Chemical constituents and technological functional properties of acerola (*Malpighia emarginata DC.*) waste flour

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Abstract

Acerola is a fruit that can be consumed in the form of juice and pulp. However, during its processing, a large amount of waste is generated (seed and bagasse). Adding value to these by-products is of great interest, since their use can enrich foods with nutrients and fiber. In this study, we performed phytochemical screening, determined the proximate and mineral composition, bioactive compounds and the technological functional properties of acerola seed flour and acerola bagasse flour. Seeds were dried in a ventilated oven at ± 45 °C and the bagasse was lyophilized. Samples were ground, stored in flasks protected from light. Phytochemical screening revealed metabolites of nutritional and pharmacological interest and no potentially toxic substances in the flours. Seed flour and bagasse flour showed high levels (g 100 g^{-1} of dry matter – DM) of soluble fiber: 4.76 and 8.74; insoluble fiber: 75.76 and 28.58, and phenolic compounds: 4.73 and 10.82, respectively. The flours also showed high absorption of water, oil and emulsion stability, presenting potential for inclusion in meat products and bakery products.

Keywords: seed; bagasse; chemical characterization.

1 Introduction

Most substances of interest in fruit are generally found in parts often discarded, such as peel, bagasse and seeds. Studies have shown that agro-industrial residues often have high contents of protein, carbohydrate, and polyunsaturated lipids, besides metabolic or physiological elements, which are beneficial to the human body, such as fibers, phenolic compounds, and antioxidant substances (NUNES et al., 2009). Thus, some residues can be used in low-cost food production. Despite numerous benefits, however, these parts are usually discarded in most consumption purposes, which results in a huge amount of waste. Moreover, some residues can also contain toxic or anti-nutritional substances such as protease inhibitors.

The processing of acerola juice generates residues of approximately 40% of production volume, consisting mainly of seeds and bagasse (peel and leftover pulp) (LOUSADA JÚNIOR et al., 2006). Considering that these residues are characterized as potential pollutants, creating alternatives for reducing this amount of residues is of great importance. However, in order to properly harness and add value to the material, it is essential to determine its chemical composition based on scientific and technological investigations.

Only a few studies about the chemical components of these residues were found in the literature review. While studying the chemical composition of acerola seed flour, Aguiar et al. (2010) observed contents of: protein (18.70); ether extract (4.33); ash (0.49); total fiber (29.29) and vitamin C (0.08) in 100 g^{-1} of dry matter (DM). In acerola residues containing seeds and bagasse, Sousa et al. (2011) reported contents of: proteins: 1.98 g 100 g^{-1} DM; ether extract: 4.30 g 100 g^{-1} DM; ash: 0.66 g 100 g^{-1} DM; vitamin C: 0.11 g 100 g^{-1} DM; phenolic compounds: 2.97 g 100 g^{-1} DM, carotenoids: 1.06 mg 100 g^{-1} DM and anthocyanins: 10.59 μg 100 g^{-1} DM.

Knowing the technological functional properties of food is essential for food industry, since they are physical and chemical aspects that contribute to achieve features desired by consumers. Thus, potential commercial by-products should be tested for these properties in order to define their intended use (NAVES et al., 2010). No studies on the technological functional properties of acerola were found in either seed flour or bagasse flour.

Therefore, this study aimed to perform the phytochemical screening, determine the proximate and mineral composition, bioactive and/or antinutritional compounds in both acerola seeds flour and acerola bagasse flour, analyzing the technological functional properties of these flours in order to ensure their incorporation into foods.

2 Materials and methods

2.1 Sampling and sample preparation

Residues (seeds and bagasse) of acerola *Malpighia emarginata* DC Variety BRS 238 Frutacor from pulp extraction were supplied by a fruit pulp company located in Perdões, State of Minas Gerais, Brazil. Three batches were collected and mixed into a single one to be used in all experiments. Seeds were washed with running water, scrubbed to remove any residual pulp and weighed still wet. The bagasse was frozen at –18 °C, and then seeds and bagasse were dehydrated as described below.

