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Effect of pretreatment with ultrasound on antioxidant properties of black glutinous rice water extracts

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Abstract

Black glutinous rice is a good source of antioxidants and has potential for making a functional beverage. The aim of this research was to examine practical ways to create extracts with high antioxidant activities from a Thai variety of dehusked black glutinous rice (BGR), with a view to develop a health drink. Water extracts from 2 extraction methods were compared for their properties. The methods were plain hot water extraction (HE) with temperatures in the range 50-100°C for 10-40 min, and similar with ultrasound pretreatment (USHE).The pretreatment might break cell walls prior to water extraction and increase yields. Various optical and physical properties of the extract filtrates were determined. After removal of suspended particles by centrifugation, determinations were done for the total polyphenol and anthocyanin contents, and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), $ABTS.^{+}$ and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) scavenging activities. A 60°C extraction temperature was near optimal, while at higher temperatures the yield decreased with time suggesting decay of the antioxidants. Without US pretreatment the yields increased with extraction time and temperature (up to 100°C) suggesting better antioxidant stability. Plain hot water extraction appears practical and competitive on pursuing high antioxidant activity health drinks extracted from BGR.

Introduction

Pigmented rice has higher antioxidants content in its seed coat or pericarp than non-colored rice (Finocchiaro *et al*., 2010; Yodmanee *et al*., 2011). The health benefits of antioxidants could add to the value of these varieties in comparison to other common rice varieties. The main antioxidants in pigmented rice seed coat are flavonoids, mainly anthocyanins, a sub-group of polyphenolic compounds (Abdel-Aal *et al.*, 2006). They are water-soluble natural colorants from the secondary synthesis in higher plants, and give red to dark purple color to rice seed coat (Abdel-Aal and Huel, 2003). These phenolic compounds are the dominant active antioxidative component in a pigmented rice grain (Iqbal *et al*., 2005; Tabaraki *et al*., 2011). The color of pigmented rice is related to its content of anthocyanin, so that a purple seed coat has more anthocyanin than a red or a brown seed coat (Yodmanee *et al*., 2011; Min *et al*., 2014), and the quantitative correlation between color and anthocyanin content is good across rice varieties (Yodmanee *et al.*, 2011). This gives pigmented rice grains potential for use in functional foods, which would provide them a competitive benefit.

The antioxidants in pigmented rice may have beneficial effects against various pathologies, reducing the risks of degenerative diseases such as cardiovascular disorder and cancer (Xia *et al.*, 2003; Chen *et al.*, 2006). They are anti-inflammatory (Wang and Mazza, 2002). The phytochemicals in pigmented rice bran can promote antioxidant enzymes and suppress tumor progression or carcinogenesis (Chiang *et al*., 2006).

Since rice is a staple food across the Asian countries, it would benefit the human health in this region if colored rice gained market share relative to the other less nutritious varieties. The color and antioxidant properties of pigmented rice could contribute in common food, in value added food products, or in food additives or beverage products. The current research is a first step towards the development of such value-added products derived from pigmented rice. The water soluble anthocyanins are the major antioxidant in pigmented rice grains, and could be extracted with water. Such water extracts could serve as the basis to alternative novel health drink products. The ideal rice extract should have high antioxidant contents and high activities in the scavenging inhibition of oxidants. Additionally, the

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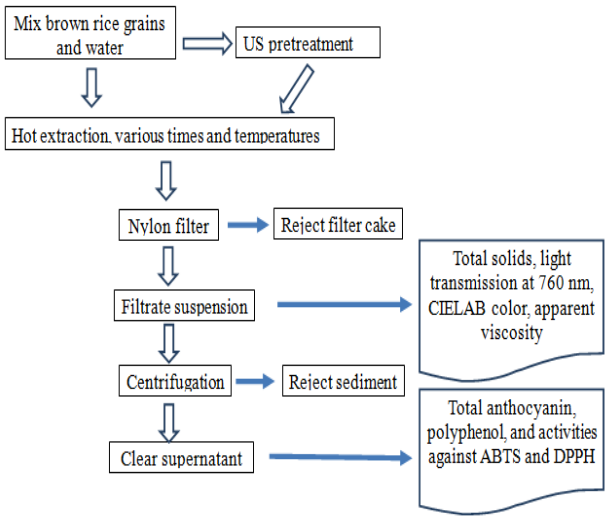
antioxidant stability in an extract is of great practical importance, as is also the low cost and simplicity of the extraction process. The temperature used for extraction needs to be considered due to anthocyanin instability with heat.

High power ultrasound (US) has become a common industrial tool in homogenization, emulsification, extraction, and also in enabling chemical reactions that otherwise require high pressure and temperature (Patist and Bates, 2008). These uses benefit from the high pressure and temperature shocks that are tightly localized at the implosions of cavitation bubbles. The shocks are so intense that they can emit photons, which is known as sonoluminescence. These shocks can break water into active radicals, contribute to other chemical reactions or the breakdown of molecules, and mechanically break surfaces such as cell walls. The lysing of cells together with the generated micro-turbulence that assists advection/diffusion has spurred a number of US applications to extractions from biological materials, as these are otherwise hindered by the cell walls or rate-limited by advection and diffusion. Power US is either applied as a pre-treatment before extraction, or it is used to actively contribute during the actual extraction in so-called ultrasound assisted extraction (UAE). In our case we chose to pursue US pretreatment to avoid breaking the rice grains and starch endosperm. For practical production of health drink consumables we chose to pursue hot water extraction, instead of using ethanol or methanol. After these choices, the extraction time and temperature need to be determined. For practical applicability in small-scale production, we chose a simple extraction in a heated vessel, at ambient pressure, with shaking but without any external flows that (by separation and recirculation, like in Soxhlet) might increase extraction yield.

