Effect of soaking on the nutritional quality of pequi (*Caryocar brasiliense* Camb.) peel flour

Beatriz dos Santos SIQUEIRA[1], Manoel Soares SOARES JÚNIOR[2]*, Kátia Flávia FERNANDES[1], Márcio CALIARI[2], Clarissa DAMIANI[2]

Abstract

Pequi peel comprises 76% of the whole fruit and it is discarded during consumption. Thus, pequi peel has been considered a solid residue, although it has potential for use in various applications. Limitations in the use of this material are mainly due to the lack of information of its nutritional composition, especially about the toxic or antinutritional factors. Soaking is often used to prepare complementary foods and has been reported to be beneficial for enhancing nutritive value. The effect of soaking on the nutritional quality of pequi peel flour was determined by measuring changes in chemical composition, antinutritional factors, total phenols and in vitro protein and starch digestibility. The results showed that 24 h of maceration increases the content of lipids (200%), protein (28.3%) and dietary fibber (31%), while carbohydrate and ash content decreases. There were no haemagglutination activity or α-amylase inhibitors, but it was detected the presence of phytic acid (0.4 g 100 g^{-1}). The soaking reduced 8.5% phenols and 19.0% tannins, 6.2% protein digestibility, and was also effective to eliminate trypsin inhibitors, and increase starch digestibility (24.2%). Soaking was efficient to improve nutritional characteristics of the pequi peel flour, opening up possibilities for its use in food formulations.

Keywords: phytates; trypsin inhibitors; hemagglutinating activity.

1 Introduction

Pequi (*Caryocar brasiliense* Camb.) is a globular fruit, drupe-type, containing from 1 to 4 stones which represent the dispersion units of the species (LORENZI, 2008). It is widely consumed in Brazil, mainly in the Cerrado, and its agro-industrial processing has increased the spread to non-producing regions (VERA et al., 2007). Due to its unique flavor and nutritive value, pequi has potential for use in feeding and survival of a significant part of the Brazilian population (MELO JÚNIOR et al., 2004), and its agro-industrial processing could be an option for the sustainable development of the Cerrado region in Brazil.

Despite the growth in consumption, the portion consumed (internal mesocarp) represents only 8.5% of the whole fruit. The external mesocarp which consists of 76% of the whole fruit (VERA et al., 2007) is normally discarded during the fruit processing, although it has potential for use in various applications, such as pectin extraction, production of flour and bakery or confectionery products (SOARES JÚNIOR et al., 2009, 2010; SIQUEIRA et al., 2012).

Pequi peel flour is a food rich in total dietary fiber, total carbohydrates, ash, magnesium, calcium, manganese and copper, but poor in lipids, zinc and iron (SOARES JÚNIOR et al., 2009). However, there are still limitations to the use of this material, mainly due to the lack of information of its nutritional composition, especially about the toxic or antinutritional factors, which can reduce the activity of some enzymes, the biological action of several chemical compounds and the absorption of nutrients.

Anti-nutritional factors can interfere with the bioavailability of nutrients or even be toxic, making important their study before application in new products. Knowledge on the nutritional composition of foods is fundamental in order to evaluate the availability of nutrients and their consumption by populations, apart from verifying the nutritional adequacy of the diet, identifying the nutritional status, developing research concerning the relationship between diet and disease, and for use in agricultural planning and by the food industry (UNIVERSIDADE..., 2004). However it is known that knowledge of the nutrients in a food is not sufficient to evaluate its nutritional quality, since not all are totally available to the organism after digestion.

Soaking is a domestic technological treatment that is often used to prepare complementary foods at home and have been reported to be beneficial for enhancing nutritive value (ELMAKI et al., 2007). Thus soaking, which represent a technological process for the removal of soluble compounds, may be an alternative to decrease the content of anti-nutritional compounds present in the *pequi* peel flour.

