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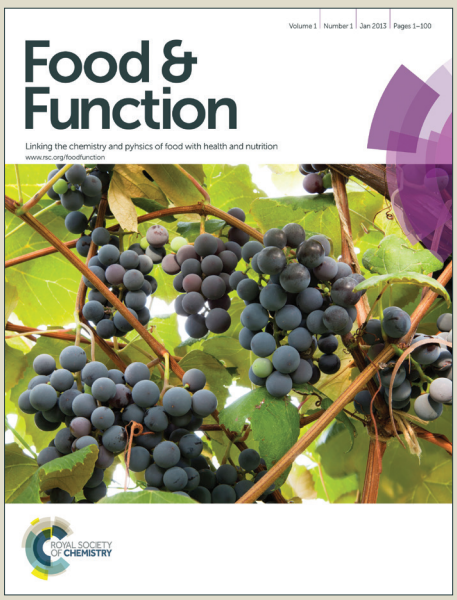

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Marigold flower-powder exhibit significant potential to inhibit lipid oxidation in rice bran tea

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Abstract

We supplemented the marigold flower-powder (MFP) in rice bran tea at different proportions as a source of natural antioxidant compounds. Changes of phenolic compounds, antioxidant activity, fatty acid composition and lipid oxidation in the rice bran tea with MFP after 30 days of storage were investigated, comparing results with the initial data. Adding MFP in rice bran tea resulted in an increased content and composition of phenolics and flavonoids along with enhanced antioxidant activities, which were increased in a dose-dependent manner. As a result, MFP supplementation of rice bran tea was able to retard the lipid oxidation as determined by peroxide value (PV), due to the protection of essential fatty acids during 30 days of storage. The PVs were strongly negatively correlated (p<0.01) with phenolic compounds, total phenolic content (TPC) and total flavonoid content (TFC), but were positively correlated with tocopherols and γ-oryzanol contents. We also found that the PV was positively correlated with PUFA (poly unsaturated fatty acid) content but adverse results were found for SFA (saturated fatty acid) and MUFA (mono unsaturated fatty acid) contents. These findings suggest that MFP could be used as a natural antioxidant in foods for preventing lipid oxidation as well as extending shelf-life of food products.

Keywords: γ-Oryzanol; tocopherol; peroxide value; food additive; essential oil

1. Introduction

Lipid oxidation in food leads to changes in the taste, flavour and nutritional value, for example, the rancidity or the loss of vitamins, bioactive compounds and other essential compounds. It also generates toxic and carcinogenic agents that pose a danger to consumers. Although the uses of commercial synthetic antioxidants as additives in food products are allowed in practice, there have been reports of their high cost and their risk to food safety. Thus, the development of natural antioxidants to substitute for synthetic ones is needed for inhibition of lipid oxidation in food.[1]

Rice bran is the most nutritious part of the rice grain. It contains large amounts of γ-oryzanol and essential fatty acids (EFAs). The γ-oryzanol is only found in rice bran, and has been found to have powerful antioxidant properties. Rice bran has several unique properties that render its suitability for niche markets like the nutraceutical and pharmaceutical industries. One such feature is the presence of significant levels of minor-elements such as oryzanol, tocotrienol and phytosterols that have a large nutraceutical application. They are used in the development of value-added healthy products. EFAs (C18:2n-6, C18:3n-6) are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them.[2] On the other hand, PUFAs are highly unstable, that can be oxidized very easily. Thus, one of the major restrictions for the use of rice bran as an ingredient or food application is its high susceptibility to rancidity during storage.

Marigold (*Tagetes erecta* L.), locally known in Thailand as Daoruang, has been reported for several important biological activities including antioxidant, anti-inflammatory, antimycotic, analgesic, anti-edematous and wound healing activities.[3] Its biological activities are related to the content of several secondary metabolites, and the most important compounds are terpenes, essential oils, flavonoids, carotenoids and polyphenols.[4]

Currently, the growing interest in new functional foods with special characteristics and health benefits has led to development of new functional foods or beverage products. These functional foods products contain a number of bioactive ingredients, which have been reported to reduce the risk of or prevent many chronic diseases.[5] Recently, we have reported that rice bran has ahigh *γ*-oryzanol and tocopherols content.[6] Marigold flower is a good source of antioxidants which could be enhanced for their bioactive compounds and antioxidant activities by far infrared radiation (FIR) drying.[7] In our previous study, we developed a functional tea product of rice bran with MFP with an overall acceptance of sensory evaluations.[8] The main purpose of this present study was to investigate the potential use of MFP at different proportions as a natural antioxidants for inhibition of lipid oxidation in rice bran tea. Changes of bioactive compounds, antioxidant properties, fatty acids contents and lipid oxidation of the products during storage were evaluated. The information obtained from our study will confirm the potential use of MFP as an active natural antioxidant and may be applied to other food products.

2. Materials and methods

2.1 Sample preparation

Paddy-rice samples (Khao Dawk Mali 105 variety) were obtained from Roi Et province, northeastern Thailand, in June 2013. The grains were milled to separate the husks from the brown rice. Then the brown rice was polished to obtain the bran. The bran was dried using optimized conditions established in our previous study by far-infrared radiation (FIR) at FIR intensity of 2 kW/m^2 (FIR energy irradiated per FIR heater surface area). The drying temperature was set at 40 °C and the drying time of 2 hr.[9] Marigold (*T. erecta* L.) flowers were bought from the market from Maha Sarakham Province, Thailand, in June 2013. Marigold flowers (MF) was cleaned and the petals were separated. The raw marigold petals were washed and kept at room temperature to drain. Afterwards, the petals were dried using the method of Wanyo *et al*. by combined far-infrared radiation with hot-air convection (FIR-HA) at FIR intensities of 5 kW/m^2, air temperature of 40 °C, air velocities of 1 m/s,[10] and a

drying time of 60 min. Dried rice bran and MFP were mixed together in various ratios by weight (rice bran: MFP were 70:30, 60:40, 50:50, 40:60 and 30:70) (Fig. 1). Moisture was determined by drying 5±0.001 g powder at 110 °C to constant mass. All analytical results were expressed on a dry matter basis and performed in triplicate. Directly after processing and a month of storage at ambient temperature (28-34 °C) the samples were subjected to analysis.

2.2 Assessment of antioxidant activity

2.2.1 Sample extraction

The extracts prepared from the rice bran with marigold teas were made by boiling the test material in distilled water for 5 min. The ratio between sample and extraction medium was 1:25 (w/v). The mixtures were then filtered through filter paper (Whatman No. 1) and the filtrate used for analyzing antioxidant activity *in vitro*. All analyses were performed in triplicate.

2.2.2 Ferric reducing/antioxidant power (FRAP) assay

The method of Butsat and Siriamornpun was used with some modifications.[6] The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl), 10 mL $FeCl_3$•6H2O (20 nM) in a ratio of 10:1:1 and 12 mL distilled water at 37°C. To perform the assay, 1.8 mL of FRAP reagent, 180 µL Milli-Q water and 60 µL sample, standard or blank were then added to the same test tubes, and incubated at 37 °C for 4 min; absorbance was measured at 593 nm, using the FRAP working solution as a blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using the $FeSO_4$•7H2O linear regression equation to calculate the FRAP values of the sample.

