International Food Research Journal 27(2): 385 - 396 (April 2020)
Journal homepage: *http://www.ifrj.upm.edu.my*

Comparative study on bioactive compounds, glucose, alcohol, and antioxidant activities of fermented rice with Thai and Chinese starter cultures and rice varieties

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Article history

Received: 23 June 2019
Received in revised form: 26 March 2020
Accepted: 19 April 2020

Keywords

phenolic acid, flavonoid, DPPH radical scavenging activity, FRAP value, fermented rice

Abstract

We investigated the bioactive compounds, glucose, alcohol, and antioxidant activities of fermented rice from two Thai rice varieties (black glutinous rice, BR; white glutinous rice, KD6) and one Chinese rice variety (white glutinous rice, CR) fermented with Thai and Chinese commercial starter cultures (TC and CC, respectively). Results showed that fermented BR contained higher contents of total phenolics and total flavonoids, and higher FRAP values as compared to fermented KD6 and CR; whereas, DPPH radical scavenging activity was slightly lower in fermented BR than in other fermented rice samples. Ferulic and sinapic acids were found in all raw and fermented rice samples; the most abundant phenolic acid in the raw rice was ferulic acid. BR fermented with CC showed the highest total amount of phenolic acids. Fermented BR had the lowest glucose content, but its alcohol content was clearly higher than that of the other fermented rice samples. Our results suggested that a combination of either Thai or Chinese rice varieties with TC and CC offers good possibilities for developing functional fermented rice products. With respect to health-promoting properties, BR may be an appropriate variety to improve the bioactivities of fermented rice. However, consumer acceptance of the products is needed for further evaluation.

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Introduction

Rice is an important staple food in Asian countries, and it is becoming more popular worldwide because of immigration and trends to multiculturalism. Besides, there are many kinds of foods made from rice including snacks, beverages, and desserts. Fermented rice, called Jiu-niang in Chinese, or Khoaw Maak in Thai, is a traditional fermented food with sweetness, plus alcohol and ester aromas. It is usually made, not only in food factories, but also at home, from white glutinous rice by solid-state fermentation.

There is an increasing interest in this fermented-rice product because it has been reported to possess high nutritive value and health-promoting qualities (Huang *et al.,* 2017). It is abundant in essential amino acids, vitamins, minerals, reducing sugars, and organic acids (Huang *et al.,* 2017). Moreover, it contains some bioactive phytochemicals, such as phenolic acids and flavonoids. These phenolic compounds have been reported to be potent antioxidants, which can prevent biological macro-molecules, such as proteins and DNA, from damage due to oxidative stress (Masisi *et al.,* 2016). Therefore, fermented rice may be helpful in reducing the risk of some chronic diseases related to oxidative stress, such as cardiovascular disease, type II diabetes, rheumatic arthritis, Alzheimer's disease, and cancer.

Generally, during fermentation, the amylase secreted by moulds decomposes gelatinised starch into fermentable sugars such as glucose, fructose, and sucrose. Then, these sugars are converted into alcohol by yeasts. Many studies (Zhang and Cao, 2007; Yang *et al.,* 2011; Wu *et al.,* 2016) have shown that the sensory characteristics and nutrient content of fermented rice products are greatly affected by the composition of starter cultures. Moreover, the sensory characteristics, bioactive-compound content, and biological activities of fermented rice products are also associated with rice variety (Manosroi *et al.,* 2011; Wang *et al.,* 2017).

Until now, white glutinous rice is still used as the usual raw material for Jiu-niang and Khoaw Maak preparation. However, other glutinous rice varieties may be superior to white rice in health benefits, thus offering a superior substitute for traditional rice. Pigmented rice such as reddish, purple, and black rice has received increasing attention because it has higher levels of bioactive compounds and more potent

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antioxidant properties as compared to white rice. Several studies (Manosroi *et al.,* 2011; Huang *et al.,* 2013) have been performed on Jiu-niang and Khoaw Maak made from pigmented rice.

Although there has been some research on the effects of microbial starter and rice variety on the quality of this popular fermented food, no work has been reported to compare the bioactive compounds and activities of Chinese rice fermented by Thai starters with those of Thai rice fermented by Chinese starters. Moreover, the profile of the phytochemicals, especially phenolics that provide health benefits during rice fermentation, has not been systematically studied. Therefore, the purpose of the present work was to investigate the possibility of improving health-promoting effects by using new combinations of rice variety and starter culture. In addition, we aimed to compare the profiles of phenolic acids and flavonoids, as well as the contents of total phenolics and total flavonoids, plus antioxidant capacities during fermentation among these fermented rice samples.

Materials and methods

Rice samples and start cultures

Three rice samples were selected, namely Thai white glutinous rice, Thai black glutinous rice (Mahasarakham Province, Thailand), and Chinese white glutinous rice (Jiangsu Province, China). They were used as the raw materials for fermented rice preparation. Two starter cultures were used during the fermentation of rice. One was Look Pang (Mahasarakham Province, Thailand) that contained three species of moulds, i.e. *Rhizopus* sp., *Aspergillus* sp., and *Mucor* sp.; and three species of yeasts, i.e. *Candida krusii, Saccharomyces cerevisiae*, and *Candida* sp. The other was rice leaven (Jiangsu Province, China), that was a mixture of rice flour and microorganisms dominated by *Rhizopus* sp.

Chemicals

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid (Trolox), 2,4,6-tripiridyl-s-triazine (TPTZ), standards of gallic, protocatechuic, p-hydroxybenzoic, vanillic, chlorogenic, syringic, *p*-coumaric, ferulic and sinapic acids, rutin, myricetin, quercetin, apigenin, and kaempferol were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). HPLC-grade methanol, acetonitrile, and acetic acid were purchased from Merck (Darmstadt, Germany). All other solvents purchased from Fisher Scientific (USA) were of the highest available purity.

