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Research article

Combination of lipidomics and gene expression of THP-1 monocytes to indicate key anti-inflammatory compounds in rice bran oil

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Abstract

Lipophilic compounds were extracted from four rice varieties: colorless (Khao Dawk Mali 105), red (Kum), purple (Riceberry) and black (Homnil). Lipidomic profiles were obtained via gas chromatography-flame ionization detection. In total, 75 peaks were identified, representing fatty acid methyl esters, free fatty acids and phytosterols. Unique lipidomic profiles were observed from each rice variety. Niosomes were fabricated from rice bran oil (RBO) using the thin film method and a ratio of RBO to Tween 80 of 1:3. RBO niosomes were measured to be approximately 200 nm in size. Lipopolysaccharide-stimulated THP-1 monocytes with different concentrations of RBO niosomes were incubated for 3 hr and 6 hr. The expressions of the pro-inflammatory genes *TNF-α* and *IL-1β* were analyzed. The results showed that RBO niosomes reduced pro-inflammatory genes in a dose-dependent manner. Principal component analysis revealed a clear correlation among three observed attributes: phytosterols, the degree of *TNF-α* and *IL-1β* gene expression reduction, and the rice variety (Homnil and Riceberry). The phytosterols responsible for RBO anti-inflammatory activity were determined using pairwise correlation, with 10 out of 12 of the identified phytosterols strongly correlated with pro-inflammatory cytokine gene reduction.

Introduction

Rice (*Oryza sativa* L.) remains one of the most popular cereals grown and consumed worldwide, especially in Asian countries (Frank et al., 2012). In 2018, rice exports from Thailand were valued at USD 5.6 billion and were second only to India worldwide (Workman, 2019). Historically, consumers have preferred colorless rice over colored rice varieties. However, health-conscious consumers are popularizing colored rice varieties that have dietary benefits. Red, black or purple rice varieties have approximately 2–3 times more anthocyanins, proanthocyanidins, vitamin E and other nutraceutical content than their colorless counterparts (Goufo and Trindade, 2014). Anthocyanin, a natural antioxidant in black and purple rice varieties, may improve blood circulation and reduce the effects of cell aging, cardiovascular disease and the incidence of ischemic stroke (Srisawat et al., 2010; Seo et al., 2013). Fatty acid composition and lipid soluble bioactive

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compounds differ among rice varieties (Goufo and Trindade, 2014) occurring in essential lipid-soluble vitamins (A, D, E, K), lipid-soluble phytochemicals (γ-oryzanol, α-tocopherol, phytosterols, carotenoid, tocotrienol) and polyunsaturated fatty acids (eicosapentaenoic acid, docosahexaenoic acid, α-linoleic acid, arachidonic acid). Therefore, rice varieties offer differing levels of bioactivity, including blood cholesterol reduction, anti-inflammatory activity (Saenjum et al., 2012; Rao et al., 2016) and anti-proliferation, and apoptosis cancer induction (Madhavi et al., 1998; Awad et al., 2003).

A powerful detection technique with both high resolution and sensitivity is required to measure the impact of the lipid profile on rice bran anti-inflammatory activity. Lipidomics, a part of metabolomics, reveals lipid profiles, structures, interactions and biological functions within cells and tissues and can measure bioactive compound changes during storage, their traceability and their structure-function relationship (Luo et al., 2019; Liu et al., 2020). In the present study, phytochemicals in rice bran oil from different rice varieties were investigated using lipopolysaccharide (LPS)-stimulated THP-1 monocytes coupled with mass spectrometry-based targeted lipidomics to indicate key anti-inflammatory compounds. The results from work should provide more comprehensive information on lipid-soluble compounds which will allow scientists to focus on anti-inflammatory activities in rice bran oil.

Materials and Methods

Chemicals

High performance liquid chromatography or analytical grade extraction and derivatization chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) and RCI Labscan Ltd. (Bangkok, Thailand). All retention time standards (undecane, hexadecane, tetracosane, triacontane and octatriacontane) were purchased from Sigma Aldrich (St. Louis, MO, USA). All reference standards for lipidomics were procured from Merck (Darmstadt, Germany) and Fluka (Taufkirchen, Germany): lauric acid methyl ester (12:0 fatty acid methyl esters or FAME), myristic acid methyl ester (14:0 FAME), pentadecanoic acid methyl ester (15:0 FAME), pentadecenoic acid methyl ester (15:1 FAME), palmitic acid methyl ester (16:0 FAME), palmitoleic acid methyl ester (16:1 FAME), stearic acid methyl ester (18:0 FAME), oleic acid methyl ester (18:1 FAME), linoleic acid methyl ester (18:2 FAME), erucic acid methyl ester (22:1 FAME), tricosanoic acid methyl ester (23:0 FAME), lauric acid (12:0 FFA), stearic acid (18:0 FFA), oleic acid (18:1 FFA), linoleic acid (18:2 FFA), linolenic acid (18:3 FFA), 1-octacosanol (28:0-OH), Δ-tocopherol, γ-tocopherol, α-tocopherol, campesterol, campestanol, stigmasterol, Δ7-campestanol, β-sitosterol, sitostanol, Δ5-avenasterol, gramisterol, cycloartenol, Δ7-avenasterol and citrostadienol.

Rice bran

The Rice Department, Ministry of Agriculture and Cooperatives, Thailand provided the *Oryza sativa* L. rice paddy samples. The colored rice varieties consisted of: (i) colorless rice (Khao Dawk Mali 105; KDML105), (ii) red rice (Kum; K), (iii) purple rice (Riceberry; RBR) and (iv) black rice (Homnil; HN). The samples were dehulled and polished using an NW1000 Turbo rice polishing machine (Natrawee Technology Ltd.; Chachoengsao, Thailand). The obtained rice bran was frozen in liquid nitrogen before being finely ground manually using a mortar and pestle. The ground rice bran was passed through an 80-mesh using a rotor mill (model ZM1000, Retsch GmbH; Haan, Germany). Then, the rice bran powder samples were freeze-dried and stored at -20°C until analysis. The sample moisture content remained at <2%.