2.2.3 DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured as described by Butsat and Siriamornpun with some modifications.[6] Briefly, sample extract (0.1 mL) was mixed with 1.9 mL of a 0.1 mM DPPH in ethanol. The mixture was vortexed (1 min), left to stand at room temperature in dark (30 min) and then the absorbance of this solution was read at 517 nm. The percent inhibition activity was calculated as [(Ao-Ae)/Ao] x 100 (Ao= Absorbance without extract; Ae = absorbance with extract).

2.3 Identification and quantification of phenolic compounds

2.3.1 Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent as followed by Abu Bakar *et al*.[11] Briefly, 300 µL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature

for 5 min; 2.25 ml of sodium carbonate (60 g/L) solution was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm using a spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.3.2 Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the colorimetric method described by Abu Bakar *et al*. with slight modification.[11] Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3 \bullet 6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 mL of 1M NaOH was added. The mixture was mixed by vortex mixer. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.3.3 HPLC–DAD system for analysis of phenolic compounds

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A diode array detection, and chromatographic separations were performed on a column Inetsil ODS-3, C18 (4.6 mm x 250 mm, 5 μm) (Hichrom Limited, Berks, UK). The composition of solvents and gradient elution conditions were described previously by Butsat and Siriamornpun with some modifications.[6] The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9% solvent B; from15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38 °C, injection volume, 20 μL and UV-diode array detection at 280 nm (hydroxybenzoic acids; HBA), 320 nm (hydroxycinnamic acids; HCA) and 370 nm (flavonoids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard methods.

2.4 Extraction and determination of γ-oryzanol and tocopherols contents

One-step equilibrium direct-solvent extraction was conducted by the method of Butsat and Siriamornpun with some modifications.[6] Each sample (1 g) was extracted with acetone at a ratio of 1:10 w/v, vortexed at maximum speed for 1 min then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residual was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate. The contents of γ-oryzanol and tocopherols were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 μm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm x 250 mm, 5 μm) with mobile phase of

acetonitrile / methanol (25:75, v/v), flow rate 1.5 ml/min and photodiode-array detector at 292 nm for the analysis of tocopherols and at 325 nm for the analysis of γ-oryzanol. Calibration curves were constructed with the external standards.

2.5 Lipid extraction

Lipids were extracted according to the method of Bligh and Dyer.[12] Approximately 5 g of well-ground samples was extracted with 50.0 mL of chloroform–methanol (2:1, v/v) containing 10 mg/L of butylated hydroxytoluene and 0.1 mg/mL of nanodecanoic acid (C19:0, Sigma) as an internal standard. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and 15 mL of 0.9% sodium chloride was added. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated and transferred into a 10-mL volumetric flask.

2.6 Fatty acid analysis

Fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by transesterfication in H_2SO_4 (0.9 M in methanol). Before injection into the gas chromatograph, the FAMEs were filtered by Sep-pak silica column (Alltech Associates, Inc., Deerfield, IL). Samples (1 mL) were analyzed quantitatively using a Shimadzu model GC-2014 system (Shimadzu, Tokyo, Japan) fitted with flame ionization detection eluted with H_2 at 30 ± 1 mL/min, with a split ratio of 1:17. A fused silica capillary column (30 m x 0.25 mm, 25 μm film thickness; DB-Wax, USA) was used. The injector and detector were maintained at 250 °C. Nitrogen was used as a carrier gas and temperature programming was from 150°C (hold 5 min) to 230 °C at 15 °C/min, then to 170 °C (hold 10 min) at 10 °C/min, then to 200 °C (hold 3 min) at 5 °C/min and then to 230 °C (hold 2 min) at 15 °C/min. Fatty acid methyl esters were identified by comparison with standard mixtutes of FAME and quantitative data were calculated using peak areas compared with the added internal standard: nanodecanoic acid (C 19:0).[13]

2.7 Peroxide value

Peroxide values (PV) of total lipids were measured by titration of liberated iodine with standardized sodium thiosulphate solution according to the AOAC official method.[14] PV was determined with known weight of samples dissolved in a mixture of acetic acid/chloroform (3:2 v/v), and saturated solution of potassium iodide was then added. The liberated iodine was titrated with sodium thiosulphate solution (0.01 N) in the presence of starch as an indicator.

2.8 Statistical analyses

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 11.5 for Windows. Analysis of variance (ANOVA) in a completely randomised design, Duncan's multiple range test and Pearson's correlation coefficients were performed to compare the data. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($p < 0.05$).

3. Results and discussion

3.1 Antioxidant activity

The FRAP and DPPH radical scavenging assays were used to evaluate the antioxidant capacities of rice bran tea with and without MFP, and the results are shown in A and B of Fig. 2. We found that both the FRAP and DPPH values in rice bran tea with MFP added significantly increased when the level of MFP increased. The addition of MFP at different levels in rice bran tea enhanced the FRAP value and DPPH scavenging activity up to 1.1 and 2.5 times, respectively. These finding indicates that the increased antioxidant potential in rice bran tea could from the antioxidant compounds present in MFP, especially phenolics. Kaisoon *et al*. reported that MF possesses strongly effective and potent antioxidant activity and serves as a good source of phenolic antioxidant, especially flavonoids.[15] Our results suggest that MFP could be used as a functional food ingredient for increasing health benefits. However, the antioxidant activity of all rice bran tea with MFP added including the control decreased significantly ($p < 0.05$) during 30 days of storage time at ambient temperature. This decrease could be due to reactions of polymerization or complexation between antioxidant compounds and other compounds, such as protein and carbohydrate that can cause the formation of new compounds with a lower antioxidant activity. Gonzalez-Molina *et al.* demonstrated that the presence of other antioxidant compounds from the samples could react in the mixtures decreasing the antioxidant activities.[16] In addition, the storage conditions, such as storage time and temperature as well as location are influence on stability of antioxidant compounds and their functional properties.[17]

3.2 Total phenolic and total flavonoid contents

In the present study, the total phenolic content (TPC) and total flavonoid content (TFC) of rice bran tea with MFP added at various levels are shown in A and B of Fig. 3. The TPC and TFC were increased in a dose dependent manner, which were increased with increases in the level of MFP. The addition of MFP increased the TPC and TFC in rice bran tea up to 12.2 and 19.7 times, respectively. During 30 days of storage, TPC in all samples were slightly decreased but not by significant differences ($p> 0.05$), whereas the content of TFC were dramatically decreased. The decreased TFC is probably a result of the oxidative degradation, precipitation, or hydrolysis of the flavonoids.[18] The poorly soluble aglycones, formed as a result of hydrolysis, can also precipitate and contribute to the decrease of the total flavonoid content in the samples. The stability of phenolic compounds is always important for the antioxidant activity. Temperature enhances the rate of degradation of ingredients due to increase in kinetic energy. Decomposition of phenolic compounds due to oxidation is nearly as probable as that with hydrolysis and the rate of oxidation

is also temperature dependent. A decrease in the phenolic compounds indicates a loss of reactive hydroxyl groups due to the oxidation of phenolic compounds.[19]