The experiments were designed to determine near optimal hot water extraction conditions, possibly with US pretreatment, for maximizing the yield of antioxidants in an extract from Thai black glutinous rice (BGR) variety. The overall experimental scheme is shown in Figure 1.To characterize the extracts after filtration, some optical properties, apparent viscosity, and total solids after evaporation were determined. The filtrate suspensions were further clarified by centrifugation, to allow spectrometric determinations of antioxidant contents and radical scavenging activities that quantify antioxidant activity. Recommendations for antioxidant extraction from BGR are provided based on the results. The current research will be followed up by a separate study of the actual health drinks high in antioxidants, and their shelf life and human palatability.



Materials and Methods

Rice samples

Black glutinous rice grains (Chormaipai variety) were obtained from Pattani Rice Research Center, Pattani, Thailand. Paddy rice grains, three months old after harvesting, were dehusked, vacuum packed in aluminum bags, and stored at -18°C. The dehusked black glutinous rice grains were used as the solid starting material for aqueous extraction.

Chemicals and reagents

All chemicals used in this study were analytical grade from Sigma Chemical Co. (St. Louis, MO, USA). These chemicals were 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{\cdot+}$), 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), folin–ciocalteu reagent, hydrochloric acid, potassium chloride, sodium acetate, sodium carbonate, and potassium sulfate.

Experiments

BGR extracts were prepared with hot extraction (HE) in water, and these were compared with similar water extraction after ultrasound pretreatment (USHE). Various characteristics of BGR extract, in particular the anthocyanin and polyphenol contents as well as antioxidant activities, were determined for these comparisons. The details of these experiments are described below (Figure 1).

Hot water extraction (HE)

A 250 mL beaker with 100 mL (g) of distilled water was placed in a water bath at the desired extraction temperature. The temperatures used were 50, 60, 70, 80, and 100°C. Once the water temperature

in the beaker had reached its target to within ±1°C, 20 g of BGR grains were added into the beaker. To limit evaporation, the beaker was covered with an aluminum foil. The extraction times used were 10, 20, 30 and 40 min, performed in a temperature-controlled water bath with shaking. The extractions were stopped by immediate filtering to separate the rice grains and collect the filtrate, which was still a cloudy suspension. The time and temperature combinations were tested in a full factorial design, with 3 replicates.

After the hot water extraction the samples were filtered through a nylon cloth to remove the rice grains, and the BGR filtrate suspension was retained for further study. Each filtrate was divided into two portions, one for determining total solids, viscosity, and light transmission at 760 nm, as well as CIE LAB color; while the other portion was centrifuged at 8000 rpm for 15 min, and the clear supernatant used in spectroscopic determinations of total anthocyanin and polyphenol contents, and antioxidant scavenging activities.

Pretreatment with ultrasound prior to heating (USHE)

The US used in this study was generated by Sonicator (VCX500S/N57083AC, Sonics Vibra Cell, USA) at a constant frequency of 20 KHz, with a 13 mm diameter solid probe, and with 500W maximum powers. The appropriate parameters for US pretreatment were determined in preliminary experiments, and then held fixed for treating rice grains before extraction. Those parameters were US power and used time, rice grain to water ratio, and the total amount of rice grain and water. The criterion used to determine the near optimal US pretreatment parameters was the anthocyanin content (Finocchiaro *et al*., 2010) determined immediately after US treatment.

The conditions that gave the highest anthocyanin content were: US nominal power set at 400W, treatment time at 30 min, and the treated mixture of 100 g water with 20 g BGR in a 250 mL beaker, with a cooling external water bath limiting the mixture temperature to below 50°C. After the US pre-treatment at this fixed condition, the pre-treated samples were immersed in a water bath to continue as with the plain HE method. Similar ranges of extraction temperature and time were covered as with the HE method, for the water extraction step. The obtained extracts were also treated and quantitatively characterized similarly as those from the HE method.

Determinations from cloudy filtrate after extraction

The filtrate from extraction remained cloudy with suspended solids. It is this filtrate that would form the basis of a BGR derived health drink. The optical properties, total solids, and viscosity of the cloudy filtrates were quantified, as described in detail below.

Total solids

Total solids were determined using a hot air oven method (A.O.A.C., 2000). The samples of BGR water extract (5 mL each) were weighed and then dehydrated in a water bath at 100°C for 30 minutes, then in an oven at 105±2°C for 2 hrs. The samples were removed from the oven, cooled in a desiccator, and weighed. The oven drying and weighing were repeated until a constant weight was obtained.

Color measurement

The color of each BGR extract was determined with Hunter-Lab (Model CQ/UNI-1600, Hunter Lab, USA). Prior to color measurements, the instrument was calibrated with a light tap and a white calibration tile. The colorimeter was set to the illuminant condition D65 and to 10° standard observer. Each 2 mL sample was pipetted into a glass cuvette and the CIELAB color parameters (a^*, b^* and L^*) were then read (Lamberts *et al.*, 2007). Three replicate determinations were done for each sample.