Preparation of fermented rice sample

The fermented rice samples were prepared following the Thai traditional procedure. Briefly, 500 g of Thai or Chinese white glutinous rice, or Thai black glutinous rice was soaked in distilled water for 3, 3, and 4 h, respectively, and then was steamed in a pot for 1 h until fully cooked. The cooked rice was washed five times with distilled water to remove viscous materials, followed by draining off excess water. To use the Thai starter culture (TC), a Look Pang ball was crushed into powder, and 1.5 g of the powder or Chinese starter culture (CC) was sprinkled on the drained cooked rice and mixed thoroughly. The mixture was put in a meal box and fermented at room temperature. The sample was withdrawn from each box every two days, followed by freezing at -40°C until analyses were performed.

Extraction of phenolics

The extraction process was done according to Kaisoon *et al.* (2012) with minor modifications. Five grams of the fermented rice sample were extracted with 50 mL of 80% aqueous ethanol using a shaking incubator (Sheldon Manufacturing, Inc., Cornelius, OR, USA) at 150 rpm and 37°C for 3 h. Following centrifugation at 3,000 rpm for 10 min, the supernatant was decanted into a 50 mL vial and stored at -18°C until used. The extraction was done in three replicates. The supernatants were combined and used for the determination of the antioxidant activity, total phenolic and total flavonoid contents.

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteau method described by Kubola *et al.* (2011). Briefly, 300 µL of the extract was mixed with 2.25 mL of Folin-Ciocalteau reagent diluted in distilled water (1:10, v/v), followed by incubation at room temperature for 5 min, following which 2.25 mL of Na_2CO_3 solution (60 g/L) was added to the mixture. After 90 min incubation at room temperature, the absorbance at 725 nm was measured using a UV-1700 spectrophotometer (Shimadzu Inc., Kyoto, Japan). TPC of the extracts was calculated based on the gallic acid standard curve and expressed as milligrams of gallic acid equivalent per one hundred grams of wet sample (mg GAE/100 g).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the colorimetric method reported by Abu Bakar *et al.* (2009). Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water, followed by the addition of 0.15 mL of 5% $NaNO_2$ solution. After

6 min incubation, 0.3 mL of 10% $AlCl_3$•$6H_2O$ solution was added and the mixture was incubated for another 5 min, followed by the addition of 1.0 mL of 4% NaOH solution, and thorough mixing, using a vortex mixer (ZX3, Velp Scientifica, Usmate, Italy). The absorbance was immediately determined at 510 nm using a UV-1700 spectrophotometer. TFC of the extracts was calculated based on the rutin standard curve, being expressed as milligrams of rutin equivalent per one hundred grams of wet sample (mg RE/100 g).

Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was determined by the method described by Braca *et al.* (2001). Briefly, 100 µL of the extract was added to 3 mL of 0.01 M DPPH in methanol. Following 30 min incubation at room temperature in the dark, the absorbance at 517 nm was determined using a UV-1700 spectrophotometer. The DPPH radical scavenging activity of the extracts was calculated based on the Trolox standard curve and expressed as milligrams of Trolox equivalent per one hundred grams of wet sample (mg TE/100 g).

Determination of ferric reducing/antioxidant power (FRAP)

The total reducing capacity was determined using the FRAP assay (Benzie and Strain, 1996). The FRAP reagent was prepared consisting of 300 mM acetate buffer (pH 3.6), 10 mM iron reagent (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3$•$6H_2O$ solution. The fresh working solution was warmed to 37°C before use. After 100 µL of the extract had reacted with 1.9 mL of the preheated FRAP reagent for 4 min, the absorbance was measured at 593 nm using the UV-1700 spectrophotometer. The results were calculated using the standard curve prepared with known concentration of $FeSO_4$, and were expressed as mg $FeSO_4$/g.

Determination of phenolic acid and flavonoid compositions by HPLC

The extraction and analysis of phenolic compounds by HPLC were performed following the method of Butsat and Siriamornpun (2010). Five grams of the sample were extracted with 50 mL of methanol/HCl (100:1, v/v) using a shaking incubator at 150 rpm and 37°C for 16 h. The extract was then centrifuged at 4,000 rpm for 20 min, and the supernatant was evaporated to dryness under vacuum at 35°C. For HPLC analysis, each residue was re-dissolved in 5 mL of methanol, and then passed through a 0.45 µm membrane (Vertical, Thailand). A 20 µL aliquot of sample solution was fractionated using a HPLC system (Shimadzu Inc., Kyoto, Japan) equipped with a diode array detector. An InertSustain C_{18} column (5 µm, 4.6 × 250 mm; GL Sciences, Tokyo, Japan) was used for fractionation at 38°C. The mobile phase consisted of solvent A (purified water containing 1% acetic acid) and solvent B (acetonitrile), and the flow rate was 0.8 mL/min. A 65 min linear gradient was performed as follows: 0 - 5 min, 5 - 9% B; 5 - 15 min, 9% B; 15 - 22 min, 9 - 11% B; 22 - 38 min, 11 - 18% B; 38 - 43 min, 18 - 23% B; 43 - 44 min, 23 - 90% B; 44 - 45 min, 90 - 80% B; 45 - 55 min, 80% B; 55 - 60 min, 80 - 5% B; and 60 - 65 min, 5% B. UV-diode array detection was done at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids), and 350 nm (flavonols). The spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of the standard compounds, and the concentration of each compound was calculated using an external standard method.

Determination of glucose and alcohol contents by HPLC

The water extraction was done following the method of Karkacier *et al* (2003). Five grams of the sample were mixed with 5 mL of water and shaken for 15 min at medium speed using vortex. The extract was then centrifuged at 5°C and 10,000 rpm for 15 min. The supernatant was filtered through the 0.45 µm membrane for HPLC analysis. Analysis of glucose and alcohol contents was performed following the method of da Cunha-Pereira *et al.* (2011). The HPLC apparatus (Shimadzu Inc., Kyoto, Japan) consisted of a LC-20AD series pumping system, SIL-20A series auto injector system, and a RID-10A series refractive index detector. Twenty microliters of each sample were injected by the auto injector and fractionated on an Aminex HPX-87H column (300 × 7.8 mm) with Bio-Rad micro-guard cartridges (30 × 4.6 mm) (Bio-Rad Inc, Richmond, CA) at 65°C. The mobile phase was 0.005 M H_2SO_4 with a flow rate of 0.5 mL/min. RI detector was used to identify and quantify glucose and alcohol.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) from three replications. Data were analysed by a one-way analysis of variance (ANOVA) test followed by Duncan's multiple range test in SPSS statistical software (version 16.0). The level of significance was set at $p < 0.05$.

