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A N I N T E R N A T I O N A L J O U R N A L

Phenolic Compounds and Antioxidant Activity of Rice

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ABSTRACT

The aim of this work was to study the phenolic compounds identified in rice, their antioxidant activity and their potential beneficial effects on health. In vitro *and* in vivo *studies evaluating the rice grains with different pericarp colour (light brown, red and black) showed potential beneficial effects on health related to the polyphenol content of the grain, such as reduction of oxidative stress, aid in the prevention of cancer, cardiovascular diseases and complications of diabetes, among others.*

Key words: rice, red rice, black rice, polyphenols, antioxidant, biological effect

INTRODUCTION

Several studies have demonstrated the importance of diet in the control of chronic diseases, such as cancer and cardiovascular problems (Birt et al., 2001; Kris-Etherton et al., 2002; Stanner et al., 2003; Houston, 2005). The consumption of fruits, legumes, vegetables and whole grains results a reduced risk of developing these diseases. This could be attributed to the presence of natural antioxidants in these foods, such as ascorbic acid, tocopherols, carotenoids and phenolic compounds (polyphenols) (Meléndez-Martínez et al., 2004; Choi et al., 2007), besides other bioactive compounds.

Among the compounds with antioxidant activity, polyphenols are important. These are found in a great variety of foods, such as apples, mulberries, cherries, grapes, raspberries, citric fruits, onions, spinach, peppers, oat, wheat, black tea, wine and chocolate, among others (Holden et al., 2005; Dimitrios, 2006). These compounds have demonstrated higher *in vitro* antioxidant capacity than other antioxidants, such as ascorbic acid and α-tocopherol (Pulido et al., 2000), emphasising the importance of polyphenols as antioxidants in the diet.

Although polyphenols are found in several foods, variation is observed in the concentration and type of these compounds due to genetic and environmental factors and processing conditions (Kris-Etherton et al., 2002). Hence, the concentration of phenolic compounds varies greatly among the diets, depending on the type and quantity of food consumed.

Rice, being one of the most produced and consumed cereals in the world, has an important role in the relation between the diet and health. Several compounds with antioxidant activity have been identified in rice, including phenolic compounds, tocopherols, tocotrienols and γ-oryzanol (Iqbal et al., 2005). The phenolic

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compounds are mainly associated with the pericarp in rice, hence, the milling process reduces the concentration of these compounds in the grain. Besides, grains with darker pericarp colour, such as red and black rice, contain higher amounts of polyphenols (Tian et al., 2004; Zhou et al., 2004).
The concentration of total phenolics in the grain has been positively associated with the antioxidant activity (Itani et al., 2002; Goffman and Bergman, 2004; Zhang et al., 2006), with potential beneficial effects on health, such as reduction of oxidative stress (Ling et al., 2001; Hu et al., 2003), aid in the prevention of cancer (Hudson et al., 2000; Hu et al., 2003; Hyun and Chung, 2004; Chen et al., 2006), in the control of blood lipids and related diseases, which may help in the prevention of cardiovascular problems (Ling et al., 2001), and in the prevention of the complications of diabetes (Morimitsu et al., 2002; Yawadio et al., 2007).
The aim of this article was to examine the phenolic compounds present in rice, their antioxidant activity and their potential beneficial effects on health.

PHENOLIC COMPOUNDS

Phenolic compounds are secondary metabolites of plants, with different activities such as protection against pathogens and predators, mechanical support, attraction of pollinating animals, and protection against ultraviolet radiation (Bravo, 1998; Parr and Bolwell, 2000). These compounds constitute a chemically heterogeneous group, containing a phenol group (a functional hydroxyl group in an aromatic ring) in its basic structure. They differ structurally from simple molecules, such as phenolic acids, to highly polymerised compounds, such as tannins, comprising different classes. However, the main phenolics in the diet are the phenolic acids, flavonoids and tannins (King and Young, 1999).
Phenolic acids consist of two subgroups, the benzoic acid and cinnamic acid derivatives (Balasundram et al., 2006). With a C_6-C_1 structure (an aromatic ring linked to a carbon atom), the benzoic acid derivatives include ρ-hidroxybenzoic, protocatechuic, vanillic, gallic and syringic acids. The cinnamic acid derivatives, with a C_6-C_3 structure (an aromatic ring linked to a three-carbon chain), include caffeic, ferulic, ρ-coumaric and sinapic acids (Simões et al., 2001). These acids may be in the free form, in the form of soluble conjugates or in the insoluble bound form (Adom and Liu, 2002; Zhou et al., 2004). Bound phenolic acids are typically involved in the structure of the cell wall, doing the cross-linking of lignin components, with effects on the growth of the cell wall, its mechanical properties and degradability (Zhou et al., 2004).
Flavonoids, the major class of phenolic compounds in plants, are formed by 15 carbons, organised in two aromatic rings linked by a three-carbon chain (structure C_6-C_3-C_6) (Rossi and Kasum, 2002). They can be divided into different classes, being the anthocyanidins the most common, responsible for the majority of the red, pink, purple and blue colours in the plants, acting as attraction for the animals for pollination and seed dispersal. Generally, the anthocyanidins are bound to glycosides, which are called anthocyanins (Kong et al., 2003).
Tannins form another group of phenolic polymers with defence properties for the plant and can be divided into condensed and hydrolysable tannins. Condensed tannins are formed by the polymerisation of flavonoid units. These compounds are frequently hydrolysed to anthocyanidins, hence, they are also called proanthocyanidins. The hydrolysable tannins are heterogeneous polymers containing phenolic acids, especially gallic acid, and simple sugars (Santos-Buelga and Scalbert, 2000; Balasundram et al., 2006).

PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

Besides their functions in vegetables, studies have demonstrated the beneficial effects of phenolic compounds from different sources in human health due to their antioxidant activity. Cells are continuously exposed to oxidants from endogenous and exogenous sources and the production of free radicals is a part of the metabolism. The organism also has antioxidant compounds from endogenous and exogenous sources participating in the maintenance of the balance between oxidants and antioxidants (Benzie and Szeto, 1999; Fogliano et al., 1999; Heim et al., 2002). The disequilibrium in this balance due to the alteration in the concentration of these compounds is called oxidative stress, which results in damage to the cells and tissues in several ways: damaging the biomolecules and cell components, activating specific signalling paths, originating toxic products, changing gene expression and enzyme activity, and interrupting normal mechanisms of cell repair. For these reasons, oxidative stress has been related to several chronic diseases, including cardiovascular diseases, aging, diabetes and cancer (Stanner et al., 2003).

Phenolic compounds may exert their antioxidant activity in different ways. They may directly scavenge some reactive species, including hydroxyl, peroxyl and superoxide radicals, acting as chain breaking antioxidants. They may suppress lipid peroxidation recycling other antioxidants, such as α-tocopherol. Some phenolic compounds may bind pro-oxidant metals, such as iron and copper, preventing the formation of free radicals from these pro-oxidants while simultaneously maintaining their capacity to scavenge free radicals (Moran et al., 1997; Kris-Etherton et al., 2003; Halliwell, 2007). Besides, the effects of some phenolics are related to the increase in the activity of antioxidant enzymes (Chiang et al., 2006) and induction of the synthesis of antioxidant proteins (Chung et al., 2006).

PHENOLIC COMPOUNDS IN RICE

Several phenolic compounds have already been identified in rice. While grains with light brown pericarp color present mainly low molecular weight phenolics (approximately 85%), in those with red and black pericarp color prevail the compounds with higher molecular weight (Goffman and Bergman, 2004). The main phenolics in rice grains with light brown pericarp color are the phenolic acids, mainly ferulic and ρ-coumaric acids (Tian et al., 2004; Zhou et al., 2004). Other compounds identified include sinapic acid, protocatechuic acid (Hudson et al., 2000; Tian et al., 2005), chlorogenic acid, hidroxybenzoic acid (Tian et al., 2005), vanillic acid, syringic acid (Zhou et al., 2004; Tian et al., 2005), caffeic acid (Hudson et al., 2000; Zhou et al., 2004; Tian et al., 2005) and gallic acid (Zhou et al., 2004), tricin (flavone) (Hudson et al., 2000) and the esters 6'-O-(E)-feruloylsucrose and 6'-O-(E)-sinapoylsucrose (Tian et al., 2004; Tian et al., 2005).

In rice grains with red and black pericarp, the main phenolics are the anthocyanins cyanidin-3-O-β-D-glucoside and peonidin-3-O-β-D-glucoside (Oki et al., 2002; Hu et al., 2003; Chen et al., 2006; Zhang et al., 2006; Yawadio et al., 2007). Other compounds identified include the anthocyanidins cyanidin and malvidin (Hyun and Chung, 2004; Zhang et al., 2006), the anthocyanins pelargonidin-3,5-diglucoside and cyanidin-3,5-diglucoside (Zhang et al., 2006) and phenolic acids, like ferulic, caffeic and protocatechuic acids (Morimitsu et al., 2002).