The objective of the current study was to analyze the composition of pequi peel flour, and verify the effect of soaking time on the chemical composition, antinutritional factors and *in vitro* digestibility

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[1] *Department of Biochemistry and Molecular Biology, Federal University of Goias – UFG, Goiânia, GO, Brazil*
[2] *Department of Food Engineering, School of Agronomy and Food Engineering, Federal University of Goias – UFG, Rod. Goiânia, Km 0, Nova Veneza, CEP 74001-970, Goiânia, GO, Brazil, e-mail: mssoaresjr@hotmail.com*
**Corresponding author*

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2 Materials and methods

2.1 Pequi peel flour preparation

Pequi fruit from São Miguel do Araguaia, Goiás State, Brazil, were acquired at Goiás State Supply Center (Ceasa-GO) in the city of Goiânia in November 11st, 2007. About 15 kg of fresh fruit were selected according to the size and absence of injury. Fruit were chemically peeled by immersion in a 5.85 g L^{-1} solution for 7 min, followed by washing with water and immersion in a 3% (v/v) acetic acid solution for 3 min (SOARES JÚNIOR et al., 2009). Then the fruit were cut into two parts to separate the external mesocarp (*pequi* peel) and the internal mesocarp, which was discarded. *Pequi* peel was sliced, blanched in boiling water for 6 min and subjected to the soaking process, that occurred by immersion of the slices in water (proportion of 1:3, w/v) at refrigerated temperature (4 °C) for different times (0, 1, 2, 3 days – treatments). Then *pequi* peel were oven-dried at 60 °C for 16 h, ground to pass through a 0.59 mm screen, sealed in plastic bags and stored at 4 °C.

2.2 Chemical composition

The proximate composition of the flours was determined according to the methods of the Association of Official Analytical Chemists (ASSOCIATION..., 2000). The moisture content was determined by drying the *pequi* peel flours on a hot air oven at 105 °C until constant weight. Crude protein content was calculated by converting the nitrogen content determined by macro Kjekdahl's method (6.25 × N). Crude lipids were extracted from the flours in a Soxhlet extractor with chloroform:methanol (2:1, v/v). The contents of crude lipids were determined gravimetrically after oven-drying (80 °C) the extract overnight. Ash content was determined by dry-ashing in a furnace at 525 °C for 24 h, and total dietary fiber was determined by the enzymatic gravimetric method.

2.3 Trypsin and α-amylase inhibitors

Samples of pequi peel flour (1 g) were diluted in a solution of sodium phosphate buffer (10 mL) 0.1 mL L^{-1} pH 7.0, shaken during 1 h at 4 °C, and then centrifuged (Excelsa II, mod 206-BL, São Paulo) at 12000 g for 15 min at 4 °C. The precipitate was discarded and the supernatant was denominated crude extract.

The activity of trypsin inhibitors was determined according to Kakade, Rackis and McGhee (1974). Samples of crude extract were incubated with 0.1 mL trypsin solution 1.0 mg mL^{-1} and phosphate buffer 0.1 mol L^{-1} pH 7.6 at 37 °C for 10 min. After that, 0.5 mL of a casein solution 1% (w/v) was added to the solution and the reaction mixture was incubated for 10 min. The reaction was interrupted by adding 1.5 mL of TCA solution 5% (v/v). One trypsin unit was arbitrarily defined as the increase of 0.1 absorbance unit at 280 nm. The unit of inhibition (TIU) was defined as the relationship between the units observed in the maximum activity and the activity of the samples containing the inhibitors.

The activity of α-amylase inhibitors was determined according to Deshpande et al. (1982). Tests were performed by adding 20 µL of crude extract to 60 µL of sodium acetate buffer 0.1 mol L^{-1} pH 5.0 and 20 µL solution of α-amylase from *Aspergillus niger*. Then, 100 µL of 0.5% starch solution (w/v) in sodium acetate buffer 0.1 mol L^{-1} pH 5.0 were added to each tube. The solution was incubated for 15 min at 40 °C. After the reaction, 100 µL of the mixture were transferred to a test tube containing 900 µL of reactive ADNS (acid 3,5-dinitrosalicylic). The system was incubated for 5 min at 100 °C. After boiling, samples were cooled to 25 °C and reading of absorbance was done at a wavelength of 550 nm. One unit of α-amylase inhibitors was defined as the amount of inhibitors that inhibits one unit of α-amylase.