Results and discussion

Total phenolic content

Table 1 shows the TPC of three rice varieties,

namely KD6, BR, and CR, and the rice fermented with Chinese or Thai starter culture, namely KD6/CC, BR/CC, CR/CC, KD6/TC, BR/TC, and CR/TC. The TPC varied greatly among these rice varieties. BR (209 mg GAE/100 g) yielded significantly higher TPC than KD6 (4.82 mg GAE/100 g) and CR (3.21 mg GAE/100 g). Consistent results are found in previous studies. Shen *et al.* (2009) reported that the average of TPC in six black rice varieties was much higher than that in 423 white rice varieties. It was also found that the TPC of black glutinous rice was significantly higher than that of the white counterpart in Chinese rice varieties (Huang and Ng, 2012) as well as in Thai rice varieties (Butsat and Siriamornpun, 2010).

Fermentation induced substantial increases in the TPC of all rice varieties. The maximum TPC of KD6/CC, BR/CC, CR/CC, KD6/TC, BR/TC, and CR/TC during fermentation was 19.7, 1.4, 37.6, 20.4, 1.6, and 39.4 times higher than the TPC of the corresponding raw rice. These data are consistent with earlier findings showing that TPC increased during fermentation with fungi (Schmidt *et al.,* 2014; Abd Razak *et al.,* 2015). The increases of TPC during

Table 1. Total phenolic contents, total flavonoid contents, and antioxidant activities of three glutinous rice samples fermented with Chinese or Thai starter culture.

Type of fermented rice	Fermentation time (d)	TPC (mg GAE/100 g)	TFC (mg RE/100 g)	DPPH (mg TE/100 g)	FRAP (mg $FeSO_4$/g)
KD6/CC	0	4.82 ± 0.61^{d}	75.45 ± 0.03^{b}	194.15 ± 0.24^{a}	0.17 ± 0.02^{d}
	2	77.06 ± 0.55^{b}	75.91 ± 0.04^{a}	188.05 ± 0.65^{c}	0.43 ± 0.05^{a}
	4	70.51 ± 0.26^{c}	75.47 ± 0.07^{b}	191.38 ± 0.86^{b}	0.26 ± 0.02^{c}
	6	94.87 ± 0.85^{a}	75.02 ± 0.42^{c}	194.80 ± 0.37^{a}	0.32 ± 0.03^{b}
BR/CC	0	209.87 ± 0.85^{c}	150.89 ± 0.04^{a}	178.21 ± 0.56^{c}	7.10 ± 0.85^{b}
	2	293.44 ± 0.43^{a}	150.27 ± 0.18^{b}	171.46 ± 0.98^{d}	9.34 ± 0.50^{a}
	4	258.67 ± 0.53^{b}	148.95 ± 0.55^{c}	184.39 ± 0.24^{b}	6.25 ± 0.39^{c}
	6	185.51 ± 1.00^{d}	144.13 ± 0.13^{d}	190.89 ± 0.78^{a}	4.33 ± 0.58^{d}
CR/CC	0	3.21 ± 0.69^{d}	75.25 ± 0.21^{b}	195.93 ± 0.99^{a}	0.17 ± 0.06^{c}
	2	120.68 ± 0.26^{a}	75.69 ± 0.03^{a}	189.02 ± 0.65^{c}	0.38 ± 0.04^{a}
	4	94.41 ± 0.46^{c}	75.19 ± 0.04^{b}	190.49 ± 0.65^{b}	0.25 ± 0.08^{bc}
	6	112.00 ± 0.30^{b}	75.69 ± 0.02^{a}	196.50 ± 0.61^{a}	0.28 ± 0.02^{ab}
KD6/TC	0	4.82 ± 0.61^{d}	75.57 ± 0.22^{a}	194.15 ± 0.24^{b}	0.17 ± 0.02^{b}
	2	85.39 ± 0.53^{c}	75.71 ± 0.19^{a}	186.75 ± 0.51^{d}	0.24 ± 0.07^{ab}
	4	94.59 ± 0.75^{b}	75.42 ± 0.08^{a}	191.54 ± 0.28^{c}	0.32 ± 0.03^{a}
	6	98.09 ± 0.87^{a}	74.77 ± 0.06^{b}	195.37 ± 0.49^{a}	0.30 ± 0.11^{a}
BR/TC	0	209.87 ± 0.85^{d}	150.89 ± 0.04^{a}	178.21 ± 0.56^{b}	7.10 ± 0.85^{b}
	2	290.97 ± 0.69^{b}	150.29 ± 0.14^{c}	168.78 ± 1.06^{d}	10.44 ± 0.76^{a}
	4	341.37 ± 0.43^{a}	150.67 ± 0.07^{b}	171.22 ± 0.42^{c}	10.87 ± 0.63^{a}
	6	217.00 ± 0.46^{c}	149.72 ± 0.02^{d}	185.53 ± 0.61^{a}	6.21 ± 0.48^{b}
CR/TC	0	3.21 ± 0.69^{d}	75.25 ± 0.21^{b}	195.93 ± 0.99^{a}	0.17 ± 0.06^{d}
	2	94.01 ± 0.70^{c}	75.94 ± 0.06^{a}	190.49 ± 0.84^{c}	0.34 ± 0.02^{a}
	4	124.59 ± 1.05^{b}	75.65 ± 0.04^{b}	193.41 ± 0.84^{b}	0.38 ± 0.07^{c}
	6	126.43 ± 0.72^{a}	75.27 ± 0.05^{c}	195.93 ± 0.56^{a}	0.32 ± 0.06^{b}

Data are means ± SD (n = 3). Means within each type of fermented rice in each column having different letters are significantly different ($p < 0.05$). TPC = total phenolic content; TFC = total flavonoid content; DPPH = DPPH radical scavenging activity; FRAP = ferric reducing antioxidant power; KD6/CC, BR/CC, and CR/CC represent Thai white glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Chinese starter culture, respectively; KD6/TC, BR/TC, and CR/TC represent Thai glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Thai starter culture, respectively; 0 d = no treatment with starter culture.

fermentation may be attributed to liberation of the free phenolics by action of enzymes derived from the starter cultures, as reported by Vattem and Shetty (2002) who found that fungal β-glucosidase and α-amylase contributed to the phenolic mobilisation.

