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Phenolic compounds in raw and cooked rice (*Oryza sativa* L.) and their inhibitory effect on the activity of angiotensin I-converting enzyme

Isabel Louro Massaretto [a], Márcio Fernando Madureira Alves [b], Nádia Valéria Mussi de Mira [a], Adriana Karaoglanovic Carmona [b], Ursula Maria Lanfer Marquez [a,*]

[a] Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, CEP 05508-900, São Paulo, SP, Brazil
[b] Department of Biophysics, Federal University of São Paulo, Rua 3 de Maio, 100, CEP 04044-020, São Paulo, SP, Brazil

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ABSTRACT

Whole rice has been widely studied due to the abundance of bioactive compounds in its pericarp. Some of the beneficial effects of these compounds on human health have been attributed to their antioxidant and other biological activities, such as enzyme inhibition. In this work, we evaluated the contents of total, soluble and insoluble phenolic compounds of 6 red and 10 non-pigmented genotypes of whole rice as well as their inhibitory effect on the activity of angiotensin I-converting enzyme (ACE). The effects of cooking on phenolics and their inhibitory activities were also investigated. Red genotypes showed high content of phenolics, mainly soluble compounds, at an average of 409.7 mg ferulic acid eq./100 g, whereas overall lower average levels (99.4 mg ferulic acid eq./100 g) at an approximate soluble/insoluble compound ratio of 1:1 were observed in non-pigmented rice. Pigmented rice displayed a greater inhibitory effect on ACE than non-pigmented rice. In fact, a significant correlation between the content of soluble phenolics and ACE inhibition was observed ($r = 0.8985$, $p < 0.05$). In addition to significantly reducing the levels of total phenolics and ACE inhibition, cooking altered the soluble/insoluble compound ratio, especially among red rice genotypes.

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1. Introduction

The angiotensin I-converting enzyme (ACE), distributed in many tissues and biological fluids, is a key component of the renin-angiotensin system (RAS) that controls blood pressure through a cascade of enzymatic reactions.

Despite some side effects that may lead to clinical treatment discontinuation, synthetic ACE inhibitors represent an important therapeutic approach to combating high blood pressure. Clinical management of hypertension requires lifelong commitment as well as drug therapy coupled with changes in lifestyle and the development of healthy eating habits (Chen et al., 2009). Although patients with pre-hypertension do not need anti-hypertensive drugs, they are likely to develop hypertension. Interestingly, they show a good response to non-medical treatment, including weight loss, smoking cessation, reduction in salt and fat intake, regular physical activity and moderate alcohol consumption (Chobanian et al., 2003).

Several food-derived peptides may contribute to reducing blood pressure, especially in pre-hypertensive patients, although flavanol-rich foods, including cocoa products, tea and red wine, as well as purified compounds, such as procyanidins and catechins have been reported to inhibit *in vitro* ACE activity (Actis-Goretta et al., 2006; Grassi et al., 2005; Ottaviani et al., 2006). The inhibitory effects on ACE activity and the activation of endothelial nitric oxide synthase (eNOS), constitute the probable mechanism since nitric oxide (NO) mediates endothelium-dependent vasodilation by acting as an intracellular and intercellular messenger (Chen et al., 2009).

Consumption of whole-grain rice, especially the red and black pigmented genotypes, has aroused considerable interest because of both its high content of flavonoids, such as proanthocyanidins and anthocyanins, and its wide range of phenolic acids and derivatives.

In general, the biological activities of phenolic compounds depend on their absorption and metabolism, which are closely related to their chemical structure ranging from simple phenolic structures to highly polymerized compounds. Therefore, from an analytical view, they can be classified into soluble (free and conjugated) and insoluble bound forms (Naczk and Shahidi, 2004; Zhou et al., 2004). In non-pigmented rice, soluble phenolics contribute with ~60% of total phenolics, whereas in pigmented

Abbreviations: Abz, ortho-aminobenzoic acid; ACE, angiotensin I-converting enzyme; Dnp, 2,4-dinitrophenyl.

* Corresponding author. Tel.: +55 11 3091 3684; fax: +55 11 3815 4410.
E-mail address: lanferum@usp.br (U.M. Lanfer Marquez).

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rice, due to the presence of high amounts of flavonoids, their contribution reaches 80% (Mira et al., 2009). In black rice, the major flavonoids are anthocyanins and in some cultivars, proanthocyanidins can also be found. In red rice the most abundant flavonoids are proanthocyanidins with different degrees of polymerization, followed by variable amounts of anthocyanins (Abdel-Aal et al., 2006; Finocchiaro et al., 2010; Oki et al., 2002). The contribution of ~40% of insoluble phenolics in non-pigmented rice is still significant while in pigmented rice it is less than 20% (Mira et al., 2009). Insoluble phenolic compounds occur mostly bound to polysaccharides of the cell wall and may be partially released and transformed in the colon by microflora enzymes before absorption. This indicates that polyphenols may exert both systemic and local antioxidant effects, modulated by the microbial metabolism (Selma et al., 2009).

Food anthocyanins are poorly absorbed from both stomach and small intestine and plasma concentrations range from nanomol to micromol/L. Methylated, glucuronidated and sulfo-conjugated anthocyanin metabolites can be found in plasma at levels more than twice as high as intact compounds. These metabolites may contribute to the reported health benefits of the consumption of anthocyanin-rich products (Manach et al., 2005).