3.3 Phenolic acids and flavonoids

The compositions of phenolic acids of rice bran tea with and without MFP are presented in Table 1. The major phenolic acids found in rice bran tea supplemented with MFP were gallic (GA), ferulic (FA) and sinapic (SA) acids, whereas protocatechuic (PCCA) and vanillic (VA) acids. These were the most dominant phenolic acids in rice bran without MFP. The total phenolic acid contents (TPA) of rice bran tea with MFP added were increased with increasing amounts of MFP. However, no significant differences ($p > 0.05$) in the content and composition of phenolic acids were observed in all the samples tested during 30 days of storage. In addition, our findings have shown that the proportions of MFP of the tea affected TPA of hydrocinnamic acid (HCA) and hydrobenzoic acid (HBA). In all samples tested, the total content of the HCA group was higher than the total content of the HBA group. We found that the HCA group was 32 μg/g in rice bran tea (control) and ranged from 123 to 157 μg/g in the tea with MFP, whereas the HBA group was 29 μg/g in rice bran and ranged from 94 to 110 μg/g in the tea with MFP (data not shown). The results obtained from this study suggest that the increases of content and composition of phenolic acids in rice bran tea with MFP added may be due to these compounds contained in MFP. A previous study has reported gallic, ferulic and sinapic acids as major phenolic acids in MF.[14] These compounds have attracted considerable attention due to their biological activities and health benefits.[20]

For flavonoids, it was possible to identify 5 flavonoids (rutin, myricetin, quercetin, apigenin and kaempferol). In the rice bran tea (control), only kaempferol was detected, whereas in rice bran teas with MFP all flavonoids were identified except for apigenin. The most abundant flavonoids found in the tea supplemented with MFP were quercetin with the values ranged from 788 to 1007 μg/g, followed by rutin (110-140 μg/g). Flavonoids of the extracts of the tea were increased significantly when the amount of MFP increased; the highest value was detected in rice bran tea supplemented with 70% MFP. Flavonoid concentrations of rice bran tea supplemented with MFP at 30 days of storage were decreased significantly when compared to the initial time of each sample (Table 1). MF is a good source of flavonoids, such as kaempferol, quercetin and rutin that have powerful antioxidant activity along with possess potential health enhancing properties.[7,15] The result indicates that phenolic acids were more stable than flavonoids in this product. In general, flavonoids are highly sensitive to the storage conditions, especially temperature and storage time in which the degradation occurred after long term of storage.[21]

3.4 γ-Oryzanol and tocopherols

The γ-oryzanol and tocopherols contents of the rice bran teas with and without MFP are shown in Table 2. For all samples, the amount of γ-oryzanol ranged from 0.72 mg/g (70% MFP added in rice bran tea) to 5.62 mg/g in rice bran tea. The three forms of α-, γ- and δ- tocopherols were detected in rice bran while none of these compounds was detected in MFP. The amounts of γ-oryzanol and tocopherols in rice bran tea with MFP increased with increasing the amount of rice bran tea or decreasing the amount of MFP. Previous study has reported that the rice bran is a

rich source of γ-oryzanol and tocopherols.[6] After 30 days of storage, the content of these compounds was slightly decreased in all samples when compared to the initial day. Although our present study did not determined tocotrienols, there has been reported that tocotrienols up to 70% in rice bran oil which consists of α-, β-, γ- and δ-tocotrienols.[22]

3.5 Fatty acid composition and concentration

Fatty acid compositions, including saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA) were analyzed in all rice bran teas at day 0 and after 30 days of storage (Table 3). The main fatty acids found in rice bran tea (control) were oleic acid (43%), followed by linoleic acid (29%) and palmitic acid (22%), respectively, plus a very small proportion of α-linolenic acid. For MFP, oleic acid was the predominant fatty acid (59%), followed by palmitic acid (18%) and α-linolenic acid (12%), respectively, while linoleic acid was not detected. We found that the fatty acid compositions of the products (rice bran tea with MFP) were varied by proportion of each ingredient. After 30 days of storage, it was observed that the fatty acid compositions were not significantly different in the rice bran with MFP samples, but of the control was significantly different; SFA was increased while MUFA and PUFA were decreased ($p < 0.05$). The fatty acid concentrations of the control and rice bran with MFP were also analyzed and the results are shown in Table 4. Similarly, the fatty acid concentrations of MFP and the rice bran tea with MFP were not significantly different after 30 days of the storage. While PUFA was significantly decreased but MUFA and SFA were remained unchanged in the control. Our findings have demonstrated that adding MFP in the rice bran tea effectively protected the degradation of fatty acids, especially for PUFA. It well knows that MUFA and PUFA contained food products are easily oxidized by both the physical and chemical factors such as enzymes, temperature and storage time. This process lead to the quality and nutritional value of food as well as health benefits for consumers decreased. The results obtained from this study suggest that the addition of MFP, which was highly contained antioxidant compounds, can be inhibited the oxidation of essential fatty acid, especially PUFA; thus the MFP could be used as an alternative ingredient in food for increasing health benefits and reducing lipid oxidation.

3.6 Peroxide value (PV)

Peroxide value (PV) is a measure of the amount of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. PV is one of the most widely used tests for the analysis of oxidative rancidity in oils and fats. In this present study, the degree of oxidation of rice bran tea samples was determined by measuring PV in the absence and presence of MFP at ambient temperature for 30 days. The effects of antioxidants from MFP during storage on PV in the rice bran tea samples are shown in Fig. 4. A significant difference ($p< 0.05$) in PV was observed between the control and rice bran tea samples containing MFP in different proportions, all of which slowed the rate of peroxide formation. The PV of rice bran tea without MFP reached a maximum value of 3.09 meq oxygen/kg during 30 days of storage. These findings showed that PVs of rice bran tea treated with MFP decreased in a dose dependent manner, which was lower than those of the control; PV decreased with increased proportions of MFP (Fig. 4). Adding MFP resulted in 80.9 to 88.4% reduction of PVs in all samples tested; rice bran tea supplemented with 70%

MFP remained the most effective and gave the lowest PV (data not shown). Our results indicated that MFP supplemented in rice bran tea was able to decompose and reduce the formation of peroxides and hydroperoxides. These results were in accordance with the results of Korimova *et al*. who reported that rosemary extract effectively inhibited the formation of PV in meat products.[23] During 30 days of storage, the PVs of rice bran tea supplemented with MFP at different proportions were slightly increased from the beginning of the storage period, indicating initial process of oxidation. However, it is clear from these data that the PV of the rice bran tea without MFP were higher than that of rice bran tea with MFP antioxidants regardless of the storage period. Previous studies have reported that antioxidant compounds showed different activities toward hydroperoxide formation and decomposition.[24] According to CODEX standards, the maximum level of PV in edible fats and oils is 15 meq oxygen/ kg oil.[25] The PV found in the tea products of our study had lower than that of CODEX standards; hence MFP could be potentially used as additives in various food products to inhibit the oxidation of those products, especially fat and oil products for human consumption.

