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Effect of sub-critical temperature during water extraction on the yield and total antioxidant properties of germinated brown rice

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Abstract: the aim of this study is to investigate the effect of temperature on the yield and total antioxidant activity of germinated brown rice variety. The brown rice was germinated, size reduced and analysed for amylose. Extraction of 25 g of the size reduced sample was done at varied sub-critical temperatures of 25°C, 100°C, 120°C, 150°C and 180°C with water for 1 h. The resulting mixtures were the filtered and evaporated to dryness. Total antioxidant activity was then evaluated using the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability assay and the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging assay. The yield of the extract was directly proportional to the extracting temperature. The extract obtained at 25°C had the lowest yield (3.15%) while that obtained at 180°C had the highest yield (61.10%). The percentage DPPH scavenging activity of the extracts and the ABTS radical cation scavenging activity of the extracts followed same pattern, they were both inversely proportional to the extracting temperature, except for the extract at 100°C which showed a slightly lower total antioxidant activity when compared to the extract at 120°C. In conclusion: Increase in temperature during extraction was directly proportional to the yield of the extracts and inversely proportional to the total antioxidant activity of the extracts.

INTRODUCTION

Brown rice and germinated brown rice have been studied extensively and have shown antidiabetic (Ling and Groof, 2009; Imam *et al*., 2012) and antioxidant potentials (Chakuton et al 2012; Sompong et al, 2011). These positive potentials have led to a wider call for a shift from white rice to better and safer alternatives like brown and germinated brown rice (Ito and Ishakawa, 2004). Better antioxidant activity and low glycaemic index has been the cornerstone behind these campaigns. Recent findings has shown that rice contains biologically active phytochemicals like tocopherols, tocotrienols and γ-oryzanol, and phenolic compounds, such as caffeic acid, sinapinic acid, chlorogenic acid, and ferulic acid (Aguilar-Garcia et al, 2007; Butsat and Siriamornpun, 2010). These phytochemicals are almost completely found in the bran which is removed during milling. During the day-to-day processing of these brown and germinated brown rice alternatives of rice, they are subjected to different sub-critical temperatures to achieve the desired end product before consumption. These temperatures might in some ways affect the total antioxidant properties of these rice. The objective of this study is to investigate the effect of these temperatures on the total antioxidant activity of a germinated brown rice variety.

MATERIALS AND METHODS

Chemicals

DPPH, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium saltdiammonium salt of ABTS and potassium persulfate were purchased from Sigma-Aldrich. All other reagents and solvents used were of analytical and HPLC grade.

Methods

Malaysian brown rice (MRQ 76) was obtained from the Malaysian Agricultural Research and Development Institute (MARDI). The rice was germinated as described by Imam *et al* (2012). Briefly the rice was washed and soaked in 0.05% of sodium hypochloride for 30 min and washed with tap water then soaked in 0.5% hydrogen peroxide for 6 h and then incubated for 18 h in an oven until it dries. The germinated brown rice (GBR) was then size reduced using a laboratory blender. The amylose content was evaluated according to the International Standard Organisation protocol (2015).

Cold and hot water extraction

Twenty five grams (25 g) of the sized reduced rice was measured on a mettler balance and poured into

two seperate conical flasks. Water at room temperature and boiling water were added to either of the sample to make up to 500 ml and extracted for 1 h at their respective temperatures. After the extraction time, all samples were centrifuged at 3000 rpg/min for 10 min after which they were filtered using whatman No 1 filter paper. They were then evaporated to dryness using a Buchi Syncore rotary evaporator.

Sub critical water extraction

Twenty five grams (25 g) of the sized reduced rice was measured on a mettler balance and poured into the extraction chamber of a Separex® subcritical water extractor machine (France). The operating procedure of the manufacturer was followed. The flow rate was set at 50 ml/min after the chiller was brought down to the set point of 3°C. The pressure was isobaric (100 bar) for all the varied temperatures (120°C, 150°C and 180°C) except for the 100°C and 25°C which were at atmospheric pressure. The extraction time was 1 h for all samples. After the extraction time, all samples were centrifuged at 3000 rpg/min for 10 min after which they were filtered using whatman No 1 filter paper. They were then evaporated to dryness using a Buchi Syncore rotary evaporator.

