Optimization of thermal processing conditions for brown rice noodles

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Abstract Based on the current thermal processing conditions for rice noodles (80–85 °C for 20–30 min), we used response surface methodology to find brown rice (BR) noodle processing conditions that maximize the noodles antioxidant activity, digestibility, and gelatinization. The experiments were designed according to the central composite design, including two independent variables (temperature and time) and six dependent variables [total phenolic contents (TPC), total flavonoid contents (TFC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activities, digestibility, and gelatinization]. Antioxidant activities decreased but digestibility and gelatinization increased with increasing temperature and time. All dependent variables suggested a quadratic model except TFC, but the probability of TPC was not applicable (0.1505), and DPPH radical scavenging activity showed a relatively low $R^2$ (0.6750). Therefore, we applied the other three dependent variables (ABTS radical scavenging activity, digestibility, and gelatinization) to the 3D response surface and proposed three optimum conditions: (1) for antioxidant activity (70 °C, 22.95 min), (2) for digestibility and gelatinization (88.18 °C, 34.89 min), and (3) for all three variables (88.5 °C, 40 min). In a validation test, all dependent variables showed values within a 5 % error range except TFC. These results show the optimum processing conditions for BR noodles to maximize antioxidant activity and provide sustainability in BR noodle processing.

Keywords Antioxidant activity · Brown rice noodles · Digestibility · Gelatinization · Response surface methodology

Introduction

Rice (Oryza sativa L.) has long been a main food crop being used in Asia, Africa, and North America. In recent years, rice with a distinctive color and flavor, such as black, red, or green rice, has been used as an energy source, along with special rice such as high amylose rice and huge sperm rice (Anderson 2003; Kong et al. 2008). Various functional components such as phytic acid, polyphenols, flavonoids, vitamins, γ-oryzanol, ferulic acid, and dietary fiber are contained in rice, and those components exhibit excellent antioxidant activity (Ha et al. 2006).

Rice noodles made of rice flour dough, which are well digested and very low-calorie compared to wheat flour noodles, have been spotlighted as a diet and globally palatable food. Rice noodles can not only be eaten fast in simple recipes, but also can easily be used in a wide variety of dishes and soups. Therefore, rice noodle is a good food that can be made palatable to many people. Addition of functional components to rice noodles could improve their palatability, and the various functional properties in brown rice (BR), such as higher antioxidant activity, could increase rice consumption. Because phenolic compounds are labile to heat above 80 °C, heat applied during
manufacturing destroys phenolic compounds and reduces antioxidant activity (Zielinski et al. 2001; Sharma and Gujral 2011).

Response surface methodology (RSM), a statistical experimental protocol used in mathematical modeling, is being extensively used as an ideal strategy for optimizing the process variables for many foods (Moon et al. 2012; Kim and Han 2012, Kim et al. 2013). Also, RSM has been successfully applied in optimizing the extraction conditions for a range of polyphenols, antioxidants, and other metabolites in plants (Karvela et al. 2011; Zhang et al. 2013; Alberti et al. 2014).

Not many applications of BR in rice noodle have been reported and the relationship between antioxidant activity and digestibility of BR in rice noodle products has been investigated. Therefore, the objective of this study is to find the optimum processing conditions (time and temperature) to minimize the loss of antioxidant activity and maximize the digestibility, based on the current manufacturing process condition (80–85 ºC) of rice noodles.

Materials and methods

Materials

BR flour was obtained from mixed BR cultivated in Gyeongnam, Korea, in 2013, and stored it at −20 ºC until use.

Sample preparation

BR flour (80 g) was mixed with corn starch (20 g) and distilled water (40 mL) and kneaded them in a bowl. Corn starch was added as a supplementary agent to form rigid dough. BR mixture was put into a polyethylene pouch and formed a thin layer for efficient heat transfer. Then we applied thermal processing as shown in Table 1. After thermal processing, we removed the BR mixture from the polyethylene pouch and dried it at 40 ºC for 24 h in a dry oven. After drying, samples were ground and stored at −20 ºC until use.