According to Yawadio et al. (2007), the main characteristic that determined the type of phenolic compounds in the grain is pericarp colour, as no differences were observed among the anthocyanins when evaluating the grains with black pericarp from the subspecies *indica* and *japonica*. Pericarp colour is also related to the concentration of phenolics in the grain and usually the concentration is higher in the grains with red and black pericarp. Goffman and Bergman (2004), evaluating different genotypes, obtained total phenolic content between 1.90 and 50.32mg GAE (gallic acid equivalent) g^{-1} bran, and between 0.25 and 5.35mg GAE g^{-1} grain, observing that for those genotypes with light brown pericarp colour these values were the lowest. Besides the difference in the content of total phenolics related to the colour of the grains, variation was also observed in the content of total phenolics among the genotypes with the same pericarp colour.

Besides the genetic characteristic, other factors may influence the concentration of phenolics. Although no influence of the growing season was observed in the content of these compounds (Goffman and Bergman, 2004), different studies have demonstrated the effect of grain processing, including polishing and germination. Polishing significantly reduces the concentration of phenolics since these compounds are localized mainly in the external layers of the grain. The bran contains between 70 and 90% of the phenolic acids in light brown pericarp rice grains (Zhou et al., 2004), and approximately 85% of the anthocyanins in the rice grains with black pericarp (Hu et al., 2003), with little variation depending on the cultivar and the compounds considered. According to Goffman and Bergman (2004), this high correlation between the content of phenolics in the grain and in the bran showed that it was

possible to select for higher or lower phenolics content through the analysis of the grain without polishing, reducing the time of sample preparation.
The germination process also affects the phenolic compounds in the grain. Tian et al. (2005) observed a reduction of approximately 70% in the concentration of feruloylsucrose and sinapoylsucrose, with an increase in the content of ferulic and sinapic acids in the grains of rice with light brown pericarp during the germination. This reduction was probably caused by the hydrolysis, indicating that the germination caused the metabolism of phenolic compounds.
Some studies have also shown that the distribution of phenolic compounds changed during the storage. Zhou et al. (2004) observed a reduction in the content of bound phenolic acids in brown and white rice during the storage; this reduction was higher at 37ºC than at 4ºC. In contrast, the concentration of free phenolic acids in white rice significantly increased during the storage, probably as a result of the enzymatic or non-enzymatic release of the bound phenolic acids.

ANTIOXIDANT ACTIVITY AND BIOLOGICAL EFFECT

The concentration of total phenolics in the rice grains has been positively correlated with the antioxidant activity (Itani et al., 2002; Goffman and Bergman, 2004; Zhang et al., 2006). In red pericarp grains, a high correlation was observed between this activity and the content of proanthocyanidins, but in the case of black pericarp grains the correlation depended on the content of anthocyanins (Oki et al., 2002). These results suggested that the phenolic compounds were among the main responsible ones for the antioxidant activity of rice grains (Goffman and Bergman, 2004).
Usually, grains with red and black pericarp presented higher antioxidant activity than those with light brown pericarp colour (Nam et al., 2005). Goffman and Bergman (2004), evaluating the genotypes with different pericarp colour, observed the values of antioxidant activity between 10.0 and 13.1µM TE (Trolox equivalent) g^{-1} bran for the grains with light brown pericarp, between 119.9 and 312.3µM TE g^{-1} bran for red pericarp grains and between 56.3 e 345.3µM TE g^{-1} bran for black pericarp grains. These results also demonstrated that, besides the variation in the antioxidant activity among grains with different pericarp colour, variation in a group of grains with the same pericarp colour was also observed.
Besides the difference in the total antioxidant activity, differences have also been observed among the genotypes in the ability to scavenge the reactive oxygen species. Nam et al. (2005) reported reduction in the concentration of superoxide anions by competitively inhibiting xanthine oxidase (enzyme that induces the formation of reactive oxygen species in the cells), and scavenged hydroxyl radicals through direct mechanism. On the other hand, another genotype scavenged superoxide anions without affecting the activity of xanthine oxidase, and reduced hydroxyl radicals through binding ferrous ion. This showed that the antioxidant compounds present in these grains might act in different ways to reduce the oxidative stress in the organism and, with that, help in the prevention of several diseases.
The reduction of the oxidative stress by polyphenols in rice grains was observed in *in vitro* and *ex vivo* studies, indicated by the reduction in the production of nitric oxide (Hu et al., 2003) and in the concentration of reactive oxygen species (Hu et al., 2003; Chiang et al., 2006). Simultaneously to the reduction in the indicators of oxidation, an increase in the antioxidant capacity was observed, including higher total antioxidant capacity and increased activity of antioxidant enzymes, such as superoxide dismutase and catalase (Chiang et al., 2006). Studies with cell cultures have shown that phenolic compounds in the rice may also be associated with antimutagenic, anticarcinogenic and antimetastasis activities, due to their ability to directly protect the DNA against the damage and affect cell proliferation (Hu et al., 2003; Chen et al., 2006). Using the extracts obtained from the rice grains with light brown pericarp colour, a reduction in the number of viable cells and colony formation of breast and colon cancer cells was observed (Hudson et al., 2000). Evaluating separately some phenolic compounds present in the rice bran, effects were observed on different types of cancer cells. Caffeic acid, ferulic acid and tricin reduced the number of cells, cell viability and clonogenicity (Hudson et al., 2000). The anthocyanins cyanidin and malvidin presented cytotoxicity in a dose-dependent way, with IC_{50} values (concentration that inhibited the growth by