2.4 Haemagglutinating activity

Haemagglutination assays, using rabbit erythrocytes, were carried out following the method described by Moreira and Perrone (1977) with modifications. The extracts (1% w/v) were diluted in 2-fold dilution series against a 0.15 mol L^{-1} NaCl solution containing $CaCl_2$ and $MnCl_2$ 5 mmol L^{-1}. 200 µL of a 2% (v/v) erythrocyte suspension was added to an equal volume of samples and the mixture incubated at 37 °C for 30 min followed by 30 min at room temperature (25 °C). The tubes were centrifuged (Excelsa II, mod 206-BL, São Paulo) at 12000 g for 1 min and the last tube that showed visible agglutination was considered the point of equivalence.

2.5 Phytic acid determination

The content of phytic acid present in the flour was determined by the method described by Latta and Eskin (1980) with modifications to the resin DOWEX-4-AGX according to Ellis and Morris (1986). To 1 g of *pequi* peel flour it was added 10 mL of 2.4% HCl and stirring was conducted at room temperature (25 °C) for 1 h. The sample was centrifuged at 5000 g for 10 min. 2 mL of supernatant were transferred to a 50 mL volumetric flask and the volume completed with distilled water. The column was prepared with 0.5 g of resin DOWEX-AGX-4, eluted with 10 mL 0.7 mol L^{-1} NaCl and 10 mL of distilled water before elution with the extract. 2 mL of the extract were applied to the column containing the resin and elution was done with 10 mL of 0.7 mol L^{-1} NaCl. 3 mL of the eluate were added to 1 mL of Wade reagent and left at room temperature (25 °C) for 15 min. Changes in absorbance were analyzed at 500 nm and compared with a standard curve of phytic acid (Sigma Aldrich, P8810). Results were expressed in mg PA g^{-1} of the sample.

2.6 Total phenols and tannins

The determination of total phenols and tannin in the *pequi* peel flour was conducted according to the method described by Hagerman and Butler (1978). Samples of *pequi* peel flour (0.1 g) were incubated with 10 mL of distilled water at 85 °C for 30 min. The solution was cooled to room temperature (25 °C) and centrifuged (Excelsa II, mod 206-BL, São Paulo) at 5000 g for 5 min. The pellet was discarded and the supernatant used as a source of phenols.

Total phenols were determined by adding $FeCl_3$ to the extract solutions under alkaline conditions to produce a colored complex with phenols, which was read at 510 nm. Tannin content determined by the Hagerman and Butler (1978) method uses Bovine Serum Albumin (BSA) solution (1 mg mL^{-1}) in 0.2 mol L^{-1} acetate buffer (pH 4.9). The extract solutions were precipitated with BSA and, after centrifugation, the precipitate was dissolved in sodium dodecyl sulfate-triethanolamine solution and the tannins were complexed with $FeCl_3$. The colored complex was then read at 510 nm. Total phenol and tannins were measured using tannic acid for standard curves and were expressed as percent tannic acid equivalents.

2.7 In vitro digestibility tests

In vitro protein digestibility was determined by a multi-enzymatic method described by Akeson and Stahmann (1964). Samples of the *pequi* peel flour were first digested with pepsin (enzyme to substrate ratio 1:10) at 37 °C for 3 h. Then, the samples were neutralized with 0.5 M NaOH solution (pH 8.0) and, after addition of pancreatin (1:5) the digestion was carried for 4 h at 37 °C. The digestion was interrupted with trichloroacetic acid and after centrifugation (Excelsa II, mod 206-BL, São Paulo) at 7000 g for 10 min, the supernatant was assayed according to the method described by Lowry et al. (1951) using bovine serum albumin as standard. The extent of hydrolysis was calculated using 1% casein solution as control (Equation 1).

$$\%\,Digestibility = \frac{\left(\begin{array}{l} BSA\ concentration\ in\ flour - \\ BSA\ concentration\ in\ hydrolyzate \end{array}\right)}{\left(\begin{array}{l} BSA\ concentration\ in\ casein\ solution - \\ BSA\ concentration\ in\ hydrolyzate \end{array}\right)} \times 100 \qquad (1)$$

