

## Effects of processing conditions on change of amino acids, reducing sugar and total polyphenols of caramelized malt produced from IR50404 rice variety

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### Article history:

Received: 16 July 2022

Received in revised form: 10 September 2022

Accepted: 21 September 2022

Available Online: 30 March 2024

### Keywords:

IR 50404,

Rice malt,

Roasting,

Tempering,

Total polyphenol

### DOI:

[https://doi.org/10.26656/fr.2017.8\(2\).982](https://doi.org/10.26656/fr.2017.8(2).982)

### Abstract

Rice beer is a value-added product from rice since it consists of enough conditions for making beer. To produce the best caramelized malt from IR50404 rice variety, understanding the effects of processing conditions on change of amino acids, reducing sugar and total polyphenols is very important for developing such rice beer processing. Changes in chemical compositions and enzyme activities in caramelized malt processed from IR50404 rice variety were understood. Suitable germination conditions were indicated, including a soaking temperature of 30°C for 24 hrs, degree of steeping of 35-36% and germination time of 4 days at 30°C. Green rice malt was incubated at 40-70°C for 30-120 mins. Then, rice malt was roasted at different temperatures of 125-200°C for 30-90 mins. The results showed that both tempering and roasting processes changed the concentration of polyphenols, reducing sugars and amino acids. When green malt was incubated at 50°C for 60 mins, reducing sugars, amino acids and polyphenol content (TPC) increased by 2 times (102.72 mg/g), 1.8 times (9.08 mg/g) and 1.5 times (4.26 mg GAE/g) respectively corresponding to the improvement of the amylase and protease activity. Moreover, there were maximum increases in phenolic compounds in roasted malt at 125 and 150°C for 45 and 75 mins for tempered malt and green malt, respectively. In contrast, higher heat treatment (roasted at 175-200°C) resulted in reducing the levels of all evaluated variables. Thus, malting with a moderate thermal treatment (125-150°C) could be considered an effective process to enrich (or enhance) antioxidants in rice grains for their further use as a functional ingredient in malt and beer production.

## 1. Introduction

In brewing, malt (germinated cereals) has substantially higher nutritional and physiological value than its raw grain counterparts. In malt, besides increasing reducing sugars, free amino nitrogen (FAN) also increases because of the proteolytic activity (Garzón and Drago, 2018). The nutritional transformation that occurs during malt and beer production is complex and involves many enzymes such as  $\alpha$ -amylase,  $\beta$ -amylase,  $\alpha$ -glucosidase, and proteinase (Gupta *et al.*, 2010). FAN (the protein degradation product) is a predictor of fermentation efficiency (Lekkas *et al.*, 2014). FAN is a crucial general measure of those yeast nutrients that essentially constitute the assimilable yeast nitrogen in a typical brewing fermentation (Lekkas *et al.*, 2014). Investigate changes in biochemical components of rice varieties during germination based on changes similar to barley malt to provide a scientific basis to increase the

use of rice to produce malt and beer. Moreover, applied studies of rice malt in production are important prerequisites for testing brewing from rice malt (Capanzana and Buckle, 1997; Usansa, 2008; Mayer *et al.*, 2016).

Malt can be classified into light and dark (special malt) (Čechovská *et al.*, 2012). The roasting of the malt helps to create beers with different aromas and colors (Hoff *et al.*, 2012). For caramelized malt, the tempering stage was performed before roasting to help hydrolyze carbohydrates and proteins to reduce sugars and amino acids in the malt (Čechovská *et al.*, 2012). In beer production, special malt was commonly used to impart distinctive color and flavor as well as to enhance antioxidant activity in beer (Floridi *et al.*, 2009). Dark malts can be considered the major product of non-enzymatic browning reactions. This degree of browning is an important factor in malt quality. It is involved not

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only in color formation in the final beer but also in antioxidant generation and flavor properties (Coghe *et al.*, 2006; Omwamba and Hu, 2009; MacLeod and Evans, 2015; Szila, 2019). Moreover, the antioxidant composition in beer, which is mainly polyphenols is largely derived from malt (about 80%) (Samaras *et al.*, 2005), and 20% is extracted from hops during boiling. Therefore, it can be said that the malt and the stages of beer production are the main sources of antioxidants in beer (Čechovská *et al.*, 2012).

