Food Research 9 (1) : 175 - 185 (February 2025)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

Effect of encapsulation of bioactive compounds from germinated black rice for rice cracker

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Article history:
Received: 2 December 2023
Received in revised form: 4 April 2024
Accepted: 1 October 2024
Available Online: 8 February 2025

Keywords:
Germinated black rice,
Encapsulation,
Rice cracker,
Fourier transform infrared spectroscopy

DOI:
https://doi.org/10.26656/fr.2017.9(1).378

Abstract

The encapsulation technique was applied to improve the stability of bioactive compounds in germinated black rice. The objective of this study was to investigate the encapsulation of bioactive compounds from germinated black rice in black rice cracker products. Germinated black rice was prepared by soaking brown rice in 37°C water for 24 hrs and watering every 4 hrs (5 times). The encapsulation was done by a coacervation technique using gelatin and maltodextrin (DE20) as coating materials. The effect of the encapsulation on bioactive compounds and the antioxidant activity degradation was evaluated. High pressure liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC) and Fourier transform infrared spectra (FTIR) analyses revealed that the bioactive compounds were successfully preserved due to the encapsulation process. Encapsulation efficiency was characterized in terms of the bioactive compounds such as α-tocopherol, γ-oryzanol, GABA, and phenolic acids. The rice cracker with encapsulation using maltodextrin and gelatin (SD2:10) showed slight decreases of α-tocopherol (38.17%%), γ-oryzanol (33.61%), and GABA (1.01%) compared to the original black rice cracker (CR). It was found that encapsulation could prevent a decrease in phenolic compounds, flavonoids, and antioxidant capacity. While the total antioxidant activities of SD2:10 decreased from CR by 2.93%, the values of ABTS and FRAP decreased by 11.33% and 11.95%, respectively. Major phenolic acids such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, and vanillic acid were slightly decreased in SD2:10. Fourier Transform Infrared spectra (FTIR) analyses suggested that encapsulation with maltodextrin and gelatin could be protected bioactive compounds from heat, light, and oxygen, and could be used to encapsulate bioactive compounds from black rice in the food industry.

1. Introduction

Black glutinous rice (Khao Hom nin) (*Oryza sativa* L.) is a local rice cultivar in Northern and Northeastern Thailand (Laokuldilok and Kanha, 2015). The major free phenolics in black rice were protocatechuic, ferulic and vanillic acids, and quercetin (Huang and Lai, 2016). Cyanidin-3-glucoside (C3G) and peonidin-3-glucoside (P3G) were the main anthocyanins found in red and black rice (Shao *et al.,* 2014). In addition, black rice contains many beneficial components, including polyphenolics, flavonoids, tocotrienols, tocopherols, phytic acid, GABA, and γ-oryzanol. These antioxidant compounds eliminate reactive oxygen species (ROS) such as lipid peroxide and superoxide anion radicals and lower cholesterol content (Kong and Lee, 2010).

Encapsulation can protect the core material such as bioactive compounds from environmental conditions. The techniques employed in encapsulation include spray drying, liposome entrapment, complexation, coacervation, co-crystallization, nanoencapsulation, and freeze-drying (Veluppillai *et al.*, 2009: Fang and Blandari, 2010). Encapsulation by coacervation generates a complex compound of liquid-liquid phase that later turns into a biopolymer coating material of the core bioactive compound. In the microencapsulation system, different natural and synthetic compounds such as native starches, maltodextrin, and hydrocolloid (gum Arabic, xanthan gum, guar gum, gelatin, carbohydrate, cellulose, carboxyl methylcellulose) are used as coating materials. The coacervation process is an interaction between hydrophobic compounds that generate ionic and

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eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

hydrogen bonds and the change in the pH. The use of maltodextrin in the encapsulation resulted in a high entrapment efficiency of the bioactive compound (Fang and Blandari, 2010; Eghbal and Choudhary, 2018). Peanparkdee *et al.* (2021) studied the stability of bioactive compounds, and antioxidants in bran extracts of Thai rice cultivars (Khao Dawk Mali 105, Kiaw Ngu, Hom Nil, and Leum Pua) encapsulated using a coacervation process with gum Arabic, gelatin, and the mixture of gelatin and gum Arabic. The encapsulation by gelatin and gum Arabic complex had the lowest release of the bioactive compound and antioxidant activity after simulated digestion. Bulatao *et al.* (2017) studied the encapsulation of anthocyanins from black rice bran using chitosan-alginate as a coating material by two processes of ionic pre-gelation and polyelectrolyte complex. The result of the study showed that the combination of chitosan-alginate and 30 mg anthocyanin extract had the highest encapsulation yield and antioxidant activity. Laokuldilok and Kanha (2016) investigated the encapsulation of anthocyanin from black glutinous rice (BGR) bran extracted using enzymatic digestion of BGR grain fractures. Maltodextrins with 3 dextrose equivalents (DE) at DE10, 20 and 30 (BRM10, BRM20 and BRM30) were used in the encapsulation by two processing techniques, freeze-drying and spray drying. Freeze-drying encapsulation with maltodextrin DE20 (BRM20) showed high antioxidant activity, microencapsulation efficiency, and high storage stability.