Total antioxidant study

Determination of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability

The DPPH radical-scavenging ability of rice extracts was evaluated according to the method reported by Brand-Williams, Cuvelier, and Berset (1995) with slight modification. The reaction mixture contained 1.5 ml DPPH working solution (4.73 mg of DPPH in 100 ml ethanol HPLC-grade) and 300 µL rice extract. The mixture was shaken and incubated for 90 min in the dark at room temperature. The absorbance was read at 515 nm relative to the control (as 100%) using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan). The percentage of radical-scavenging ability was calculated by using the formula:

$$\text{Scavenging ability (\%)} = [\text{Absorbance}_{515\text{ nm of control}} - \text{Absorbance}_{515\text{ nm of sample}} / \text{Absorbance}_{515\text{ nm of control}}] \times 100$$

ABTS Radical Cation Scavenging Assay

The ABTS radical cation scavenging assay was analysed following a modified method of Pellegrini *et al* (2003) and Moore *et al* (2005). A stable stock solution of ABTS radical cation was produced by reacting a 7 mM aqueous solution of ABTS with 2.45 Mm solution of potassium persulfate in the dark at room temperature for 12–16 h before use. Rice extract (120 µL) was allowed to react with 1.5 ml of a diluted ABTS radical cation solution (absorbance of 0.70 ± 0.02 AU at 734 nm). The absorbance at 734 nm of the mixture was measured after 1 min reaction time. The gradient of the plot of percentage inhibition of absorbance vs. concentration plot for each sample was then divided by the gradient of the plot for trolox to get Trolox equivalent antioxidant capacity. Results were expressed as Trolox equivalents antioxidant capacity (TEAC) in mmol of Trolox per 100 g of flour.

Statistical analysis

Data were reported as mean ± standard deviation for at least triplicate analyses of the same sample. All statistical analyses were carried out using the SPSS software package (version 16). Analysis of variance was performed by the general linear model (GLM) procedure. Multiple mean comparisons within the sample set were carried out at the 5% significance level using the Duncan's multiple range test. Statistical significance was considered for $p < 0.05$.

RESULTS

Amylose content

The amylose content was evaluated as 14.1% of the total starch content.

Yield

Table 1 below gives the yield of the extracts. The sub-critical water extract at 180°C had the highest yield while the cold water extract at 25°C had the lowest yield.

Total antioxidant activity

The concept of the total antioxidant capacity, which describes the ability of different food antioxidants in scavenging preformed free radicals, has been suggested as a tool for investigating the health effects of antioxidant-rich foods.

Percentage DPPH scavenging activity of the extract

The stable DPPH radical is frequently used to investigate free radical-scavenging activities of hydrogen donating antioxidants in many plant materials. Figure 1 shows the percentage DPPH scavenging activity of various concentrations of the different extracts. The cold water extract had the highest percentage DPPH scavenging activity while the sub-critical water extract at 180°C had the lowest.

ABTS radical cation scavenging activity in TEAC

A concentration response curve of the reference antioxidant (Trolox) was plotted to compare the equivalent antioxidant activity of the various extract to that of the standard reference (Figure 2).

Table 1: Yield of extraction at varying sub-critical temperatures

Temperature (°C)	% yield	Pressure
25	3.15 ± 0.12	Atmospheric pressure
100	3.78 ± 0.14	Atmospheric pressure
120	6.93 ± 0.23	100 bar
150	28.08 ± 2.76	100 bar
180	61.10 ± 3.15	100 bar

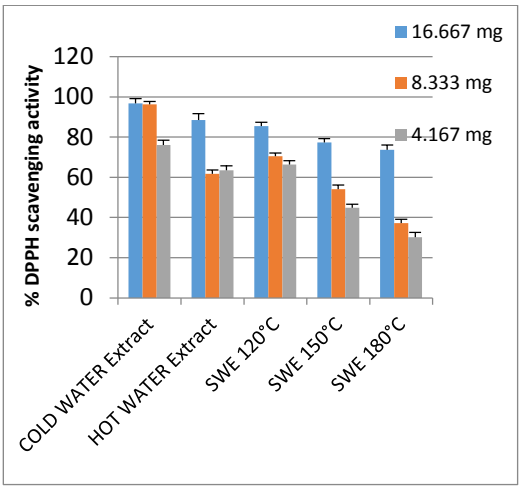

Figure 1. Scavenging activity of varying concentrations of the extracts on DPPH radical