Recent research has shown that only several rice varieties grown in the Mekong Delta such as IR50404 are promising for seed germination and malt. IR50404 rice variety is prevalent in the Mekong Delta due to its good alum tolerance, high growth rate, and high yield (Tran *et al.*, 2016). In Vietnam, there have been initial studies on the processing of malt from rice as well as changes in nutrient composition during immersion and germination of some common rice varieties (Nguyen and Trinh, 2013) or on germinated brown rice (Tran *et al.*, 2016), as a basis for malt processing from rice and towards testing beer production from rice. Therefore, the objectives of this work were to determine the antioxidant activity and the colour of roasted malt and to monitor changes in the composition (phenolic compounds, sugars, amino acids) of roasted malt. Therefore, the objective of this work was to monitor changes in the nutritional composition (sugars, amino acids, phenolic compounds, antioxidant activity and color) of caramelized rice malt during processing.

## 2. Materials and methods

### 2.1 Materials

Rough rice of *Oryza sativa L.*, cultivar IR50404 was purchased from Mekong Rice Research, Can Tho city, Viet Nam.

### 2.2 Sample preparation

#### 2.2.1 Prepare green rice malt

Raw seeds were washed and soaked in tap water at room temperature for 24 hrs (change water every 12 hrs, allow to aerate 30 mins). The seeds were germinated in a germinating oven (SANYO MCO-5AC, Japan) for 4 days at 30°C.

#### 2.2.2 Production of caramel malt

For the saccharification stage, the green malt was contained in a closed plastic bag in the oven, and the outside temperature bag was increased to 40-70°C for 30-90 mins. In the subsequent roasting phase, tempered malt was roasted by a Coffee roaster (JMS-270, China) at 125-200°C for 30 - 90 mins. After roasting, malted rice was allowed to cool to ambient temperature in a

desiccator. Each roasting condition was performed in triplicate. Roasted malt processing is shown in Figure 1.

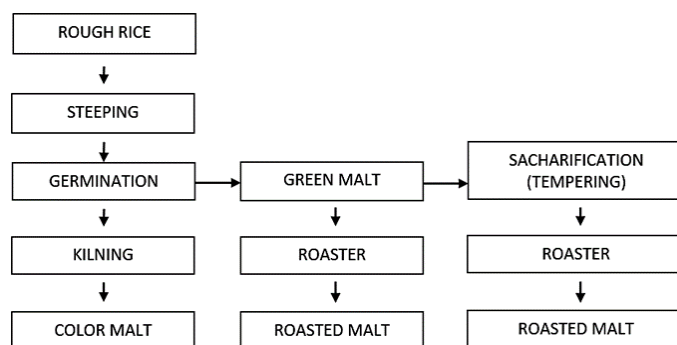


Figure 1. Diagram of roasted rice malt processing.

### 2.3 Analytical methods

#### 2.3.1 Total phenolic compounds

Using the Folin-Ciocaltue method, the total phenolic compounds (TPC) in raw and malted rice were extracted twice with 80% ethanol, were pooled, filtered and stored at -18°C (Moongngarm and Khomphiphatkul, 2011; Carciochi *et al.*, 2016). The 0.2 mL extract was mixed with 0.8 mL of Folin-Ciocaltue (1:10) reagent (freshly prepared diluted) and 2 mL of 7.5% sodium carbonate, diluted to 10 mL with deionized water. The mixture was kept in the dark at ambient temperature for 30 mins. The absorbance at 765 nm was measured, using a UV-Vis spectrophotometer (Visible spectrophotometer 722, Korea). The results of TPC were expressed as mg Gallic Acid Equivalents (GAE) per g of malt.

#### 2.3.2 Antioxidant activity

Radical scavenging activity (DPPH) was measured in the same conditions described in previous work by Zhao (2015).

#### 2.3.3 Reducing sugar

One mL of diluted wort was mixed with 1 mL of DNS solution (10 g of 3, 5-dinitrosalicylic acid, 300 g of  $\text{KNaC}_4\text{H}_4\text{O}_6$  in 200 mL of 2N NaOH and adjusted to 1 L with RO water). The mixtures were mixed thoroughly, and the development of colour was conducted by boiling the reaction tube for 5 mins. The concentration of reducing sugar was calculated against the standard glucose concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 g/L (Miller, 1959).

#### 2.3.4 $\alpha$ -Amylase

The  $\alpha$ - Amylase activity was measured according to the method by Watanabe *et al.* (1998). A total of 10.0 g of rice malt was extracted with 0.5% NaCl solution (50 mL in 3 hrs). The 0.1 mL rice malt extract was added to 2.0 mL of 40 mM acetate buffer (pH 5.0) containing 1.0% soluble starch at 40°C. 0.1 mL of the enzyme

reaction mixture was added to 10 mL of  $2.5 \times 10^{-4}$  M iodine solution, and the per cent transmission of the resulting starch-iodine colour was measured at 670 nm.