3.7 Correlation analysis

Statistical correlations have been studied between PV and bioactive components including fatty acids and compounds as well as antioxidant activity, as shown in Table 5.The PVs in the rice bran tea with MFP were strongly positively correlated with PUFA content but negatively correlated with SFA and MUFA contents. These findings indicated that the PUFA content was the key determinant that was responsible for primary lipid oxidation as determined by PV in rice bran tea with MFP added. PUFA contains at least two double bonds which are sensitive to free radical damage and are targets for reactive oxygen species. As a result, the free radical attack causes lipid peroxidation, producing lipid hydroperoxides and several other oxidation products.[26] To evaluate the potential use of MFP as a natural antioxidant for prevention of primary lipid oxidation, the correlations between PV and phenolic-antioxidants in rice bran tea with MFP were studied. Undoubtedly, the PV were strongly negatively correlated with phenolic compounds (HBA, HCA and flavonoids), TPC, TFC and consequently with antioxidant activity (DPPH and FRAP assay) ($p< 0.01$). Phenolic antioxidants inhibit lipid peroxidation by trapping the lipid alkoxyl radical or able to chelate metals such as iron.[27, 28] This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules.[29] In contrast, the PVs in the rice bran tea with MFP were strongly positively correlated with tocopherols and γ-oryzanol contents ($p< 0.01$). These findings indicated that both tocopherols and γ-oryzanol contents were responsible for PV in rice bran tea with MFP. Tocopherols can act as antioxidants and pro-oxidants depending on temperature, pH, concentration, and their chemical characteristics.[30,31,32] The pro-oxidant effect of α-tocopherol reported *in vitro* studies appears to be related to its tocopheroxy radicals.[33] When the concentration of the tocopheroxy radical is high, the radical may react reversibly with unperoxidized lipids and hydroperoxides by chain transfer and generate alkyl and peroxy radicals, respectively.[32,34] Although in our previous study, the γ-oryzanol exhibits antioxidant properties, including free radical scavenging and lipid peroxidation prevention,[29] in this present study it was found the higher content of γ-oryzanol resulted in higher PV of the products indicating that γ-oryzanol content in the tea samples might be insufficient to exert significant systemic antioxidant effects. To our opinion, the reason might be similar to those of α-tocopherol. However, further research is needed on the preventation of lipid oxidation by various concentration of γ-oryzanol.

Conclusions

The present study has demonstrated that MFP exhibited high antioxidant activity and effectiveness on controlling lipid oxidation in rice bran tea during 30 days of storage. MFP increased the antioxidant compounds such as phenolics and flavonoids making them available in the rice bran tea, thus reducing the formation of oxidation products and exhibiting a higher protection against unsaturated fatty acid loss during 30 days of storage. In addition, MFP effectively inhibited the formation of peroxides and hydroperoxides in these products. The PVs were strongly negatively correlated with phenolic compounds, TPC, TFC and with antioxidant activities. Unexpectedly, it was found that tocopherols and γ-oryzanol contents were strongly positively correlated with the PV; however the explanation for this result is not yet understood. Therefore, MFP could be used as a natural antioxidant supplement in foods and drinks, where it would improve colour and flavour as well as extending the shelf-life of those foods, and promoting human health.

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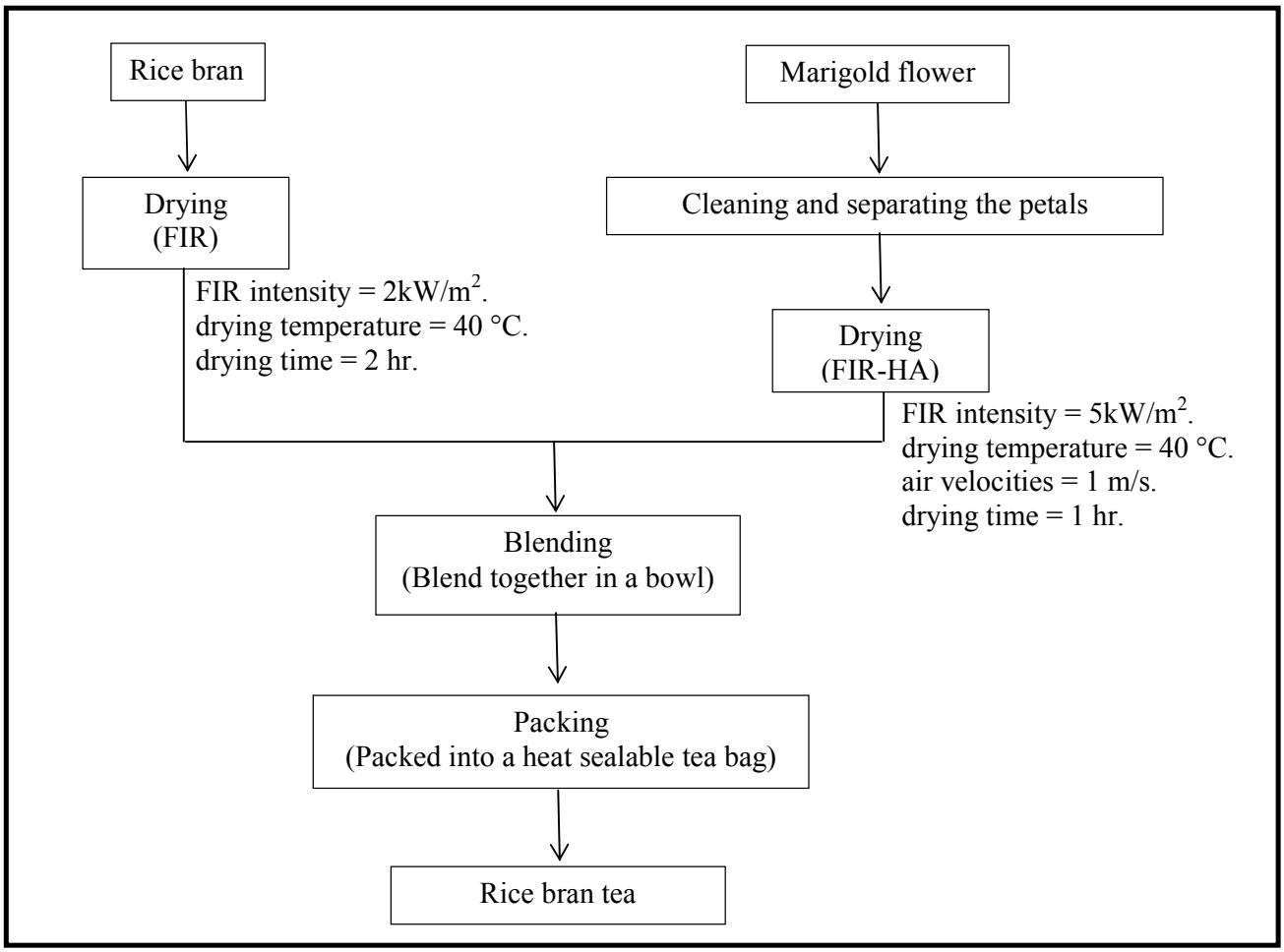