Apparent viscosity

The viscosity of BGR water extract samples was determined in a controlled temperature room at 26+1°C by a Brookfield viscometer used at 60 rpm with spindle No. LV2, and the apparent viscosity measured was expressed in centipoises. (1cP= 1 mPa.s)

This measurement would give a well-defined viscosity for a Newtonian liquid, but in our case the particulate suspensions with dissolved polymers (mainly amylose and amylopectin forms of starch) are likely non-Newtonian. Therefore, the viscosity measurement result may strongly depend on the measurement conditions, and the result obtained here should only be considered an apparent viscosity value, specific for the conditions above. However, this is implicitly understood in the rest of this manuscript, and we shall simply refer to "viscosity".

Light transmission

The transmission of BGR water extract samples was determined by a spectrophotometer (LibraS22, Biochrom, England) in the transmission mode for absorbance measurement at 760 nm. Each sample was measured in a plastic cuvette, and the transmission parameters were read (Palou *et al.*, 1999) with distilled water as the blank.

Determinations from clear supernatant of centrifuged filtrate

The antioxidant content and activity determinations were based on spectroscopy, and for this reason the cloudy filtrate was further centrifuged to retain a clear supernatant solution. Thus, only the soluble antioxidants were determined, while a BGR extract may have possessed higher contents and activities with its suspended solids included.

Total anthocyanin content

Total anthocyanin content was determined using the pH-differential method, slightly modified from Finocchiaro *et al.* (2010).Samples of BGR extracts were diluted with potassium chloride buffer, pH 1.0 (0.025 M), and separately with sodium acetate buffer, pH 4.5 (0.4 M), and left for 15 min before taking absorbance measurements. The absorbance in each buffer was measured at λ_{max} (550nm) and at 700 nm with a spectrophotometer (Libra S22, Biochrom, England). Distilled water was used as a blank. All analyses were done in triplicates (n = 3).

Total anthocyanin content was calculated as equivalent cyanindin-3-glucoside according to the following equation:

Total anthocyanin content = ($\Delta A \times MW \times DF \times 1000$) / e)

Where

$\Delta A = (Abs_{\lambda 550} - Abs_{\lambda 700})_{pH\ 1.0} - (Abs_{\lambda 550} - Abs_{\lambda 700})_{pH\ 4.5}$

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside

DF = dilution factor

e = is the molar extinction coefficient, equaling 26,900 L/mol cm for cyanidin-3-glucoside.

1000 = conversion factor from g to mg.

The total anthocyanins are expressed as cyanidinglucoside equivalents in mg per 100 g grain weight.

Total polyphenol content

Total polyphenol content was assayed using the folin–ciocalteu method, as used by Aguilar-Garcia *et al.* (2007). Briefly, the rice extract sample (60 µL) was added into 2.5 mL of water-diluted folin-ciocalteu reagent (1: 9 v/v). After 2 min of incubation at room temperature, 2 mL of sodium carbonate solution (75 g/L) was added. The mixture was incubated for 15 min at 50°C, and cooled quickly in an ice-water bath. The absorbance at 760 nm was read within 15 min by a spectrophotometer (Libra S22, Biochrom, England), and distilled water was used as the blank. The measured absorbance was converted to polyphenol content (mg GAE/100 g grain weight) using a standard curve equation, fit by regression to gallic acid standards.

Radical $DPPH^{\cdot}$ scavenging activity

$DPPH^{\cdot}$ scavenging activity was determined according to a method available in the literature (Butsat and Siriamornpun, 2010). A BGR water extract sample was diluted to suit the absorbance determination. Then 5 mL of the diluted sample was added to 1 mL of $DPPH^{\cdot}$ solution (200 µMol), mixed well and left at room temperature for 30 min. The absorbance of the mixture was determined at 517 nm using a spectrophotometer (LibraS22, Biochrom, England) with water blank. In addition, a $DPPH^{\cdot}$ solution without added sample was measured for its absorbance, as the control case. The percentage of inhibition was calculated as follows:

$DPPH^{\cdot}$ inhibition (%) = $(Abs_{control} - Abs_{sample}) \times 100 / Abs_{control}$

where:

$Abs_{control}$ is the absorbance of DPPH solution, without sample.

Abs_{sample} is the absorbance of sample in $DPPH^{\cdot}$ solution

A standard curve was created from the absorbancies for various concentrations of trolox as calibration samples in $DPPH^{\cdot}$ solution. Each $DPPH^{\cdot}$ radical scavenging activity of an actual sample was determined from the trolox standard curve and expressed as µM trolox.

Radical $ABTS^{\cdot+}$ scavenging activity

The total antioxidant capacity of a BGR water extract sample was determined using a spectrophotometer (Libra S22, Biochrom, England) with the method described by Choi *et al*. (2007). The $ABTS^{\cdot+}$ solution was prepared by adding 7 mM $ABTS^{\cdot+}$ into 2.45 mM potassium persulfate, and the mixture was kept in dark overnight. The 414 nm absorbance of the $ABTS^{\cdot+}$ solution was adjusted to be within the range 1.4-1.5 by dilution with distilled water. 0.2 mL (5 mg/mL) rice extract sample was added into 2 mL $ABTS^{\cdot+}$ solution and mixed thoroughly. The reaction mixture was kept at room temperature for 60 min, and the absorbance was then immediately recorded at 414 nm. Distilled water was used as the blank. Absorbance of the $ABTS^{\cdot+}$ solution without sample was also determined, as control. The percentage inhibition was calculated as follows:

Inhibition% = $(Abs_{control} - Abs_{sample}) \times 100 / Abs_{control}$

Where:

Abs_{sample} is the absorbance of sample in $ABTS^{\cdot+}$ solution

$Abs_{control}$ is the absorbance of $ABTS^{\cdot+}$ without sample.