Extraction of antioxidant materials

Extraction of antioxidant materials from the BR mixture was performed using 70 % ethanol. Two grams of ground BR mixture was mixed with 20 mL of 70 % ethanol at 40 ºC for 2 h in a water bath. After 30 min of extraction, samples were filtered through a filter paper (Whatman No. 2, Sigma-Aldrich Co., St. Louis, MO, USA).

Experimental design for processing conditions

The experiment was designed based on the known processing conditions of commercial product line for rice noodles (80–85 ºC). The independent factors are time (20–40 min) and temperature (70–90 ºC).

We used RSM with central composite design (CCD) to minimize the loss of antioxidant activity by considering six dependent variables: total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity), digestibility, and gelatinization. The range and central point values of the two independent factors are shown in Table 1.

Total phenolic content

TPC of BR mixture was measured using the Folin & Ciocalteu’s assay (Singleton and Rossi 1965). We mixed extracts (200 µL) with 2.6 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. After mixing for 6 min, we added 2 mL of 7 % Na2CO3 to the mixture and measured the absorbance at 750 nm after 90-min standing. Gallic acid was used as the calibration standard and results were expressed as gallic acid equivalent (GAE)/g solid sample.

Total flavonoid content

TFC of BR mixture was measured according to the method of (Zhishen et al. 1999). We mixed the extracted sample (0.5 mL) with 3.2 mL of distilled water and 0.15 mL of 5 % NaNO2. After 5 min, we added 0.15 mL of 10 % AlCl3 and 1 mL of 1 M NaOH consecutively and then immediately measured the absorbance at 510 nm. Catechin was used as the calibration standard and results were expressed as catechin equivalent (CE)/g solid sample.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

DPPH radical scavenging activity assay was performed according to the method of (Brand-Williams et al. 1995),
with a slight modification. We adjusted the absorbance of the DPPH solution to 0.650 ± 0.020 using 80 % methanol at 517 nm. We mixed the extracted sample (0.5 mL) with 2.95 mL of DPPH solution and left it in a dark place at 23 °C for 30 min. We then measured the absorbance at 517 nm. Ascorbic acid was used as the calibration standard and results were expressed as vitamin C equivalent (VCE)/g solid sample.

**ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt) radical scavenging activity**

ABTS radical scavenging activity assay was carried out according to the method of van den Berg et al. (1999). We added 1 mM of 22.117 mg 2,2′-azobis-(2-amidino-propane)HCl and 137.175 mg of 2.5 mM ABTS− to 100 mL of 100 mM phosphate buffer saline (PBS buffer). The mixture was incubated with shaking at 70 °C for 30 min and then cooled down to 25 °C. The supernatant was filtered using an Acrodisc LC13 PVDF 0.45 μm (Pall Life Sciences, Ann Arbor, MI, USA). We adjusted the absorbance of the ABTS radical solution at 0.650 ± 0.020 using 100 mM PBS buffer at 734 nm. Then we mixed 20 μL of the extracted sample with 980 μL of the ABTS radical solution at 37 °C for 10 min and measured the absorbance at 734 nm. Ascorbic acid was used as the calibration standard and results were expressed as VCE/g solid sample.

**Digestibility measurement**

Digestibility was measured according to (Leegwater and Luten 1971) with a slight modification. To prepare a pancreatin stock solution, 100 mg pancreatin, 500 mg NaCl, and 200 mg NaNO₂ were dissolved in 50 mL of pH 6.85 phosphate buffer solution (10.2 g of KH₂PO₄, 10.65 g Na₂HPO₄, and 1000 mL of distilled water). Dried BR mixture (100 mg) was suspended in 1 mL distilled water and 250 μL of pancreatin stock solution was added. The mixtures were incubated in a 37 °C water bath for 15 h with shaking. Then we centrifuged the mixture at 1500×g for 15 min, and collected and dried the residue. Digestibility was calculated using the following equation:

\[
\text{Digestibility (\%)} = \frac{\text{weight of final brown rice mixture after incubation}}{\text{weight of initial brown rice mixture}} \times 100
\]

