

Assessment of antioxidant capacity and liquid chromatography-mass spectrometry chemical profiling in MRQ74 and MRQ76 rice varieties

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ABSTRACT

Rice is a common, nutritious grain that is widely consumed globally as a staple food. The purpose of this study was to comparatively analyze a new variety of fragrant rice in Malaysia, MRQ74 and MRQ76: fresh polished; unpolished, and 3-month-aged polished; unpolished using total phenolic content (TPC), total flavonoid content (TFC), antioxidant assay, and liquid chromatography-mass spectrometry (LC-MS) chemical profiling. In this study, the Paddy and Rice Research Centre, Malaysian Agricultural Research and Development Institute, Seberang Perai, Pulau Pinang, provided the new cultivars of fragrant rice varieties, MRQ74 and MRQ76. The TPC and TFC assays were performed using a Folin Ciocalteu's reagent and an aluminum chloride colorimetric technique, respectively. The antioxidant activity was measured using the Ferric Reducing Antioxidant Power colorimetric test kit from Abcam, China. Chemical profiling was carried out by the Integrative Pharmacogenomics Institute, Universiti Teknologi Mara, Selangor by utilizing a LC-MS system equipped with an Agilent 1200 liquid chromatography system. Each rice variety sample had extractable TPC, TFC, and antioxidant content, with 76 FUP having the greatest phenolic value (261.35 ± 0.01 mg GAE/100 g), and 76 FP having the highest antioxidant (0.37 ± 0.01 mM ferrous equivalent) and flavonoid value (1715.00 ± 0.01 mg CE/100 g). Based on the chromatography analysis data, 48 compounds with Db scores ranging from 90% to 100% were identified as minor organic acids, amino acids, vitamins, flavonoid derivatives, and phenolic acids. As a result, the data were able to highlight the health-benefits worth of the MRQ74 and MRQ76 rice types, allowing for improved future exposure to consumers.

INTRODUCTION

A decade ago, the Malaysian Agricultural Research and Development Institute as a statutory body in Malaysia had declared two new varieties of fragrant rice namely, MRQ74 and MRQ76. The rice variety of MRQ76 was declared in 2012 for its quality of being fragrant, soft, and slightly sticky [1]. While rice

variety of MRQ74 had been declared in 2005 for its properties like Basmati rice [2]. However, the production of these fragrant rice varieties facing a decline because consumers have become accustomed to using imported fragrant rice as staple food.

The previous study proved that phytochemicals which are naturally occurring compounds found in crops could offer health benefits for humans recognized to its micronutrients [3]. The most highlighted phytochemicals found in grains are phenolic acids, flavonoids, anthocyanins, anthocyanidins, tocopherols, tocotrienols, and phytic acid [4]. Phenolics, as the richest phytochemicals in grains are natural antioxidants, which act as radical scavengers to reduce the incidence of oxidative

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stress-induced damage [5]. Then, flavonoids could also prevent the production of radicals by scavenging free radicals and/or chelating metal ions [6].

To be known, rice-derived polyphenols and their antioxidant properties in global rice cultivars have been broadly researched. However, there is not yet research on the chemical profiling and antioxidant potential of new fragrant rice varieties of MRQ74 and MRQ76. Acting a key role in the biological effects of rice, phenolics, and flavonoids had been recognized as a focus target for modulation of antioxidant activity from MRQ74 and MRQ76 varieties. Therefore, the study was conducted to investigate the phytochemicals profiling towards their phenolic content, flavonoid content, and antioxidant capacity. The present findings aim to expose the health benefits of these new varieties for consumer acceptance and gradually increase crop production in the industry.

MATERIALS AND METHODS

Chemicals and reagents

The list of chemicals and reagents in this study are listed in the following Table 1.

Sample collection of MRQ74 and MRQ76 varieties

New fragrant rice varieties of MRQ74 and MRQ76 were obtained from the Paddy and Rice Research Center, Malaysian Agricultural Research and Development Institute (MARDI), Seberang Perai, Pulau Pinang. The authentication rice varieties letter had been provided from MARDI with reference number MDI/PR/PA/001/ for fresh polished and unpolished samples and 3-months aged polished and unpolished samples (Appendix

1). The rice grain samples were cautiously separated from any physical residue and put aside in a closed container.

Extraction procedure of MRQ74 and MRQ76 varieties

The method introduced by Rohin *et al.* [7] was used in the extraction process with slight adjustments (Appendix 2). In this study, each of the rice grain varieties were grinded using an electric blender (Waring Commercial, Thermo Fisher Scientific, Massachusetts). Then, 50 g of each sample going through with soaking process (1:10; w/v) using an aqueous solution (deionized water) at 25°C for 24 hours. Afterward, each of the solvent extracts was filtered by using vacuum filtration (Favorit Filtration, Thermo Fisher Scientific, Massachusetts) with nylon filter paper (0.45 µm) and concentrated in a rotary evaporator (RE-1020, Biobase Group, Shandong, China) at 50°C. The concentrated extracts were then placed in the oven at 40°C to allow for complete solvent evaporation. Finally, all the crude extracts were kept in -20°C freezer until they were used for the next analysis.