In addition, the TPC varied in the six types of fermented rice. BR/TC contained the highest TPC (341 mg GAE/100 g), whereas the lowest TPC (71 mg GAE/100 g) was found in KD6/CC. Similar findings were reported by Manosroi *et al.* (2011), who showed that the TPC of the fermented pigmented rice (purple plain rice, purple glutinous rice, and brown plain rice, 3.89, 2.31, and 8.38 mg GAE/g, respectively) was much higher than that of the fermented non-pigmented rice (white plain rice and white glutinous rice, 0.16 and 0.14 mg GAE/g, respectively). The TPC of the fermented rice was also associated with fermentation time. KD6/CC, KD6/TC, and CR/TC had the highest TPC on the 6th day, while the highest TPC in BR/TC, and BR/CC and CR/CC was observed on the 4th and 2nd day, respectively. Moreover, the type of starter culture also affected TPC. Generally, between the same rice variety, TC led to a greater increase in the TPC than CC. For example, KD6/TC contained higher TPC than KD6/CC throughout the fermentation. The results indicated that TC may be more effective for phenolic mobilisation than CC, probably due to there being more abundant strains in the Thai starter culture than in the Chinese one. As reported by Abd Razak *et al.* (2015), the TPC of rice bran fermented with a single culture was lower than that of rice bran fermented with mixed cultures.

Total flavonoid content

The TFC of the three rice varieties and six types of fermented rice is shown in Table 1. For the unfermented rice, BR contained the highest TFC (151 mg RE/100 g), about two times higher than that in KD6 (75.5 mg RE/100 g) and CR (75.3 mg RE/100 g). The observation of TFC being higher in pigmented rice compared to white rice is in good agreement with the results from other studies (Shen *et al.,* 2009; Huang and Ng, 2012). In contrast to TPC results, TFC in all samples was almost constant during fermentation, with the exception that for BR/CC, TFC slightly decreased from 151 to 144 mg RE/100 g. These results are inconsistent with reports in previous studies, which indicated that fermentation led to increases in TFC with fungi such as *Aspergillus niger*, *Rhizopus oligosporus*, and *A. oryzae* (Liu *et al.,* 2017), or with bacteria such as lactic acid bacteria (Liu *et al.,* 2017) and *Bacillus pumilus* (Cho *et al.,* 2009).

Antioxidant activities

The antioxidant activities of the raw and fermented rice were evaluated by DPPH radical scavenging activity and ferric reducing/antioxidant power (FRAP). As displayed in Table 1, KD6 (194 mg TE/100 g) and CR (196 mg TE/100 g) had comparable activity of DPPH radical scavenging. Although BR yielded much higher contents of TPC and TFC than KD6 and CR, the lower DPPH radical scavenging activity was found in BR (178 mg TE/100 g). Meanwhile, fermented rice had considerably higher TPC and TFC than raw rice, but the latter showed comparable radical scavenging activity to or even slightly higher than the former. These results did not agree with the positive relationship between DPPH radical scavenging activity and TPC or TFC reported in previous studies (Choi *et al.,* 2008; Zhang *et al.,* 2015). However, Abd Razak *et al.* (2015) found that the radical scavenging activity of fermented rice bran was poorly related to TPC. It could be inferred that the composition of phenolic compounds, such as phenolic acids and flavonoids as well as other antioxidant secondary metabolites in our samples, may also be responsible for the DPPH radical scavenging activity besides TPC and TFC. Similar to TPC and TFC, the FRAP of BR (7.10 mg $FeSO_4$/g) was remarkably higher than that of KD6 (0.17 mg $FeSO_4$/g) and CR (0.17 mg $FeSO_4$/g). This result is in line with a positive correlation between FRAP and TPC reported by Butsat and Siriamornpun (2010). The contradictory results between DPPH radical scavenging activity and FRAP may be attributed to the different mechanisms of the two methods used in determining antioxidant activities.

Fermentation resulted in changes in the antioxidant activity of the rice, particularly the FRAP of fermented BR. BR/CC and BR/TC showed evident changes in FRAP during fermentation, with the ranges from 4.33 to 9.34 mg $FeSO_4$/g and from 6.21 to 10.87 mg $FeSO_4$/g, respectively. These results are consistent with those reported by Abd Razak *et al.* (2015) that fermentation could increase the antioxidant activity evaluated by the FRAP method. Nevertheless, the highest scavenging activity of fermented KD6 and CR was still comparable to that of the corresponding non-fermented rice. These results are similar to those reported by Abd Razak *et al.* (2015), who found that DPPH radical scavenging activity in all fermented rice brans was not significantly improved.

Profile of phenolic acids

Phenolic acids, the second most popular group of phenolic compounds, exist widely in rice as

well as in vegetables and fruits, and can be divided into two categories, i.e. the derivatives of hydroxybenzoic and of hydroxycinnamic acids. To understand the effects of the rice variety and starter culture on the profile of phenolic acids, the contents of five hydroxybenzoic acid derivatives (gallic, protocatechuic, p-hydroxybenzoic, vanillic, and syringic acids) and four hydroxycinnamic acid derivatives (*p*-coumaric, ferulic, sinapic, and chlorogenic acids) that are commonly in rice were determined by reversed-phase HPLC. The phenolic acid composition of raw and fermented rice is presented in Table 2. The results revealed that the type and content of phenolic acids in the raw rice varied depending on the rice variety. BR had six types of phenolic acids; gallic, protocatechuic, syringic, *p*-coumaric, ferulic, and sinapic acids, while besides ferulic and sinapic acids, KD6 and CR only contained vanillic and chlorogenic acids, respectively. The total amount of the phenolic acids detected in BR (124 mg/g) was substantially higher than that in KD6 (47 mg/g) and CR (51 mg/g). These results agree well with the findings by Goufo and Trindade (2014) that pigmented rice varieties are more abundant in phenolic acids than non-pigmented ones regardless of the rice variety; consistent with earlier findings (Goufo and Trindade, 2014; Zhang *et al.,* 2015), ferulic acid was the most abundant phenolic acid in all of the three rice varieties, while the ferulic acid content of BR was 59.23 mg/g, 2.6 and 2.4 times higher than that of CR (22.59 mg/g) and KD6 (24.81 mg/g), respectively. Sinapic acid was another phenolic acid present in all the three rice varieties, with KD6 (12.53 mg/g) yielding the highest amount of sinapic acid, followed by BR (9.75 mg/g) and CR (9.68 mg/g).