Lipidomics of rice bran oil

Sample extraction

Freeze-dried rice bran powder (600 mg) was weighed into a disposable cartridge (3 mL column volume, VWR International; Darmstadt, Germany), sealed with plastic frits on the top and bottom of the powder layer. Then, the cartridge was connected to a vacuum solid-phase extraction manifold (Waters Corp.; Milford, MA, USA) with valves to regulate the flow rate. The valves remained closed while the rice bran powder soaked in 300 µL methanol for 20 min at ambient temperature (25°C). Methanol was removed by vacuum (25 mbar) via the cartridge top for 30 min. The lipid fraction was then eluted by gravity flow with 4 mL of dichloromethane for 20 min. The 150 µL of each internal standard, including tetracosane and 5α-cholestan-3β-ol (Sigma-Aldrich; St. Louis, MO, USA), were added to 4 mL of the lipid fraction, and then evaporated until dry under vacuum, using a parallel evaporator at 40°C (Syncore Buchi; Flawil, Switzerland) (Na Jom et al., 2016).

Lipid fractionation

A modified lipid fractionation technique was utilized (Limwiwattana et al., 2016). For transesterification, the dried

lipid fraction was re-dissolved with a mixture consisting of 500 µL of methyl tert-butyl ether (MTBE), 300 µL of methanol and 50 µL of sodium-methylate. Selective hydrolysis was performed by adding 1 mL of dichloromethane followed by 2 mL of 0.35 M hydrochloric acid solution. The upper phase was separated by 2 mL of 0.35 M hydrochloric acid solution. The lower phase, containing transmethylated lipids, was collected and evaporated until dry. The residue was re-dissolved with 250 µL of dichloromethane. Transmethylated lipids, FAME (fraction 1) and polar lipids (fraction 2), were fractionated using elution with different ratios of hexane:MTBE solution. The ratio for fraction 1 was 100:2 volume per volume (v/v) and 80:20 v/v for fraction 2 using a solid-phase microextraction C18-LP cartridge (Vertical Chromatography Co. Ltd.; Nonthaburi, Thailand). All eluents were evaporated using a parallel evaporator at 50°C. The dried fraction 1 residue was re-dissolved with 300 µL of hexane and the solution was subsequently stored in a glass amber vial. The dried fraction 2 residue was silylated by adding 250 µL of pyridine and 50 µL of N-trimethylsilyl-N-methyl trifluoroacetamide at 70°C for 15 min. All lipid fractions were immediately stored at -20°C until gas chromatography-flame ionized detection (GC-FID) analysis.

Gas chromatography with flame ionized detection analysis

All samples were analyzed using an HP Gas Chromatograph 6890 Plus with a flame ionization detector (Hewlett Packard; Palo Alto, CA, USA). Separation was performed using a DB-1 capillary column (60 m × 0.32 mm × 0.25 µm film thickness) with a 100% dimethylpolysiloxane stationary phase (Agilent Technologies; Santa Clara, CA, USA). One microliter of each sample was injected into the GC-FID equipment using spitless mode with helium as a carrier gas at a constant flow rate of 1.8 mL/min. The inlet temperature was 280°C. The oven temperature program started at 100°C, ramped to 320°C at a rate of 4°C/min and then was held at 320°C for 25 min. The detector temperature was 320°C. The lipophilic compound peaks were measured using the HP-ChemStation A.06.03 program (Hewlett Packard; Palo Alto, CA, USA). Lipophilic compounds were identified using the reference standards comparison technique. The concentration of each identified metabolite was reported as milligrams per milliliter related to the internal standard of each fraction.

Anti-inflammatory activities of rice bran oil niosomes

Fabrication of rice bran oil niosomes

Rice bran oil niosomes were fabricated with a slight modification regarding the ratio between rice bran oil (RBO) and Tween 80 (1:3). The particle size distribution of the RBO niosomes was measured using a Zetasizer Nano-ZS (Hunthayung et al., 2019).

Endotoxin quantification assay

The presence of lipopolysaccharide (LPS) in the RBO niosomes was determined using a ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript; Piscataway, NJ, USA.).

Cytotoxicity of rice bran oil niosomes

THP-1 monocytes were seeded in 8.2×10^4 cells per well of a 96-well cell culture plate. Cells were exposed to a range of RBO niosome concentrations for 6 hr. Cytotoxicity was determined using a methyl thiazol tetrazolium. Values were expressed relative to non-stimulated cells which were set as 100%.

Inflammatory gene expression

The human monocyte THP-1 cell line (American Type Culture Collection; Rockville, MD, USA.) was grown in an RPMI 1640 medium supplemented with a 10% fetal bovine serum and 1% penicillin/streptomycin while in a humidified incubator at 37°C and 5% CO_2. The exponential phase THP-1 monocytes with passage numbers less than 25 were stimulated with 50 ng/mL LPS from *Escherichia. coli* (O111:B4) for 3 hr. Then, different concentrations of RBO niosomes (50 ng/mL and 250 ng/mL) were added and further incubated for 3 hr and 6 hr, respectively. Pro-inflammatory gene expressions *Tumor Necrosis Factor-alpha (TNF-α)* and *Interleukin-1-beta (IL-1β)* were measured using quantitative real-time polymerase chain reaction with primer sequences as indicated in Chanput et al. (2010). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and time 0 hr of non-stimulated cells were used for ΔΔCt normalization.

Statistical analysis

Treatments from gene expression were compared using one-way analysis of variance with Duncan's post hoc comparison test. A p value < 0.05 was considered statistically significant. Relative concentrations of lipophilic compounds and the expression of immune modulating genes were subjected to principal component analysis (PCA) using the XLSTAT-base version 2018.3 software (Addinsoft; New York, NY, USA).