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2.2 Dehydrating acerola residues and preparing the flours

Seeds were placed in ten fine mesh metallic material baskets containing 400 g, and dehydrated at a temperature of 45 °C in a forced air oven. Readings for loss of seed weight were performed every 24 hours. The baskets were removed from the oven, weighed on a 3 decimal place semi analytical balance and quickly returned to the oven until constant weight. The bagasse was freeze-dried in twelve beakers containing 250 g portions, and protected from light until constant weight.

After dehydration, seeds and bagasse were ground in a knife mill (TE 631 Tecnal®) for 3 minutes, packaged in hermetically sealed vials and protected from light in a refrigerator. Then, the flours underwent analysis in triplicate.

2.3 Analyses

Particle size of flours

A sieve shaker was used to determine the particle size of both acerola seed flour (ASF) and acerola bagasse flour (ABF). A 30 g-portion of each sample was sieved for 10 minutes, using a set of five round sieves, openings 10, 40, 60, 80 and 100-mesh and a base. The contents retained in each sieve were weighed and expressed in retention percentages.

Phytochemical screening

Acerola seed flour (ASF) and acerola bagasse flour (ABF) underwent phytochemical screening process. Specific reagents were applied to each chemical group in chemical reactions resulting in change of color or precipitate formation, or both, which is particular for each substance class as tannins, catechins, flavonoids, cardiac glycosides, lactones, carotenoids, steroids, depsides and depsidonas, saponins and alkaloids (MATOS, 1997).

Proximate and mineral composition

Proximate composition (moisture, ether extract, crude protein (N × 6.25), ashes, dietary fiber and nitrogen-free extract) was determined with the method of the Association of Official Analytical Chemists - AOAC (2005).

For quantifying minerals (Fe, Zn, Mn, Cu, Ca, Mg, P, K and S), ASF and ABF were subjected to nitric perchloric digestion in digester blocks with temperature control. P and S were determined by colorimetry, K by flame photometry and Ca, Mg, Cu, Mn, Zn and Fe by atomic absorption spectroscopy (MALAVOLTA; VITTI; OLIVEIRA, 1997).

Vitamin C

Vitamin C was determined by the colorimetric method described by Strohecker and Henning (1967). Ascorbic acid was extracted with 0.5% oxalic acid, in the ratio 1:30 (w/v). After filtration, vitamin C was dosed in the extract, using ascorbic acid as standard.

Nitrate

Nitrate was extracted with distilled water at 45 °C, in the ratio 1:20 (w/v). In the dosage, a complex is formed by nitration of salicylic acid under highly acidic conditions, read at 410 nm in basic solutions (pH above 12). Potassium nitrate was used as standard (CATALDO et al., 1975).

Saponin

Saponin was extracted with 99% ethanol, in the ratio 1:10 (w/v), under stirring for 1 hour at room temperature. The extract was centrifuged at 10,000 × g/10 minutes and the supernatant was collected. The level of saponin was determined colorimetrically (λ = 430 nm) by reaction with anisaldehyde in acidic media, using digitonin as standard (BACCOU; LAMBERT; SAUVAIRE, 1977).

Oxalic acid

The method of Loures and Jokl (1990) was applied for oxalic acid quantification, in which oxalic acid was extracted with hot HCl, in the ratio 1:5 (w/v) and the precipitate quantified by titration with calcium oxalate potassium permanganate.

Trypsin inhibitors

Trypsin inhibitors were extracted with NaOH 0.1 mol L^{-1} with magnetic stirring in the ratio 1:20 (w/v) (KAKADE et al., 1974). After centrifugation, an aliquot of the supernatant was used in the enzyme assay. Trypsin activity was determined according to the methodology proposed by Erlanger, Kokowsky and Cohen (1961).

Phytate

Phytate was extracted with HCl in the ratio 1:20 (w/v) for one hour at room temperature, the pH adjusted to 6.0 and the sample centrifuged. The extract was eluted through an anion exchange resin to remove the inorganic phosphorus compounds and other interferences. The phytate content was determined using the Wade reagent and sodium phytate as a standard (LATTA; ESKIN, 1980; FRÜHBECK et al., 1995).