**Gelatinization measurement**

Differential scanning calorimeter (DSC4000, Perkinelmer Inc., Waltham, MA, USA) was used to investigate the gelatinization of the BR mixture. We weighed 10 mg of BR mixture suspension (30 % water content) into a stainless steel pan. The temperature range was from 20 to 100 °C, and the heating rate was 10 °C/min. An empty stainless steel pan was used as a reference. The degree of gelatinization was calculated using the following equation:

\[
\text{Degree of gelatinization (\%)} = \left( \frac{\Delta H_{nb} - \Delta H_{hb}}{\Delta H_{nb}} \right) \times 100
\]

where \(\Delta H_{nb}\) is the native BR mixture melting enthalpy and \(\Delta H_{hb}\) is the heated BR mixture melting enthalpy.

**Model fitting using the six dependent variables**

The data of six dependent variables were fitted using the following mathematical model corresponding to the CCD:

\[
Y = b_0 + \sum_{i=1}^{2} b_i X_i + \sum_{i=1}^{2} b_{ij} X_i X_j
\]

where \(X\) represents the independent factors (time and temperature), \(Y\) represents the dependent variables (TPC, TFC, DPPH, ABTS, digestibility, and gelatinization), and \(\beta\) represents the model coefficients (linear and quadratic forms). We analyzed the experimental design data and calculated the predicted responses using the Design-Expert 7 software (Version 7.0, Stat-Ease, Inc., Minneapolis, MN, USA).

**Statistical analysis**

Triplicate samples were used in each experiment, and each sample was analyzed at least three times. Statistical significance test was conducted to compare the responses obtained by optimal conditions using Duncan’s multiple range test (SAS version 9.1.3, SAS Institute, Inc., Cary, NC, USA) with a 95 % confidence level. We performed RSM using Design-Expert 7 (Stat-Ease, Inc., Minneapolis, MN, USA).

**Results**

**Experimental design and model fitting**

Experimental design was shown in Table 2 based on the known processing condition of rice noodles (80–85 °C). Independent factors were time and temperature, which temperature range was 70–90 °C and time range was 20–40 min. Experiments were carried out 11 running times including three replications of the center point (80 °C, 30 min). These CCD of experiments referenced the extract conditions of puffed ginseng (time and pressure), (Lee...
et al. 2014) and deer antler (time, temperature, and solvent ratio), (Jin et al. 2015). The range of the each result with six dependent variables was as follows; 0.577–0.770 mg GAE/g solid sample of TPC, 0.195–0.251 mg CE/g solid sample of TPC, 0.028–0.361 mg VCE/g solid sample of DPPH, 0.666–0.816 mg VCE/g solid sample of ABTS, 51.00–66.63 % of digestibility, and 57.63–100 % of gelatinization, respectively.

Linear or quadratic model for six responses was evaluated by using the F-test and lack of fit test (Ilaiyaraja et al. 2015). The results are shown in Table 3. The dependent variables suggested a quadratic model except for the TFC, which suggested the two factor interaction model. The probabilities of ABTS radical scavenging activity, digestibility, and gelatinization were significant at 95 % confidence level ($p < 0.05$), which indicated that the optimized conditions could be applied; the lack of fit values for those three dependent variables were 0.011, 0.0997, and 0.0001, respectively. The $R^2$ values, which represent the model adequacies (Karacabey and Mazza 2010), were 0.9021, 0.9908, and 0.9945 for ABTS radical scavenging activity, digestibility, and gelatinization, respectively. Therefore, three dependent variables were appropriated to the model applied and it was confirmed that digestibility was the best condition considering F-test and lack of fit test.

For DPPH radical scavenging activity, the lack of fit value was 0.1496 with low $R^2$ value of 0.6375. Thus, the adequacy of the model applied to DPPH radical scavenging activity was considered inappropriate.