Fig. 1 Flow sheet for the preparation of rice bran tea samples

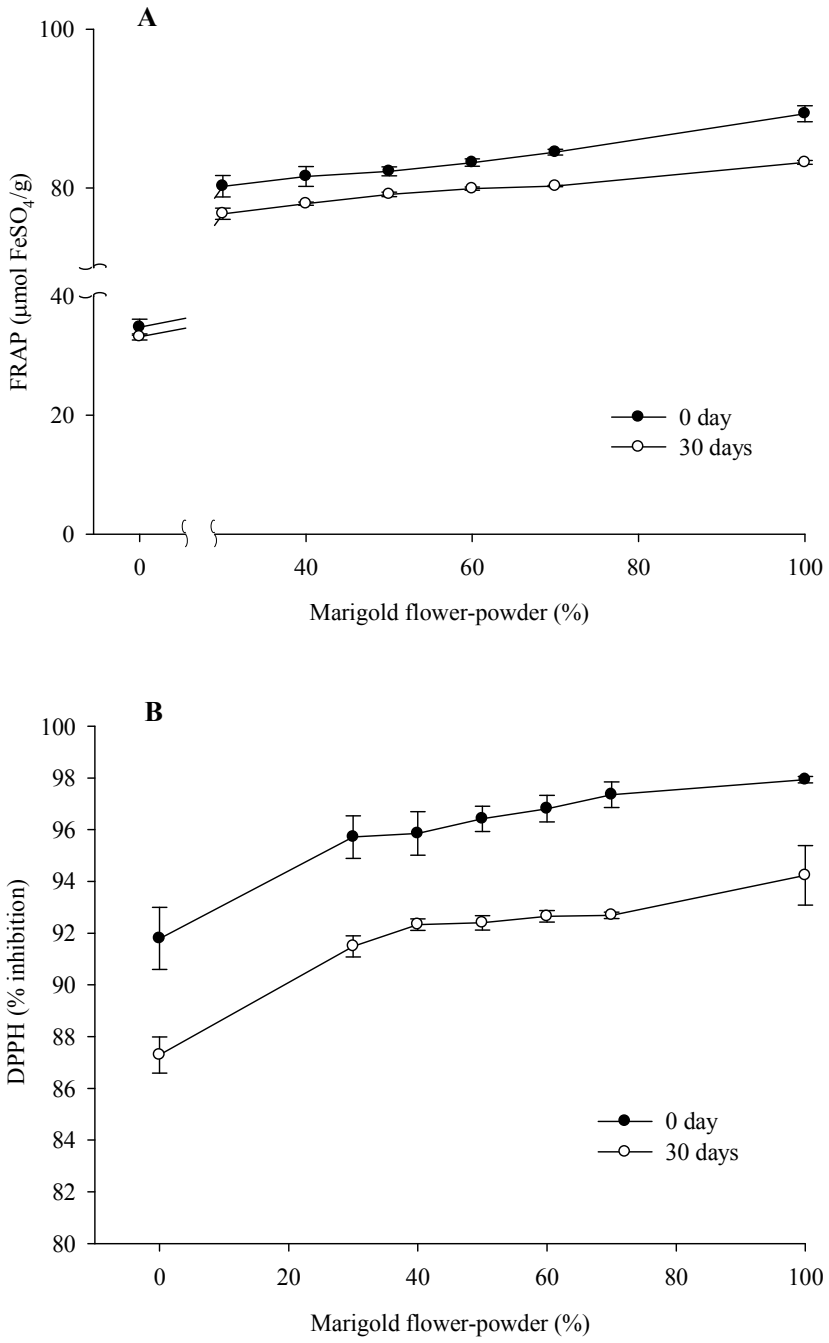

Fig. 2 Effects of marigold flower powder addition in rice bran tea on (A) ferric reducing/antioxidant power (FRAP) and (B) DPPH radical scavenging activity (significantly at $p < 0.05$ level)

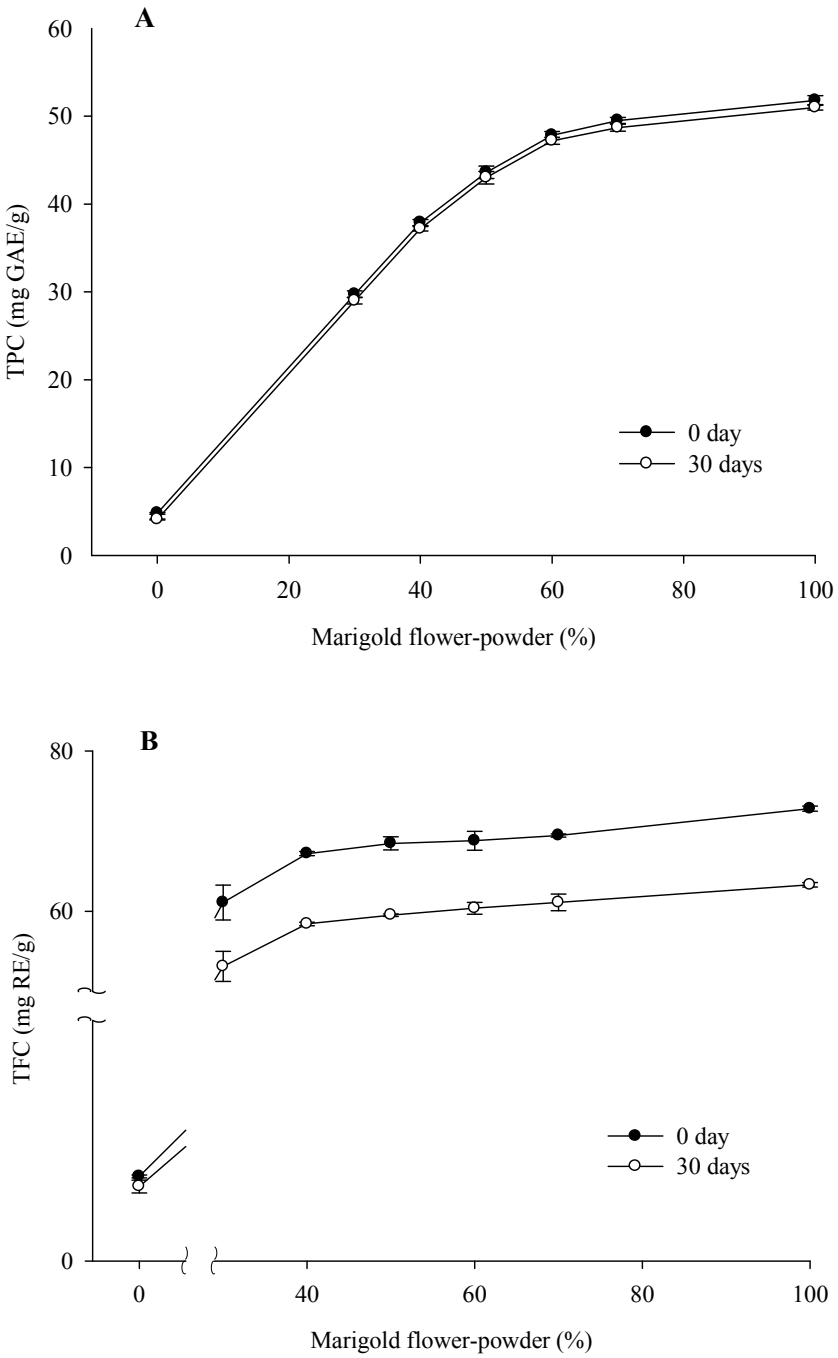

Fig. 3 Effects of marigold flower powder addition in rice bran tea on (A) total phenolic content (TPC) and (B) total flavonoid content (TFC) (significantly at $p < 0.05$ level)