Phenolic compounds

The extraction of phenolic compounds was performed with 50% methanol in reflux for three consecutive times at 80 °C, in the ratio 1:25 (w/v). The extracts were combined, evaporated to 25 mL and subjected to the determination, using the Folin-Denis (ASSOCIATION..., 2005). The tannic acid was used as standard.

Technological functional properties

In order to determine technological functional properties, the Mallory Robot Classic mixer was used at its maximum speed.

Absorption of water and oil

The ASF and ABF were suspended in oil or water, homogenized for 60 seconds with mixer and then centrifuged

at 2,000 × g for 10 minutes. The volume of the supernatant was measured and the amount of absorbed water or oil was multiplied by their respective specific gravity to convert into grams (OKEZIE; BELLO, 1988).

Emulsion stability

The ASF and ABF were dispersed in distilled water and the oil was gradually added at moderate agitation for 30 seconds. Then, the solution was stirred for another 60 seconds to homogenize the system. The homogenate was transferred to a beaker and changing volume of the foam, oil and aqueous phase was determined after 0.5, 2.0 and 6.0 hours (OKEZIE; BELLO, 1988).

Foam volume

The ASF and ABF were suspended in distilled water and homogenized with mixer for 3.5 minutes. Subsequently, the mixture was transferred to a beaker for determining volumes of foam at different times (0, 30, 60 and 120 minutes after the agitation for 3.5 minutes). The volume of the foam remained over time was calculated considering 100% the specific foam volume at time zero (WANG; CABALLERO-CORBOBA; SGARBIERI, 1992).

Emulsifying activity

It was weighed 5 g of flour and suspended in distilled water (40 mL) and the pH was adjusted to 7.0 with NaOH or HCl. Afterwards, the suspension was stirred for 15 minutes. The pH of the suspension was checked and adjusted again and the final volume was completed to 50 mL with distilled water. To this suspension was added 50 mL of soybean oil, keeping it under stirring for 3 minutes. The new emulsion obtained was centrifuged at 2,000 × g for 5 minutes (DENCH; RIVAS; CAYGILL, 1981). After visual reading of the graduated centrifuge tubes, the emulsifying activity was calculated by dividing the height of the layer by the total height of the emulsifying fluid x 100.

3 Results and discussion

Most flour particles were retained on sieves sized 40 mesh (0.425 mm) to 80 mesh (0.180 mm). According to Zanotto and Bellaver (1996), the uniformity index indicates the relative proportion between coarse, medium and fine particles, which are defined according to diameters larger than 2 mm, between 2 and 0.60 mm, and lower than 0.60 mm, respectively. Therefore, ASF and ABF particles were classified as fine.

The results of phytochemical screening indicated the presence of different metabolic groups of nutritional and pharmacological interest in both flours, such as tannins, flavonoids, depsides, depsidones, and coumarins. Cardiac glycosides, steroids and alkaloids were not detected in any of the flours.

Both flours showed low contents of crude protein (ASF: 8.51 g 100 g^{-1}; ABF: 11.55 g 100 g^{-1}), ether extract (ASF: 5.27 g 100 g^{-1}; ABF: 5.61 g 100 g^{-1}) and ashes (ASF: 1.65 g 100 g^{-1}; ABF: 3.46 g 100 g^{-1}) (Table 1).

While studying ASF, Aguiar et al. (2010) found 18.70 g 100 g^{-1} of proteins in dry matter (DM), a far higher level than registered in this study. Regarding ether extract, Aguiar et al. (2010) and Lousada Júnior et al. (2005) reported 4.33 g 100 g^{-1} DM and 3.20 g 100 g^{-1} DM in ASF, respectively. Sousa et al. (2011), while analyzing residues of acerola (seeds, small amount of peel and bagasse) observed 3.59 g 100 g^{-1} DM of ether extract.

The content of ash detected in ASF and ABF were higher than reported by Aguiar et al. (2010) that recorded 0.49 g 100 g^{-1} DM in ASF, and by Sousa et al. (2011), who found 0.55 g 100 g^{-1} DM in residues of acerola (seeds, small amount of peel and bagasse).