50%) for human leukaemia cells as 60 and 40μg ml^{-1}, respectively. This activity was attributed to the effect of these compounds on cell cycle, interrupting it at G_2/M phase and inducing the apoptosis. However, no cytotoxic activity was observed when these compounds were evaluated in the form of glucosides, suggesting that this activity was due to the aglycone part of the molecule (Hyun and Chung, 2004). Peonidin-3-glucoside and cyanidin-3-glucoside showed inhibition of cell invasion and motility of human hepatocellular carcinoma cells, without apparent toxicity (Chen et al., 2006).

The extracts obtained from the rice with red and black pericarp may also have positive effects in the prevention of the complications of diabetes. Morimitsu et al. (2002) observed an inhibitory effect on the lens opacity in lens organ culture obtained from the rats; this might help in the prevention of cataract in diabetics. According to them, this effect might be related to the inhibition of the enzyme aldose reductase. Some compounds were isolated from the black rice and tested *in vitro*, including cyanidin, peonidin, ferulic acid and α-tocopherol, which demonstrated an inhibitory effect on this enzyme with a dose-dependent effect (Yawadio et al., 2007).

Although *in vitro* studies can give some information about the antioxidant activity and possible biological effects of rice phenolics, the relevance of this information to antioxidant effectiveness in the organism is very limited without data on their bioavailability and metabolism (Collins, 2005). The bioavailability differs greatly from one polyphenol to another due to several factors, such as food matrix, concentration of the polyphenol in the food, background diet and interindividual variations, affecting the concentration of active metabolites in the organism (Manach et al., 2005; Zhao and Moghadasian, 2008). However, few studies have been developed to evaluate the antioxidant properties of rice phenolics *in vivo*, and most of them used animal models instead of humans. Studies with animals have shown beneficial effects of the consumption of the colored fraction (pericarp) of rice grains on the control of blood lipids and related diseases, helping in the prevention of cardiovascular problems. In these studies, with apolipoprotein (apo)E-deficient mice (Xia et al., 2003) and hypercholesterolemic rabbits (Ling et al., 2001), the supplementation of the diet with the pericarp of the rice reduced the occurrence of atherosclerotic plaques. This effect was related to different mechanisms, including the increase in the antioxidant capacity of the organism (Ling et al., 2001), reduction in the total cholesterol concentration in the blood (Xia et al., 2003), reduction in the ratio between LDL and HDL cholesterol (Ling et al., 2001; Xia et al., 2003), reduction in the accumulation of cholesterol in the aorta tissue and reduction in the oxidation of LDL cholesterol (Xia et al., 2003).

A study with humans was developed by Wang et al. (2007) aiming at evaluating the effect of diet supplementation of black rice fraction in the patients with coronary heart disease. The patients consuming the black rice fraction showed an increase in the plasma antioxidant status and reduction in inflammation. This could benefit the patients with coronary heart disease. According to the authors, the higher amount of anthocyanins contained in the black rice compared to white rice might be the major component responsible for the cardioprotective effects observed. The absorption of cyanidin-3-O-β-D-glucoside (the dominant anthocyanin in black rice) was also observed, which appeared in the plasma and reached maximum level (21.5±4.48 ng/ml) at 1.5h, but disappeared quickly after 4h.

CONCLUSIONS

Phenolic compounds, due to their antioxidant activity, present potential beneficial health effects. The same variability observed in the concentration of these compounds in the foods is also observed in the diets, and the inclusion in the diet of foods rich in these compounds may change the balance between the oxidants and antioxidants in the organism, which may help in the maintenance of health. Rice possesses different compounds with antioxidant activity, including polyphenols, but variations are observed in the concentration of these compounds in the grains, mainly due to genotype, pericarp colour and processing.

In vitro and *in vivo* studies have shown potential beneficial effects of rice phenolics on the health. However, the majority of these studies have evaluated the effect of fractions rich in phenolics, and not the grain itself, as it is usually consumed. Hence, more studies should be done evaluating the effect of the consumption of the grain, helping to understand if the consumption of rice in the diet can have beneficial effects on the health. Although rice is not among the foods with higher concentrations of phenolic compounds, it may be an important source of these compounds due to its wide utilization in feeding, justifying the studies in this area, especially in *in vivo* studies, to evaluate the bioavailability and metabolism of rice polyphenols and their effect on the organism.

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