The germination process of rice stimulates and enhances the bioactivities of α-amylase, β-amylase, and proteinase enzymes that break down macromolecules resulting in increases of free amino acid, reducing sugar, and GABA. Rice germination can create a variety of health benefits due to the higher nutritional value and the increase of bioactive compounds, i.e., α-tocopherol, α-tocotrienol, γ-oryzanol, γ-aminobutyric acid (GABA), polyphenolic compounds, vitamin B complex. The germination could also increase phenolic compounds including protocatechuic acid, p-coumaric acid, and ferulic acid which is mostly founding the form of cell wall-bound phenolic acids (Moongngarm and Saetung, 2010; Sompong *et al*., 2011).

This study explored the encapsulation of bioactive compounds from germinated black rice by coacervation complex technique using maltodextrin and gelatin. The encapsulates were used to produce seasoned rice crackers. The antioxidant activity, phytochemicals such as α-tocopherol, α-tocotrienol, γ-oryzanol, γ-aminobutyric acid (GABA), total phenolic compounds, total flavonoids, phenolic acids in germinated black rice flour and germinated black rice cracker were analyzed using High-pressure liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC), and the change in the chemical structures was evaluated by Fourier transform infrared spectra (FTIR).

2. Materials and methods

2.1 Rice sample

Black glutinous rice (Khao Hom Nin, *Oryza sativa* L.) was obtained from the local market in Surin Province, Thailand. Germinated rice was prepared using the method described by Moongngarm and Saetung (2010) by soaking brown rice in 37°C water for 24 hrs, then watering it every 4 hrs (5 times) after drying it in the hot air oven at 80°C for 3 hrs (Ger BR).

2.2 Chemicals and reagents

Potassium hydrogen phosphate (KH_2PO_4) and ferric sulphate ($FeSO_4$) were obtained from Univar Solutions Inc., USA. Folin–Ciocalteu reagent was obtained from LOBA Chemie Pvt. Ltd., India. Ferric chloride ($FeCl_3$) and trichloroacetic acid were acquired from POCH SA, Sowinskiego, Poland. All other reagents and solvents used were of HPLC grades. HPLC grade methanol, isopropanol, dichloromethane, formic acid, acetonitrile, and ammonium molybdate used for extraction and purification were obtained from BDH, Middle East, FZ-LLC, UK. Gallic acid was obtained from Fluka Analytical, USA. Trolox, hydroxy naphthaldehyde (HN), 2-2-Diphenyl-2-picrylhydrazyl (DPPH), Vitamin E (α-tocopherol), standard vitamin B, g-aminobutyric acid and phenolic acid standards were purchased from Sigma Aldrich Chemical Co., USA.

2.3 Sample preparation

Rice flour was produced by dried milling and sieved through an 80-mesh sieve. The rice cracker was produced by adapting the recipe of traditional crispy rice (Khao Kriap Wow) from the Northeast of Thailand. The method used was a slight modification from Qadri *et al.* (2018). A total of 100 g of germinated black rice flour, 25 g of sugar, 1 g of salt, 1 g of gelatin, and 250 g of water were mixed and cooked using an electric cooker for 5 mins at 80°C, after that the mixture was pasted on an aluminum tray with the thickness of 0.1 mm. Rice paste was dried in the hot air oven at 70°C for 5 hrs, and cut into 2´2 cm samples. The rice cracker was seasoned and deep-fried in 160°C palm oil (CR). Encapsulation of germinated black rice cracker was done by mixing in maltodextrin and gelatin in the rice paste in the tray before drying. The ratio of maltodextrin: germinated black rice flour was 20:80 g with 1 g gelatin. The sample of encapsulated rice crackers was referred to as SD2:10.

https://doi.org/10.26656/fr.2017.9(1).378

2.4 Ferric reducing antioxidant power

The FRAP assay is a method measuring the formation of blue color of Fe^{2+}–TPTZ complex (Fe^{2+} tripyridyl triazine) that increases the absorbance at 593 nm. The FRAP assay followed the method by Thaipong *et al.* (2006). The stock solutions to be used in the method included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution, and 40 mM ferric chloride ($FeCl_3.6H_2O$) solution. The fresh working solution was prepared by mixing 2.5 mL TPTZ solution, 25 mL acetate buffer, and 2.5 mL of $FeCl_3.6H_2O$ solution together in the test tube. Then, 0.25 ml of rice extract (or rice cracker extract), and 4.75 mL of the FRAP solution were mixed in and kept for 30 mins in the dark room. The absorbance of colored product was taken at 593 nm by UV 1800 Shimadzu spectrophotometer. The standard curve was Trolox equivalent (0-50 µg/mL).

2.5 ABTS radical scavenging activity

The ABTS radical scavenging activity of the germinated black rice, rice cracker, and rice cracker with SD2:10 extract was measured using a method adapted from Thaipong *et al*. (2016). The stock solutions of 2 mM of ABTS solution and 70 mM of $K_2S_2O_8$ solution were prepared. The working solution was then prepared by mixing the two stock solutions. The ABTS solution incubating the solution for 48 h in a dark room at room temperature. The solution was diluted by mixing the ABTS solution with a 0.1 M phosphate buffer solution added with NaCl (0.818%) and KCl (0.0015%) at a pH of 7.4 (1:6 v/v). Approximately, 0.1 mL rice extracted and 3 mL ABTS solution were mixed and kept in the dark condition for 6 min. The solution was measured at 734 nm using the UV 1800 Shimadzu spectrophotometer. For each experiment, a fresh ABTS solution was prepared. The standard curve was Trolox equivalent (0-50 mg/mL).