In vitro starch hydrolysis was determined according to Zabidi and Aziz (2009) with modifications. Two hundred mg of sample were incubated with 1 mL of α-amylase of *Aspergillus niger* on water bath at 90 °C for 15 min under agitation. Then, 200 µL of amyloglucosidase solution was added (Sigma-Aldrich) and incubated at 55 °C for 120 min under continuous agitation. The reducing sugar was determined as described by Miller (1959). Flour starch digestion was expressed as a percentage of the reducing sugar obtained after hydrolysis, using 1% (w/v) starch solution as control (Equation 2).

$$\%\,Digestibility = \frac{\left(\begin{array}{l} [reducing\ suggar]in\ hydrolyzate - \\ [reducing\ suggar]in\ flour \end{array}\right)}{\left(\begin{array}{l} [reducing\ suggar]in\ hydrolyzate - \\ [reducing\ suggar]\ in\ starch\ solution \end{array}\right)} \times 100 \qquad (2)$$

2.8 Statistical analysis

A completely random experimental design was used with four treatments and five repetitions, evaluating the content of moisture, ash, protein, lipids, total, soluble and insoluble dietary fiber, phytic acid, haemagglutinating activity. All analysis was run on triplicate. Statistica 6.0 (StatSoft Inc., Tulsa, USA) was used to run ANOVA followed by Tukey's test to determine the significance among the means. The level of significance used was 5%. The total phenols, tannins, protein and starch digestibility were determined only at zero and 24 h of soaking time. Descriptive statistics was used for this data evaluation.

3 Results and discussion

3.1 Chemical composition

The mean moisture contents of the *pequi* peel flours were low [0.48-1.54 g (100 g)$^{-1}$] and decreased over maceration time (Table 1). The flours differed ($P < 0.05$) with respect to moisture content, except the flour obtained with a maceration time of 48 h, which was not significantly different ($P > 0.05$) from those obtained with 24 and 72 h of maceration. *Pequi* peel flours can be considered products with good physical and chemical stability, as long as stored adequately in hermetically sealed packages.

The data obtained allow affirming that the *pequi* peel flour is a good source of nutrients, especially soluble and insoluble fiber contents. There was no significant difference ($P > 0.05$) in the protein contents of the *pequi* peel flours subjected to different times of maceration, but these were different from the non-macerated flour ($P < 0.05$). The protein contents of the macerated *pequi* peel flours were close to the values found by Vera et al. (2007) for *pequi* pulp, which varied from 3.18% to 3.89%. On the other hand, the non-macerated flour presented a protein content close to that found by Roesler et al. (2007) in the flour from the peel of *Anonna crassiflora* Mart. [2.14 g (100 g)$^{-1}$] and in the flour from the peel of *Solanum lycocarpum* St. Hil.-lobeira [2.51 g (100 g)$^{-1}$].These are fruits from the Brazilian savanna with potential for commercialization as flour, mainly due to their abundance and functional characteristics.

The total lipids varied between [1.3-4.0 g (100 g)$^{-1}$]. The lowest value for lipids was found in the non-macerated flour ($P < 0.05$). However there was an increase in the lipid content with maceration as also for the protein and fiber contents (Table 1). Soares Júnior et al. (2010), studying the *pequi* peel flour, found lower total lipid content [0.85 g (100 g)$^{-1}$] than that found in the non-macerated flour in the present study, probably due to the non-removal of the epicarp (lipid-rich) in the elaboration of the flour. The lipid content for the flours obtained with different maceration times were higher than verified by Soares Júnior et al. (2009) in *pequi* peel flour [0.88 g (100 g)$^{-1}$], principally in those underwent maceration. The increase in the lipid content, as also in the protein and dietary fiber content, after maceration of the *pequi* peel, was probably related to the decrease in the content of carbohydrates and water soluble salts by leaching into the maceration water, resulting in a proportional increase in the contents of both components.

Unlike proteins and lipids, the ash content decreased with maceration due to the high water solubility of the minerals. The highest ash content was found in the non-macerated *pequi* peel flour, significantly different from the others ($P < 0.05$). There was no significant difference in the mineral content between macerated flours ($P > 0.05$). Soares Júnior et al. (2010), in a study with *pequi* peel flour, registered [2.86 g (100 g)$^{-1}$] of ash, a value higher than found in the non-macerated flour in the present study. This was probably due to the removal of the epicarp by

leaching, which is a tissue rich in ash as well as lipids. However these values were lower than in the macerated *pequi* peel flours, due to leaching during maceration.