ASF showed a higher content of insoluble fibers (75.66 g 100 g^{-1} DM), whereas ABF had higher content of soluble fibers (8.74 g 100 g^{-1} DM). It is not possible to compare the results of fibers of the present study with the literature, because the methodology used was not the same. These differences in the proximate composition are probably due to several factors affecting the crops and to fruit variety. In addition, the differences are due to the use of all residues from acerola processing and not only seeds or bagasse.

IOM (INSTITUTE..., 2005) recommends consuming 25 to 38 g fibers per day. Thus, consuming approximately 17 to 26 g of ASF and 38 to 57 g of ABF supplies half the recommended intake. Thus, the presence of high contents of fibers in both ASF and ABF may contribute to a better use of these residues, since the evolution of scientific knowledge allowed us to conclude that dietary fiber ingestion is related to health maintenance and prevention of some diseases such as constipation, colon disease, diverticular disease, and colon cancer as well as systemic diseases such as hyperlipidemia, cardiovascular disease, diabetes, obesity (INSTITUTE..., 2005). Since several studies have demonstrated fiber effectiveness in the treatment and prevention of some diseases, ASF and ABF may provide beneficial effects to the human body. Nitrogen-free extract consists primarily of sugars. Thus, the highest content was found in ABF.

Table 1. Proximate composition in g 100 g^{-1} of dry matter of acerola seed flour (ASF) and acerola bagasse flour (ABF).

Sample	Moisture	Crude Protein[1]	Ether Extract[1]	Ashes[1]	Dietary Fiber[1]		NFE[1]*
					Soluble	Insoluble	
ASF	9.20 ± 0.18	8.51 ± 0,11	5.27 ± 0.18	1.65 ± 0.05	4.76 ± 0.98	75.66 ± 1.58	4.15 ± 1.48
ABF	10.90 ± 0,40	11.55 ± 0,09	5.61 ± 0.98	3.46 ± 0.05	8.74 ± 0.53	28.58 ± 1.24	42.06 ± 2.49

[1]Data represent the mean of triplicate determinations ± standard deviation. *NFE: Nitrogen-free extract.

Minerals participate in several functions in the human body, such as the regulation of metabolism. Calcium and potassium presented the highest contents (Table 2). Calcium showed higher content in ASF (264.32 mg 100 g^{-1} DM) whereas potassium content was higher in ABF (791.25 mg 100 g^{-1} DM). ASF also showed higher levels of copper, manganese, iron and zinc.

Iron is an important mineral for health of the human body, as it helps to form red blood cells. However, despite its abundance in food, iron deficiency anemia is still common today. The recommended daily intake of iron for adults is 18 mg (INSTITUTE..., 2002), therefore, taking about 85 g of ASF would be sufficient to satisfy this need. But, it is important not to infer that all the iron present in food is absorbed by the body, so it can not refer to the ASF as a food rich in iron, it is necessary to check the availability of this mineral.

Mineral results in ASF differs from those reported by Aguiar et al. (2010), who used plasma mass spectrometry to register calcium: 46.09 mg 100 g^{-1} DM, potassium: 45.68 mg 100 g^{-1} DM, magnesium: 24.55 mg 100 g^{-1} DM, phosphorus: 0.09 mg 100 g^{-1} DM, copper: 1.66 µg 100 g^{-1}, manganese: 0.82 mg 100 g^{-1} DM, iron: 41.09 mg 100 g^{-1} DM, and zinc: 0.10 mg 100 g^{-1} DM. These differences are probably due to many factors affecting the crops, to acerola variety (acid variety), and even to the experiment methods.

The vitamin C content of the ASF and of the ABF were 457.32 and 10,282.45 mg 100 g^{-1} DM, respectively. Sousa et al. (2011) while studying the residue of acerola (seed, small amount of bark, and pulp rest) recorded a significantly lower content of vitamin C: 89.55 mg 100 g^{-1} DM and Correia et al. (2012) in acerola residue (seed, small amount of bark, and pulp rest) reported 2,748.03 mg 100 g^{-1} DM, higher than the ASF and lower than the ABF of this work. Aquino et al. (2010), though, verified 10,448.15 mg 100 g^{-1} DM of vitamin C in the acerola residues flour (seed, small amount of bark, and pulp rest), the approximate content of the ABF. The recorded differences are probably related to the use of all the residue of the acerola processing, and not only the seeds or the bagasse.