2.6 Total antioxidant capacity

The total antioxidant capacity was the measurement of the transformation of Mo (VI) to Mo (V) according to the formation of a phosphate Mo (V) complex at acid pH described by Dasgupta *et al.* (2004). 0.3 mL of rice extract (or rice cracker extract) was combined with 0.1 mL of 0.6 mM H_2SO_4, 0.1 mL of 28 mM sodium phosphate and 0.1 mL of 4 mM ammonium molybdate in a test tube, then incubated in a water bath at 95°C for 90 mins. The absorbance of the solution was measured at 659 nm using the UV 1800 Shimadzu spectrophotometer. The percentage of inhibition activity was calculated with the following equation;

$$\text{Inhibition Activity} = [(A_0 - A_e)/A_0]\times 100\%$$

where A_0 is an absorbance of the solution without the extract, and A_e is an absorbance of the solution with the extract.

2.7 Total phenolic compounds

Total phenolic compounds were analyzed using Folin-Ciocalteu reagent with the method adapted from Dasgupta *et al*. (2004). A total of 10 g of germinated black rice (or rice cracker) was extracted using 100 mL of 75% ethanol. The supernatant was filtered by a Whatman filter membrane No. 1 and 4, respectively. Briefly, Folin-Ciocalteu reagent was diluted with water (1:1 v/v). Approximately 1.25 mL of this reagent was combined with 300 µL of the sample extract and after 30 mins incubation at room temperature, 3.75 mL sodium carbonate solution (0.75% w/v) was added. The mixture was incubated for 120 mins in the dark room. The absorbance at 765 nm was measured using the UV 1800 Shimadzu spectrophotometer. The standard curve was created using gallic acid 0-1.0 mg/mL.

2.8 Flavonoids content

The total flavonoid content (TFC) was determined by the colorimetric method described by Wanyo *et al.* (2014) with slight modification. Briefly, 0.5 mL of the sample extract was mixed with 2.25 mL of distilled water in a test tube followed by the addition of 0.15 mL of 5% $NaNO_3$ solution and mixed by a vortex mixer. After 6 min, 0.3 mL of a 10% $AlCl_3.6H_2O$ solution was added then mixed with a vortex mixer for another 5 mins before 1.0 mL of 1 M NaOH was added and mixed well. The absorbance was measured at 510 nm using the UV 1800 Shimadzu spectrophotometer. Rutin (0-1 mg/mL) was used to create the standard curve of the flavonoids.

2.9 GABA

The HPLC analysis of GABA was performed on a Shimadzu 20A UV detector using the method adapted from Jannoey *et al.* (2010). Approximately 10 g of ground samples were vigorously mixed in 50 mL of 70% ethanol. The mixture was shaken for 10 mins in an ultrasonic bath and then centrifuged at 8,000 rpm at 4°C for 10 mins. The supernatant in the upper layer was collected and then rotary evaporated at 40°C (Buchi Rotary Evaporators, R124, B480, Japan), and 4 mL of 70% ethanol was added to re-dissolve the extract, the extraction process was repeated three times. The supernatant was collected and combined with the first supernatant. The obtained crude extract containing GABA was then injected through the 0.45 mm Whatman nylon filter and analyzed by HPLC UV detector (Shimadzu, Co, Japan) which consisted of a pump (20 A pump), a variable wavelength UV-Visible detector 20A, auto sampler 20A and a guard column NH_2 size 4.6 mm

https://doi.org/10.26656/fr.2017.9(1).378

× 25.0 cm, and analyzed column Intertil-NH_2 column size 4.5×220 mm pore size 0.45 mm, HPLC oven at 40°C. HPLC with pre-column derivatization with 2-hydroxynaphthaldehyde (HN) was done using the following conditions; UV detection at 330 nm. Mobile phase containing acetonitrile (mobile phase A): The mobile phase consists of A (acetonitrile) and B (0.1% formic acid) by isocratic method 80:20 v/v, the flow rate is 0.5 mL/min and held for 15 mins for washing the column. The spike with GABA and HN standard followed by derivatization.

2.10 Alphα-tocopherol and gamma-oryzanol

The α-tocopherol and γ-oryzanol were analyzed by HPLC according to Chen and Bergman (2005). 10 grams of germinated black rice (or rice cracker) was extracted using 50 mL of 2-propanol: dichloromethane (2:1) and homogenized for 10 mins, after that ultrasonicated for 20 mins, then filtered using Whatman membrane No 1 and No 4, respectively. The supernatant was collected and then rotary evaporation at 40°C (Buchi Rotary Evaporators, R124, B480, Japan), and diluted by extracted solvent 4 mL. The solution was filtered through the Whatman nylon membrane 0.45 mm before being injected into the HPLC system. The injection volume was 10 mL using an autosampler. 20A pump, A guard column; Shimadzu ODS, pore size 5 mm, 3.9 × 20 mm, and the analyzed column; Shimadzu ODS, pore size 5 mm, 4.5 × 150 mm were used. The mobile phase consisted of methanol (A) and deionized water (B) (90:10) with a flow rate of 0.8 mL/min by HPLC UV detector 20 A (Shimadzu, co, Japan), at 325 nm, 40°C. The content of α-tocopherol and γ-oryzanol were quantified using external standards and identified peak as a spike standard.