Fermentation led to fundamental changes in the phenolic acid composition of each rice. Vanillic, *p*-hydroxybenzoic, and chlorogenic acids, which could not be detected in BR, appeared after fermentation. Similarly, protocatechuic, *p*-hydroxybenzoic, chlorogenic, and *p*-coumaric acids could be detected in the fermented KD6, but not in KD6. This phenomenon was also found by Abd Razak *et al.* (2015), who reported differences in the phenolic acid composition among non-fermented, single strain, and mixed strains of *A. oryzae* and *R. oryzae* fermented rice bran.

In addition, the phenolic acid content of the rice samples showed different variation patterns with the extension of fermentation time. Most of phenolic acids in the raw rice showed evident variation due to fermentation. For example, gallic acid in BR/CC increased remarkably from 11.30 to 52.57 mg/g at the first two days of fermentation followed by a slight decrease, and then increased again up to 84.83 mg/g on the 6th day. These results are similar to previous studies (Moore *et al.,* 2007; Cai *et al.,* 2011; Schmidt *et al.,* 2014), which indicated that solid-state fermentation of cereals causes changes in the contents of phenolic acids as compared to non-fermented counterparts.

The reasons for the changes in the phenolic acid profiles may be as follows: (i) phenolic acids in rice are known to exist in two forms, namely, free and bound, and the bound accounts for the major part of phenolic acids. During fermentation, free phenolic acids are released due to enzymatic hydrolysis, e.g., β-glucosidase, which is found in fungi, including *A. oryzae, A. niger, R. oryzae,* and *Saccharomyces cerevisiae*. This enzyme can catalyse the hydrolysis of glucosides. In addition, phenolic acids can be converted to each other, and (ii) two sources of starter cultures, i.e. CC and TC, used to ferment the rice in our study, affected the phenolic acid content. For example, BR/CC contained a much higher amount of total phenolic acids than BR/TC throughout fermentation. Furthermore, KD6/CC contained 1.6 and 1.4 times of the total phenolic acids higher than KD6/TC on the 2nd and 6th days of fermentation, respectively. Considering the specific phenolic acids, the gallic acid content of BR/CC increased substantially during fermentation up to 7.5 times higher than that of BR, whereas that of BR/TC displayed only slight changes. In addition, on the 2nd and 4th days, the ferulic acid content of BR/CC (67.06 and 62.60 mg/g, respectively) was significantly higher than that of BR/TC (28.22 and 27.47 mg/g, respectively). Generally, BR fermentation with CC induced greater amounts of phenolic acids than with TC.

Profile of flavonoids

Flavonoids are a broad class of bioactive compounds consisting of flavanol, flavone, flavonol, flavanone, isoflavone, and anthocyanidin. High consumption of dietary flavonoids has been demonstrated to reduce the risk of chronic diseases, such as cardiovascular diseases (Grosso *et al.,* 2017), type II diabetes (Liu *et al.,* 2014), and several types of cancers such as ovarian (Rossi *et al.,* 2008) and colon (Kyle *et al.,* 2010). Two kinds of flavones (rutin and apigenin) and three kinds of flavonols (myricetin, quercetin, and kaempferol) normally present in rice were investigated in the present work. Table 3 shows the levels of these five individual flavonoids in the three commercially available rice varieties and their fermented products.

Similar to phenolic acids, the total content of these five individual flavonoids was highest in BR

Table 2. Phenolic acid profile of three glutinous rice fermented with Chinese or Thai starter culture.