Results

Lipidomics of rice bran oil

The physical appearance of each of the dehulled colored and colorless rice varieties is shown in Fig. 1. All rice samples were *Oryza sativa* L. Indica species, which are normally grown in tropical and subtropical climates. Individual rice kernels were approximately 7.05 ± 0.50 mm in length and were smooth and slender.

The lipidomics profile from the GC-FID analysis identified 75 peaks capable of being divided into three major groups: (i) fatty acid methyl esters (FAME) (38 peaks), (ii) free fatty acids (FFA) (25 peaks) and (iii) phytosterols (12 peaks), as shown in Fig. 2. Indications of each peak are designated in Tables 1 and 2. The relative quantification of lipophilic metabolites in the lipid fractions for the four rice varieties was displayed in a Microsoft Excel heat plot as shown in Fig. 3. The results revealed that the red rice (K) and purple rice (RBR) contained greater amounts of palmitic acid methyl ester (C16:0 FAME), oleic acid methyl ester (C18:1 FAME) and linoleic acid methyl ester (C18:2 FAME) than the black rice (HN) and colorless rice (KDML105). Among the analyzed phytosterols, β-sitosterol was the most abundant in the RBR variety compared to HN, K, and KDML105. The same pattern was also obtained with campesterol and stigmasterol (Fig. 3).

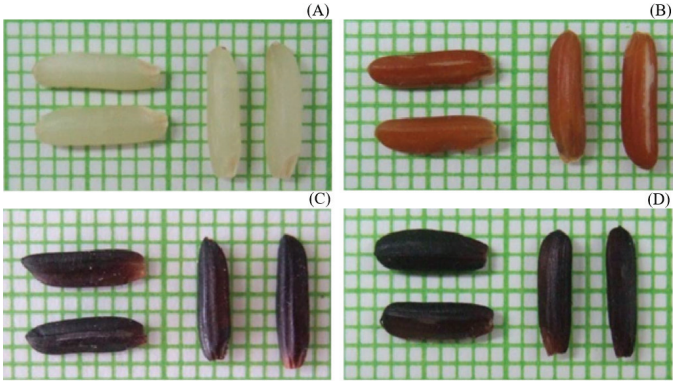

Fig. 1 Physical appearance of (A) colorless rice Khao Dawk Mali 105; (B) red rice Kum; (C) purple rice Riceberry; (D) black rice Homnil, where grid scale = 1 mm × 1 mm

Anti-inflammatory activity of rice bran oil niosomes

RBO oil from the four rice varieties was fabricated in a niosome delivery system with a particle size of approximately 200 nm and a polydispersity index (PDI) in the range 0.173–0.243 (Table 3). The stability of the RBO system was longer than 4 wk without any significant change in particle size and appearance (data not shown). Niosomes containing RBO were investigated for cytotoxicity on THP-1 monocytic cells. Cell viability up to 95% was observed after stimulation for 6 hr with RBO niosomes with concentrations of up to 250 ng/mL (data not shown). The presence of lipopolysaccharide endotoxin in RBO niosomes was quantified using a ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript; Piscataway, NJ, USA.). The LPS concentrations in all RBO niosome samples were less than 10 pg/mL in both testing concentrations (50 ng/mL and 250 ng/mL).

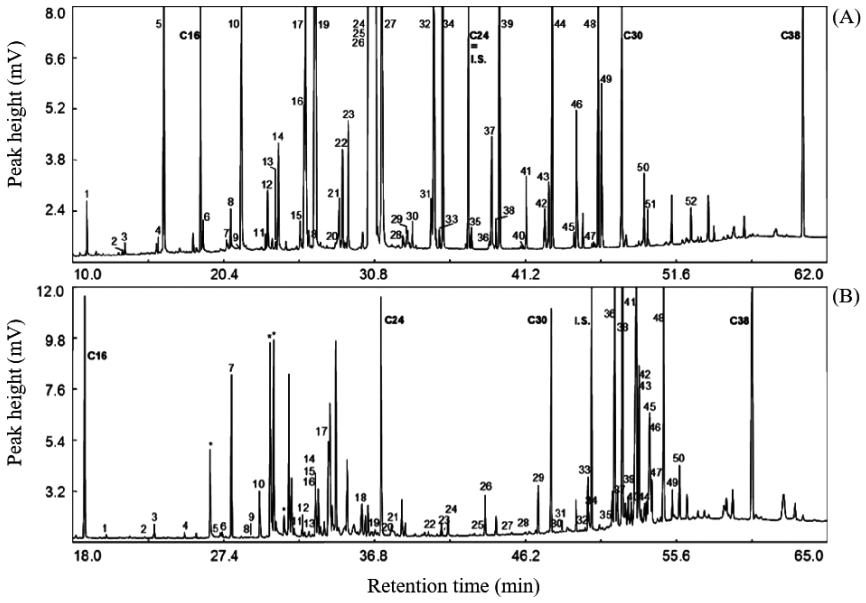

Fig. 2 Gas chromatography-flame ionized detection chromatograms based on lipidomics obtained from rice sample: (A) fatty acid methyl esters; (B) polar lipids, where I.S. = internal standards tetradecane and C16, C24, C30 and C38 = retention time standards

Table 1 Compound identification of fatty acid methyl esters (FAME) obtained from rice samples