2.3.5 Protease activity

The enzyme activity (U) was defined as the amount of 1.0 micromole of tyrosin enzyme released from 10 g/L casein in 1 hr at pH 7.2 and 50°C. The activity of malt protease is presented as U/g dry malt powder.

2.3.6 Free amino nitrogen

The free amino nitrogen (FAN) was determined by the conventional ninhydrin base according to Lie (1973). The results were expressed as mg FAN/g dry wt malt.

2.3.7 Moisture content

Moisture content was examined by AOAC 925.40 (AOAC, 1998). Samples were dried at 105°C to constant weight.

2.3.8 Extract colour

Spectrophotometric Method (EBC-U), EBC method 8.5 (Viggiano, 2006). Extract colour was measured at a wavelength of 430 nm at room temperature, distilled water served as a blank. The colors for sample were calculated according to EBC8.5 (1998) using the formula: Color (EBC units) = A. f. 25 (where: A = absorbance of the sample at 430 nm in 10 mm cell, f = dilution factor).

3. Results and discussion

3.1 Physical change of the reducing sugars and free amino acids during intensive tempering

Caramel malts (dark malts) are the malt with a significant part of the conversion of starch to sugars that occurred before mashing because of amylase enzyme activity. During the processing of caramel malts, stewing is carried out before high-temperature roasting, which hydrolyzes two important storage components, carbohydrates and proteins into reducing sugars and soluble amino acids (Čechovská *et al.*, 2012). Green malts (with high moisture content > 40%) are tempered and heated to a temperature of about 62–68°C for 30–60 mins in a closed drum (Hertrich, 2013).

Figure 2(A-D) illustrates data on how temperature and time of incubation affected  $\alpha$ -amylase enzyme activity, reducing sugars, protease activity and amino acids of rice malts. In general, the tempering conditions (temperature and time of incubation) have a significant impact on the nutritional ingredients of the extracts. Also, the reduced sugar content increased strongly in the first stage before showing a falling trend from 60 mins onwards and corresponded to the change in  $\alpha$ -amylase enzyme activity (Figure 2A).  $\alpha$ -amylase enzyme activity was the highest at 50° for 60 mins (56.26 U/g). The reduced sugar content (Figure 2B) peaked at 70°C for 60 mins (188.71 mg/g). According to Coghe *et al.* (2006), when green malts were processed inside the closed drum, and the grain temperature increased to about 70°C, at a