Type of fermented rice	Fermentation time (d)	Phenolic acid (mg/g)									Total
		Gallic acid	Protocatechuic acid	p-Hydroxybenzoic acid	Vanillic acid	Chlorogenic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	
KD6/CC	0	nd	nd	nd	10.11 ± 0.15^{c}	nd	nd	nd	24.81 ± 0.01^{a}	12.53 ± 0.89^{a}	47.45
	2	nd	43.42 ± 1.14^{a}	13.78 ± 0.05	nd	55.82 ± 0.24	nd	nd	10.95 ± 0.12^{c}	9.64 ± 0.04^{c}	133.61
	4	nd	11.53 ± 0.25^{c}	nd	10.49 ± 0.08^{b}	nd	nd	nd	12.38 ± 0.28^{b}	9.66 ± 0.06^{c}	44.06
	6	nd	24.45 ± 0.15^{b}	nd	35.48 ± 0.10^{a}	nd	nd	9.86 ± 0.19	13.71 ± 1.36^{b}	9.90 ± 0.15^{b}	93.40
BR/CC	0	11.30 ± 0.13^{d}	25.31 ± 0.48^{c}	nd	nd	nd	9.63 ± 0.13^{c}	9.11 ± 0.04^{b}	59.23 ± 0.09^{c}	9.75 ± 0.01^{b}	124.33
	2	52.57 ± 0.26^{b}	27.85 ± 0.53^{b}	10.72 ± 0.05^{c}	nd	28.74 ± 1.15^{a}	10.08 ± 0.02^{b}	9.07 ± 0.04^{c}	67.06 ± 0.73^{a}	9.81 ± 0.02^{a}	215.90
	4	45.84 ± 0.37^{c}	38.74 ± 0.48^{a}	36.10 ± 0.70^{a}	nd	19.25 ± 0.16^{b}	10.37 ± 0.08^{a}	9.20 ± 0.14^{a}	62.60 ± 0.05^{b}	9.57 ± 0.02^{c}	231.67
	6	84.83 ± 0.33^{a}	20.30 ± 0.48^{d}	20.80 ± 0.89^{b}	23.60 ± 1.24	28.94 ± 1.38^{a}	nd	9.20 ± 0.02^{a}	54.52 ± 0.16^{d}	9.55 ± 0.02^{c}	251.74
CR/CC	0	nd	nd	nd	nd	18.76 ± 1.07	nd	nd	22.59 ± 0.25^{a}	9.68 ± 0.03^{b}	51.03
	2	nd	44.15 ± 0.93^{b}	14.07 ± 0.36	28.71 ± 0.20^{b}	nd	nd	nd	18.54 ± 1.32^{c}	9.67 ± 0.12^{b}	115.14
	4	14.03 ± 0.01^{a}	15.11 ± 0.05^{c}	nd	11.36 ± 0.07^{c}	nd	nd	nd	11.98 ± 0.56^{d}	11.65 ± 0.27^{a}	64.13
	6	10.99 ± 0.14^{b}	60.69 ± 0.88^{a}	14.37 ± 1.56	50.18 ± 0.77^{a}	nd	nd	nd	20.12 ± 0.25^{b}	9.76 ± 0.17^{b}	166.11
KD6/TC	0	nd	nd	nd	10.11 ± 0.15^{b}	nd	nd	nd	24.81 ± 0.01^{a}	12.53 ± 0.89^{a}	47.45
	2	nd	34.85 ± 0.97^{a}	11.29 ± 0.30^{a}	nd	17.57 ± 0.22^{a}	nd	nd	10.72 ± 0.10^{d}	9.82 ± 0.01^{d}	84.25
	4	nd	14.85 ± 0.32^{c}	10.14 ± 0.04^{c}	12.84 ± 0.25^{a}	14.39 ± 0.33^{b}	nd	nd	12.32 ± 0.04^{c}	11.86 ± 0.05^{b}	76.40
	6	nd	18.46 ± 0.23^{b}	10.33 ± 0.02^{b}	nd	12.45 ± 0.03^{c}	nd	nd	15.43 ± 0.52^{b}	10.39 ± 0.31^{c}	67.06
BR/TC	0	11.30 ± 0.13^{a}	25.31 ± 0.48^{a}	nd	nd	nd	9.63 ± 0.13^{a}	9.11 ± 0.04^{b}	59.23 ± 0.09^{a}	9.75 ± 0.01^{a}	124.33
	2	10.61 ± 0.01^{c}	12.11 ± 0.11^{d}	nd	nd	nd	9.42 ± 0.01^{b}	9.06 ± 0.01^{c}	28.22 ± 0.55^{c}	9.58 ± 0.01^{b}	79.00
	4	10.98 ± 0.10^{b}	17.12 ± 0.55^{b}	nd	nd	48.65 ± 0.15	9.41 ± 0.01^{b}	9.10 ± 0.05^{bc}	27.47 ± 0.38^{d}	9.62 ± 0.07^{b}	132.35
	6	10.89 ± 0.19^{b}	15.60 ± 0.24^{c}	nd	nd	nd	nd	9.32 ± 0.02^{a}	56.84 ± 0.68^{b}	9.67 ± 0.09^{ab}	102.32
CR/TC	0	nd	nd	nd	nd	18.76 ± 1.07	nd	nd	22.59 ± 0.25^{a}	9.68 ± 0.03^{a}	51.03
	2	nd	12.06 ± 0.70^{c}	nd	13.45 ± 0.65^{c}	nd	nd	nd	15.78 ± 1.35^{d}	9.59 ± 0.02^{b}	50.88
	4	nd	31.49 ± 0.19^{b}	13.75 ± 0.34^{b}	51.16 ± 0.71^{a}	nd	nd	nd	16.26 ± 0.59^{c}	9.60 ± 0.01^{b}	122.26
	6	nd	45.99 ± 0.37^{a}	15.92 ± 0.72^{a}	31.57 ± 1.21^{b}	nd	nd	nd	18.49 ± 0.38^{b}	9.60 ± 0.01^{b}	121.57

Data are means ± SD (n = 3). Means within each type of fermented rice in each column having different letters are significantly different ($p < 0.05$). 0 d = no treatment with starter culture; nd = not detected; KD6/CC, BR/CC, and CR/CC represent Thai white glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Chinese starter culture, respectively; KD6/TC, BR/TC, and CR/TC represent Thai glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Thai starter culture, respectively.

Table 3. Flavonoid profile of three glutinous rice fermented with Chinese or Thai starter culture.