2.11 Identification and quantification of phenolic acids

Phenolic compounds were extracted using the method reported by Shao *et al.* (2014) with minor modifications. Briefly, 10 g of geminated black rice, or rice cracker was mixed with 100 mL of 80% methanol and was ultrasonicated for 30 min at 40°C. The mixture was then centrifuged at 10,000×*g* for 10 min at 4°C, and the supernatants were collected. After adjusting the pH to about 1.5-2.0 by 2 M HCl, the supernatant was concentrated by removing methanol using a rotary evaporator at 50°C. The dried extracts were dissolved in 5 mL of 80% methanol used as crude-free phenolic extracts and transferred to vials. The study of free phenolic by ultra-performance liquid chromatography (UPLC) with a photodiode array detector. A guard column (Waters, symmetry C18, 5 µm, 3.9 × 20 mm), and the column with the following specifications 150 mm × 4.6 mm, 5 µm symmetry C18, were used for separation. The UPLC system includes Waters Acquity TM Autosampler, UPLC Waters Acquity TM column, and DAD Waters detector Acquity. The sample was filtered through a 0.2 µm microfilter membrane. The injection volume was 10 µL using an autosampler. The mobile phase consists of A (0.1% acetic acid in deionized water) and B (acetonitrile; ACN); the flow rate is 0.8 mL/min. The gradient condition was performed as follows: 7% ACN (initial step), ramped to 15% ACN (0-10 mins), ramped to 35% ACN (10-15 mins), and then ramped to 55% ACN (15-20 mins) after that ramped to 100% ACN (20-25 mins) and held for 15 mins for washing the column. The phenolic acids were detected at a wavelength of 280 nm. The contents of phenolic acids were quantified using external calibration curves and identified peaks by a spike of standard phenolic compounds.

2.12 Fourier-transformed infrared spectroscopy

Changes in the phytochemicals in germinated black rice, rice cracker (CR), and rice cracker with 2SD:10 were investigated by the Fourier transform infrared spectroscopy (FTIR) according to Silva *et al*. (2014). The infrared spectrum of the sample was recorded using Bruker ATR-FTIR Analysis. The spectral data within the range of 400-4000 cm^{-1} was recorded. The air was used as a reference cell. The amide I band was used with transmittance mode. The spectral data was processed using the Bruker ATR-FTIR program operator (Bruker ATR-FTIR ID 7, Germany).

2.13 Statistical analysis

All experiments were repeated twice, and each assay was done at least in duplicate. Results are presented as mean ±standard deviation (SD). One-way analysis of variance was performed (ANOVA) to determine whether significant differences existed among treatments. Duncan's test was used to separate significant differences between means at the 95% level.

3. Results and discussion

3.1 Antioxidant activity and chemical consistent of germinated rice

The result of total antioxidant activity was demonstrated in Table 1. Samples containing relative amount of bioactive compounds by FRAP assay (Table 1) i.e. Trolox, Ger BR, CR, and SD2:10 tended to have weaker antioxidant activity (97.86 to 75.72% inhibition). Phenolic acids and bioactive compounds seemed to have a significant influence on inhibition of ABST activity as they had lower values (97.47 to 76.25% inhibition) (Table 1). These concluded that the antioxidant activities (ABTS radical scavenging activity and FRAP) of the germinated rice and its bioactive compounds were highly related to flavonoids, phenolic acid, GABA, α-tocopherol, γ-oryzanol content. The results indicated that the process of producing rice crackers, and encapsulation could decrease antioxidant activity ($p<0.05$). On the other hand, Konwatchara and Ahromrit (2014) studied germinated black rice cooking using a pressure cooker and microwave and found that the germinated black rice KKU-ULR012 gave a high antioxidant activity of DPPH 33.74±0.15 mg Trolox/100 g DW. Bulatao *et al*. (2017) studied the encapsulation of anthocyanins in chitosan-alginate nanoparticles by ionic pre-gelation formation and polyelectrolyte complex. The mass ratio of chitosan and alginate polymers of 100:10 gave the highest encapsulation efficiency (68.9%) and antioxidant scavenging activity (38.3%). Chaiyasut *et al.* (2017) reported that the antioxidant capacity by ABTS, DPPH, FRAP, and lipid peroxidation methods of germinated brown rice was significantly improved due to the increase of phenolic acids, anthocyanins, GABA, and α-tocopherol after germination process.

Table 1. Antioxidant activity of Ger BR, CR, SD2:10 and Trolox.

Treatment	FRAP (% inhibition)	ABTS (% inhibition)	Total antioxidant activity (% inhibition)
Ger BR	91.16±1.04^{b}	91.16±1.04^{b}	79.9±1.66^{b}
CR	85.71±3.47^{c}	85.71±1.47^{b}	77.76±1.75^{bc}
SD2:10	75.72±2.22^{d}	76.25±2.22^{c}	75.72±0.55^{c}
Trolox	97.86±1.04^{a}	97.47±0.07^{a}	97.86±0.42^{a}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). Ger BR: germinated black rice, CR: black rice cracker, SD2:10: black rice cracker with encapsulation by maltodextrin.