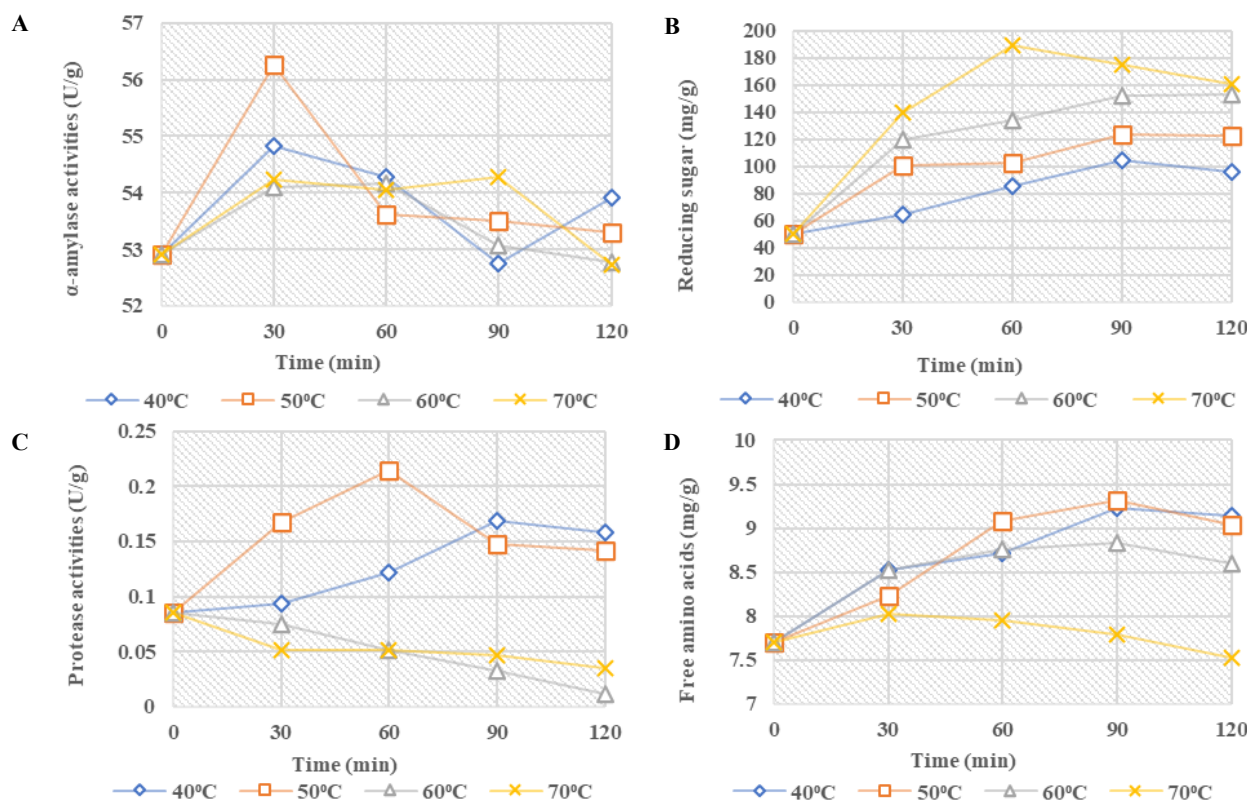


Figure 2. Change of the  $\alpha$ -amylase activity (A) reducing sugars, (B) protease activity, (C) free amino acids, and (D) in during tempering. The concentrations of all rice malt extracts are on the same dry weight basis.



constant moisture level of about 43% was the optimal temperature for amylase activity, which hydrolyzes a significant portion of the starch in the grain. Thus, the tempering conditions had a great influence on reducing sugar content in malt.

Similar to amylase activity, the protease enzyme activity showed a sharp rise during the first 60 mins too corresponding to the improvement in amino acids content. Protease enzyme activity was the highest at 50°C for 60 mins and the peak of amino acids for 90 mins with 0.214 U/g and 9.32 mg/g (increased by 1.21 times compared to green malts), respectively. At high temperatures (60°C and 70°C), the enzyme activity was the lowest and declined steadily. This result showed that amino acid content was affected by temperature and tempering time because some amino acid components were converted from one form to another and partly for rice development of plants (Okolo and Ezeogu, 1996). The reduction in amino acids in the early stages can be explained by participation in Maillard reactions - a series of complex reactions after the reaction between sugars and amino acids. Most of the non-enzymatic browning during the heat treatment process of malt forms the products of this reaction which enhances the colour of malt (Coghe *et al.*, 2004).

### 3.2 Total polyphenol content during intensive tempering

Phenolic compounds were a major group of compounds that contributed to the antioxidant activity (Zhao, 2008). In general, the incubation time (30-90 mins) had a significant effect on the total phenol content. TPC of the different conditions tempering was higher for unprocessed green malt and ranged from 3.44 to 5.05 mg GAE/g (Figure 3A). The differences in TPC content at 120 mins for four tempering temperatures were not significant ( $P>0.05$ ), but a higher amount of TPC in line 0 mins was detected.

The cause of the increase in TPC during malting could be explained by the action of endogenous esters synthesized during germination leading to the release of the initial phenolic compound associated with the formation of products of the Maillard reaction during incubation (Carciochi *et al.*, 2016). The tempering process modified the antioxidant activity, measured by its free radical scavenging capacity, of rice malt studied (Figure 3B). After a tempering period from 30 to 120 mins, tempered rice malt showed higher antioxidant activity than green malt by 25.23-46.21% with percentage inhibitor 30.87 and 36.04%, respectively. Time and temperature were factors that seemed to influence this capacity, as it reached the highest values of antioxidant activity from 60-90 mins of tempering.

In short, during the saccharification phase, the activity of the two enzyme groups, protease and amylase increased in the appropriate temperature zone and hydrolyzed the nutrient components of protein and starch in rice to form amino acids and sugars reducing some other products. However, at higher temperatures, treatment inhibited hydrolysing enzymes. Thus, the suitable temperature and incubation time for the creation of reducing sugars and amino acids of 50°C for 60 mins period were selected for the next roasting experiment.