No.	Compound	Identity[a]	No.	Compound	Identity[a]	No.	Compound	Identity[a]
Saturated FAME			Unsaturated FAME			Hydrocarbons		
1	C10:0	A	8	C14:1	A	2	C14	A
3	C11:0	A	12	C15:1	A	4	C15	A
5	C12:0	A	16	C16:1	C	9	C17	A
6	C13:0	A	17	C16:1 cis	A	13	C18	A
10	C14:0	A	21	C17:1	A	18	C19	A
14	C15:0	A	24	C18:1	A	22	C20	A
19	C16:0	A	28	C19:1	B	29	C22	A
23	C17:0	A	32	C20:1	A	33	C23	A
27	C18:0	A	37	C22:1	A	38	C25	A
30	C19:0	A	42	C24:1	A	40	C26	A
34	C20:0	A	7	C14:2	C	43	C27	A
35	C21:0	A	11	C15:2	C	45	C28	A
39	C22:0	A	15	C16:2	C	46	squalene	A
41	C23:0	A	20	C17:2	C	47	cholestane	C
44	C24:0	A	25	C18:2	A	48	C29	A
49	C26:0	A	31	C20:2	A	50	C31	A
51	C28:0	A	36	C22:2	A			
52	C30:0	A	26	C18:3	A			

[a] identification according to: A = mass spectrometric data and retention time of reference compounds; B = mass spectrometric data and retention index of the Golm metabolome database (Kopka et al., 2004); C = NIST 02 MS library

Table 2 Compound identification of polar lipids obtained from rice samples

no.	compound	ident.[a]	no.	compound	ident.[a]	no.	compound	ident.[a]
free fatty acids			fatty alcohols			sterols and triterpenic alcohols		
1	C12:0	A	7	C16:0	A	34	cholesterol	A
2	C13:0	A	12	C18:0	A	36	campesterol	A
4	C14:0	A	13	phytol	A	37	campestanol	A
6	C15:0	A	19	C20:0	A	38	stigmasterol	A
8	C16:1	C	22	C22:0	A	39	Δ7-campestanol	E
9	C16:1 (*cis* 9)	A	26	C24:0	C	41	ß-sitosterol	A
10	C16:0	A	29	C26:0	A	42	sitostanol	A
11	C17:0	A	33	C28:0	A	43	Δ5-avenasterol	A
14	C18:3	A	40	C30:0	D	44	gramisterol	F
15	C18:2	A	50	C32:0	D	45	Δ7-stigmastenol	F
16	C18:1	A				46	cycloartenol	A
17	C18:0	A	phenolic compounds			47	Δ7-avenasterol	F
18	C19:0	A	3	methyl *p*-hydroxy-		48	24-methylene-	
20	C20:1	A		cinnamate	A		cylcoartanol	A
21	C20:0	A	5	methyl ferulate	A	49	citrostadienol	F
23	C22:1	A						
24	C22:0	A	tocopherols					
25	C23:0	A	28	δ-tocopherol	A			
27	C24:0	A	30	γ-tocopherol	B			
31	C26:0	B	32	α-tocopherol	A			
35	C28:0	A						

[a] Identification according to: A = mass spectrometric data and retention time of reference compounds; B = mass spectrometric data and retention index of the Golm metabolome database (Kopka et al., 2004); C = NIST 02 MS library; D = MS data; E = Xu and Godber, 1999; F = Kamal-Eldin et al., 1992

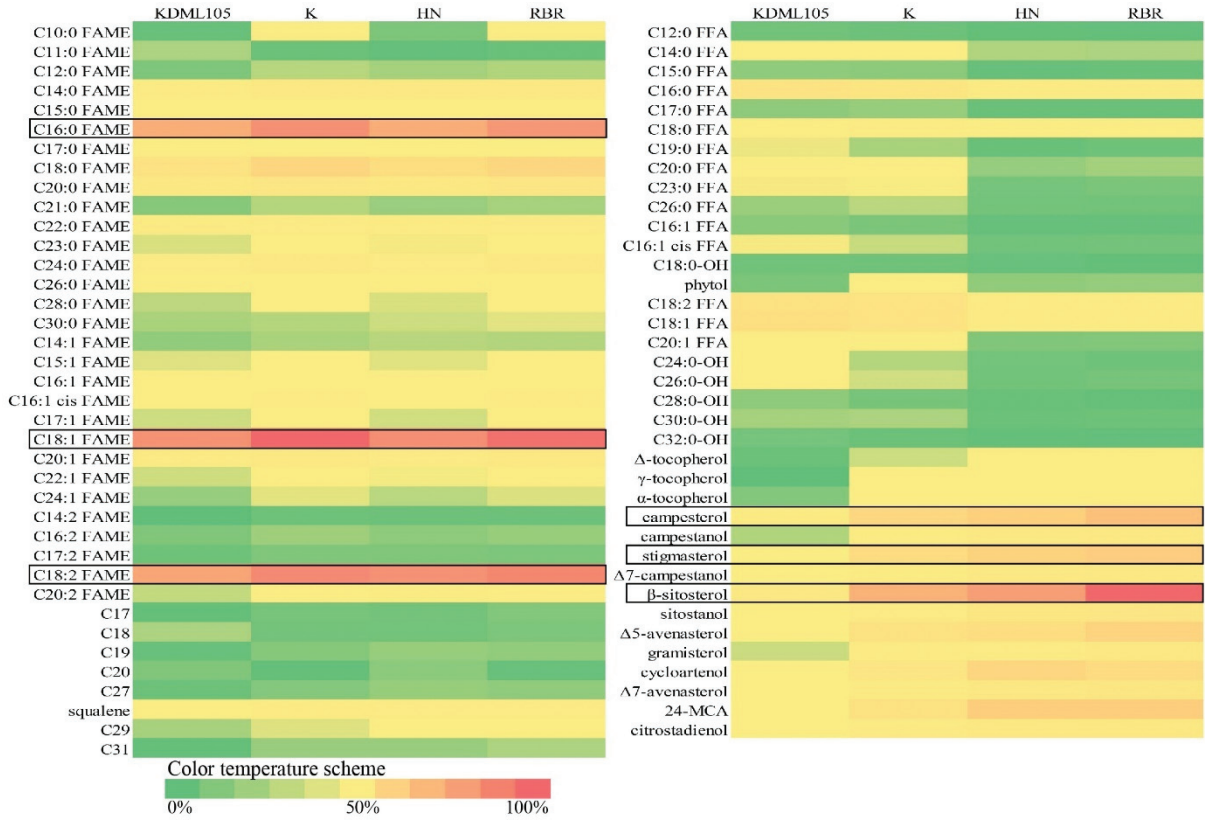

Fig. 3 Heat plot of lipophilic metabolites, including fatty acid methyl esters (FAME), free fatty acids (FFA) and phytosterols in lipid fractions from four rice varieties: red rice Kum (K), purple rice Riceberry (RBR), black rice Homnil (HN) and Khao Dawk Mali 105 (KDML105), where red hues indicate greater amounts and green hues less