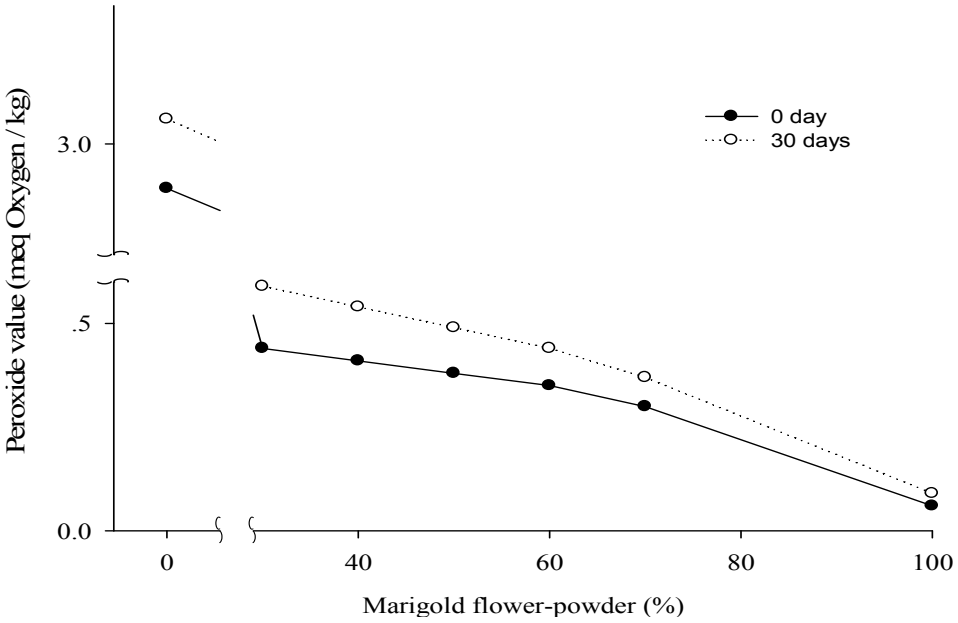

Fig. 4 Peroxide value (meq oxygen / kg) of rice bran tea supplemented with marigold flower powder at different proportions (significantly at $p < 0.05$ level)

Table 1 Phenolic acids and flavonoids of rice bran tea with and without marigold flower powder (μg/g dry weight)

Compounds	Rice bran tea		MFP		30% MFP		50% MFP		70% MFP	
	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
Hydrobenzoic acids										
GA	6.79±0.05^{e}	6.74±0.08^{e}	146.53±1.11^{a}	145.84±0.28^{a}	67.11±0.22^{d}	66.99±0.26^{d}	74.14±0.54^{c}	74.07±0.42^{c}	89.99±0.13^{b}	89.96±0.09^{b}
PCCA	20.04±0.20^{a}	19.97±0.11^{a}	12.01±0.06^{e}	12.01±0.07^{e}	19.38±0.71^{b}	19.28±0.53^{b}	18.22±0.10^{c}	18.05±0.38^{c}	12.19±0.12^{d}	12.16±0.17^{d}
p-OH	Nd	Nd	7.90±0.13^{a}	7.88±0.10^{a}	3.31±0.04^{d}	3.31±0.04^{d}	3.70±0.09^{c}	3.70±0.09^{c}	4.31±0.05^{b}	4.29±0.10^{b}
VA	4.79±0.01^{a}	4.79±0.00^{a}	3.93±0.06^{d}	3.92±0.06^{d}	4.74±0.03^{a}	4.73±0.03^{a}	4.53±0.06^{b}	4.52±0.05^{b}	4.06±0.02^{c}	4.05±0.01^{c}
Hydrocinnamic acids										
ChA	3.17±0.01^{d}	3.17±0.02^{d}	31.25±0.53^{a}	31.09±0.81^{a}	16.16±0.12^{c}	16.14±0.16^{c}	17.15±0.11^{bc}	17.10±0.20^{bc}	17.58±0.30^{b}	17.57±0.32^{b}
CFA	3.52±0.02^{e}	3.52±0.04^{e}	26.62±0.35^{a}	26.41±0.33^{a}	11.56±0.16^{d}	11.52±0.22^{d}	13.61±0.38^{c}	13.60±0.39^{c}	14.81±0.37^{b}	14.80±0.37^{b}
SyA	3.16±0.01^{e}	3.15±0.03^{e}	11.10±0.07^{a}	11.07±0.02^{a}	5.04±0.05^{d}	5.03±0.06^{d}	7.13±0.05^{c}	7.08±0.14^{c}	9.72±0.04^{b}	9.71±0.07^{b}
p-CA	3.07±0.02^{e}	3.07±0.01^{e}	15.00±0.18^{a}	14.89±0.16^{a}	8.31±0.09^{d}	8.29±0.12^{d}	8.89±0.09^{c}	8.88±0.09^{c}	9.19±0.21^{b}	9.18±0.22^{b}
FA	15.55±0.24^{e}	15.54±0.24^{e}	86.29±0.91^{a}	86.13±0.70^{a}	45.28±0.41^{d}	45.21±0.51^{d}	50.57±0.20^{c}	50.46±0.39^{c}	52.65±0.10^{b}	52.62±0.15^{b}
SNA	3.54±0.01^{e}	3.54±0.01^{e}	80.07±0.65^{a}	80.05±0.65^{a}	37.55±0.29^{d}	37.50±0.37^{d}	41.26±0.68^{c}	41.24±0.71^{c}	53.85±0.79^{b}	53.83±0.83^{b}
Total phenolic acids	62.36±0.41^{e}	62.49±0.38^{e}	420.69±1.29^{a}	419.29±0.30^{a}	223.93±1.12^{d}	218.00±1.16^{d}	239.18±1.45^{c}	238.69±1.14^{c}	268.35±0.35^{b}	268.16±0.49^{b}
Flavonoids										
Rutin	Nd	Nd	158.80±0.56^{a}	151.58±0.25^{b}	113.34±0.42^{g}	109.74±0.40^{h}	128.36±0.40^{e}	124.29±0.39^{f}	140.36±0.51^{c}	135.90±0.50^{d}
Myricetin	Nd	Nd	6.31±0.02^{a}	6.09±0.04^{b}	5.90±0.01^{g}	5.88±0.01^{h}	5.98±0.01^{e}	5.95±0.01^{f}	6.06±0.01^{c}	6.03±0.01^{d}
Quercetin	Nd	Nd	1159.78±3.92^{a}	1025.25±2.87^{b}	804.05±3.29^{g}	788.35±3.23^{h}	919.35±0.31^{e}	901.39±0.30^{f}	1007.80±1.87^{c}	988.10±1.84^{d}
Apigenin	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Kaempferol	1.75±0.01^{h}	1.69±0.01^{i}	3.73±0.02^{a}	3.51±0.02^{b}	1.77±0.02^{g}	1.74±0.01^{h}	2.67±0.02^{e}	2.58±0.01^{f}	3.12±0.03^{c}	3.06±0.02^{d}
Total flavonoids	1.75±0.01^{i}	1.69±0.01^{j}	1328.62±4.11^{a}	1186.43±2.80^{b}	925.07±3.69^{g}	905.71±3.60^{h}	1056.37±0.48^{e}	1034.20±0.44^{f}	1157.36±1.94^{c}	1133.09±1.93^{d}

