Ciência Rural, Santa Maria, v.48:09, e20180076, 2018

http://dx.doi.org/10.1590/0103-8478cr20180076

ISSNe 1678-4596

FOOD TECHNOLOGY

Passion fruit and apple: from residues to antioxidant, antimicrobial and anti-Alzheimer's potential

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ABSTRACT: *The waste in the fruit production chain, including the juice and pulp industries, produces large quantities of leftover husks, seeds and bagasse. This volume of waste generates huge environmental and economic impact. The objective of this research was to determine the potential of using residues from passion fruit (Passiflora edulis) and apple (Malus domestica) varieties in the production of functional flours. Passion fruit flour showed greater reduction of DPPH ($EC_{50\%}$: 50.4µg/mL) radicals, showing antioxidant potential, as well as a more efficient inhibition of Staphylococcus aureus (71.3±1.2µg/mL), with a modest; however efficient, inhibition of acetylcholinesterase (10%). All Apple flours were good antioxidants and the fuji apple flour stood out inhibiting Pseudomonas aeruginosa (78.6±3.1µg/mL). All the residues showed potential for use as a functional product either as a source of antioxidants, a natural (antimicrobial) preservative for dry foods or supplementary use by patients with Alzheimer's disease.*
Key words: *Acetylcholinesterase, agroindustrial residues, antioxidant, natural preservative.*

Maracujá e maçã: de resíduos ao potencial antioxidante, antimicrobiano e anti-Alzheimer

RESUMO: *O desperdício na cadeia produtiva de frutos, incluindo as indústrias de suco e polpa, produz grandes quantidades de resíduos, como cascas, sementes e bagaços. Esse volume de resíduos gera impacto ambiental e econômico. O objetivo deste trabalho foi determinar o potencial de aproveitamento de resíduos de maracujá (Passiflora edulis) e de variedades de maçã (Malus domestica) na produção de farinhas funcionais. A farinha de maracujá apresentou maior redução de radicais DPPH ($EC_{50\%}$: 50,4µg/mL), demonstrando potencial antioxidante, maior inibição de Staphylococcus aureus (71,3±1,2µg/mL) e da acetilcolinesterase (10%). Todas as farinhas de maçã foram boas antioxidantes e a fuji destacou-se inibindo Pseudomonas aeruginosa (78,6±3,1µg/mL). Todos os resíduos mostraram potencial para aproveitamento como produto funcional, seja como fonte de antioxidantes, conservante natural (antimicrobiano) para alimentos secos ou uso suplementar no tratamento de Alzheimer.*
Palavras-chave: *Acetilcolinesterase, antioxidantes, conservante natural, resíduos agroindustriais.*

Different extraction techniques, including the use of adjuncts of pressing and enzymatic systems, optimize the process, improve its performance and decrease solid waste from conventional extraction systems (DHILLON et al., 2013). However, juice and pulp industries discard about 70% of the fruits (husks, seeds and bagasse). This residual material cannot be used without further processing (by-product) and produces growing indexes of agro-industrial residues, generating undesirable environmental impacts. A strategy that can be used to minimize this problem is to promote the reuse of such fruit residues in human food, since they have fibers, vitamins and minerals, as well as bioactive compounds, mainly in the fruits and vegetables peels (DHILLON et al., 2013; ROTILI et al., 2013).

Fruits residues also have phyto-elements important for the health, which exercise biological functions on the metabolism besides nutrition; these compounds also have antioxidant activity, like vitamin C and polyphenols. Studies show that the consumption of foods with such compounds can help in the treatment of diabetes mellitus, by doing glycemic control, as well as in the significant decrease of the lipidemic index, promoting reduction of total cholesterol and LDL fraction (CAZARIN & SILVA, 2014). They are also capable of reducing free radicals, such as reactive species of oxygen and nitrogen, which come from the metabolic processes (mitochondria, cytoplasm) or from exogenous sources. Polyphenols are supporting acts in the

Received 02.05.18 Approved 07.16.18 Returned by the author 08.25.18
CR-2018-0076.R1

prevention of cardiovascular disease, because they promote the increase of oxidative defenses; they also improve the levels of acetylcholine in the brain, reducing the action of acetylcholinesterase (AChE) enzyme, which is involved in Alzheimer's disease (SOUSA et al., 2011).

Many polyphenols present in fruit peels are secondary metabolites which help control *in vivo* environmental stresses and combat phytopathogens; therefore these compounds usually exhibit antimicrobial activity (CAZARIN & SILVA, 2014). In view of the antimicrobial properties of phenolic compounds present in fruit husk residues, they could be employed as an auxiliary technology for the conservation of food, aiming to extend its shelf-life (SANDE et al., 2016).