A standard curve was created from absorbancies of various concentrations of trolox calibration samples in $ABTS^{\cdot+}$.solution. The $ABTS^{\cdot+}$ radical scavenging activity was determined from the trolox standard curve and expressed as μM trolox.

Statistical analysis

All data were subjected to the analysis of variance (ANOVA). Significant differences between the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% confidence level ($p<0.05$). The differences of water extract properties between HE and USHE methods were tested by T-test.

Results and Discussion

Preliminary US experiments

In the preliminary experiments, various rice/water blend ratios were sonicated at nominal power settings of 100, 200, 300, 400, and 500W, for durations of 10, 20, 30, 40, or 50 minutes, in a full factorial design. The total anthocyanin contents of samples were determined from clear centrifuged supernatants in the same way as in the primary experiments. In these results, the anthocyanin content increased with extraction time for the US power settings 100-200W. However, at higher US power settings the anthocyanin content tended to decrease with extraction time. For example, at 500W US power the anthocyanin content decreased at 40 min, while at 400W it started to decrease the earlier 30 min extraction time. This indicates an US treatment of BGR in water may cause deterioration of anthocyanins, making them sensitive to thermal degradation. This agrees well with Ashokkumar *et al.* (2008). Our highest 32 mg Cy-3-G per 100 g grains total anthocyanin yield was obtained with 400W power setting and 30 minutes of sonication, with the weight ration of rice to water = 1:5, for a 120 g total. These settings were held fixed for the US pretreatment over the experiments that followed.

Determinations from cloudy filtrate

Color, total solids, viscosity and light transmissions

Overall with USHE method, the color of GBR extracts showed much lower a^*, b^* and L^* values than those from HE method at a similar extraction temperature and time. This is attributable not only to the anthocyanin content but also to the total solids in the extracts (data not shown).

Increasing the extraction temperature and time significantly increased the total solids, and hence increased the viscosity of the BGR extract. Heating rice grains in water is like cooking rice, and will not only release phytochemical substances from the rice grains, but gelatinizes starch in the grains. Once the starch is cooked, starch polymers from the rice endosperm would be released to the water. The US agitation may have both broken cell walls and pitted the solid surfaces, releasing particles, as well as aided in solubilization by its mixing effects. It is then no surprise that the USHE gave almost up to 7% total solids, while the plain HE maximally reached only about 2%. The overall trends in light transmission are those that one would expect from the development of total solids, i.e. providing no new insights (data not shown).

Determinations from clear solution

Anthocyanin

The total anthocyanin yields for hot water extraction, with and without US pretreatment, are shown in Figure 2. The yield with plain HE consistently increased nearly linearly with both extraction time and temperature. The thermal instability of anthocyanins did not reduce yield even at the highest 100°C temperature and the longest 40min time used in this study for hot extraction. The current results indicate that hot extraction in ambient pressure is advantageous also for the antioxidant yield. Overall, practical small-scale production would be easy to set up and to scale up later, when only using hot water extraction in non-pressurized vessels.

The time trends obtained for extractions at 50°C and 60°C temperatures with the USHE method were similar to those with the HE, but had higher yields than HE at equal extraction times. The lowest 50°C water extraction temperature gave lower yields than 60°C. In contrast, with the US pretreatment the total anthocyanin yield decreased with extraction times exceeding 10 minutes, when the temperature was 70°C or above. This indicates that either the anthocyanin was bound to the solids removed by centrifuging before determinations, or it was degraded due to instability. The latter option seems likely because at 60°C the extraction time consistently increases yield, showing no loss from binding to solids. At 60°C with 40 min of extraction, the USHE method yielded the highest anthocyanin content (43.5 mgCy-3-G/100 g grain weight), about 3 times higher than the HE method. Therefore, the pretreatment of BGR with US