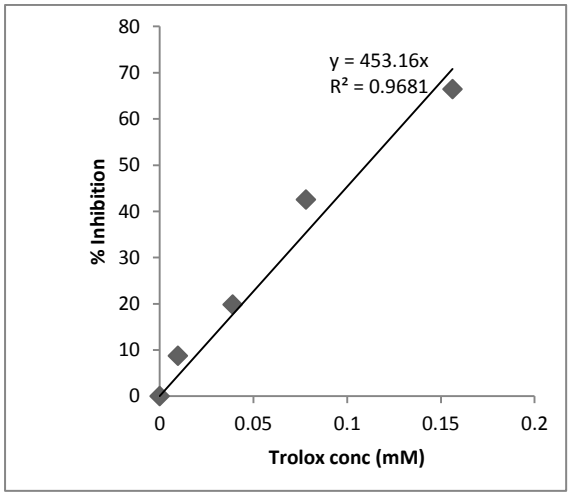

Figure 2: Concentration response curve for the absorbance at 734 nm for ABTS$^{\bullet+}$ as a function of concentration of standard trolox solution.

Because of its simplicity and the fact that it can be used in lipid and aqueous phases, the Trolox equivalent antioxidant capacity (TEAC) assay has become routine practice in evaluating phytochemicals. Similar to the result of the DPPH scavenging assay above, the cold water extract had the highest Trolox equivalence while the sub-critical water extract at 180°C had the lowest Trolox equivalence (Figure 3).

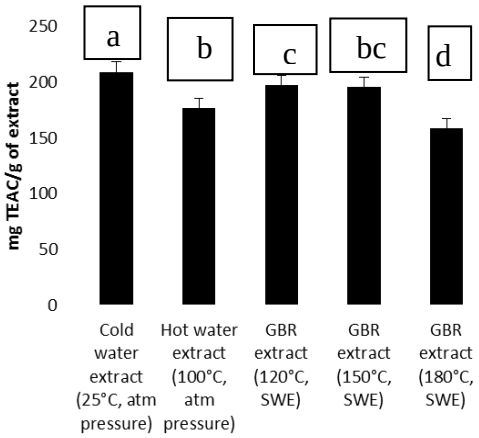

Figure 3. ABTS radical scavenging ability of extracts in trolox equivalence

DISCUSSION

The amylose content result was unremarkable. The rice variety with amylose content of 14.1% falls under the low amylose rice based on the classification of Coffman and Juliano (1987). The amylose content of a rice variety could affect the glycaemic index and subsequently the systemic glucose handling capacity of an individual (Denardin et al., 2012) which would, in the long run affect free radical-antioxidant homeostasis (oxidative stress).

The yield varied significantly between 3.15% for the room temperature extraction to 61.10% for the sub critical water extraction at 180°C. These yield results is in line with the fact that temperature is the most important parameter below critical temperature (374°C), as pressure has little or no effect on water (incompressible) below this critical point. As the extracting temperature increases, hydrolysis of the gelatinised starch increases simultaneously making the starch component to go into solution and increasing the yield. The yield was more than doubled with every 30° rise in temperature.

The DPPH assay method is among the most routinely employed method for total antioxidant activity evaluation (Chew et al., 2008). The cold water extract exhibited the highest percentage inhibition of the DPPH radicals at all concentrations. The sub-critical water extract at 180°C exhibited the lowest percentage inhibition of the DPPH radicals. An inverse relationship was observed between the extracting temperature and the percentage inhibition of DPPH radical. Except for the lower concentrations of the hot water (100°C), as the extracting temperature rises from 25°C through 180°C, the DPPH radical scavenging activities was reducing proportionately. An insight into such relationship was highlighted by the study of Parnsakhorn and Noomhorm (2008) which showed that the concentration of Vitamin E (an established antioxidant) was lost after a parboiling process on brown rice at 105°C followed by

steaming. Also, a more recent study by Reblova (2012) showed similar inverse relationship between extracting temperature and antioxidant activity.

Due to the simplicity of the assay and the fact that it can be used in aqueous and lipid phases the Trolox equivalent antioxidant capacity (TEAC) assay has become routine practice in evaluating plant materials. There was a positive correlation between the pattern of ABTS radical scavenging properties of the extracts and the DPPH radical scavenging properties. The cold water extract had the highest ABTS radical scavenging ability in TEAC while the sub-critical water extract at 180°C had the lowest. The decrease in total antioxidant activity observed in the DPPH assay and the ABTS assay methods as the temperature increases might not be unconnected to the denaturing of the antioxidants as the temperature increases. A second reason could be that the hydrolysis of starch at high temperatures ensured increased yield (6.93% - 61.1%) thereby reducing the total antioxidant concentration per gram of the extract as against the cold water extract where starchy component is not extracted.

CONCLUSION

As the sub-critical temperature increases during water extraction of germinated brown rice, the yield increases while the total antioxidant property decreases.

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