The recommended daily intake of vitamin C is 90 mg for men and 75 mg for women (INSTITUTE..., 2000). Thus, the ingestion of 19.68 g and 16.40 g of ASF or 0.88 g and 0.73 g of ABF represents an intake of 90 mg and 75 mg of vitamin C, respectively, which would practically supply the recommended daily need. The ASF and the ABF are, thus, good sources of vitamin C with potential for use, and can be used to enrich foods, cosmetics and contribute to its antioxidant activity.

In addition to the nutrients present in these flours, antinutritional and bioactive compounds can also be found. Although some are health hazards, others provide benefits depending on the concentration, such as polyphenols and saponins.

ABF showed the highest content of nitrate, whereas ASF had the highest levels of saponins (Table 3). The acceptable daily intake of nitrate set by the World Health Organization is 5 mg kg^{-1} body weight. Thus, a 50 kg person could ingest 250 mg of nitrate, which is only found in amounts above 312 g ASF and 125 g ABF. Hence, nitrate contents in these flours pose no health hazards.

The major adverse effects of saponins are changes in reproduction and growth and decrease in nutrient absorption due to changes in cell membrane permeability (FRANCIS et al., 2002). No references to acceptable daily intake of saponins were found in the available literature.

Oxalic acid and trypsin inhibitors were not detected in the flours (Table 3).

ASF showed the highest content of phytate (Table 3). Phytate levels in ASF and ABF are within the range reported for groups of cereals and legumes, which varies from 0.19 g to 1.37 g 100 g^{-1} (JOUNG et al., 2004).

Phytate can act as anti-nutritional factor, as it chelates minerals and prevents bioavailability (FREDLUND et al., 2006); however, some studies have suggested beneficial effects for health, e. g. protective role in carcinogenesis (NORAZALINA et al., 2010); antioxidant function (FREDLUND et al., 2006) and glycemic control (KIM et al., 2010).

No levels of nitrate, saponins, oxalic acid, trypsin inhibitors or phytate were found in acerola or its fractions in the published literature for comparison.

The content of phenolic compounds was high in both flours (Table 3). The major adverse effects of phenolic compounds are the inhibition of certain digestive enzymes such as trypsin, composition of protein complexes and decrease in protein digestibility (SATHE, 2002). No references as for

Table 2. Mineral composition in mg 100 g^{-1} of dry matter in acerola seed flour (ASF) and acerola bagasse flour (ABF).

Minerals[1]	ASF	ABF
Calcium	264.32 ± 9	86.98 ± 5.61
Potassium	178.96 ± 5,51	791.25 ± 26.72
Magnesium	77.09 ± 0	106.62 ± 6.48
Phosphorus	99.12 ± 0	151.52 ± 6.48
Copper	0.81 ± 0.01	0.44 ± 0
Manganese	0.21 ± 0.01	0.11 ± 0.03
Iron	21.15 ± 0.59	5.88 ± 0.98
Sulfur	115.64 ± 6,36	140.29 ± 9.83
Zinc	4.24 ± 0.16	1.72 ± 0.07

[1]Data represent the mean of triplicate determinations ± standard deviation. Moisture content of flours in g 100 g^{-1}: ASF: 9.20 and ABF: 10.90.

Table 3. Bioactive compound contents in g 100 g^{-1} of dry matter in acerola seed flour (ASF) and acerola bagasse flour (ABF).

Samples	Nitrate[1]	Saponins[1]	Oxalic acid[1]	Tripsin inhibitors[1]	Phytate[1]	Phenolic compounds[1]
ASF	0.08 ± 0,02	0.49 ± 0.01	ND*	ND*	0.23 ± 0,04	4.73 ± 0.07
ABF	0.20 ± 0.06	0.26 ± 0.01	ND*	ND*	0.18 ± 0.04	10.82 ± 0.09

[1]The data are mean of triplicate determinations± standard deviation. *ND: Not Detected. Moisture content of flours in g 100 g^{-1}: ASF: 9.20 and ABF: 10.90.