**ANOVA analysis using the three dependent variables**

ANOVA analysis was also carried out for three dependent variables, such as ABTS radical scavenging activity,

| Run  | Coded variable levels | Observed | Digestibility (%) | Gelatinization (%) |
|------|-----------------------|----------|-------------------|-------------------|
|      | $X_1$ (°C) | $X_2$ (min) | TPC (mg GAE/g solid sample) | TFC (mg CE/g solid sample) | DPPH (mg VCE/g solid sample) | ABTS (mg VCE/g solid sample) |      |          |
| 1    | 80 | 30 | 0.579 | 0.230 | 0.308 | 0.666 | 63.06 | 97.91 |
| 2    | 65.86 | 30 | 0.770 | 0.195 | 0.361 | 0.816 | 44.82 | 54.63 |
| 3    | 90 | 20 | 0.615 | 0.234 | 0.313 | 0.743 | 64.51 | 100  |
| 4    | 90 | 40 | 0.577 | 0.236 | 0.327 | 0.753 | 66.63 | 100  |
| 5    | 80 | 15.86 | 0.659 | 0.203 | 0.349 | 0.722 | 63.88 | 97.96 |
| 6    | 80 | 14.4 | 0.621 | 0.199 | 0.351 | 0.705 | 62.10 | 98.20 |
| 7    | 80 | 30 | 0.584 | 0.229 | 0.288 | 0.669 | 63.47 | 97.96 |
| 8    | 94.14 | 30 | 0.644 | 0.230 | 0.353 | 0.707 | 65.94 | 100  |
| 9    | 70 | 20 | 0.647 | 0.219 | 0.338 | 0.795 | 51.00 | 71.51 |
| 10   | 80 | 30 | 0.581 | 0.232 | 0.298 | 0.672 | 63.87 | 97.94 |
| 11   | 70 | 40 | 0.594 | 0.251 | 0.318 | 0.750 | 51.42 | 72.16 |

$X_1$ temperature, $X_2$ time

Table 3 Model fitting of the brown rice paste from the result of six dependent variables (TPC, TFC, DPPH, and ABTS radical scavenging activities, digestibility, and gelatinization)

| Response               | Model     | Prob > $F$ | Lack of fit | $R^2$ | Equation in term of coded variable levels |
|-----------------------|-----------|------------|-------------|-------|----------------------------------------|
| Total phenolic contents | Quadratic | 0.1505     | 0.0018      | 0.6643 | $Y_1 = 0.58 - 0.028x_1 - 0.018x_2 + 0.004x_1x_2 + 0.047x_1^2 + 0.013x_2^2$ |
| Total flavonoid contents | 2FI       | 0.0994     | 0.0001      | 0.4787 | $Y_2 = 0.25 - 0.031x_1 + 0.041x_2 - 0.082x_1x_2$ |
| DPPH radical scavenging activity | Quadratic | 0.0710     | 0.1496      | 0.6750 | $Y_3 = 0.3 - 0.003x_1 - 0.004x_2 + 0.009x_1x_2 + 0.022x_1^2 + 0.019x_2^2$ |
| ABTS radical scavenging activity | Quadratic | 0.0062     | 0.0110      | 0.9021 | $Y_4 = 0.67 - 0.025x_1 - 0.007x_2 + 0.014x_1x_2 + 0.052x_1^2 + 0.028x_2^2$ |
| Digestibility         | Quadratic | 0.0004     | 0.0977      | 0.9908 | $Y_5 = 63.47 - 7.32x_1 + 0.033x_2 + 0.42x_1x_2 - 4.24x_1^2 - 0.44x_2^2$ |
| Gelatinization        | Quadratic | 0.0001     | 0.0001      | 0.9945 | $Y_6 = 97.94 - 15.06x_1 + 0.13x_2 - 0.16x_1x_2 - 10.75x_1^2 + 0.38x_2^2$ |
digestibility, and gelatinization, and they were determined to be appropriate for the model applied. The ANOVA results indicating the statistical significance of the three dependent variables (ABTS radical scavenging activity, digestibility, and gelatinization) are shown in Table 4.