The total, soluble and insoluble dietary fiber contents of the non-macerated *pequi* peel flours were high, 59.67, 24.27 and 35.4 g (100 g)$^{-1}$, respectively. However, after maceration of the peel during processing, the values were even higher, reaching 91 g (100 g)$^{-1}$ total dietary fiber, 36.5 g (100 g)$^{-1}$ soluble dietary fiber and 54.4 (100 g)$^{-1}$ insoluble dietary fiber (dwb). Thus there was an increase in the total, soluble and insoluble dietary fiber contents with increased maceration time up to 48 h. The *pequi* peel flours differed ($P < 0.05$) with respect to the total, soluble and insoluble dietary fiber contents, with the exception of those obtained with 48 h and 72 h of maceration ($P > 0.05$).

There are currently many studies being carried out with fruit peels with the objective of verifying the nutritional and functional value of these residues, which for decades were usually discarded by the consumers. Fruit peels such as those of the passion flower (*Passiflora edulis flavicarpa* DEG.) show a total dietary fiber content of 57.32 g (100 g)$^{-1}$ (CORDOVA et al., 2005); and the bran composed of *pera* orange peel and bagasse contains 11.04 g (100 g)$^{-1}$ dietary fiber (RUVIARO et al., 2008). The insoluble fiber content in the fibrous residues from a cassava starch factory reached maximum values of 25.1 g (100 g)$^{-1}$ (RAUPP et al., 1999). All the above mentioned residues showed much lower total, soluble and insoluble dietary fiber contents than the *pequi* peel flour [59.67 g (100 g)$^{-1}$].

Dietary fiber is composed of an insoluble part and a soluble part. The insoluble fraction is related to increasing the fecal ball, thus guaranteeing intestinal peristalsis, avoiding constipation and annulling the appearance of hemorrhoids and diverticulitis. The soluble fraction, on the other hand, has proven beneficial effects on the metabolism of insulin and cholesterol. Thus the ingestion of *pequi* peel flour, whether pure or in the form of bread, cake or pasta, could contribute considerably to the good functioning of the intestine and prevent increases in blood cholesterol levels. It can also be consumed by diabetics, since the water soluble fibers exert a hyperglycemic effect by delaying gastric emptying, shortening the intestinal transit and reducing glucose absorption (CORRÊA, 2002).

3.2 Antinutritional factors

The presence of antinutritional factors in plant products must be analyzed to ensure that the nutritional quality is not compromised by any of these components. As observed in Table 2, haemagglutination activity and α-amylase inhibitors were not detected in the pequi peel flour.

In the assay for lectins, there was no hemagglutination of erythrocytes in any of the tubes, characterizing the absence of hemagglutinins in the extracts. To the contrary, the erythrocytes were completely hydrolyzed. In this way, the assay was repeated using an extract previously heated at 100 °C for 5 min, thus determining the presence of hemolysins, due to the non-hydrolysis of the erythrocytes. Accordingly, thermal treatments above 100 °C such as cooking and roasting, are sufficient to eliminate this anti-nutritional component, present in the *pequi* skin flours. On the other hand, hemolysins are important compounds from the biotechnological point of view, given the potential bactericidal and anticancer activities (YULDASHEVA et al., 2005).

From the nutritional point of view, the absence of an α-amylase inhibitor in the *pequi* peel flour was interesting because these antinutritional factors are responsible for reducing the rate of starch digestion in the mouth and small intestine, affecting the release of glucose into the blood (FUNKE;

Table 1. Moisture, protein, lipid, ash, total dietary, soluble and insoluble fiber value (dwb) of *pequi* peel flours as a function of soaking time (h).