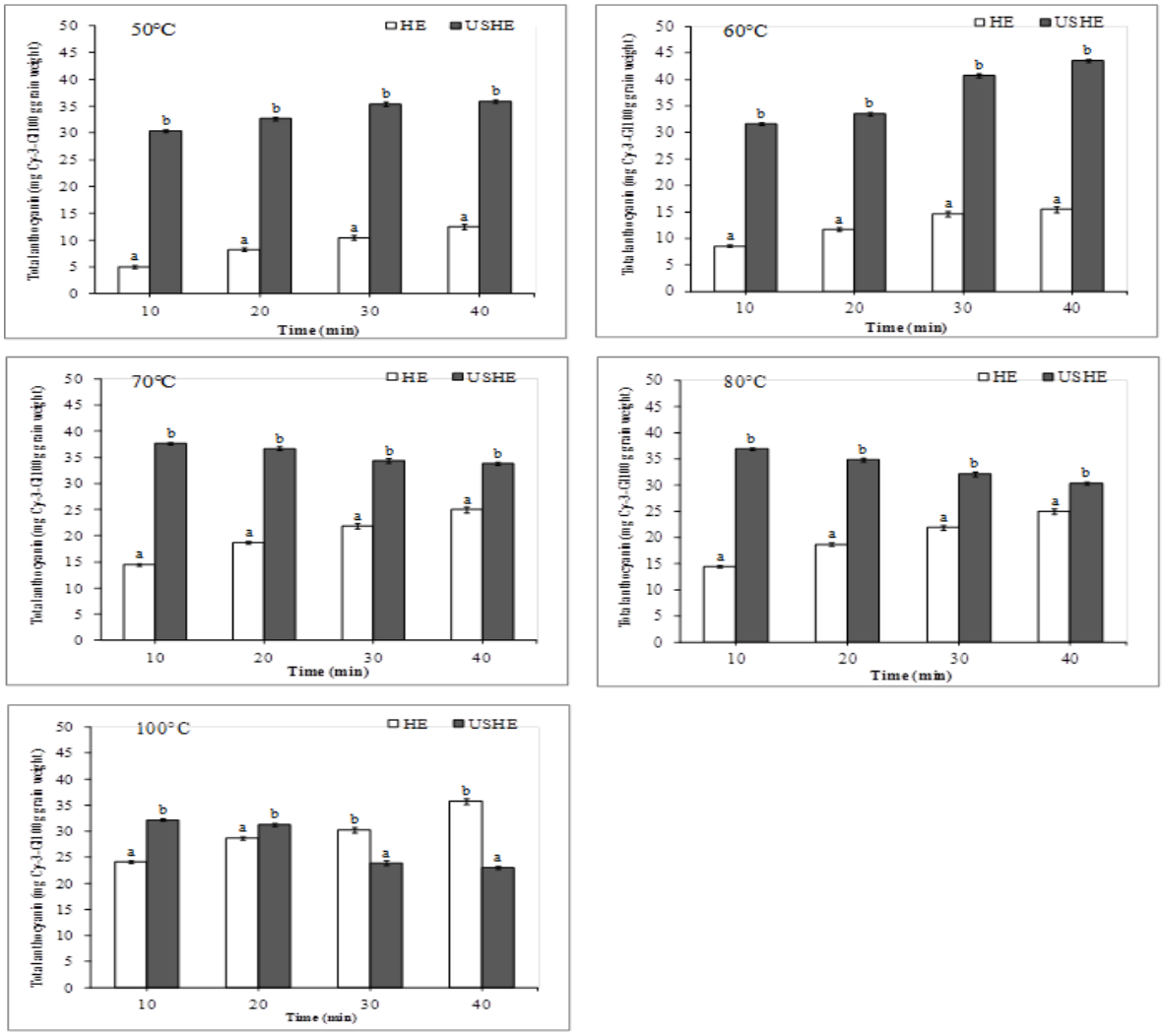


Figure 2. Comparisons of anthocyanin contents between BGR water extracts obtained from HE and USHE methods. Different letters indicate statically significant differences ($p \leq 0.05$). The vertical bars indicate the standard deviations (n = 3)

before hot water extraction enhanced the release of anthocyanin from the grain seed coats and increased the yield, as well as reduced the required extraction temperature. However, the total processing time for the USHE method was 30 min longer than for the HE method, the difference being the duration of pretreatment. At 100°C and 40 min, which gave the highest yield of the HE method (36.2 mgCy-3-G/100 g grain weight), the USHE method gave 30% less anthocyanin. This suggests that the US pretreatment converted anthocyanin to forms with lower thermal stability. Possibly the sonication generated H. and OH. radicals in the water medium (Henglien, 1993), and these radicals may destabilize anthocyanins by hydroxylation (Riesz and Kondo, 1992). Another potential mechanism, lowering thermal stability, is direct action of the high shear shocks from cavitation that might mechanically degrade the anthocyanin molecules. We did not design and execute further experiments to assess the relative effects of various mechanisms, as that was not warranted by the scope and goals of the current project.

Polyphenol

The total polyphenol yields, also measured from clear supernatants after centrifuging, had qualitatively similar trends to the anthocyanin yields (Figure 3). It is likely that the mechanisms causing these trends are also similar, and these were discussed above.

On using plain hot water extraction (HE) without sonication, the polyphenol yield consistently increases with extraction time and temperature, up to the atmospheric boiling point of water. However, sonication apparently made these antioxidants prone to thermal instability starting from about 70°C. Hence, with US pretreatment the water extraction at 60°C for 40 min was near optimal, within the current range of experiments.

The HE method with the same 40 min of extraction, but at 100°C, gave about similar total polyphenol yield, and in such forms that were more thermally stable. Overall, this suggests that an investment