This study aimed to determine the potential use of passion fruit and apple for the production of functional flours with biological activity. In this way, three types of apple (*Malus domestica*): Fuji, gala, green; and passion fruit (*Passiflora edulis*) were obtained in Belo Horizonte city, Minas Gerais State (Brazil). Those fruits were washed and sanitized (sodium hypochlorite 2% v/v, 15min). Apples were processed in a centrifuge for obtaining the bagasse and the passion fruit pulp has been manually removed to get the peels, which were crushed using a blender. Passion fruit and apple residues were dried at 60°C for 10h and 90°C for 30h, respectively. After this period, the dry residues were crushed (in a blender) and sieved (8 mesh) to obtain four homogeneous flours. Then ethanol extracts were prepared from the four flours by adding 150mL of ethanol (95%) to 50g of each flour. Those mixtures were shaken by 1.5h at 25°C. Then, the mixtures were filtered and the solvent was removed in rotary evaporator (SOUSA et al., 2011).

Evaluation of radical's (DPPH) capture antioxidant activity was evaluated using SANDE et al. (2016) methodology. This DPPH method consists in gently mixing ethanol solution of extracts in different concentrations (500-50µg/mL), with DPPH 0.3% in methanol. The reaction took place for 30min in a dark place. Absorbance at 492nm was measured. Ascorbic acid was used as positive control. A blank was made with ethanol, instead of the extracts. Extract concentration (µg/mL) required for capturing 50% of DPPH radical ($EC_{50\%}$) was obtained from a concentration x inhibition curve.

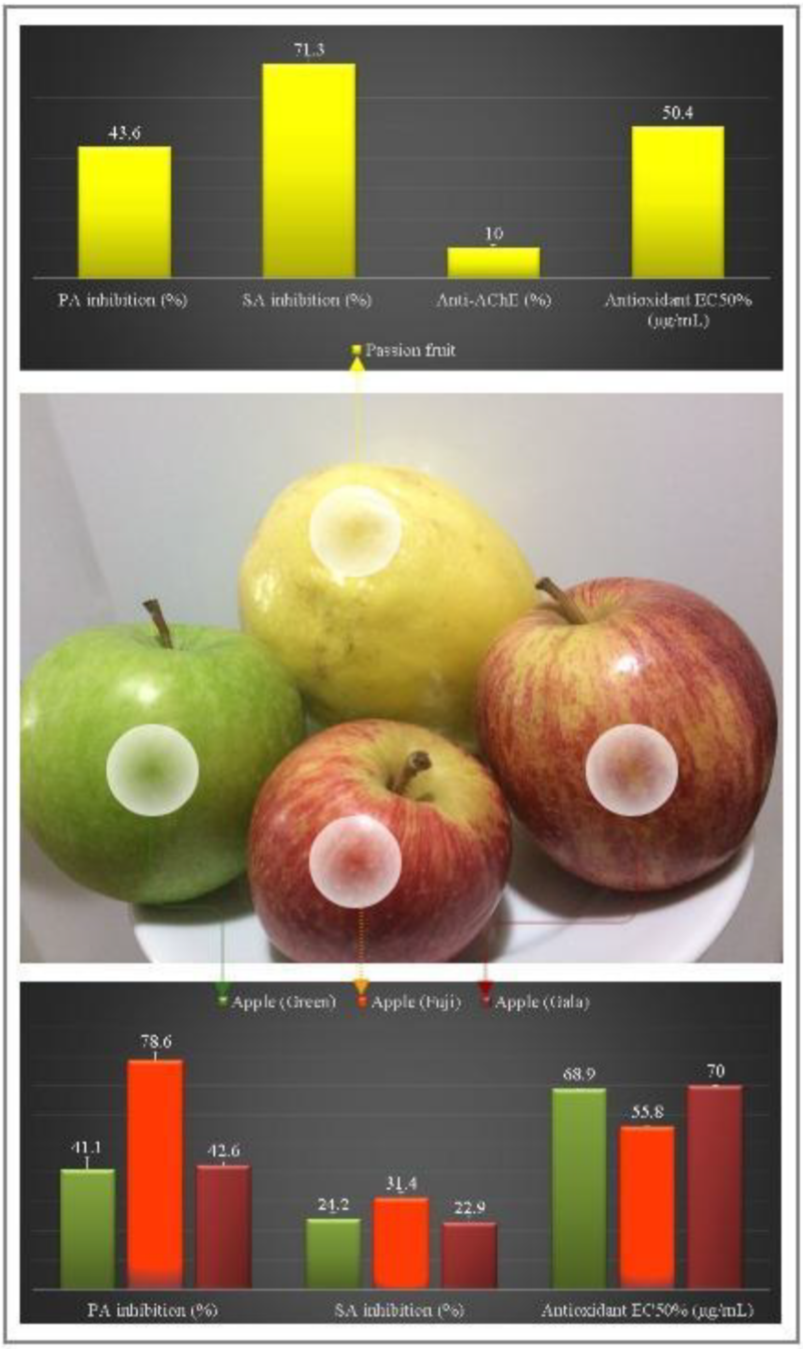
Antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were performed using Clinical and Laboratory Standards Institute (2012) methodology. Different concentration of extracts (1000-0.49µg/mL) were dissolved in dimetylsulfoxide (DMSO). The bacterial inocula had their concentration adjusted at 600nm, corresponding to 0.5 of the McFarland scale. Ampicillin was used as a positive control. Plates were incubated at 36°C for 24h, then absorbance readings were made at 490nm. The result is given as % of inhibition.