Table 3 RBO niosome particle size distribution fabricated from different rice varieties and polydispersity index

Rice variety	Niosome size (nm)	Polydispersity index
Khao Dawk Mali 105	236.3±8.4 [b]	0.222±0.08 [a]
Homnil	176.3±2.0 [d]	0.243±0.03 [a]
Kum	256.6±4.8 [a]	0.173±0.02 [b]
Riceberry	217.9±2.2 [c]	0.224±0.03 [a]

Values (mean ± SD) of two independent replications, where different lowercase superscripts in the same column denote significantly different ($p < 0.05$) among treatments.

LPS-stimulated THP-1 monocytes were incubated with 50 ng/mL or 250 ng/mL of RBO niosomes for 3 hr and 6 hr, respectively. Fig. 4 shows the reduction of *TNF-α* and *IL-1β* gene expression for both incubation times compared to the LPS treatment (except for Kum rice variety 50 ng/ml at 6 hr). The expression of both the *TNF-α* and *IL-1β* genes significantly decreased in a dose-dependent manner in all samples (Fig. 4).

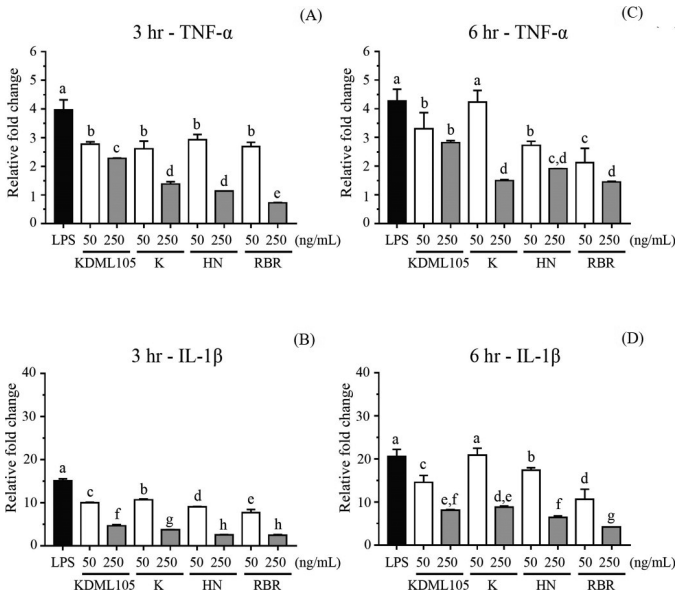

Fig. 4 Pro-inflammatory gene expression of 3 hr-LPS stimulated THP-1 monocytes incubated with RBO niosomes at 50 ng/mL and 250 ng/mL: (A, B) 3 hr and (C, D) 6 hr, where gene expression expressed relative to glyceraldehyde-3-phosphate dehydrogenase and non-stimulated cells at 0 hr, data (mean ± SD) from two biological and two technical replications and different lowercase letters above bars represent significant ($p < 0.05$).

Correlation between lipidomics and inflammatory gene expression

PCA revealed the correlation between the lipidomic results and the reduction of pro-inflammatory gene expression, *TNF-α* and *IL-1β*. As shown in Fig.5, principal components (PC) 1 and 2 covered 95.79% of the total variance. PC quadrant II had a high correlation with 12 phytosterols, *TNF-α* 3 hr, *IL-1β* 3 hr and *IL-1β* 6 hr (but not *TNF-α* 6 hr) representing 66.66% of the variation, meaning that phytosterols and the reduction of *TNF-α* and *IL-1β* are clearly associated, as well as black rice (RBR) and purple rice (HN). Red rice (K) was in the same quadrant as FAME, but not white rice (KDML). This might explain the high content and similar profile of phytosterols in RBR and HN (Fig. 3) which contribute to anti-inflammatory activity.

Further exploration was undertaken to confirm the degree of correlation between the reduction of *TNF-α* and *IL-1β* gene expression and phytosterols. The XLSTAT program allowed pairwise correlation to provide insights into these two attributes (Fig. 6). Campesterol, campestanol, stigmasterol, Δ7-campestanol, β-sitosterol, sitostanol, Δ5-avenasterol, gramisterol, Δ7-avenasterol and citrostadienol were perfectly correlated with the anti-inflammatory activity of the THP-1 monocytes at 3 hr of incubation (as shown by r = 1). This was not true for cycloartenol, 24-MCA and 6 hr of incubation.

Discussion

Additional lipophilic substances were found in a group of phytosterols apart from the dominant FAME and FFA. The lipidomics results corresponded with previous findings where RBO contained mainly palmitic acid, oleic acid and linoleic acid. Frei and Becker (2005) reported that unsaturated fatty acids accounted for the largest proportion of the lipid fraction, indicating that the colorless rice varieties were not significantly different in unsaturated fatty acid content compared to the colored rice varieties, which was in line with the present findings. FAME are a type of fatty acid ester which is derived from the transesterification of fats with methanol. This reaction occurs during sample preparation for the analysis of GC-FID. The content of FAME represents fatty acids in the triglyceride chain, with β-sitosterol being the most common phytosterol (Jiang and Wang, 2005; Friedman, 2013; Hunthayung et al., 2019), whereas campesterol and stigmasterol represented the second greatest amount of phytosterols found in RBO (Sawadikiat and Hongsprabhas, 2014). It is considered that environmental factors play a role in the nutritional quality of grain such as their proteins and minerals, but that genotype is a major factor determining the lipid profile for example, of fatty acids, phytosterols and α-tocopherol (Juliano, 1993; Frei and Becker, 2005).