Type of fermented rice	Fermentation time (d)	Flavonoids (mg/g)					Total
		Rutin	Myricetin	Quercetin	Apigenin	Kaempferol	
KD6/CC	0	17.21 ± 1.22^{b}	31.74 ± 0.47^{a}	11.90 ± 0.35^{b}	9.17 ± 0.68^{ab}	25.05 ± 1.36^{a}	95.07
	2	15.22 ± 0.06^{c}	21.77 ± 0.03^{b}	13.67 ± 1.02^{a}	10.17 ± 0.25^{a}	24.96 ± 1.22^{a}	85.79
	4	25.23 ± 0.12^{a}	15.29 ± 0.07^{d}	9.78 ± 0.09^{c}	8.08 ± 0.28^{ab}	9.75 ± 0.47^{b}	68.13
	6	24.25 ± 1.94^{a}	16.86 ± 0.90^{c}	10.77 ± 0.45^{bc}	7.62 ± 1.56^{b}	8.06 ± 1.21^{c}	67.56
BR/CC	0	24.67 ± 0.50^{a}	35.39 ± 0.99^{a}	10.20 ± 0.14^{a}	13.32 ± 1.31^{b}	24.54 ± 0.31^{a}	108.12
	2	23.60 ± 0.30^{a}	31.98 ± 0.07^{c}	9.96 ± 0.04^{b}	17.54 ± 0.03^{a}	11.21 ± 0.71^{b}	94.29
	4	20.13 ± 0.08^{b}	29.29 ± 0.15^{d}	10.04 ± 0.01^{b}	6.64 ± 0.13^{c}	12.41 ± 0.09^{b}	78.51
	6	10.02 ± 1.13^{c}	33.11 ± 0.10^{b}	9.92 ± 0.01^{b}	7.33 ± 0.66^{c}	12.99 ± 1.13^{b}	73.37
CR/CC	0	19.85 ± 0.05^{b}	20.72 ± 0.01^{b}	17.73 ± 0.28^{b}	10.02 ± 0.23^{b}	24.44 ± 0.27^{b}	92.76
	2	16.88 ± 0.32^{c}	25.86 ± 0.06^{a}	19.04 ± 0.33^{a}	9.72 ± 0.12^{b}	25.99 ± 0.21^{ab}	97.49
	4	13.84 ± 0.25^{d}	15.54 ± 0.05^{c}	10.14 ± 0.01^{c}	9.57 ± 0.30^{b}	26.66 ± 1.08^{a}	75.75
	6	23.74 ± 0.75^{a}	19.84 ± 0.98^{b}	10.13 ± 0.18^{c}	11.17 ± 0.40^{a}	19.13 ± 0.33^{c}	84.01
KD6/TC	0	17.21 ± 1.22	31.74 ± 0.47^{a}	11.90 ± 0.35^{a}	9.17 ± 0.68^{a}	25.05 ± 1.36^{a}	95.07
	2	18.82 ± 0.25	21.05 ± 0.94^{b}	10.92 ± 0.59^{b}	7.67 ± 0.46^{ab}	7.28 ± 0.06^{b}	65.74
	4	17.83 ± 0.31	15.35 ± 0.05^{c}	9.75 ± 0.01^{c}	7.17 ± 0.90^{b}	5.06 ± 0.12^{c}	55.16
	6	17.39 ± 0.01	16.06 ± 0.80^{c}	9.72 ± 0.02^{c}	8.40 ± 0.15^{ab}	5.29 ± 0.53^{bc}	56.86
BR/TC	0	24.67 ± 0.50^{a}	35.39 ± 0.99^{a}	10.20 ± 0.14	13.32 ± 1.31^{a}	24.54 ± 0.31^{a}	108.12
	2	19.48 ± 0.20^{b}	33.09 ± 0.29^{b}	10.40 ± 1.06	11.15 ± 0.78^{ab}	6.83 ± 0.15^{c}	80.95
	4	20.44 ± 1.22^{b}	29.14 ± 0.25^{c}	10.50 ± 0.02	8.79 ± 1.16^{b}	7.43 ± 0.45^{c}	76.30
	6	19.43 ± 0.32^{b}	28.98 ± 0.01^{c}	9.88 ± 0.34	9.60 ± 0.23^{b}	16.96 ± 1.06^{b}	84.85
CR/TC	0	19.85 ± 0.05^{c}	20.72 ± 0.01^{a}	17.73 ± 0.28^{a}	10.02 ± 0.23^{a}	24.44 ± 5.49^{a}	95.07
	2	28.68 ± 1.29^{a}	17.25 ± 1.24^{b}	10.04 ± 0.07^{b}	8.72 ± 0.26^{b}	5.49 ± 0.05^{c}	70.18
	4	21.41 ± 0.07^{bc}	15.57 ± 0.07^{b}	9.93 ± 0.23^{b}	7.01 ± 0.16^{c}	6.08 ± 0.42^{c}	60.00
	6	21.74 ± 0.31^{b}	20.10 ± 0.18^{a}	9.68 ± 0.07^{b}	7.47 ± 0.14^{c}	10.93 ± 1.31^{b}	69.92

Data are means ± SD (n = 3). Means within each type of fermented rice in each column having different letters are significantly different ($p < 0.05$). 0 d = no treatment with starter culture; KD6/CC, BR/CC, and CR/CC represent Thai white glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Chinese starter culture, respectively; KD6/TC, BR/TC, and CR/TC represent Thai glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Thai starter culture, respectively.

among the three rice varieties, with 13.7 and 16.6% higher than that in KD6 and CR, respectively, while KD6 had comparable content to CR. Moreover, the flavonoid profile in the raw rice varied by rice variety. The most abundant flavonoid in KD6 was myricetin, followed in descending order by kaempferol, rutin, quercetin, and apigenin, while kaempferol was shown to have the highest content in CR, followed by myricetin, rutin, quercetin, and apigenin. Similar to KD6, myricetin was the most abundant flavonoid in BR, but the amount of quercetin was the lowest. Differences in flavonoid profile among rice varieties were also reported in previous studies (Goufo and Trindade, 2014).

Regardless of the rice variety, fermentation induced an evident decrease in the total amount of these flavonoids. The value of KD6/CC, BR/CC, CR/CC, KD6/TC, BR/TC, and CR/TC during fermentation decreased up to 28.9, 32.1, 18.3, 42.0, 29.4, and 36.9%, respectively, as compared to the corresponding raw rice. Meanwhile, the rice fermented by CC had higher total amounts of these five

flavonoids than that fermented by TC during fermentation. Furthermore, the individual flavonoid content at the time of fermentation when maximal was not significantly higher than that at the beginning, particularly for KD6/TC and BR/TC. The changes in the flavonoid profiles induced by fermentation may be associated with the bioconversion of flavonoids into their metabolites by different pathways such as glycosylation, deglycosylation, and ring cleavage (Huynh *et al.,* 2014).

Glucose and alcohol contents

During fermentation, starch in steamed rice decomposes into various fermentable monosaccharides including glucose, which can be further transformed into alcohol by microorganisms. Furthermore, the alcohol reacts with organic acids to form various kinds of flavouring esters. Therefore, as the key compound during the fermentation process, the glucose level may affect the alcohol level, and be closely related to the taste and flavour of fermented rice. As shown in Table 4, the glucose content of the three rice varieties was 0.64 - 1.53 μg/g, much lower than that of the corresponding fermented rice samples (67 - 190 μg/g). Our results indicated that the type of starter culture influenced glucose content. For the same rice variety, rice fermented with CC had a higher glucose content than that fermented with TC throughout the fermentation. For instance, the glucose content of KD6/CC was 15.2, 17.4, and 5.8% higher than that of KD6/TC on the 2nd, 4th, and 6th days of fermentation, respectively. These results may be due to a stronger activity of glucoamylase and/or a lower capacity of converting sugar to alcohol for the microorganisms in CC than those in TC.