Table 4. Stability of emulsion: mean volumes of foam, oil and aqueous phase in times: 0.5; 2.0 and 6.0 hours after stirring of acerola seed flour (ASF) and acerola bagasse flour (ABF).

Samples	Time after stirring (hours)	Mean volumes (mL)		
		Foam[1]	Oil[1]	Aqueous Phase[1]
ASF	0.5	1.00 ± 0.01	17.67 ± 2.08	37.33 ± 1.15
	2.0	0	12.67 ± 2.08	41.33 ± 0.58
	6.0	0	11.33 ± 0.58	42.67 ± 0.58
ABF	0.5	0	53.07 ± 2.48	2.33 ± 2.08
	2.0	0	47.67 ± 2.89	8.33 ± 3.06
	6.0	0	45.33 ± 1.53	10.33 ± 1.53

[1]Data represent the mean of triplicate determinations ± standard deviation.

acceptable daily intake of phenolic compounds were found in the published literature. Thus, the use of these flours in feeding must be performed with caution, owing to the high levels of phenolic compounds.

The mean percentages of water and oil absorption were 300% and 373.33% in ASF, respectively. Oil absorption in ASF was higher than water absorption, possibly due to the presence of a higher number of hydrophobic groups which can bind to oil. In ABF, water and oil absorption were 591.67% and 320%, respectively. This high rate of water absorption may be related to the high content of soluble fibers in this flour, as these fibers have high capacity of water retention. Therefore, ABF can be used in meat and bakery products, as it enables more addition of water to the dough thus improving its handling characteristics.

Cheftel, Cuq and Lorient (1985) suggested that water absorption and water retention play an important role in the texture quality of a wide variety of foods, particularly meat products. Similarly, the capacity of oil absorption is also required in meat formulation, meat substitutes and analogues (ABBEY; IBEH, 1988). The technological functional properties of protein-water interaction (water retention) are important in the formulation of certain foods, especially meat products (cold meats, sausages, meatballs, and savory nibbles) in which proteins also play an important role in thickening, providing greater consistency to the product (CHEFTEL; CUQ; LORIENT, 1985). Thus, both flours analyzed in this study showed satisfactory values which make them function in industrialized food products requiring this feature.

ASF and ABF showed no foam formation either during the mixing process or after. According to Cheftel, Cuq and Lorient (1985) an explanation for no foam formation is related to protein content, as foam formation requires flexible-chain proteins, which are poor in secondary and tertiary structures that adapt quickly in the air-liquid phase. Furthermore, these proteins must have the possibility to form hydrophobic bonds on their surface. Therefore, we believe that the proteins had their structures changed in both flours. Stability is important in formulations requiring foam formation, such as meringues, mousses and cakes (OKEZIE; BELLO, 1988; FENNEMA, 2000).

Emulsifying properties are technological functional properties important in food formulations such as meat products, mayonnaise, sauces, soups, cream cheese, and others (CHEFTEL; CUQ; LORIENT, 1989). According to this study, the emulsifying activity of ASF and ABF were 23.3% and 3.39%, respectively. For emulsion stability, the mean volumes of foam, oil and aqueous phase in ASF and ABF are listed in Table 4.

However, when the stability of each phase was analyzed (foam, oil, and water) considering the time after mixing process, we observed a slight reduction in oil volume and a little increase in water volume over time. Hence, ASF and ABF showed high emulsion stability, as water and oil phases were not completely separated 6 hours after sample mixing.

It is greatly difficult to compare the results obtained in this study with others mentioned in previous researches due to the lack of both methodology standardization and conditions for assessing emulsion properties, since they are affected by several factors such as pH, temperature, type and geometry of the equipment used in the experiment, rate of oil addition, and emulsifying properties of proteins (FENNEMA, 2000).

4 Conclusions

ASF and ABF showed high levels of dietary fibers and minerals, as well as high emulsion stability and water and oil absorption capacity. Therefore, the flours have potential to be incorporated into meat and bakery products.

Among antinutritional factors, phenolic compounds stood out with high levels. Since they can bring benefits to the human body and also be related to nutritional deficiencies, these flours should be used with caution.

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