3.2 Chemical composition

3.2.1 Total flavonoids

The total flavonoid contents shown in Figure 1 of Ger BR, were significantly the highest ($p<0.05$) (332.25±5.86 mg quercitrin/g DW), followed by CR of 120.77±4.88 mg quercitrin/100 g DW. Wu *et al.* (2022) reported that the total phenolic and total flavonoid content of the rice germinated in the form of paddy and the rice germinated in the form of brown rice for 18-72 hrs germination by bound form and free form. The germinated paddy rice has the highest total flavonoid content at 36 hrs for germinated time (94.97±2.82 mg RE/100 g DW). The total flavonoid content of rice germination in free form during 18-72 hrs increased from 33.98 to 35.01 mg RE/100 g DW but decreased at 72 hrs. The flavonoids in bound increased from 68.18 to 81.43 mg RE/100 g DW at 18-54 hrs. During rice germination chacone isomerase and phenylalanine ammonia-lyase were a key enzyme of rice grain activated germination time can break down bound flavonoids to free form.

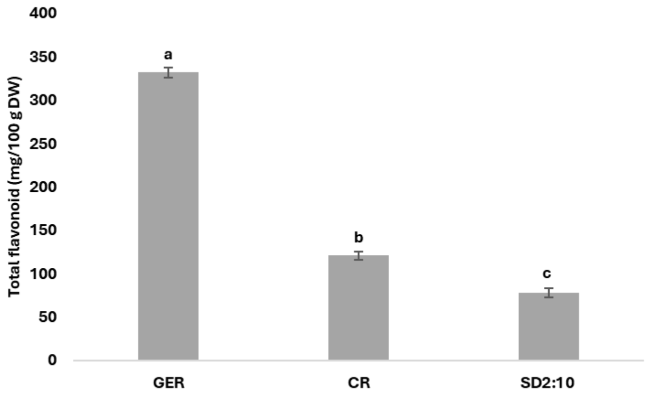

Figure 1. Effect of encapsulation of flavonoid content. Ger: germinated black rice, CR: black rice cracker, SD2:10: black rice cracker with encapsulation by maltodextrin. Values are presented as mean ± SD (n = 3). Bars with different notations are statistically significantly different ($p<0.05$) between groups determined using one-way ANOVA with Duncan's post-hoc test.

3.2.2 Total phenolic compounds

Figure 2 shows the total phenolic content determined using the Folin–Ciocalteu assay expressed in terms of mg GAE/100 g of germinated black rice (Ger BR), rice cracker (CR), and germinated black rice cracker encapsulated using maltodextrin (SD2:10) and gelatin. The highest total phenolic content was observed in the germinated black rice sample (134.71 mg GAE/kg). The TPC had decreased along the high temperature when the maltodextrin and gelatin encapsulation was low (160°C, at 5 mins) while increasing and then decreasing at higher extraction temperatures. A significant encapsulation effect between the fried and the temperature was also observed, encapsulation by maltodextrin and gelatin may have protected the phenolic compounds' decomposition from their high temperature processing. Moongngarm and Saetung (2010) studied a bioactive compound in KD6 rice (glutinous rice) and germinated KD6 rice. It was found that the germinated KD6 rice had a high value of phenolic compound of 84.2 mg GAE/100 g DW, while the non-germinated rice had a lower value of 70.3 mg GAE/100 g DW. Ti *et al.* (2016) reported that germinated rice had a total phenolic compound between 106.6 mg GAE/100 g DW and 177.2 mg GAE/100 g DW. According to Shao *et al.* (2014), the germination process helped increase the process of transforming phenolic compounds in the bound form on the cell wall into the free form. (During the process of germination, the enzymes in the rice grain such as a α-amylase, and β-amylase are activated causing the decomposition of starch to sugar and the cleavage of phenolic-pectin, phenolic-carbohydrate bonds off the cell wall, leaving these phenolic compounds in the free-form (Sompong *et al.,* 2011; Wattananapakasem *et al.* 2021). Wu *et al.* (2022) reported that free phenolic content in germination time at 18-72 hrs increased during germination, and phenolic bounds increased by 17.44-45.66 mg GAE/100 g DW, respectively.

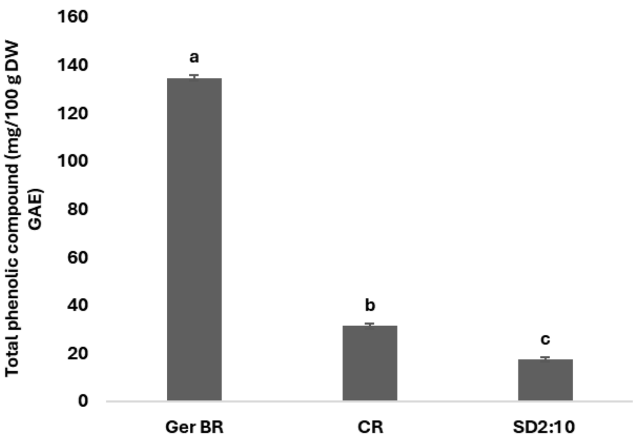

Figure 2. Effect of encapsulation of total phenolic content. Ger BR: germinated black rice, CR: black rice cracker, SD2:10: black rice cracker with encapsulation by maltodextrin. Values are presented as mean ±SD (n = 3). Bars with different notations are statistically significantly different (p<0.05) between groups determined using one-way ANOVA with Duncan's post-hoc test.