In the $F$-test at the quadratic term, ABTS radical scavenging activity, digestibility, and gelatinization were significantly affected by temperature ($p < 0.05$). On the other hand, ABTS radical scavenging activity was significantly affected by time ($p < 0.05$), but the other two dependent variables were not significantly influenced ($p > 0.05$). This result suggested that processing temperature used in this study was enough to achieve certain levels of digestibility and gelatinization and resulting in no significant effect.

In case of the lack of fit test, ABTS radical scavenging activity and gelatinization were significant ($p < 0.05$). In the previous model fitting result, the lack of fit value was less than 0.05, suggesting that it can be considered in RSM as the model applied (Ilaiyaraja et al. 2015). Considering the $R^2$ values, ABTS radical scavenging activity (0.9021), digestibility (0.9908) and gelatinization (0.9945) were adequate to the model applied in this result. Therefore, above three variables were analyzed by three-dimensional (3D) response surface.

Three-dimensional (3D) response surface results for the three dependent variables

The 3D response surface results for ABTS radical scavenging activity and gelatinization were shown in Fig. 1. They confirmed high ABTS radical scavenging activity at 70 $^\circ$C for 20 min (red region). Cereal grains such as rice contain special phenolic acids (ferulic acid, $p$-coumaric, diferulate, etc.) that are not present in fruits and vegetables (Adom and Liu 2002), and the structure of those phenolic acids affects antioxidant activity (Cao et al. 1997). At constant time, as the temperature increased from 70 to 80 $^\circ$C, ABTS radical scavenging activity decreased because phenolic compounds with antioxidant activity were destroyed (Kwak et al. 2013). However after 80 $^\circ$C, ABTS radical scavenging activity increased, which can be explained by the depolymerization of polyphenols. It has been reported that polyphenols, which strongly bind with polysaccharides and oligosaccharides with ester bonds, convert to free polyphenols by heating (Xu and Chang 2009). Polyphenol compounds can be converted to low molecular weight compounds by heating, and new phenolic compounds can be produced (Woo et al. 2006). Moreover, many studies have reported that the antioxidant activity of flavonoids and tannins increases when plant materials are heated, such as chicory (Hong et al. 1998), Polygonatum odoratum tea (Ryu et al. 1997), chrysanthemum indicum L. (Yu et al. 2008), and horseweed (Woo et al. 2009).

The 3D response surface results for digestibility are shown in Fig. 2. Digestibility was greatly affected by temperature, with a high value at 90 $^\circ$C (red region).

Table 4 ANOVA analysis of the brown rice mixture from the results of three dependent variables (ABTS radical scavenging activity, digestibility, and gelatinization)

| Source | ABTS | Digestibility | Gelatinization |
|--------|------|---------------|---------------|
|        | $Prob > F$ ($p$ value) | Significance | $Prob > F$ ($p$ value) | Significance | $Prob > F$ ($p$ value) | Significance |
| Model  | 0.0146 | * | 0.011 | * | 0.0011 | * |
| A-Temp | 0.0228 | * | 0.0282 | * | 0.0002 | * |
| B-Time | 0.389 | ns | 0.832 | ns | 0.833 | ns |
| AB     | 0.269 | ns | 0.344 | ns | 0.618 | ns |
| $A^2$  | 0.0026 | * | 0.0002 | * | 0.0001 | *** |
| $B^2$  | 0.0301 | * | 0.3445 | ns | 0.6189 | ns |
| Residual | 0.011 | * | 0.0977 | ns | 0.0001 | *** |

*p < 0.05; **p < 0.01, ***p < 0.001
this point, digestibility was 67 %, which indicates that BR flour has a relatively high amount of dietary fiber (Pih and Kim 2013). It has been reported that the digestibility of normal cooked rice is about 71 % (Leegwater and Luten 1971; Hu et al. 2004).

The 3D response surface results for gelatinization are shown in Fig. 3. Gelatinization showed a tendency similar to that of digestibility. Gelatinization also showed its highest value at 90 °C, with 100 % gelatinization.