Component [g (100 g)$^{-1}$]	Soaking time (h)			
	0	24	48	72
Moisture	$1.54^{a} \pm 0.03$	$0.82^{b} \pm 0.16$	$0.58^{bc} \pm 0.02$	$0.48^{c} \pm 0.10$
Protein	$2.65^{b} \pm 0.08$	$3.40^{a} \pm 0.10$	$3.39^{a} \pm 0.09$	$3.48^{a} \pm 0.04$
Lipid	$1.32^{c} \pm 0.02$	$3.97^{a} \pm 0.05$	$3.76^{b} \pm 0.07$	$3.93^{a} \pm 0.03$
Ash	$2.09^{a} \pm 0.02$	$1.21^{b} \pm 0.01$	$1.16^{b} \pm 0.07$	$1.11^{b} \pm 0.04$
Total dietary fiber	$59.67^{c} \pm 0.97$	$78.17^{b} \pm 0.87$	$91.0^{a} \pm 0.75$	$91.47^{a} \pm 0.82$
Soluble dietary fiber	$24.27^{c} \pm 1.01$	$31.47^{b} \pm 1.01$	$36.5^{a} \pm 0.6$	$37.0^{a} \pm 0.61$
Insoluble dietary fiber	$35.4^{c} \pm 0.1$	$46.7^{b} \pm 0.17$	$54.4^{a} \pm 0.17$	$54.47^{a} \pm 0.21$

Means followed by the same letter in the same row are not significantly different ($P < 0.01$) according to Tukey's test.

Table 2. Hemagglutinating activity, α-Amylase and Trypsin inhibitor (UI), and Phytic acid content of *pequi* peel flours as a function of soaking time (h).

Component [g (100 g)$^{-1}$]	Soaking time (h)			
	0	24	48	72
Haemagglutination	-	-	-	-
α-Amylase inhibitor	-	-	-	-
Trypsin inhibitor	1.50 ± 0.56	-	-	-
Phytic acid[1]	$0.40^{a} \pm 0.03$	$0.33^{a} \pm 0.05$	$0.11^{b} \pm 0.002$	0.00^{b}

[1]Means followed by the same letter in the same row are not significantly different ($P < 0.01$) according to Tukey's test

MELZING, 2006). This is common in cereals such as wheat, barley, corn, rye and rice; in leguminous seeds such as beans and also in fruit such as mango. After forming the inhibitor-substrate complex, this is quite thermally stable and can even resist cooking (MIZUBUTI; IDA, 1999). The presence of α-amylase is necessary from a technological point of view, since when mixed with other flours for the elaboration of bread, cakes and pasta in general, it can act on the α 1,4 bonds of the starch producing oligosaccharides, which serve as a fermentable substrate for yeast, conferring shorter times on the fermentation period (DAMODARON; PARKIN; FENNEMA, 2010).

On the other hand, the trypsin inhibitor content decreased considerably after soaking. The reduction of trypsin inhibitors by this process may be due to the water-soluble nature of the inhibitors that permits migration from the flour into the soaking medium (EL-HADY; HABIBA, 2003) or to the extraction of ions essential for the inhibitors activity (PIERGIOVANNI; GATTA, 1994). This result calls attention to the possibility of consuming non-macerated *pequi* peel flour, since this kind of inhibitor can cause indigestibility if consumed in large amounts, thus prejudicing the gastrointestinal tract of the organism (SGARBIERI, 1996). The ingestion of large amounts of trypsin inhibitor leads to a super-production of pancreatic proteolytic enzymes, which can represent an important loss in sulfur amino acids (LIENER, 1994). Trypsin inhibitors are usually inactivated by thermal treatment (VEN; MATSER; BERG, 2005). However, this procedure is costly and may result in the loss of essential amino acids, thus, soaking should be an option to reduce costs and preserve nutritional quality.

There was a significant reduction in the phytic acid content with increased maceration time. After three days, the presence of phytic acid was no longer detected (Table 2), indicating that the process was efficient to eliminate this type of antinutritional substance. According to Hossain and Becker (2002), the decrease in the phytic acid content during the soaking of *Sesbania* spp. seed flour was caused by leaching of the phytate ions into the water due to the influence of a concentration gradient, and the loss is a function of the cell permeability acquired by the grain. Oliveira et al. (2001) observed a reduction in the phytate content (85%) in beans using soaking. The phytate content of oat flour was found to be 0.74 g (100 g)$^{-1}$ and that of rice flour 0.55 g (100 g)$^{-1}$ (GARCÍA-ESTEPA; GUERRA-HERNÁNDEZ; GARCÍA-VILLANOVA, 1999), values higher than found in the non-soaked *pequi* peel flours. On evaluating the bioavailability of iron in meals consisting of rice, beans, meat and tomato, Fantini et al. (2008) reported that the values of 0.09 g (100 g)$^{-1}$ and 0.2 g (100 g)$^{-1}$ for Phytic acid played no inhibitory effect.