3.3 GABA, alphα-tocopherol, and gamma-oryzanol content

The results of γ-aminobutyric acid (GABA), α-tocopherol, and γ-oryzanol using high-performance liquid chromatography (HPLC) are shown in Table 2. The α-tocopherol in Ger BR detected was significantly highest (p<0.05) with the value of 45.8±1.44 mg/100 g DW, followed by CR and SD2:10 of the values 15.59±0.42 mg/100 g DW, and 9.64±0.44 mg/100 g DW, respectively (the value of CR with SD2:10 was 38.17% less than that of CR). While γ-oryzanol of Ger BR was found to be 65.49±0.76 mg/100 g DW, CR and SD2:10 held the values of 12.85±0.26 mg/100 g DW and 8.53±0.16 mg/100 g DW, respectively. GABA in Ger BR was found to be the highest at 75.57±1.41 mg/100 g DW, GABA in CR and CR with SD2:10 were not significantly different, of 6.77±0.02 mg/100 g DW and 6.86±0.16 mg/100 g DW, respectively. In the previous study reported by Wu *et al*. (2022) the GABA during 18-72 hrs germination time were 9.35-19.21 mg/100g DW, γ-oryzanol values at 0-36 hrs 90.90-121.76 mg/100 g DW. Rice grain during germination, L-glutamic acid can be catalyzed to GABA by decarboxylase (GAD). The GAD produced a GABA and activated lipase activity breakdown lipid substance to γ-oryzanol. The encapsulation process using the coacervation technique by mixing maltodextrin and gelatin in the germinated black rice flour paste with the seasoning effectively prevented the loss of bioactive compounds. α-tocopherol, γ-oryzanol, and GABA of CR with SD2:10 were 38.17%, 33.61%, and 1.01% less than CR, respectively. Chaiyasut *et al.* (2017) reported the changes in the GABA, and the phytochemical content of Kum Payao achieves maximum GABA content (0.2029 mg/g of germinated rice). The results were in accordance with Norkaew *et al.* (2019) who explored the encapsulation by the microencapsulation method using spray drying of anthocyanin of Thai sticky rice Leum Pua with the wall materials comprising of gum arabic, whey protein isolate, maltodextrin, and their combinations. The encapsulation efficiencies of maltodextrin were 88% and its combination with whey protein isolate exhibited the best anthocyanin retention. Laokuldilok and Kanha (2016) investigated the protection of encapsulation on the flavor and bioactive compounds of germinated sticky black rice and found that the use of maltodextrin could decrease the loss of bioactive compounds including GABA, α-tocopherol, γ-oryzanol, polyphenols, and flavonoids. The encapsulation efficiently protected bioactive compounds from light, oxygen, and high temperatures from the frying process of rice crackers. This prevented the migration of flavor and the decrease of phytochemicals that generally occurred as they were exposed to the previously mentioned factors (Madene *et al.,* 2006; Laokuldilok and Kanha. 2016; Norkaew, *et al.,* 2019).

3.4 Phenolic profiles

Phenolic acids identification and quantification by ultra-performance liquid chromatography (UPLC). The results are shown in Table 3 from Ger BR, CR, and SD2:10 rice crackers. Ger Br had the highest values in all types of phenolic acid compared to CR and SD2:10: major phenolic acids including gallic acid, and

Table 2. The bioactive compounds in Ger BR, CR and SD2:10.

Treatment	α-tocopherol (mg/100 g WB)	% Decreased α-tocopherol	γ-oryzanol (mg/100 g WB)	γ-oryzanol % Decreased	GABA (mg/100 g WB)	% Decreased GABA
Ger BR	45.8±1.44^{a}		65.49±0.76^{a}		75.57±1.41^{a}	-
CR	15.59±0.42^{b}	-	12.85±0.26^{b}	-	6.77±0.02^{b}	-
SD2:10	9.64±0.44^{c}	38.17±0.45	8.53±0.16^{c}	33.61±0.35	6.86±0.16^{b}	1.01± 0.23

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (p<0.05). Ger BR: germinated black rice, CR: black rice cracker, SD2:10: black rice cracker with encapsulation by maltodextrin.

https://doi.org/10.26656/fr.2017.9(1).378

Table 3. The phenolic compounds of rice extracted using Ultra-performance liquid chromatography (UPLC) (mg/100 g DW).

Phenolic acid	Ger BR	CR	SD2:10
Gallic acid	28.97±1.48^{a}	25.10±0.31^{b}	17.56±0.22^{c}
Trans – ferulic acid	3.63±0.08^{a}	2.79±0.08^{b}	2.45±0.05^{b}
Protocatechuic acid	44.33±1.30^{a}	28.10±1.04^{b}	22.04±0.05^{c}
Vanillic acid	6.87±0.77^{a}	6.61±0.35^{b}	5.27±0.22^{c}
Quercetin	3.69±0.03^{a}	2.86±0.04^{b}	2.50±0.08^{c}
Coumaric acid	1.20±0.02^{a}	0.88±0.02^{b}	0.72±0.01^{c}
Hydroxy-benzoic acid	9.46±0.49^{a}	7.80±0.39^{b}	5.92±0.15^{c}
Syringic acid	4.01±0.20^{a}	3.26±0.07^{b}	2.94±0.05^{c}
Sinapic acid	0.35±0.03^{a}	0.28±0.01^{b}	0.30±0.05^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). Ger BR: germinated black rice, CR: black rice cracker, SD2:10: black rice cracker with encapsulation by maltodextrin.