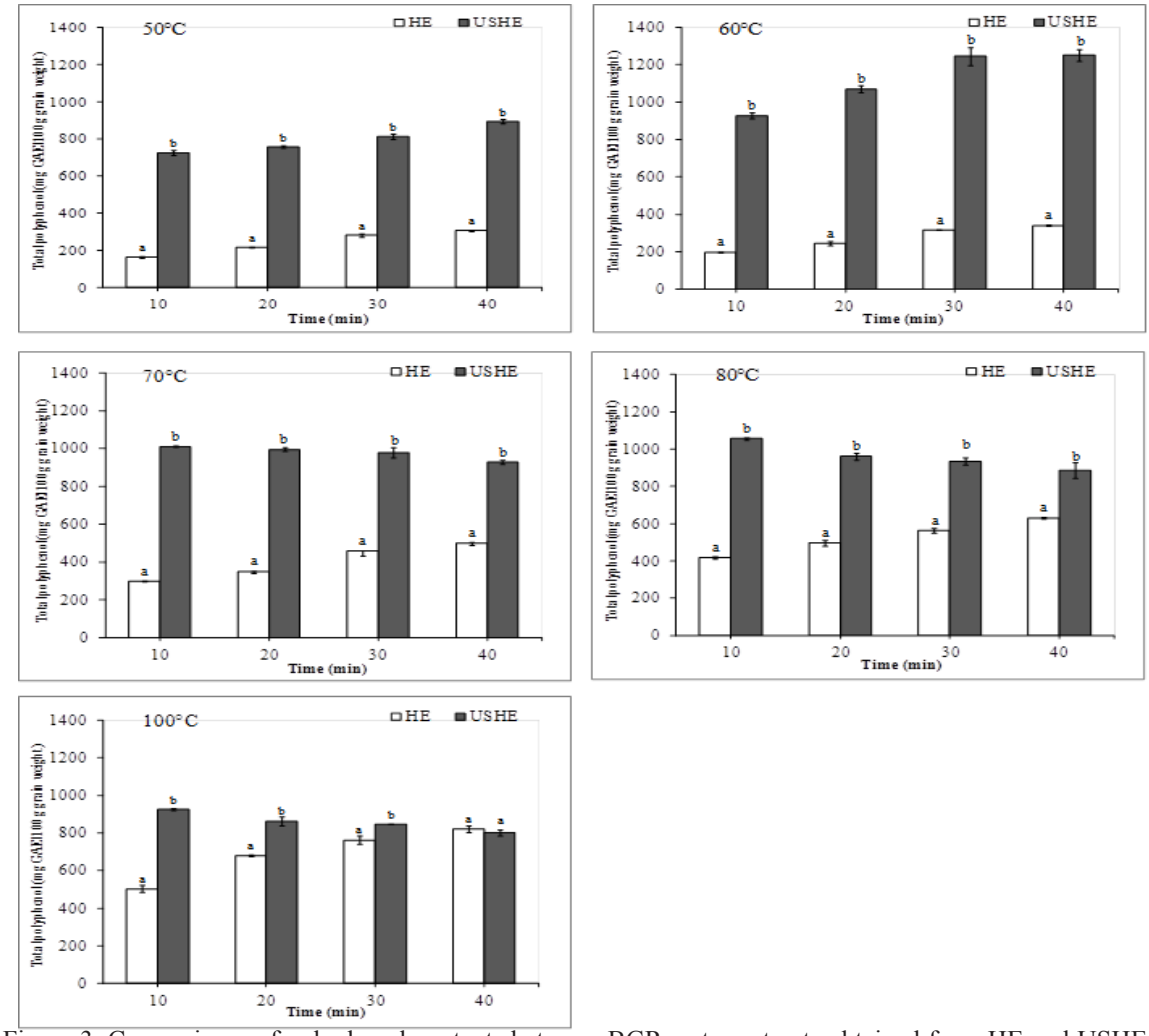


Figure 3. Comparisons of polyphenol contents between BGR water extracts obtained from HE and USHE methods, each graph representing fixed extraction temperature and time. Different letters indicate statically significant differences ($p \leq 0.05$). The vertical bars indicate the standard deviations ($n = 3$)

to a US pretreatment would not be economically competitive compared to plain hot water extraction at ambient pressure. The US pretreatment would add investment, maintenance, and energy costs, as well as processing time, while reducing stability and probably the effective shelf life of the final product.

DPPH˙ and ABTS˙+

The radical scavenging activities were also measured from the clear supernatants after centrifugation, posing the potential of losses with the removed suspended solids. The activities against DPPH˙ and ABTS˙+ are shown in Figures 4 and 5, respectively. The trends seen in antioxidant yields are reiterated in these activities. Again with plain HE both activities consistently increase with extraction time and temperature, while with the US pretreatment the time-trend is decaying at 70°C or above. The highest radical scavenging activity of the BGR extracts obtained from USHE method was found at 60°C and 30 min of extraction, which matches well our observations on anthocyanin and poloyphenol contents.

On comparing the HE and the USHE at100 °C water extraction for 40min, the BGR extract with HE showed higher antioxidant capacities for both DPPH˙ and ABTS˙+ than the extract with USHE, matching also the contents analyzed. It can be noted that the BGR extract with USHE method at its optimal conditions (60°C and 40min) had much higher antioxidant contents than the BGR extract from optimal conditions with HE method (100°C and 40min), but these extracts had similar radical scavenging activities for both DPPH˙ and ABTS˙+.This again corroborates the degradation of antioxidants at high temperatures, when sonication was used as a pretreatment, now by loss of activities relative to contents.