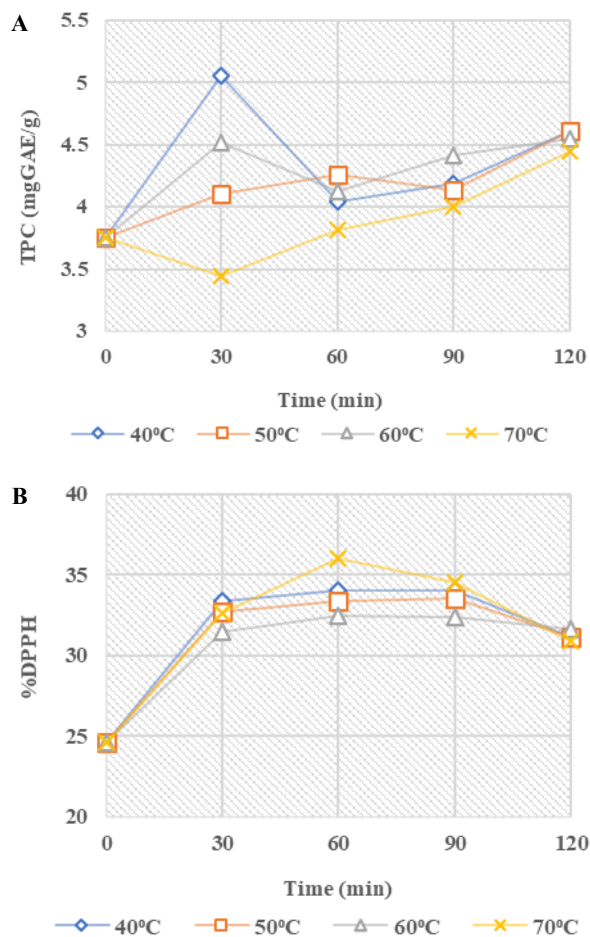


Figure 3. Change of (A) TPC and (B) DPPH during tempering. The concentrations of all rice malt extracts are on the same dry weight basis.

### 3.3 Change of the reducing sugars and free amino acids content in a roasting

The reducing sugars and amino acid content generally decreased at all roasting temperature levels. Figure 4(A-D) illustrates data on how temperature and time of roasting affected reducing sugars and amino acids of roasted malts for both tempered malt and green malt. The decline of most pronounced at the beginning of the roasting process by 30 mins for roasted malt from tempered malt (Figures 4A and 4C) and green malt (Figures 4B and 4D).

In general, the reduction of FAN and reducing sugars also depended on the roasting temperature. At the higher roasting temperature (175 and 200°C), a faster reduction

