of different extracts from leaves of *Pereskia bleo* (Cactaceae). *Journal of Medicinal Plants Research, 6*(15), 2932-2937. DOI: 10.5897/JMPR11.760

Institute of Medicine. (1997). *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride.* Washington, D.C.: National Academy Press.

Institute of Medicine. (2002). *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington D.C.: National Academy Press.

Institute of Medicine. (2004). *Dietary reference intakes for water, potassium, sodium, chloride, and sulfate.* Washington D.C.: National Academy Press.

Institute of Medicine. (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.* Washington D.C.: National Academy Press.

Institute of Medicine. (2006). *Dietary reference intakes the essential guide to nutrient requirements*. Washington, D.C.: National Academy Press.

Jani, G. K., Shah, D. P., Prajapati, V. D., Jain, V. C. (2009). Gums and mucilages: versatile excipients for pharmaceutical formulations. Asian J Biomed Pharmaceut. Sci., 4(5), 308-322. ISSN: 2249 - 622X.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern Food Microbiology* (7th ed.). New York: Springer.

Konica Minolta. (2019). Identifying Color Differences Using L*a*b* or L*C*H* Coordinates. Retrieved from https://sensing.konicaminolta.us/us/blog/identifying-color-differences-using-l-a-b-or-l-c-h-coordinates/

Marchylo, B. A., Dexter, J. E., Clarke, F. R., Clarke, J. M., & Preston, K. R. (2001). Relationships among bread-making quality, gluten strength, physical dough properties, and pasta cooking quality for some Canadian durum wheat genotypes. *Canadian Journal of Plant Science*, *81*, 611-620. https://doi.org/10.4141/P00-133

Martin, A. A., Freitas, R. A., Sassaki, G. L., Evangelista, P. H. L., & Sierakowski, M. R. (2017). Chemical structure and physical-chemical properties of mucilage from the leaves of *Pereskia aculeata*. *Food Hydrocolloids,* *70*(1), 20-28. https://doi.org/10.1016/j.foodhyd.2017.03.020

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *The Japanese Society for Food Science and Technology, 39*(10), 925-928. https://doi.org/10.3136/nskkk1962.39.925

Pinto, N. C. C., Machado, D. C., Silva, J. M., Conegundes, J. L. M., Gualberto, A. C. M., Gameiro, J., Chedier, L. M., Castañon, M. C. M. N., & Scio, E. (2015). *Pereskia aculeata* Miller leaves present in vivo topical anti-inflammatory activity in models of acute and chronic dermatites. *Journal of Ethnopharmacology, 173*(1), 330-337. https://doi.org/10.1016/j.jep.2015.07.032

Re, R., Pelegrini, N., Proteggente, A., Pannala, A., Yang, M., & Riceevans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*. Washington, D.C.: ILSI.

Sibanda, T., Ncube, T., & Ngoromani, N. (2015). Rheological properties and bread making quality of white grain sorghum-wheat flour composites. *International Journal of Food Science and Nutrition Engineering, 5*(4), 176-182. DOI: 10.5923/j.food.20150504.03

Silva, A. P. G., Spricigo, P. C., Freitas, T. P., Acioly, T. M. S., Alencar, S. M., & Jacomino, A. P. (2018). Ripe Ora-pro-nobis (*Pereskia aculeata Miller*) fruits express high contents of bioactive compounds and antioxidant capacity. *Revista Brasileira de Fruticultura, 40*(3), e-749. https://doi.org/10.1590/0100-29452018749

Stahl, W., & Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta, 1740*(2), 101-107. https://doi.org/10.1016/j.bbadis.2004.12.006

Swain, T., & Hills, W. E. (1959). The phenolic constituents of *Punnus domestica*. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 19*, 63-68. https://doi.org/10.1002/jsfa.2740100110

Takeiti, C. Y., Antonio, G. C., Motta, E. M. P., Collares-Queiroz, F. P., & Park, K. J. (2009). Nutritive evaluation of a non-conventional leafy vegetable (*Pereskia aculeata* Miller). *International Journal of Food Sciences and Nutrition*, *60* (Suppl. 1), 148-160. https://doi.org/10.1080/09637480802534509

Weickert, M. O., & Pfeiffer, A. F. H. (2018). Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *Journal of Nutrition*, *148*(1), 7-12. https://doi.org/10.1093/jn/nxx008

Zhang, L., Cheng, L., Jiang, L., Wang, Y., Yang, G., & He, G. (2010). Effects of tannic acid on gluten protein structure, dough properties and bread quality of Chinese wheat. *Journal of the Science of Food and Agriculture, 90*, 2462-2468. DOI: 10.1002/jsfa.4107

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*, 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Percentage of contribution of each author in the manuscript

Micheli Mayara Trentin – 40%

Marlene Bampi – 30%

Andréia Zilio Dinon – 30%