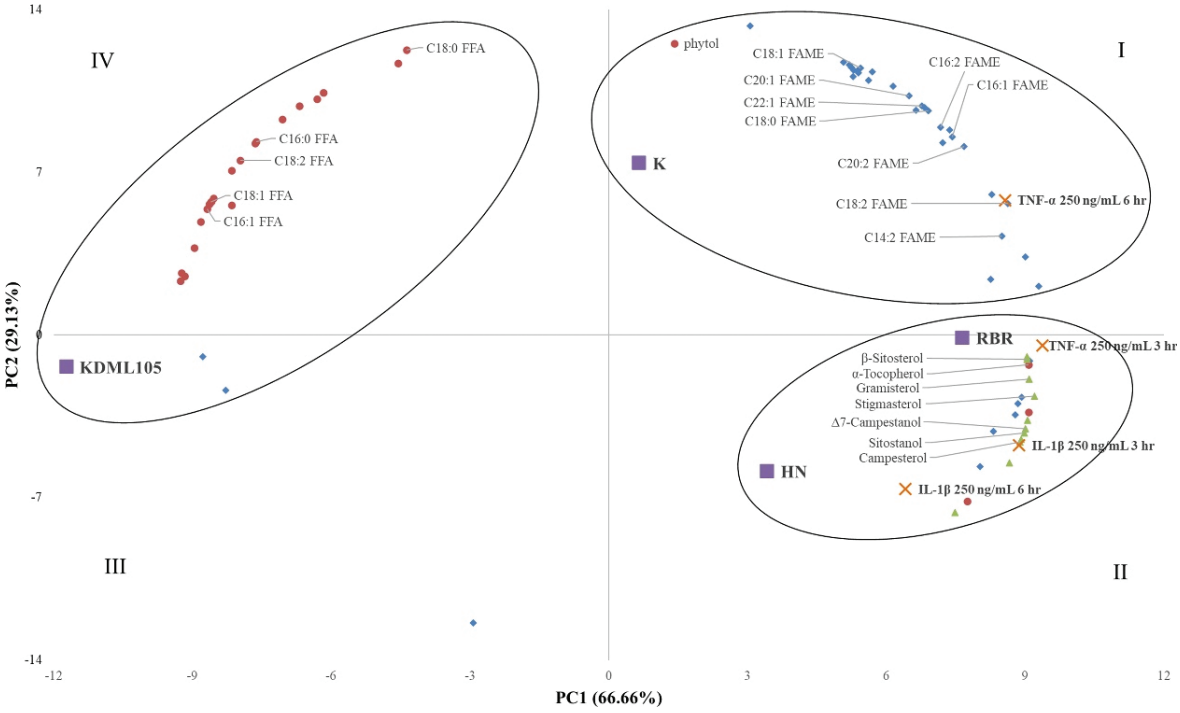

Fig. 5 Correlation loading of principal component (PC)1 and PC2 using a biplot of principal component analysis from all identified lipophilic components: fatty acid methyl esters (♦), free fatty acids (●), phytosterols (▲) and inflammatory gene expression (x) where rice varieties are bold: KDML105 = Khao Dawk Mali 105, K = Kum, HN = Homnil, and RBR = Riceberry

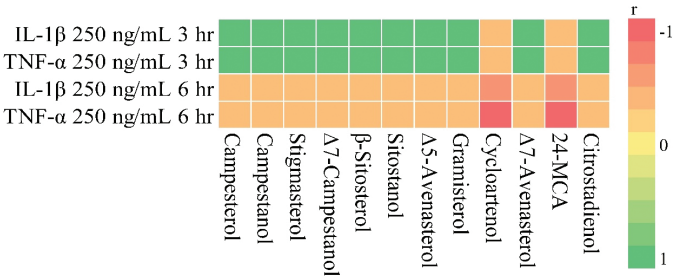

Fig. 6 Pairwise correlation analysis using XLSTAT between *TNF-α* and *IL-1β* gene expression reduction and rice phytosterols, where positive correlations (r > 0.3) are shown using green scale (r > 0.7) indicating a strong positive correlation, orange-red scale indicates negative correlations (r < -0.3) and red scale (r < -0.7) indicates a strong negative correlation.

Huntayung et al. (2019) reported the average size of cold-pressed RBO niosome was 200 nm. Their results were similar to the present study, although a different oil extraction method and different rice varieties were used. The PDI was lower than 0.25 in the present study, indicating a monomodal size distribution of particles (Tamjidi et al., 2014). Tween 80 is a biocompatibility surfactant, implying low toxicity and low-immunogenicity; thereby no cytotoxicity of the RBO niosome was observed (Jiao, 2008). LPS is a Gram-negative bacterial endotoxin which can bind to toll-like receptor-4 and stimulates NF-κB cascade and subsequently up-regulation of a set of pro-inflammatory genes such as *TNF-α*, *IL-1β*, *IL-6* and *IL-8* (Andreakos et al., 2004; Chanput et al., 2010). As earlier mentioned in the present results, the LPS concentrations in all RBO niosome samples were less than 10 pg/mL. An LPS value lower than 10 pg/mL could not up-regulate pro-inflammatory genes (Hunthayung et al., 2019). In the present study, the RBO niosomes of all rice varieties could reduce the expression of *TNF-α* and *IL-1β* genes in the LPS-stimulated THP-1 monocytes. PCA was applied to elucidate the relationship between the reductions in the *TNF-α* and *IL-1β* genes and lipophilic compounds in the RBO niosomes, which showed a strong correlation between rice phytosterols and a reduction in the observed genes. PCA is widely used to investigate the relative importance of individual variables for data determination; for example, antioxidative versus anti-inflammatory activity (Chanput et al., 2016) and antioxidative activity versus chemical composition in Thai plants (Maisuthisakul et al., 2008).