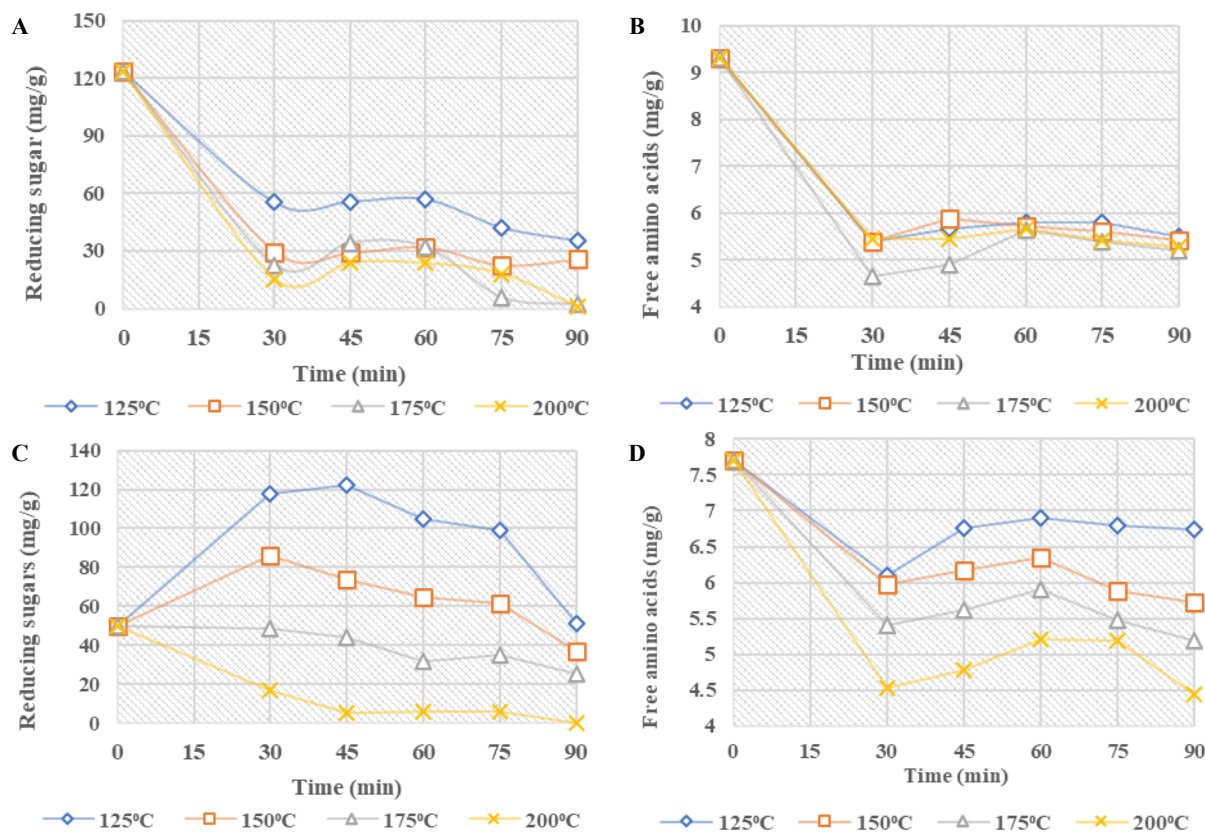


Figure 4. Change of reducing sygars and free amino acids in roasting from tempered malt (A-B) and green malt (C-D). The concentrations of all rice malt extracts are on the same dry weight basis.

of reduced sugar was created/released because reducing sugar is the primary substrate for pyrolysis reaction (temperature > 200°C) and caramelization (Coghe *et al.*, 2004). In addition, according to Benzing-Purdie *et al.* (1985), there was an increase in the rate of the Maillard reaction with increasing temperature with different reaction patterns between sugars and amino acids.

However, at the lower roasting temperatures (125 and 150°C), there was an increase in reducing sugars during the first period (the first 30 mins) for roasted malt from green malt. This variation can be explained that, at the beginning of the roasting process, the green malt received heat from the roasting equipment and increased the heat inside the grain, stimulating the active amylase enzyme system to decompose many starches reserve to form reducing sugar at an early stage. After this period, when the temperature of malt increased, leading to the destruction of these enzymes and the formation of non-enzymatic browning reactions – Maillard and Caramelization reactions take place strongly. Most of the non-enzymatic browning during the heat treatment process of malt forms the products of this reaction which enhances the colour of malt, and these reactions have started at temperatures of 50°C (Coghe *et al.*, 2004). The change in reducing sugar and amino acid content due to these two components is the main substrate involved in the Maillard and Caramelization reaction leading to an increase in malt colour and malt extract colour.

### 3.4 Change of the polyphenol contents, moisture and color

TPC in roasted malt are shown in Figure 5A and Figure 5B. Compared with the raw grain (3.25 mg GAE/g), TPC increased significantly in green malt (3.75 mg GAE/g), and tempered malt (4.26 mg GAE/g), and continued to increase after roasting. For malt roasted from tempered malt, TPC was highest when tempered malts were roasted at 125°C for 90 mins (5.95 mg GAE/g) and at 150°C for 45 mins from green malt (7.19 mg GAE/g). However, increasing the roasting temperature (175-200°C) will reduce TPC in received malt. The cause of this decline may be the breakdown of some phenolic compounds. This result is similar to that reported by Carciochi *et al.* (2016) when roasting quinoa seeds, they noted that the highest phenolic compounds content was recorded when the seeds were roasted at 145°C and gradually decreased at 190°C.

The increase in TPC during malting can be explained by the activity of endogenous esterases synthesized during germination, which release the initial phenolic compounds (Maillard *et al.*, 1996; Carciochi *et al.*, 2016) and changes in plant cell wall conformation during high-temperature roasting lead to the release of previously glycosylated/esterified phenolic compounds (Dewanto *et al.*, 2002; Maillard *et al.*, 2007; Chandrasekara and Shahidi, 2011).

When roasting at higher temperatures (175 and 200°

C) will significantly reduce the TPC value in malt. The cause of this phenomenon is the depletion of some phenolic compounds and the thermal decomposition of the reactants under conditions of high temperature and low moisture (Yahya *et al.*, 2014). According to Samaras *et al.* (2005), catechin and ferulic acid are the most abundant phenolic compounds in yellow malt and there is a decrease in ferulic acid content when roasting at high temperature. Moreover, the concentration and type of participating substances are essential for Maillard's reaction rate. The Maillard reaction usually takes place strongly in the early stages of the heating process. Monosaccharides have a stronger reaction than di or oligosaccharides. The nature of amino compounds also affects the Maillard reaction rate (Coghe *et al.*, 2004). Under some conditions, essential amino acids such as serine and threonine, contain aliphatic hydroxyl groups and have the highest reaction with  $\alpha$ -dicarbonyl

compounds. In contrast, non-polar amino acids and acids have the lowest reaction (Samaras *et al.*, 2005).