CFA: caffeic acid; ChA: chorogenic acid; *p*-CA: *p*-coumaric acid; FA: ferulic acid; GA: gallic acid: *p*-OH: *p*-hydroxybenzoic acid; PCCA: protocatechuic acid; SyA: syringic acid; SNA: sinapic acid; VA: vanillic acid.
Values are expressed as mean ± standard deviation (n = 3). Nd (not detected).
[a,b,c] Means with different letters in the same row were significantly different at the level $p < 0.05$.

Table 2 γ-Oryzanol and tocopherols of rice bran tea with and without marigold flower powder (mg/g dry weight)

Samples	γ-oryzanol (mg/g)		α-tocopherol(μg/g)		γ-tocopherol (μg/g)		δ-tocopherol (μg/g)	
	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
Rice bran tea	5.62±0.01^{a,A}	5.57±0.01^{a,B}	95.78±3.81^{a,A}	88.15±1.74^{a,B}	5.14±0.09^{a,A}	4.99±0.01^{a,B}	7.84±0.12^{a}	7.69±0.03^{a}
MFP	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
30% MFP	4.82±0.03^{b,A}	4.70±0.04^{b,B}	44.06±0.15^{b,A}	40.48±0.34^{b,B}	2.01±0.01^{b,A}	1.90±0.01^{b,B}	1.28±0.00^{b,A}	1.07±0.00^{b,B}
50% MFP	2.77±0.02^{c,A}	2.74±0.01^{c,B}	41.52±0.20^{c,A}	35.72±0.17^{c,B}	1.92±0.01^{c,A}	1.81±0.00^{c,B}	1.13±0.00^{c,A}	1.00±0.01^{c,B}
70% MFP	0.72±0.01^{d,A}	0.61±0.01^{d,B}	38.72±0.13^{d,A}	30.23±0.38^{d,B}	1.74±0.01^{d,A}	1.68±0.01^{d,B}	0.96±0.01^{d,A}	0.86±0.03^{d,B}

Values are expressed as mean ± standard deviation (n = 3). Nd (not detected).
[a,b,c] Means with different letters in the same column were significantly different at the level $p < 0.05$.
[A,B,C] Means with different letters in the same row (between group) were significantly different at the level $p < 0.05$.

Table 3 Fatty acid compositions of rice bran tea supplemented with marigold flower powder at different proportions (% of total fatty acids)

Fatty acids	Rice bran tea		MFP		30% MFP		50% MFP		70% MFP	
	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
14:0	0.56±0.00^{e}	0.57±0.01^{e}	0.66±0.01^{a}	0.65±0.02^{a}	0.59±0.00^{d}	0.59±0.00^{d}	0.60±0.00^{c}	0.61±0.01^{c}	0.63±0.01^{b}	0.63±0.00^{b}
15:0	0.04±0.00^{e}	0.04±0.00^{e}	0.13±0.01^{b}	0.13±0.00^{a}	0.04±0.00^{e}	0.04±0.00^{e}	0.05±0.00^{d}	0.05±0.00^{d}	0.07±0.00^{c}	0.07±0.00^{c}
16:0	22.78±0.71^{b}	23.15±0.21^{a}	18.30±0.15^{f}	18.23±0.16^{f}	22.08±0.01^{c}	22.08±0.00^{c}	21.08±0.00^{d}	21.08±0.00^{d}	20.51±0.00^{e}	20.51±0.01^{e}
17:0	Nd	Nd	0.13±0.01^{a}	0.13±0.01^{a}	0.01±0.00^{cd}	0.01±0.00^{cd}	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{b}	0.01±0.00^{b}
18:0	1.67±0.04^{e}	1.69±0.03^{e}	8.03±0.02^{a}	7.99±0.12^{a}	1.72±0.00^{de}	1.72±0.00^{de}	1.90±0.00^{c}	1.90±0.01^{c}	2.43±0.02^{b}	2.43±0.02^{b}
20:0	Nd	Nd	1.50±0.03^{a}	1.47±0.05^{b}	0.04±0.01^{e}	0.04±0.01^{e}	0.07±0.00^{d}	0.07±0.00^{d}	0.12±0.00^{c}	0.12±0.00^{c}
SFA	25.06±0.75^{c}	25.45±0.24^{b}	28.75±0.18^{a}	28.60±0.08^{a}	24.47±0.01^{d}	24.47±0.00^{d}	23.71±0.01^{e}	23.71±0.02^{e}	23.77±0.02^{e}	23.77±0.03^{e}
16:1	0.04±0.00^{e}	0.04±0.00^{e}	0.66±0.01^{a}	0.65±0.02^{a}	0.05±0.00^{d}	0.05±0.00^{d}	0.06±0.00^{c}	0.06±0.00^{c}	0.08±0.00^{b}	0.08±0.00^{b}
17:1	Nd	Nd	0.14±0.01^{b}	0.15±0.00^{a}	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{c}	0.01±0.00^{c}
18:1	43.12±0.04^{g}	43.41±0.06^{f}	58.65±0.20^{b}	59.09±0.16^{a}	44.13±0.10^{e}	44.13±0.11^{e}	46.76±0.01^{d}	46.76±0.02^{d}	48.75±0.01^{c}	48.76±0.01^{c}
MUFA	43.16±0.04^{g}	43.45±0.06^{f}	59.44±0.19^{b}	59.89±0.14^{a}	44.19±0.10^{e}	44.19±0.11^{e}	46.84±0.01^{d}	46.83±0.02^{d}	48.85±0.01^{c}	48.85±0.01^{c}
18:2 n-6	29.36±0.97^{a}	28.80±0.31^{b}	Nd	Nd	29.12±0.11^{ab}	29.11±0.12^{ab}	26.82±0.01^{c}	26.82±0.01^{c}	24.05±0.02^{d}	24.05±0.03^{d}
18:3 n-3	2.42±0.17^{e}	2.29±0.08^{ef}	11.81±0.02^{a}	11.51±0.21^{b}	2.23±0.02^{f}	2.23±0.01^{f}	2.65±0.00^{d}	2.64±0.01^{d}	3.33±0.02^{c}	3.33±0.02^{c}
PUFA	31.79±0.72^{a}	31.09±0.23^{a}	11.81±0.02^{d}	11.51±0.21^{d}	31.35±0.10^{a}	31.34±0.11^{a}	29.47±0.01^{b}	29.45±0.02^{b}	27.39±0.02^{c}	27.38±0.02^{c}

14:0 (myristic acid); 15: (pentadecanoic acid); 16:0 (palmitic acid); 17:0 (margaric acid); 18:0 (stearic acid); 20:0 (arachidic acid); 16:1 (palmitoleic acid); 17:1 (heptadecenoic acid); 18:1 (oleic acid); 18:2 n-6 (linoleic acid); 18:3 n-3 (α-linolenic acid).
SFA (saturated fatty acid); MUFA (mono unsaturated fatty acid); PUFA (poly unsaturated fatty acid)
Values are expressed as mean ± standard deviation (n = 3). Nd (not detected).
[a,b,c]Means with different letters in the same row were significantly different at the level $p < 0.05$.