Meanwhile, regardless of the starter culture, fermented CR (174 - 190 and 152 - 185 μg/g for CC and TC, respectively) contained the highest glucose content throughout the fermentation, followed in descending order by fermented KD6 (135 - 152 and 125 - 132 μg/g for CC and TC, respectively) and fermented BR (93 - 136 and 67 - 82 μg/g for CC and TC, respectively). Glucose content after fermentation may be affected by the amylose content of the original rice sample. Because amylose is easier to age than amylopectin, and the aging starch is difficult to be hydrolysed to sugars by amylase, this may induce the lower glucose content of the fermented rice with higher amylose content.

There were different trends in the changes of glucose level among the rice varieties during fermentation with different starter cultures. The glucose content of all samples increased sharply after two days of fermentation, but glucose contents were low for KD6/CC, BR/CC, and KD6/TC during the remaining times of fermentation as compared to those on the 2nd day. On the contrary, there was a continuous increase in the glucose content of CR/CC, BR/TC, and CR/TC from the 2nd to 6th day. These different results may be associated with the combination of rice variety with starter culture, possibly determined by the equilibrium of production between glucose and ethanol.

Table 5 shows the level of alcohol in the three rice varieties and their corresponding fermented rice samples. No alcohol was detected in any of the raw rice samples, while the alcohol content in all samples significantly increased until the end of fermentation. On the 2nd day, all the rice fermented with TC showed higher levels of alcohol than those fermented with CC, with the highest occurring in BR/TC (6.0 μg/g), followed by CR/TC (3.21 μg/g), and then KD6/TC (1.56 μg/g). Meanwhile, on the 4th and 6th days, the highest alcohol content was found in BR/CC (10.07 and 20.19 μg/g, respectively), 17.9 and 63.6% higher than that in BR/TC, respectively; whereas, the alcohol contents of KD6/CC and CR/CC were lower than those of KD6/TC and

Table 4. Glucose content of three glutinous rice fermented with Chinese or Thai starter culture.

Fermentation time (d)	Glucose (μg/g)					
	KD6/CC	BR/CC	CR/CC	KD6/TC	BR/TC	CR/TC
0	0.64 ± 0.11^{d}	1.53 ± 0.05^{d}	0.91 ± 0.01^{d}	0.64 ± 0.11^{d}	1.53 ± 0.05^{d}	0.91 ± 0.01^{d}
2	152.40 ± 0.61^{a}	136.46 ± 0.48^{a}	174.45 ± 0.33^{c}	132.26 ± 0.09^{a}	67.26 ± 0.42^{c}	151.55 ± 0.08^{c}
4	146.75 ± 0.05^{b}	92.52 ± 0.12^{c}	182.80 ± 0.46^{b}	125.05 ± 0.24^{c}	78.09 ± 0.01^{b}	153.69 ± 0.12^{b}
6	135.24 ± 0.24^{c}	100.31 ± 0.36^{b}	190.18 ± 0.06^{a}	127.81 ± 0.37^{b}	81.91 ± 0.10^{a}	185.03 ± 0.90^{a}

Data are means ± SD (n = 3). Means within the same column with different letters are significantly different ($p < 0.05$). 0 d = no treatment with starter culture; KD6/CC, BR/CC, and CR/CC represent Thai white glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Chinese starter culture, respectively; KD6/TC, BR/TC, and CR/TC represent Thai glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Thai starter culture, respectively.

Table 5. Alcohol content of three glutinous rice fermented with Chinese or Thai starter culture.

Fermentation time (d)	Alcohol (µg/g)					
	KD6/CC	BR/CC	CR/CC	KD6/TC	BR/TC	CR/TC
0	nd	nd	nd	nd	nd	nd
2	0.14 ± 0.16^{c}	0.06 ± 0.06^{c}	0.03 ± 0.06^{c}	1.56 ± 0.16^{c}	6.00 ± 0.12^{c}	3.21 ± 0.21^{c}
4	2.11 ± 0.08^{b}	10.07 ± 0.14^{b}	0.45 ± 0.02^{b}	6.14 ± 0.01^{b}	8.54 ± 0.11^{b}	7.36 ± 0.40^{b}
6	6.95 ± 0.03^{a}	20.19 ± 0.68^{a}	2.02 ± 0.03^{a}	9.92 ± 0.06^{a}	12.35 ± 0.36^{a}	9.82 ± 0.17^{a}

Data are means ± SD (n = 3). Means within the same column with different letters are significantly different ($p < 0.05$). 0 d = no treatment with starter culture; nd = not detected; KD6/CC, BR/CC, and CR/CC represent Thai white glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Chinese starter culture, respectively; KD6/TC, BR/TC, and CR/TC represent Thai glutinous rice, Thai black glutinous rice, and Chinese glutinous rice fermented with Thai starter culture, respectively.

CR/TC, respectively. These results indicated that the varieties of rice and starter cultures had complementary impacts on the production of alcohol.

Conclusion

For adding value to traditional fermented foods, much more importance has been attached on their health-promoting effects. As one of these kinds of promising foods, Jiu-niang or Khoaw Maak has always been made from white glutinous rice both in China and in Thailand since its emergence. Based on our findings, rice variety and starter culture as well as fermentation conditions (such as fermentation time) are closely related to the bioactive compounds and bioactivities of fermented foods. Our experiments have shown the effects of two popular commercial microbial cultures on the phenolics and antioxidant capacities of two traditional white and one pigmented glutinous rice. With respect to bioactive-compound content and antioxidant activity, the pigmented rice (BR sample) was shown to be a good source of raw material for this fermented rice product. The results also demonstrated the possibilities of modifying and developing fermented rice products using raw-material combinations involving Chinese and Thai rice varieties and cultures. However, consumer acceptance is also a major concern. Further investigation of consumer acceptability is needed.

Acknowledgement

We thank Colin Wrigley, Honorary Professor at the University of Queensland, Australia for his valuable suggestions on the preparation of the manuscript as well as English proofreading.

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