protocatechuic acid were 28.97±1.48 mg/100 g DW and 44.33±1.30 mg/100 g DW, respectively. Hydroxy-benzoic acid, quercetin, and syringic acid were 9.46±0.49, 3.69±0.03, and 4.01±0.20 mg/100 g DW, respectively. The minor phenolic acids including vanillic acid, coumaric acid, and sinapic acid were 6.87±0.77, 1.20±0.02, and 0.35±0.03 mg/100 g DW, respectively. All phenolic acid in CR and CR with SD2:10 tended to decrease due to the decomposition at the high temperature because CR and CR with SD2:10 needed to be fried in hot oil at 160°C for 5 min. In the same way, Jun *et al.* (2012) reported that the major phenolic acids identified in pigmented rice brans were vanillic acid, ferulic acid, and p-coumaric acids. While the most abundant bound free phenolic compounds in the rice grain include ferulic acid, proto-catechuic acid, and vanillic acid, the bound forms include gallic acid, hydroxy-benzoic acid, vanillic acid, and *p*-coumaric acid (Sompong *et al.,* 2011; Shao *et al.,* 2014; Wattananapakasem *et al.*, 2021). During the germination, water was absorbed into the rice grain and the embryo, the physical and biochemical changes occurred activating gibberellic acid, α-amylase, and β-amylase enzymes that enhanced carbohydrate metabolism. The endogenous esterase in the germination process can break down bound phenolic acid to free phenolic increased by polymerization (Wu *et al.,* 2022). The activity of hydrolytic enzymes such as α-amylase caused the decomposition of starch into glucose and maltose. Therefore, germination of rice enhanced a γ-aminobutyric acid (GABA), α-tocopherols, phenolic acids, γ-oryzanol, α-tocotrienols, respectively. The phenolic compounds break down the bonds from the cell wall of rice cells to free forms (Moongngarm and Saetung, 2010; Ng *et al.,* 2013; Ti *et al.,* 2014; Cho and Lim, 2016; Chaiyasut *et al.*, 2017). Therefore, encapsulation could protect the flavor and bioactive compounds of germinated black rice. This protection is against evaporation, migration of flavor, and phytochemical decrease (Pedro *et al.,* 2016). Many active compounds such as polyphenol, anthocyanin, vitamin B complex, volatile compounds, and phytochemical food products can be degraded by physicochemical factors i.e., light, moisture, and oxygen (Madene *et al.,* 2006).

3.5 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy has emerged as an attractive alternative technique. The development of attenuated total reflectance (ATR) as a sampling handling technique has revitalized the use of FTIR spectroscopy (Rohman and Man, 2012). Conventional techniques of vitamins and bioactive compounds analysis are based on High-Pressure Liquid Chromatography, which is costly and laborious. This study evaluated the potential of the ATR-Fourier transform infrared spectroscopy (ATR-FTIR) technique in predicting GABA, γ-oryzanol, α-tocopherol and phenolic acid in germinated black rice (Ger BR) and black rice cracker (CR), and black rice cracker with encapsulation by maltodextrin (SD2:10). In Figure 3A which is the spectra of Ger BR, the sharp peak at 1744 cm^{-1} has been assigned to the bending of $–CH_3$ absorption while in rice cracker (Figure 3B), the peak is broader at 1742-1629 cm^{-1} associated with symmetric in-plane absorptions due to CH and phenols, and OH deformation vibrations. The spectra of rice cracker encapsulation with SD2:10 (Figure 3C) show the peak at 1708-1632 cm^{-1} of the stretch of phenolic group in the sample. The peak at 2922 cm^{-1} showed a γ-oryzanol and the peak at 2853 cm^{-1} of α-tocopherol (symmetric stretch) was associated with vibrations of the CH_2 and CH_3 in Ger BR. At the same time, in CR, these peaks appear at 2923 cm^{-1} for γ-oryzanol, and 2853 cm^{-1} for α-tocopherol For SD2:10 rice cracker, the deformation vibrations of γ-oryzanol is at 2921 cm^{-1}, and the absorption band stretching of α-tocopherol is at 2951 cm^{-1}, respectively. In a previous study by Silva *et al.* (2014) signal from the phenols could be found in the region of 1680-900 cm^{-1} and the peaks at the stretching band of