Polymeric proanthocyanidins are flavanols with molecular weights ranging from approximately 500 to 18,000 Da are not absorbed as such, since animal and *in vitro* studies have confirmed that polymerization greatly impairs their intestinal absorption (Déprez et al., 2001; Oki et al., 2002). Indeed, these compounds may have direct protective effects on the intestinal mucosa against oxidative stress or the action of carcinogens.

Cooking may cause complex physical and chemical changes in phenolic compounds, including release from bound forms, degradation, polymerization, oxidation and the formation of Maillard reaction products. Few studies have investigated the effect of thermal processing on cereals other than rice and generally found a significant reduction in total phenolic content (Finocchiaro et al., 2007; Parra et al., 2007; Zielinski et al., 2006).

To date, little is known about the effects of cooking on the content of phenolic compounds of pigmented and non-pigmented rice, as well as on their ACE inhibitory capacity. Therefore, this research was conducted to evaluate the contents of phenolic compounds of red pigmented and non-pigmented rice genotypes, their inhibitory effect on ACE activity and alterations brought about by the cooking process.

2. Materials and methods

2.1. Reagents

Captopril (C-4042), purified rabbit lung ACE 1U (A-2580), ferulic acid (12,870-8), Folin-Ciocalteau reagent and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich Co. The fluorescence resonance energy transfer substrate Abz-FRK(Dnp)P-OH was synthesized at UNIFESP (Universidade Federal de São Paulo) according to Araujo et al. (1999). Other chemicals and solvents were analytical grade.

2.2. Rice samples

Ten non-pigmented (Epagri 108, Epagri 109, SCS 115 CL, SCSBRS TIO TAKA, SCS 112, SCS 114 Andosan, L 230, Empasc 104, SC 354, SC 339) and six red pigmented (red rice, ITJ 8, ITJ 31, ITJ 75, ITJ 80, ITJ 82) rice genotypes (*Oryza sativa* L ssp *Indica*) were grown in an irrigated system in the experimental rice fields at the Institute of Agronomy at Epagri (Itajaí Experiment Station) in Santa Catarina, Brazil and harvested in 2006. The non-pigmented genotypes were selected to be representative of high-quality and highly productive grains while the reddish genotypes were experimentally cultivated from populations of red rice that infested the rice fields in Santa Catarina and Rio Grande do Sul (Brazil).

Soluble and insoluble phenolic contents of all sixteen samples were evaluated, but ACE inhibition activity was only measured for six pigmented and six non-pigmented genotypes (Epagri 108, SCS 115 CL, SCS 112, Empasc 104, SC 354, SC 339), randomly chosen among the ten.

2.3. Cooking tests

Thirty grams of each rice sample was cooked for 30 min in a partially covered beaker containing 120 mL distilled water. This water-to-rice ratio was adequate to ensure complete water absorption and cooking time was enough to guarantee a soft texture. The cooked rice samples were freeze-dried and stored in vacuum-packed polyethylene pouches at room temperature until milling.

2.4. Sample preparation

Whole grains of raw and cooked rice were milled in an Analytical Mill A10, (Kinematica AG, Luzern, Switzerland) and sieved (20-mesh sieve) to uniform size. Samples were stored in vacuum-packed polyethylene pouches at room temperature until analysis. The samples were used within four days of milling.

Moisture was determined by drying about 5 g of rice flour in an oven at 105 °C to constant weight to allow comparison of data on a dry weight basis. Moisture content ranged from 12.3% to 14.4% (average 13.3%) and from 2.0% to 5.6% (average 3.5%) for raw and cooked rice samples, respectively.

2.5. Extraction of soluble (free and conjugated) phenolic compounds

Extraction was performed according to Adom and Liu (2002) and Zhou et al. (2004) with minor modifications: amount of sample and number of extractions. Rice flour (2 g) was extracted twice with 10 mL ethanol (80%) and once with 5 mL ethanol (80%) for 10 min each at room temperature. The suspensions were centrifuged at 7000 g for 10 min and the supernatants collected. The final volume was brought to 25 mL with 80% ethanol and the extract was stored at −20 °C until analysis. Each sample was extracted in triplicate.

2.6. Extraction of insoluble (bound) phenolic compounds

Insoluble, bound phenolics were solubilized according to Liyana-Pathirana and Shahidi (2006) and Zhou et al. (2004) with slight modifications. The residue obtained after the extraction of the free and conjugated compounds was washed with 10 mL hexane to remove lipids, centrifuged at 7000 g for 10 min and the supernatant was discarded. The bound phenolics were then released by hydrolyzing the residue with 60 mL aqueous 4 M NaOH solution at room temperature with stirring under nitrogen atmosphere for 4 h. The resulting clear mixture was acidified to pH 1.5–2.0 by gradual addition of ice-cold 6 M HCl. After centrifugation at 7000 g for 30 min, the supernatant was extracted five times with 30 mL ethyl acetate. The pooled ethyl acetate fractions were dried using anhydrous sodium sulfate, filtered and concentrated under reduced pressure at 35 °C. The residue was then dissolved in 80% ethanol to a final volume of 10 mL. All extracts obtained were stored at −20 °C until analysis.

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