Compared with yellow pils (pilsner), special dark malts are subjected to a higher impact temperature, resulting in a dark brown colour. According to Coghe *et al.* (2006), a variety of malts of different dark and light colors (dark, from pale yellow to amber to brown to almost black) can be produced depending on the degree of heat during roasting. As shown in Figure 6C and Figure 6D, the colour of roasted malt extract in EBC colour units varies widely in roasting temperature and time as well as roasted malt materials. The higher the roasting temperature, the darker the malt extract colour is shown in the tropical EBC unit with roasting temperatures of 175 and 200°C.

For malts roasted at a lower temperature, this increase in the colour index represents less (150°C) and is virtually unchanged over time for roasted temperatures of 125°C. Because rice contains only a very low concentration of natural pigments, the malt colour mainly develops during malt production. For dark malt, the Maillard reaction is the main source of colour formation. Colours formed in the production range from light yellow to very dark brown, depending on the range of reactions. One of the key ingredients in the colour of black malt is macromolecular melanoidin (Coghe *et al.*, 2004). This argument is consistent with the results of the survey on the fluctuation of amino acids and reducing sugars during roasting due to Maillard reaction and Caramelization. In beer brewing, roasted malt is used as a low percentage additive to conventionally dried pale malts which enhances the final beer color (Yahya *et al.*, 2014).

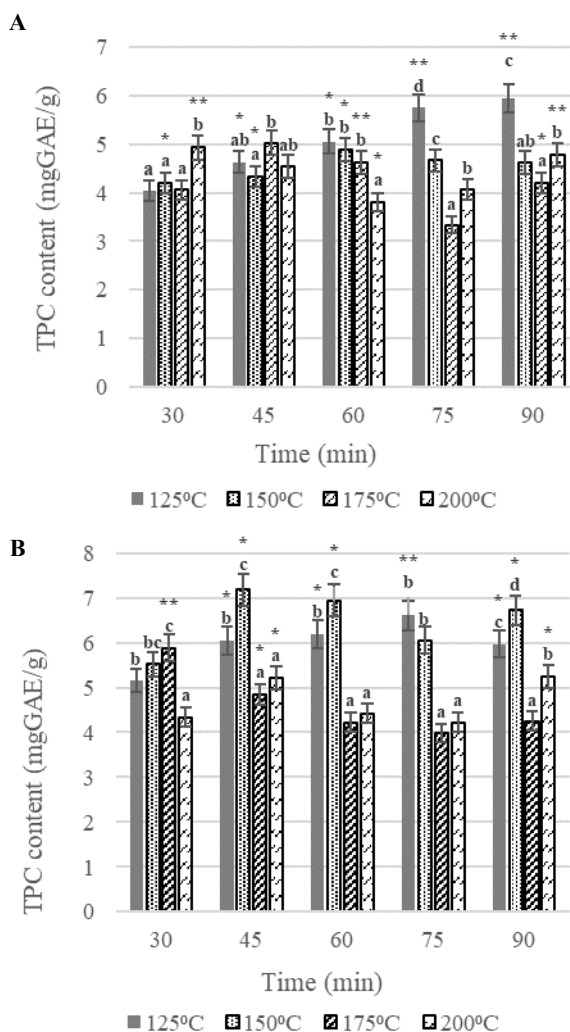


Figure 5. Change of TPC in roasting from tempered malt (A) and green malt (B). The concentrations of all rice malt extracts are on the same dry weight basis. Error bars represent the standard deviation of each data point (n = 3). Bars with different alphabets are statistically significantly different ( $P < 0.05$ ) for roasting temperature while bars with different asterisks (\*) are statistically significantly different ( $P < 0.05$ ) for roasting time.

#### 4. Conclusion

The study of the effect of caramel malt processing (tempering and roasting) on the nutritional ingredients of rice malt allowed us to find suitable incubation and roasting conditions: Tempering trials performed on green rice malt obtained under suitable conditions showed the increases in reducing sugars, free amino acids and phenolic compounds at 50°C for 60 mins. During roasting, there was an unequal change in the quality of roasted malt for samples of roasted malt from green malt and tempered malt. However, roasting temperature and time had a relatively similar effect between the two roasted malt groups. Specifically, the higher the roasting temperature and the longer compound time, which leads to the loss of many nutrients in roasted malt. In more intensive thermal treatment (200°C) decreased the levels of all evaluated variables. Therefore, malt roasted at a moderate temperature (125-150°C) can be considered an