The inhibitory activity of AChE was conducted using ELLMAN et al. (1961) methodology. Eserin (10mg/mL) and DMSO were used as positive and negative controls, respectively. Extracts were dissolved in DMSO (10mg/mL), and 25µL of those mixtures were added to 25µL of acetylthiocholine iodide solution (15mM), 125µL of 5,5'-dithiobis-[2-nitrobenzoic acid] (3mM) and 50µL of Tris/HCl (50mM, pH 8.0). Absorbance measures at 405nm were made. Then AChE solution (0.22U/mL) was added and new absorbance measures were taken.

Statistical analyses were performed using Graphpad prism 5.02 and averages were compared by analysis of variance (ANOVA) and Dunett test (5% significance level). All values statistically different from each other are shown in the figure 1.

All of the flours presented antioxidant activity (Figure 1), with emphasis on the passion fruit (lower $EC_{50\%}$), demonstrating that a smaller mass of this residue already captures 50% of the free radicals, i.e, it presents greater efficiency and, therefore, its antioxidant activity is bigger. The second best antioxidant activity was presented by fuji apple (Figure 1). This antioxidant action can be based on the high content of ascorbic acid (vitamin C) and polyphenols, such as anthocyanin, present in the fruits (DHILLON et al., 2013; SANDE et al., 2016).

In relation to the antimicrobial assay (Figure 1), the passion fruit husk flour presented higher percentage of inhibition against *S. aureus*, being about 40% more active than the other samples. The fuji apple variety stood out against *P. aeruginosa*, inhibiting approximately 35% more than the other flours as well as against *S. aureus*, presenting higher percentage of inhibition among apple flours. Gala and green apple flours also were active against both tested bacteria (Figure 1). *S. aureus* is a relevant pathogen in quality control, because it produces pyrogenic toxins, which can cause toxic shock and are usually associated with food poisoning (FAGUNDES & OLIVEIRA, 2004). The capacity shown by passion fruit flour to fight this species highlights the potential of this residue as a natural preservative. The inhibition of *P. aeruginosa*, promoted especially by fuji apple, also reinforces this potential, since this bacteria can deteriorate foods, provoking alteration of flavor and/



PA Inhibition (%) = Inhibition of ***P. aeruginosa*** growth; SA Inhibition (%) = Inhibition of ***S. aureus*** growth; EC 50% = Extract concentration required to capture 50% of free radical (DPPH); Anti-AChE(%)= Inhibition of Acetylcholinesterase (%). * Apple flours did not show any acetylcholinesterase inhibition (Anti-Alzheimer). Value of ampicillin in inhibition of ***P. aeruginosa*** was 93.4 ± 1.3 and inhibition of ***S. aureus*** was 97.1 ± 1.2. All values shown in the figure are statistically different from each other. Yellow = Passion fruit; Green = Apple Green; Orange = Apple Fuji; Red = Apple Gala.

Figure 1 - Antimicrobial, antioxidant and anti-Alzheimer activities of passion fruit and apple residues.

or color. The antimicrobial activity of the flours prepared with the fruit residues is possibly attributed to the combined effects of bacterial adsorption of polyphenols, which lead to the rupture of the membrane (leakage of cellular contents), interfering with active transport and metabolic enzymes, provoking cytoplasmic coagulation, causing cell death (NEGI, 2012).

When evaluating the inhibitory activity of acetylcholinesterase, it was observed that only the passion fruit flour extract showed an inhibition of the enzyme AChE (10%) (Figure 1). Anti acetylcholinesterase agents commonly used in the treatment of Alzheimer's disease have limitations of use due to undesirable effects. Natural plant compounds that have anti-AChE action can be used on the search for new drugs with fewer side effects or as a supplementary therapy (as a dietary supplement) in the treatment of the disease, providing the patient with a better quality of life.

In this study passion fruit and fuji apple stood out as potential antioxidants, suggesting that the consumption of those flours can act in the prevention of cardiovascular diseases and of premature aging. Fuji apple and passion fruit flours were also highlighted as a natural antimicrobial agent. Its incorporation in food products, individually or in a mix, could prolong the products' shelf life. Lastly; although, the passion fruit flour presented a low inhibition of the AChE enzyme, this activity associated with its great antioxidant capacity points out its potential as a functional food, applicable in the supplementary treatment for patients with Alzheimer's disease. Results of this study showed the reuse potential of passion fruit and apple residues.

ACKNOWLEDGMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Centro Universitário de Belo Horizonte (UNIBH) and the Universidade Federal de Minas Gerais (UFMG) for the support.

DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

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