It has been reported that rice bran oil contains bioactive phytochemicals such as γ-oryzanol, γ-aminobutyric, tocopherols, tocotrienols and phytosterols (Mingyai et al., 2017; Park et al., 2017). In particular, phytosterols have been well documented as immune-modulating agents most frequently among the lipophilic metabolites (Alappat et al., 2010; Vilahur et al., 2019). Huntayung et al. (2019) revealed that an anti-inflammatory mechanism of cold-pressed RBO was caused by converting M1 pro-inflammatory macrophages into M0 resting macrophages. Furthermore, Nagasaka et al. (2007) reported the ability of phytosterols and γ-oryzanol to inhibit the translocation of NF-κB into the nucleus in RAW264.7 macrophages. In mice studies, it has been mentioned that phytosterols may play a role in the different signaling pathways involved in liver inflammation as antioxidants (Wang et al., 2013; Rocha et al., 2016). The present findings reflected the fact that concentrations of β-sitosterol, campesterol and stigmasterol play a vital role in the anti-inflammatory activity of RBO. This may explain why RBR and HN appeared in the same quadrant as the reduction of pro-inflammatory genes. To the best of the authors' knowledge, the present study is the first to report indicating other phytosterols (campestanol, Δ7-campestanol, sitostanol, Δ5-avenasterol, gramisterol, Δ7-avenasterol and citrostadienol) which also exhibit anti-inflammatory action in RBO and are probably as strong as the well-known ones. The findings from the present study could be used as a baseline for further study on anti-inflammatory agents in rice bran oil.

In conclusion, lipidomics in combination with the gene expression profile successfully indicated key bioactive compounds in rice bran oil which act as anti-inflammatory agents. Among lipophilic compounds, phytosterol plays a vital role to mitigate the inflammatory situation in the THP-1 monocyte cell model. As a result of the higher phytosterol content in the black and purple rice varieties, they exhibited stronger anti-inflammatory activity than the red and colorless rice varities. Not only commonly known anti-inflammatory rice phytosterols, such as, β-sitosterol, campesterol and stigmasterol, but another seven compounds in the phytosterol group were also responsible for the anti-inflammatory activities of rice bran oil.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Alappat, L., Valerio, M., Awad, A.B. 2010. Effect of vitamin D and β-sitosterol on immune function of macrophages. Int. Immunopharmacol. 10: 1390–1396. doi.org/10.1016/j.intimp.2010.08.003

Andreakos, E., Sacre, S.M., Smith, C., et al. 2004. Distinct pathways of LPS-induced NF-kappa B activation and cytokine production in human myeloid and nonmyeloid cells defined by selective utilization of Myd88 and Mal/Tirap. Blood 103: 2229–2237. doi.org/10.1182/blood-2003-04-1356

Awad, A.B., Roy, R., Fink, C.S. 2003. β-sitosterol, a plant sterol, induces apoptosis and activates key caspases in Mda-Mb-231 human breast cancer cells. Oncol. Rep. 10: 497–500. doi.org/10.3892/or.10.2.497

Chanput, W., Krueyos, N., Ritthiruangdej, P. 2016. Anti-oxidative assays as markers for anti-inflammatory activity of flavonoids. Int. Immunopharmacol. 40: 170–175. doi.org/10.1016/j.intimp.2016.08.038

Chanput, W., Mes, J., Vreeburg, R.A.M., Savelkoul, H.F.J., Wichers, H.J. 2010. Transcription profiles of LPS-stimulated THP-1 monocytes and macrophages: A tool to study inflammation modulating effects of food-derived compounds. Food Funct. 1: 254–261. doi.org/10.1039/C0FO00113A

Frank, T., Reichardt, B., Shu, Q.Y., Engel, K.H. 2012. Metabolite profiling of colored rice (*Oryza sativa* L.) grains. J. Cereal Sci. 55: 112–119. doi.org/10.1016/j.jcs.2011.09.009

Frei, M., Becker, K. 2005. Fatty acids and all-trans-β-carotene are correlated in differently colored rice landraces. J. Sci. Food. Agric. 85: 2380–2384. doi.org/10.1002/jsfa.2263

Friedman, M. 2013. Rice brans, rice bran oils, and rice hulls: Composition, food and industrial uses, and bioactivities in humans, animals, and cells. J. Agric. Food Chem. 61: 10626–10641. doi.org/10.1021/jf403635v

Goufo, P., Trindade, H. 2014. Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, gamma-oryzanol, and phytic acid. Food Sci. Nutr. 2: 75–104. doi.org/10.1002/fsn3.86

Hunthayung, K., Klinkesorn, U., Hongsprabhas, P., Chanput, W. 2019. Controlled release and macrophage polarizing activity of cold-pressed rice bran oil in a niosome system. Food Funct. 10: 3272–3281. doi.org/10.1039/C8FO01884G

Jiang, Y.Z., Wang., T. 2005. Phytosterols in cereal by-products. J. Am. Oil Chem. Soc. 82: 439–444. doi.org/10.1007/s11746-005-1090-5

Jiao, J. 2008. Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery. Adv. Drug Deliv. Rev. 60: 1663–1673. doi.org/10.1016/j.addr.2008.09.002

Juliano, B.O. 1993. Rice in Human Nutrition. Int. Rice Res. Inst. Rome, Italy.