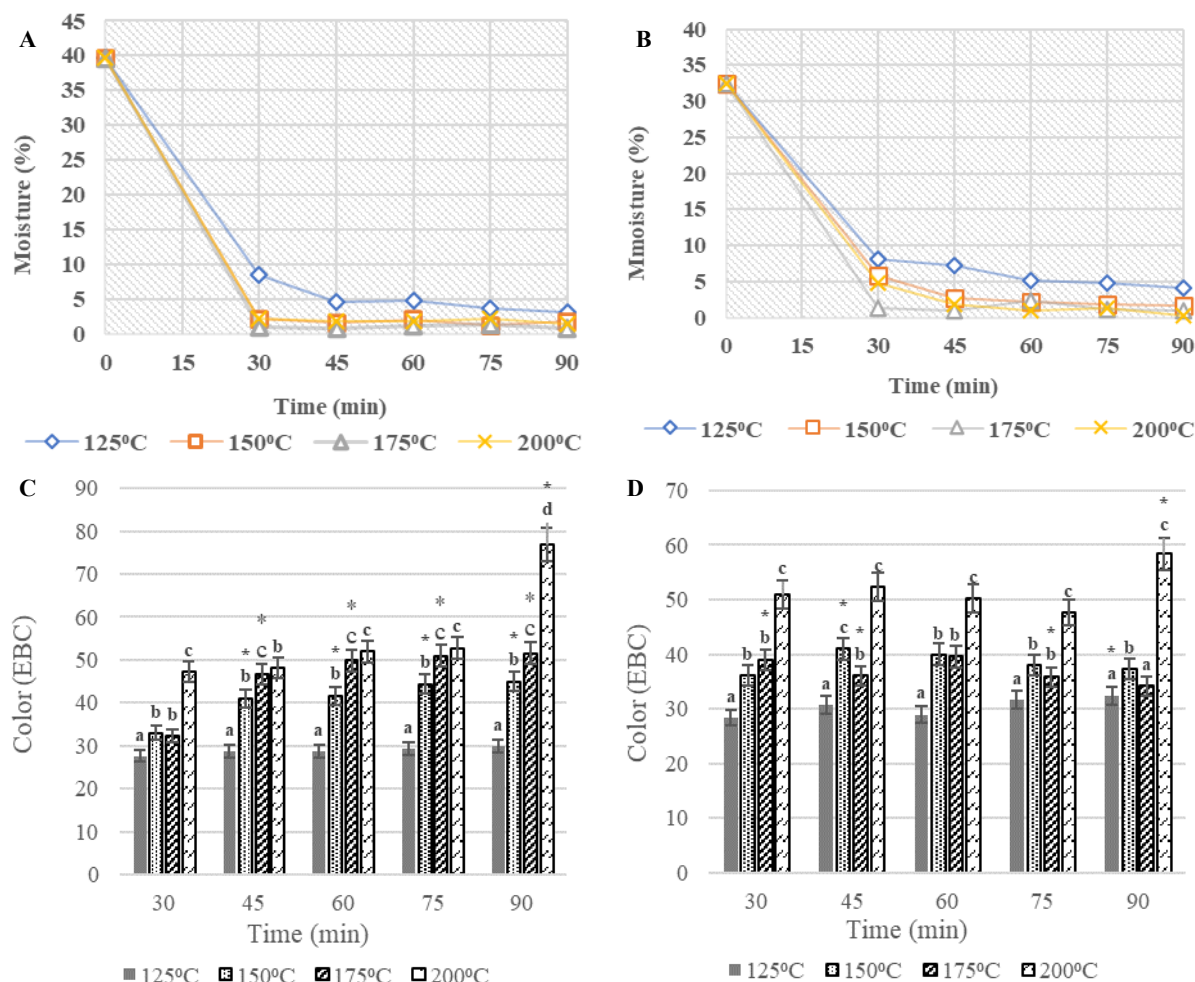


Figure 6. Change of moisture and color in roasting from tempered malt (A, C) and green malt (B, D). The concentrations of all rice malt extracts are on the same dry weight basis. Vertical bars represent the standard deviation of each data point (n = 3). Bars with different alphabets are statistically significantly different ( $P < 0.05$ ) for roasting temperature while bars with different asterisks (\*) are statistically significantly different ( $P < 0.05$ ) for roasting time.

effective process to help increase TPC, which has an antioxidant role in raw rice, which can then be used as a functional ingredient for the production of gluten-free foods and beverages.

**Conflict of interest**

The authors declare no conflicts of interest.

**Acknowledgements**

This study is funded in part by the Can Tho University Improvement Project VN14-P6, supported by a Japanese ODA loan, Program A15.

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