Our hypothesis is that the US pretreatment creates free H and OH radicals in the water medium (Hengleim, 1993), so the antioxidant molecules were hydroxylated, and this decreased their ability to

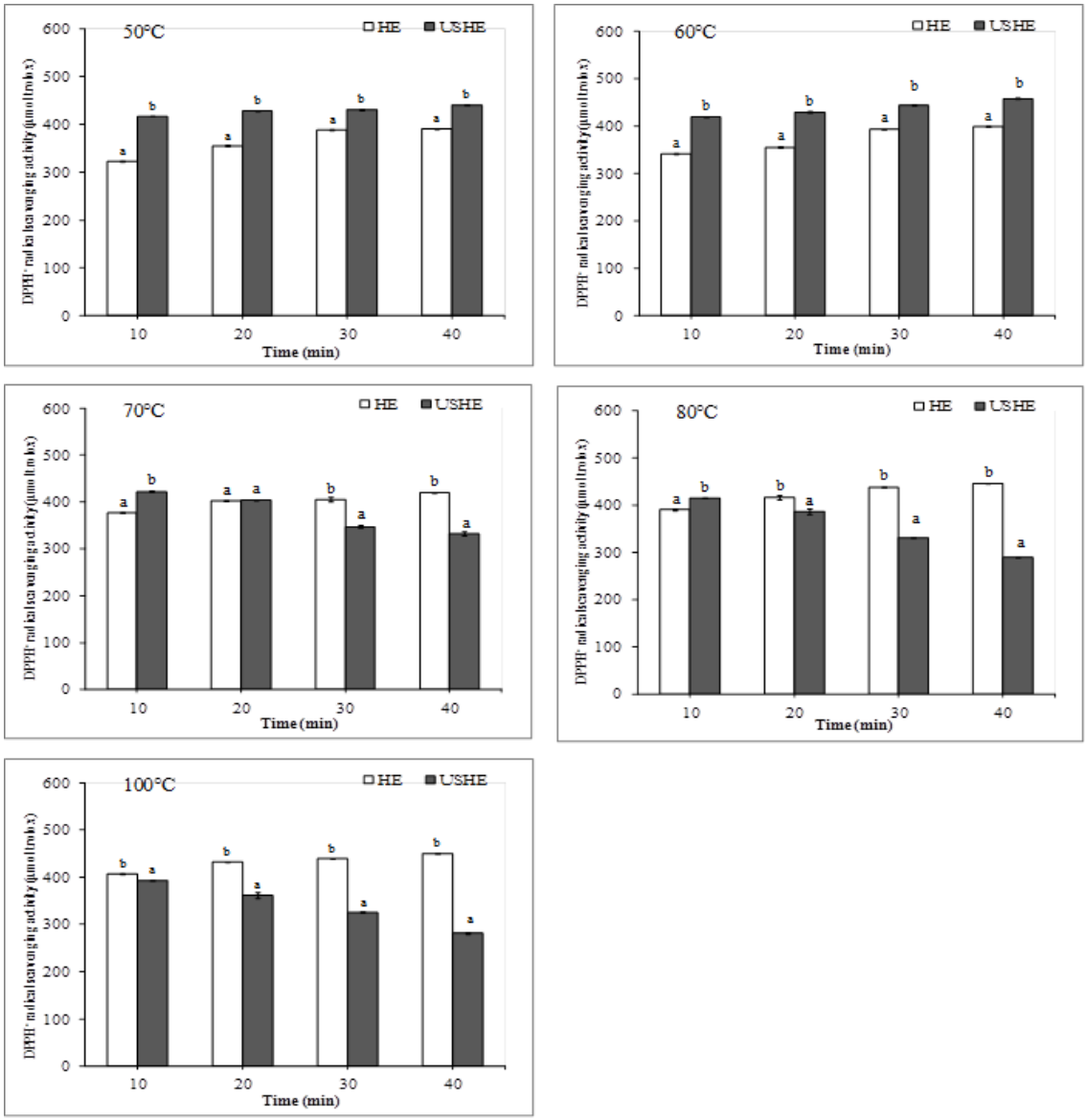


Figure 4. Comparisons of DPPH• radical scavenging activities between BGR water extracts obtained from HE and USHE methods, each graph representing fixed extraction temperature and time. Different letters indicate statically significant differences ($p \leq 0.05$).The vertical bars indicate the standard deviations (n = 3)

scavenge $ABTS^{\cdot+}$ or $DPPH^{\cdot}$ radicals. However, this hypothesis is not supported by all prior observations. In fact, hydroxyl radicals can be used to enhance the antioxidant properties of food materials by hydroxylation (Wanasundara *et al*., 1997). The use of sonochemical hydroxylation in a strategy to manage phenolic compounds has been also proposed (Ashokkumar *et al*., 2008). With this uncertainty, it is reasonable to keep in mind the alternative mechanism of direct chemical degradation effected by the shear and the thermal shocks from US cavitation.

Antioxidant compounds extracted from plants tend to be unstable and have a limited shelf life. Their decay can be catalyzed by light, access to oxygen, or elevated temperatures. The prevailing pH and the hydroxylation degree of the structure affect stability, with hydroxylation promoting instability (Cavalcanti *et al*., 2011). In particular the free OH radicals, generated by power US that splits water molecules (Soria and Villamiel, 2010) may harm the stability of these antioxidants and alter their chemical structures.

Also, in terms of the radical scavenging activities, even a 10-minute extraction with 100°C water temperature gave activities that practically (within about 10%) equal the best among all the experimental cases. This further supports plain hot water extraction at atmospheric pressure, as a cost-effective and non-wasteful approach to recover antioxidants from BGR.