Kamal-Eldin, A., Appelqvist, L.A., Yousif, G., Iskander, G.M. 1992. Seed lipids of sesamum indicum and related wild species in Sudan. The sterols. J. Sci. Food Agric. 59: 327–334. doi.org/10.1002/jsfa.2740590309

Kopka, J., Schauer, N., Krueger, S., et al. 2004. Gmd@Csb.Db: The Golm metabolome database. Bioinformatics 21: 1635–1638. doi.org/10.1093/bioinformatics/bti236

Limwiwattana, D., Tongkhao, K., Jom, K.N. 2016. Effect of sprouting temperature and air relative humidity on metabolic profiles of sprouting black gram (*Vigna Mungo* L.). J. Food Process. Preserv. 40: 306–315. doi.org/10.1111/jfpp.12608

Liu, H., Guo, X., Zhao, Q., Qin, Y. Zhang, J. 2020. Lipidomics analysis for identifying the geographical origin and lactation stage of goat milk. Food Chem. 309: 125765. doi.org/10.1016/j.foodchem.2019.125765

Luo, L., Zhou, J., Zhao, H., Fan, M., Gao, W. 2019. The anti-inflammatory effects of formononetin and ononin on lipopolysaccharide-induced zebrafish models based on lipidomics and targeted transcriptomics. Metabolomics 15: 153. doi.org/10.1007/s11306-019-1614-2

Madhavi, D.L., Bomser, J., Smith, M.A.L. Singletary, K. 1998. Isolation of bioactive constituents from *Vaccinium myrtillus* (bilberry) fruits and cell cultures. Plant Sci. 131: 95–103. doi.org/10.1016/S0168-9452(97)00241-0

Maisuthisakul, P., Pasuk, S., Ritthiruangdej, P. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. J. Food Compos. Anal. 21: 229–240. doi.org/10.1016/j.jfca.2007.11.005

Mingyai, S., Kettawan, A., Srikaeo, K., Singanusong, R. 2017. Physicochemical and antioxidant properties of rice bran oils produced from colored rice using different extraction methods. J. Oleo Sci. 66: 565–572. doi.org/10.5650/jos.ess17014

Na Jom, K., Lorjaroenphon, Y., Udompijitkul, P. 2016. Differentiation of four varieties of germinating Thai colored indica rice (*Oryza sativa* L.) by metabolite profiling. Food Sci. Technol. Res. 22: 65–73. doi.org/10.3136/fstr.22.65

Nagasaka, R., Chotimarkorn, C., Shafiqul, I.M., Hori, M., Ozaki, H., Ushio, H. 2007. Anti-inflammatory effects of hydroxycinnamic acid derivatives. Biochem. Biophys. Res. Commun. 358: 615–619. doi.org/10.1016/j.bbrc.2007.04.178

Park, H.Y., Lee, K.W., Choi, H.D. 2017. Rice bran constituents: Immunomodulatory and therapeutic activities. Food Funct. 8: 935–943. doi.org/10.1039/C6FO01763K

Rao, Y.P.C., Sugasini, D., Lokesh, B.R. 2016. Dietary gamma oryzanol plays a significant role in the anti-inflammatory activity of rice bran oil by decreasing pro-inflammatory mediators secreted by peritoneal macrophages of rats. Biochem. Biophys. Res. Commun. 479: 747–752. doi.org/10.1016/j.bbrc.2016.09.140

Rocha, V.Z., Ras, R.T., Gagliardi, A.C., Mangili, L.C., Trautwein, E.A. Santos, R.D. 2016. Effects of phytosterols on markers of inflammation: A systematic review and meta-analysis. Atherosclerosis 248: 76–83. doi.org/10.1016/j.atherosclerosis.2016.01.035

Saenjum, C., Chaiyasut, C. Chansakaow, S., Suttajit, M., Sirithunyalug, B. 2012. Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. J. Medicinal Plants Res. 6: 1070–1077. doi.org/10.5897/JMPR11.1247

Sawadikiat, P., Hongsprabhas, P. 2014. Phytosterols and γ-oryzanol in rice bran oils and distillates from physical refining process. Int. J. Food Sci. Technol. 49: 2030–2036. doi.org/10.1111/ijfs.12506

Seo, W.D., Kim, J.Y., Song, Y.C. 2013. Comparative analysis of physicochemicals and antioxidative properties in new red rice (*Oryza sativa* L. Cv. Gunganghongmi). J. Crop Sci. Biotech. 16: 63–68. doi.org/10.1007/s12892-012-0057-3

Srisawat, U., Panunto, W., Kaendee, N., Tanuchit, S., Itharat, A., Lerdvuthisopon, N., Hansakul, P. 2010. Determination of phenolic compounds, flavonoids, and antioxidant activities in water extracts of Thai red and white rice cultivars. J. Med. Assoc. Thai. 93: 83–91. doi.org/10.1055/s-0030-1264431

Tamjidi, F., Shahedi, M., Varshosaz, J., Nasirpour, A. 2014. Design and characterization of astaxanthin-loaded nanostructured lipid carriers. Innov. Food Sci. Emerg. Technol. 26: 366–374. doi.org/10.1016/j.ifset.2014.06.012

Vilahur, G., Ben-Aicha, S., Diaz-Riera, E., Badimon, L., Padro, T. 2019. Phytosterols and inflammation. Curr. Med. Chem. 26: 6724–6734. doi.org/10.2174/0929867325666180622151438

Wang, Y., Chun, O.K., Song, W.O. 2013. Plasma and dietary antioxidant status as cardiovascular disease risk factors: A review of human studies. Nutrients 5: 2969–3004. doi.org/10.3390/nu5082969

Workman, D. 2019. Rice exports by country: World's top exports. http://www.worldstopexports.com/rice-exports-country/, 4 May 2021.

Xu, Z., Godber, J.S. 1999. Purification and identification of components of gamma-oryzanol in rice bran oil. J. Agric. Food Chem. 47: 2724–2728. doi.org/10.1021/jf981175j