Table 4 Fatty acids concentration of rice bran tea supplemented with marigold flower powder at different proportions (mg / 100 g)

Fatty acids	Rice bran tea		MFP		30% MFP		50% MFP		70% MFP	
	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days	0 day	30 days
14:0	58.53±0.54^{a}	59.05±1.40^{a}	3.18±0.05^{e}	3.14±0.09^{e}	43.98±0.03^{b}	44.03±0.10^{b}	34.81±0.08^{c}	34.78±0.10^{c}	23.19±0.01^{d}	23.24±0.10^{d}
15:0	4.24±0.20^{b}	4.41±0.03^{a}	0.63±0.04^{g}	0.65±0.02^{g}	2.88±0.02^{c}	2.89±0.003^{c}	2.64±0.01^{e}	2.65±0.01^{de}	2.48±0.01^{f}	2.49±0.01^{f}
16:0	2385.30±5.24^{a}	2388.68±18.63^{a}	88.48±1.24^{e}	87.97±0.81^{e}	1654.34±0.70^{b}	1654.06±0.89^{b}	1206.56±1.80^{c}	1205.77±1.06^{c}	752.76±0.75^{d}	752.61±0.85^{d}
17:0	Nd	Nd	0.63±0.04^{a}	0.63±0.03^{a}	0.32±0.00^{d}	0.32±0.00^{d}	0.35±0.01^{c}	0.35±0.01^{c}	0.51±0.01^{b}	0.51±0.02^{b}
18:0	175.55±0.12^{b}	176.53±0.35^{a}	38.79±0.14^{f}	38.53±0.76^{f}	128.76±0.30^{c}	128.98±0.18^{c}	108.71±0.49^{d}	108.75±0.44^{d}	89.19±0.59^{e}	89.21±0.64^{e}
20:0	Nd	Nd	7.25±0.20^{a}	7.07±0.19^{a}	2.95±0.49^{d}	2.96±0.48^{d}	4.00±0.01^{c}	4.01±0.03^{c}	4.25±0.04^{b}	4.26±0.03^{b}
SFA	2623.61±5.01^{a}	2628.68±8.41^{a}	138.69±1.70^{e}	137.99±1.04^{e}	1833.23±1.21^{b}	1833.24±1.09^{b}	1357.08±1.94^{c}	1356.32±0.72^{c}	872.39±0.65^{d}	872.33±0.89^{d}
16:1	4.22±0.07^{b}	4.45±0.04^{a}	3.19±0.06^{e}	3.15±0.11^{ef}	3.93±0.01^{c}	3.93±0.00^{c}	3.62±0.05^{d}	3.65±0.02^{d}	3.09±0.02^{f}	3.10±0.04^{ef}
17:1	Nd	Nd	0.67±0.01^{b}	0.69±0.01^{a}	0.29±0.01^{e}	0.29±0.00^{e}	0.35±0.00^{d}	0.35±0.01^{d}	0.39±0.00^{c}	0.40±0.01^{c}
18:1	4456.88±11.68^{a}	4455.01±8.75^{a}	283.50±0.78^{e}	284.04±0.45^{e}	3306.46±8.40^{b}	3306.03±9.32^{b}	2676.29±4.43^{c}	2674.81±2.94^{c}	1789.51±2.04^{d}	1789.28±2.45^{d}
MUFA	4461.11±11.70^{a}	4459.45±8.74^{a}	287.36±0.87^{e}	288.93±1.78^{e}	3310.68±8.41^{b}	3310.25±9.32^{b}	2680.27±4.48^{c}	2678.81±2.94^{c}	1793.00±2.05^{d}	1792.78±2.46^{d}
18:2 n-6	3045.77±13.72^{a}	2971.84±12.98^{a}	Nd	Nd	2181.63±8.01^{b}	2180.66±8.20^{b}	1535.15±1.79^{c}	1533.89±1.35^{c}	882.87±0.35^{d}	882.57±0.39^{d}
18:3 n-3	240.58±3.30^{a}	237.72±4.57^{a}	57.09±0.42^{e}	56.56±0.74^{e}	167.27±1.22^{b}	166.99±1.18^{b}	151.41±0.17^{c}	150.88±0.63^{c}	122.32±1.00^{d}	122.22±0.95^{d}
PUFA	3286.36±17.01^{a}	3209.33±14.76^{b}	57.09±0.42^{f}	55.50±1.12^{f}	2348.90±7.28^{c}	2347.64±7.46^{c}	1686.55±1.94^{d}	1684.77±1.90^{d}	1005.19±1.24^{e}	1004.79±1.20^{e}
Total	10371.07±24.68^{a}	10297.69±19.73^{b}	483.42±2.96^{f}	482.42±3.07^{f}	7492.80±2.74^{c}	7491.13±3.42^{c}	5723.90±8.26^{d}	5719.90±4.69^{d}	3670.57±3.60^{e}	3669.91±4.03^{e}

14:0 (myristic acid); 15: (pentadecanoic acid); 16:0 (palmitic acid); 17:0 (margaric acid); 18:0 (stearic acid); 20:0 (arachidic acid); 16:1 (palmitoleic acid); 17:1 (heptadecenoic acid); 18:1 (oleic acid); 18:2 n-6 (linoleic acid); 18:3 n-3 (α-linolenic acid).
SFA (saturated fatty acid); MUFA (mono unsaturated fatty acid); PUFA (poly unsaturated fatty acid)
Values are expressed as mean ± standard deviation (n = 3). Nd (not detected).
[a,b,c]Means with different letters in the same row were significantly different at the level $p < 0.05$.

Table 5 Pearson's correlation between peroxide values (PV) and bioactive components

variables	correlation
SFA	-0.820**
MUFA	-0.675**
PUFA	0.941**
γ-oryzanol	0.702**
α-tocopherol	0.895**
γ- tocopherol	0.936**
δ- tocopherol	0.999**
HBA	-0.828**
HCA	-0.825**
Flavonoids	-0.983**
TPC	-0.922**
TFC	-0.995**
DPPH	-0.822**
FRAP	-0.998**

SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); HBA (hydrobenzoic acids); HCA (hydrocinnamic acids); TPC (total phenolic content); TFC (total flavonoid content).
** Significantly correlated at $p < 0.01$, n = 21.