The structure of the phytic acid suggests a tremendous chelating potential, so it is believed to interfere with mineral bioavailability in humans (GRAF; EATON, 1990). On the other hand, phytic acid has been reported to possess various significant health benefits including its potential as antioxidant and anticancer agent (JARIWALLA, 2001). Anticancer activity of phytic acid has been demonstrated both *in vivo* and *in vitro*, which is based on the hypothesis that exogenously administered phytic acid may be internalized, dephosphorylated to IP(1-5) and thus can inhibit cell growth. Furthermore, due to its relatively high binding affinity for iron, phytic acid arouses great interest as a potential food preservative (STODOLAK et al., 2007).

Pequi peel flour presented high levels of total phenols and tannins, whose content decreased slightly with soaking by 24 h (Table 3). Reduction of phenols and tannins was around 8.5% and 19.0%, respectively, probably due to the low solubility of these compounds in water (WATERMAN; MOLE, 1994). This low reduction may be also related to the binding of polyphenols with other organic substances, such as proteins or carbohydrates, forming insoluble aggregates (SAXENA; CHADHA; SHARMA, 2003).

Tannins also present toxic activity, probably due to their ability to bond to the proteins and other macromolecules. They are considered to be nutritionally undesirable since they precipitate proteins, inhibit digestive enzymes and affect the use of the vitamins and minerals (MONTEIRO et al., 2005). However, they are capable of reacting with free radicals, which can be formed naturally by the metabolism, during physical exercise or even by exposure to sunlight. They also show the capacity to scavenge radicals such as the hydroxyl, superoxide and peroxyl radicals, compounds of importance in the pro-oxidant cell state (PIMENTEL; FRANCKI; GOLLUCKE, 2005).

3.3 In vitro protein and starch digestibility

The value of *in vitro* protein digestibility of soaked flour for 24 h was 6.2% lower than the non-soaked one (Table 3). The small reduction in protein digestibility on PPFS may be related to the polyphenol content of samples, since high-molecular-weight structures, usually designated as tannins, have the ability to interact with proteins. Hydrogen bonding is thought to be augmented by hydrophobic phenomena in which the aromatic nuclei of the tannin interact with hydrophobic regions in proteins, rendering them unavailable for absorption by the human body (HASLAM, 1989). The results agree with those of Oliveira et al. (2001) who observed reduction in protein digestibility of soaked common bean compared to non-soaked ones.

Pequi peel flour presented reduced starch digestibility, but the soaking process was effective for improving 24% of its value (Table 3). Starch digestibility depends on the amount of susceptible starch to the α-amylase and amyloglucosidase activity and on the structural characteristics of the starch grain. The amylose/amylopectin rate and the molecular structure of amylopectin can also interfere with starch digestibility

Table 3. *In vitro* protein and starch digestibility (%), total phenols and tannins (mg g^{-1} of flour) in *pequi* peel flours as a function of soaking time (h).

Component	Soaking time (h)	
	0	24
Protein digestibility	79.80 ± 0.25	74.82 ± 0.25
Starch digestibility	36.19 ± 1.28	44.94 ±1.07
Total Phenols	85.60 ± 0.78	78.34 ± 0.23
Tannins	14.51 ± 0.12	11.75 ± 0.31

(SANDHU; LIM, 2008). High amylose content leads to greater amount of resistant starch, which negatively influences the digestibility (THARANATHAN; MAHADEVAMMA, 2003).

4 Conclusions

Soaking constitutes a viable process with several technological applications. In this study, soaking was important to reduce antinutritional factors, such as trypsin inhibitors and phytic acid, and improve starch digestibility, thus conferring improvement of nutritional characteristics to the *pequi* peel flour. Soaking, which consists of a simple and inexpensive treatment, was efficient to improve the nutritional characteristics of the *pequi* peel flour, opening up possibilities for its use in food formulations.

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