Optimized processing conditions

The objective of this study is to find the conditions that minimize the loss of antioxidant activity during the manufacturing of BR noodles. Therefore, three optimum conditions that minimize the loss of antioxidant activity and maximize the digestibility and gelatinization are shown in Tables 5, 6 and 7. Table 5 gives conditions considering only ABTS radical scavenging activity; Table 6 gives processing conditions considering only digestibility and gelatinization; and Table 7 gives conditions considering all three dependent variables. At each optimum condition, we maximized the appropriate dependent variables while keeping the others in range.

When considering only ABTS radical scavenging activity, the optimum processing condition is 70 °C and 22.95 min (Table 5). At that optimum condition, the predicted ABTS radical scavenging activity is 0.775 mg VCE/g solid sample, with digestibility and gelatinization of 51.98 % and 71.73 %, respectively. Under those conditions, the desirability was 0.727.

When considering only digestibility and gelatinization, the optimum processing condition is 88.18 °C and 34.89 min (Table 6). That optimum condition maximized digestibility and gelatinization at predicted values of 66.69 and 102.97 %, respectively. Under those conditions, ABTS
radical scavenging activity was 0.692 mg VCE/g solid sample, and desirability was 1.000. When considering all three variables, the optimum processing condition is 88.50°C and 40 min (Table 7). This optimum condition maximized ABTS radical scavenging activity, digestibility, and gelatinization. Predicted values for ABTS radical scavenging activity, digestibility, and gelatinization are 0.717 mg VCE/g solid sample, 66.55 and 102.58 %, respectively, with a desirability of 0.698. The latter two optimum processing conditions are similar. As mentioned previously, antioxidant activity can increase after certain heat treatment. Both digestibility and gelatinization also increase after enough heat treatment. It has been reported that the antioxidant activity of BR and germinated BR increased when they were gelatinized (Kim et al. 2013). Therefore, certain heat treatment in BR processing could improve both digestibility and antioxidant activity.

Validation tests

The results of the validation tests, based on the optimum processing conditions presented in Tables 5, 6 and 7, are shown in Table 8. Throughout the validation tests, the predicted and experimental values for all dependent variables were within the 5 % error range except TFC (p < 0.05).

Discussion

The aim of this study was to find the optimum processing conditions (temperature and time) to minimize the loss of antioxidant activity, based on current manufacturing process conditions (80–85 °C) for rice noodles. We considered six dependent variables (TPC, TFC, DPPH, and ABTS radical scavenging activity, digestibility, and gelatinization), and suggest three optimum conditions. It is important to not only minimize the loss of antioxidant activity but also maximize digestibility. Considering only antioxidant activity could result in insufficient digestibility and gelatinization. Our research suggests that sufficient heat treatment (88.50 °C) can increase both antioxidant activity and digestibility.

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Table 8 Comparison between predicted value and experimental value for the optimum conditions

| Run | Process variables | Predicted value | Experimental value |
|-----|-------------------|----------------|--------------------|
|     | X1 (°C) | X2 (min) | TPC (mg GAE/g solid sample) | TFC (mg CE/g solid sample) | DPPH (mg VCE/g solid sample) | ABTS (mg VCE/g solid sample) | Digestibility (% d.b) | Gelatinization (% w.b) |
| 1   | 70     | 22.95  | 0.678              | 0.195               | 0.339              | 0.775              | 51.98               | 71.73               |
|     |        |        | 0.687 ± 0.04       | 0.257 ± 0.02        | 0.353 ± 0.01       | 0.768 ± 0.07       | 50.51 ± 0.43        | 66.80 ± 0.06        |
| 2   | 88.18  | 34.89  | 0.585              | 0.212               | 0.318              | 0.692              | 66.69               | 102.97              |
|     |        |        | 0.621 ± 0.04       | 0.268 ± 0.01        | 0.356 ± 0.03       | 0.713 ± 0.09       | 63.21 ± 0.49        | 100 ± 0.00          |
| 3   | 88.5   | 40     | 0.589              | 0.195               | 0.337              | 0.717              | 66.55               | 102.5               |
|     |        |        | 0.590 ± 0.04       | 0.253 ± 0.04        | 0.329 ± 0.03       | 0.715 ± 0.09       | 66.81 ± 0.21        | 100 ± 0.00          |
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