Conclusions

The quality of black glutinous rice (BGR) water extracts was compared between plain hot water extraction (HE) and similar extraction with ultrasound pretreatment (USHE). The extracts were

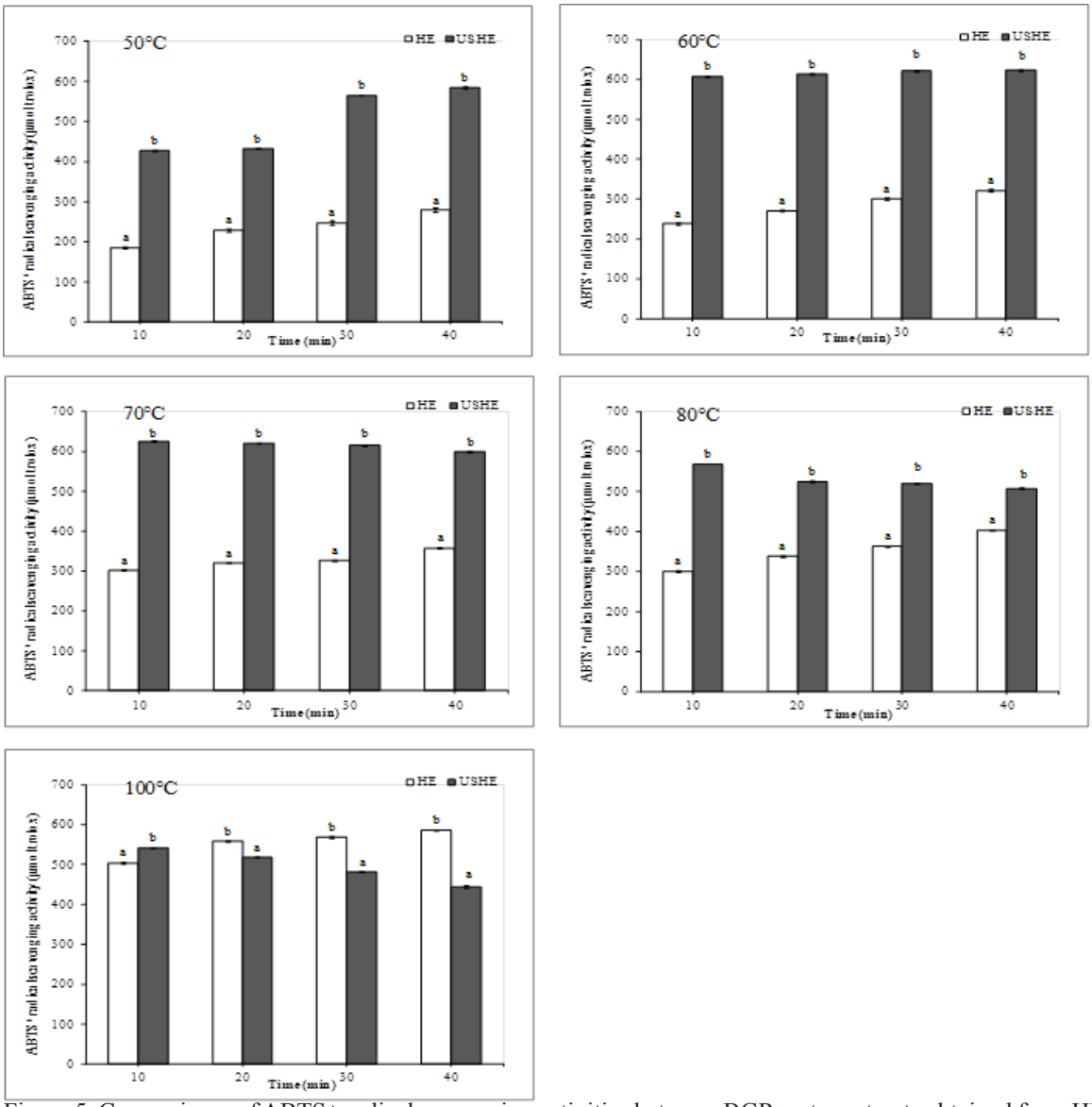


Figure 5. Comparisons of ABTS+ radical scavenging activities between BGR water extracts obtained from HE and USHE methods, each graph representing fixed extraction temperature and time. Different letters indicate statically significant differences ($p \leq 0.05$).The vertical bars indicate the standard deviations (n = 3)

particulate suspensions, characterized in terms of color parameters (a^*, b^* and L^*), total solids, an apparent viscosity, and light transmission. Among these, the light transmission is perhaps the easiest and the quickest to monitor as a quality indicator in an implemented production process. It correlates well with the other two determinations, though nonlinearly. It predicted the total solids well, irrespective of whether US pretreatment was used, and it predicted the viscosity fairly well in a fixed process with or without US.

With the HE method the yields of total anthocyanins and total polyphenols, as well as the antioxidant activities, consistently increased with temperature (50-100°C) and time (10-40 min). However, the benefit in the activities with continued extraction after 10 minutes was very minor at 100°C. The US pretreatment apparently modified the antioxidants so that they became less stable at temperatures of 70°C or above, the modification potentially being hydroxylation.

Based on experimental results, we recommend for aqueous antioxidant extraction from dehusked BGR is to use an about 1/5 weight ratio of rice/water, 100°C water temperatures at atmospheric pressure and about 10 minutes or longer extraction time. This could be applied for small-scale "cottage industry" to create new health drink products that would add value to black glutinous rice, with an inexpensive production cost.

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