https://doi.org/10.26656/fr.2017.9(1).378

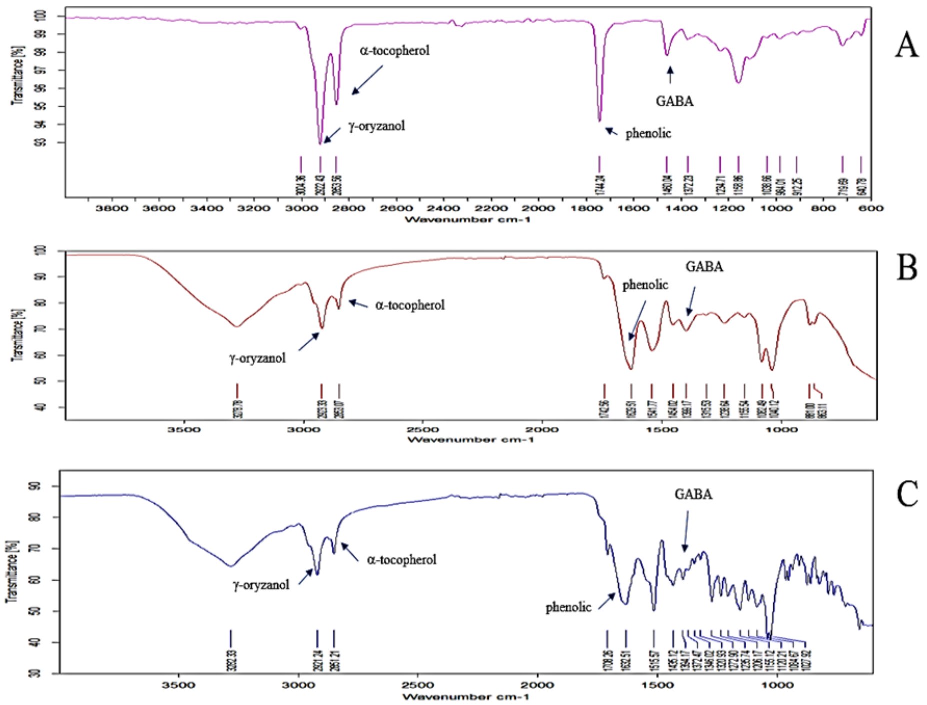

Figure 3. Effect of encapsulation on bioactive compounds changed of spectra of FTIR: (A) FTIR spectra of germinated black rice (Ger BR), (B) FTIR spectra of black rice cracker (CR) and (C) FTIR spectra of black rice cracker with encapsulation by maltodextrin (SD2:10).

carbonyl groups (1712-1704 cm^{-1}) and C stretching bands (1609-1608 and 1519-1516 cm^{-1}) were typical of aromatic molecules. Lucarini *et al.* (2020) reported the bands around 1600 cm^{-1} associated with the stretching of C=OO−and aromatic C=C groups, e.g., in pectin and phenolic compounds, but also with the bending vibrations of OH groups. According to Olale *et al.* (2019), mango fruits in the ripening stage showed an α-tocopherol exhibited peaks at 2922 cm^{-1}, and 2862 cm^{-1} of γ-oryzanol (symmetric stretch) associated with vibrations of the CH_2 and CH_3 respectively. Zareie *et al.* (2020) proposed that the peak at around 1401 cm^{-1} in the GABA spectrum might be ascribed to the presence of C-O stretching in carboxyl groups attached to the amide linkage in the amide I. Rodsuwan *et al.* (2020) studied a γ-oryzanol interaction of nanoparticles of zein protein and pure γ-oryzanol in rice bran oil by FTIR. The hydrogen bond of the amine group of glutamic amino acid in protein and hydroxyl group (-OH) in γ-oryzanol were associated with the peaks at 3420 cm^{-1}.

However, in the process of producing rice crackers and rice crackers with the encapsulation of maltodextrin, there was an exposure of the product to the high temperature of 160°C for 5 min which could cause the vibration of the functional units of the bioactive chemicals, the cleavage and stretch of the bonds in molecules. The encapsulation could reduce the change of the amount of the bioactive compounds which could be recognized from the difference between the FTIR spectra that show the peaks specifically belonging to these key bioactive compounds of Ger BR, CR, and SD2:10. Microencapsulation by coacervation technique coats the core material with polymeric compounds by the interaction between them. The interaction between liquid -liquid core-coating compounds induces either ionic bonding, hydrogen bonding, hydrophobic forces, or polarization. This was influenced by pH, ionic values, concentration, and the ratio of biopolymers. The hydrocolloids that are generally used for microencapsulation include gelatin, starch, pectin, cellulose, alginate, chitosan, glucan, gum arabic, xanthan gum, dextran, and cellulose derivatives. These compounds are used to generate complex materials such as gelatin-gum arabic, gelatin-carboxymethylcellulose, alginate-polylysine, alginate-chitosan, albumin-gum arabic, and glycan-cellulose (Madene *et al.*, 2006; Eghbal and Choudhary, 2018). This process minimizes the decomposition of α-tocopherol, γ-oryzanol, GABA, and phenolic compounds.

4. Conclusion

The encapsulation process could slightly protect bioactive compounds from degradation. The results showed that maltodextrin and gelatin-coating material could effectively protect bioactive compounds from environment and processing factors. The experiment demonstrated that maltodextrin and gelatin, as coating materials, could dissolve well in water when heat was applied due to their negative-charged property. They formed interactions with bioactive compounds including phenolics, flavonoids, anthocyanin, GABA, and α-tocopherol with Van der Waals and hydrogen forces. The

https://doi.org/10.26656/fr.2017.9(1).378

stability of these bioactive compounds depended on pH, temperature, and their compatibility with the coating materials. Microencapsulation could prevent the degradation of phenolic compounds. This study exhibited that microencapsulation with gelatin and maltodextrin could effectively protect the bioactive compounds from processing and environmental factors. This research suggested that the selection of appropriate coating materials could preserve bioactive compounds and could be applied as solutions and practices at the food industry level.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was also supported by Research and Technology Transfer Affairs, Khon Kaen University.

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