Determination of total phenolic content (TPC)

TPC assay was determined using a Folin Ciocalteu's reagent [8]. A stock solution of 1 mg/ml was prepared by dissolving 1 mg of sample with 1 ml of 100% methanol High performance liquid chromatography grade (HPLC grade). Six data points with concentrations of 0, 20, 40, 60, 80, and 100 µg/ml were used to analyze the calibration curve of gallic acid. Each of the 100 µl extract samples and gallic acid standard solutions were added to 0.4 ml distilled water and 0.5 ml Folin-Ciocalteu reagent and was left for 5 minutes. After that, 1 ml of 7.5% sodium carbonate (w/v) were added and was left in a dark place for another 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Massachusetts). This process was conducted in triplicates for each of the samples. The results were expressed as milligrams of gallic acid equivalents (GAEs) per 100 g of sample (mg GAE/100 g of sample).

Determination of total flavonoid content (TFC)

TFC assay was determined by the aluminum chloride colorimetric method [9] and modifications were done based on Pithonah *et al.* [10]. A stock solution of 1 mg/ml was prepared by dissolving 1 mg of sample with 1 ml of 100% methanol (HPLC grade). Six data points with concentrations of 0, 10, 20, 30, 40, and 50 µg/ml were used to analyze the calibration curve of catechin. Each of the 100 µl extract samples and catechin standard solutions was added to 500 µl of distilled water and 100 µl of 5% sodium nitrate and was left for 6 minutes. Next, 150 µl of 10 % aluminum chloride solution and 200 µl of 1M sodium hydroxide were added and were left for another 5 minutes. The absorbance was measured and recorded at 510 nm on a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Massachusetts). This process was conducted in triplicates for each of the samples. The results were expressed as catechin equivalents (mg CE/100 g).

Antioxidant activity assay

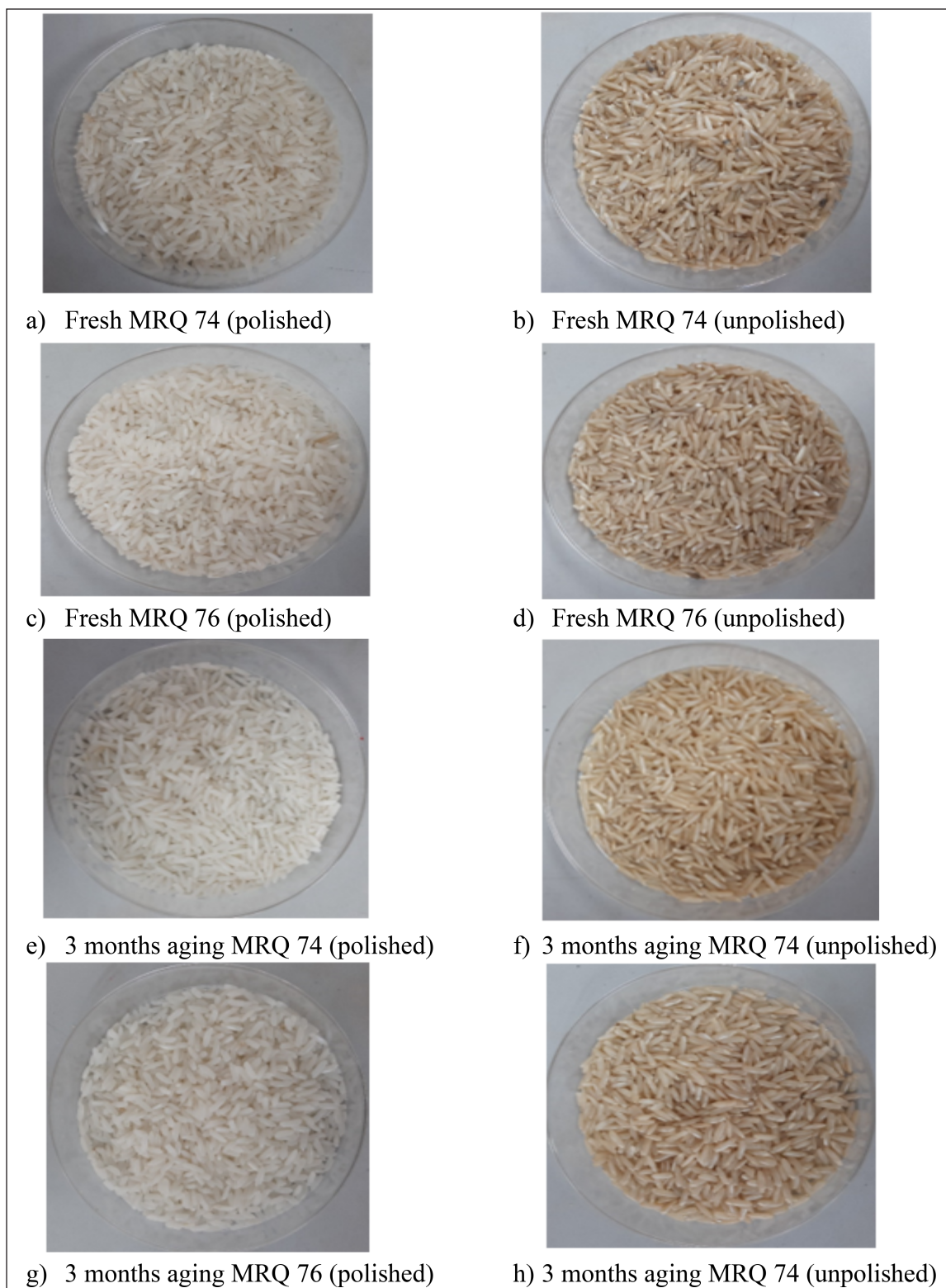
The antioxidant activity of extract samples was determined using a ferric reducing antioxidant power (FRAP) colorimetric assay kit from Abcam (ab234626), China. Standard solutions

Table 1. Chemicals and reagents used in this study.

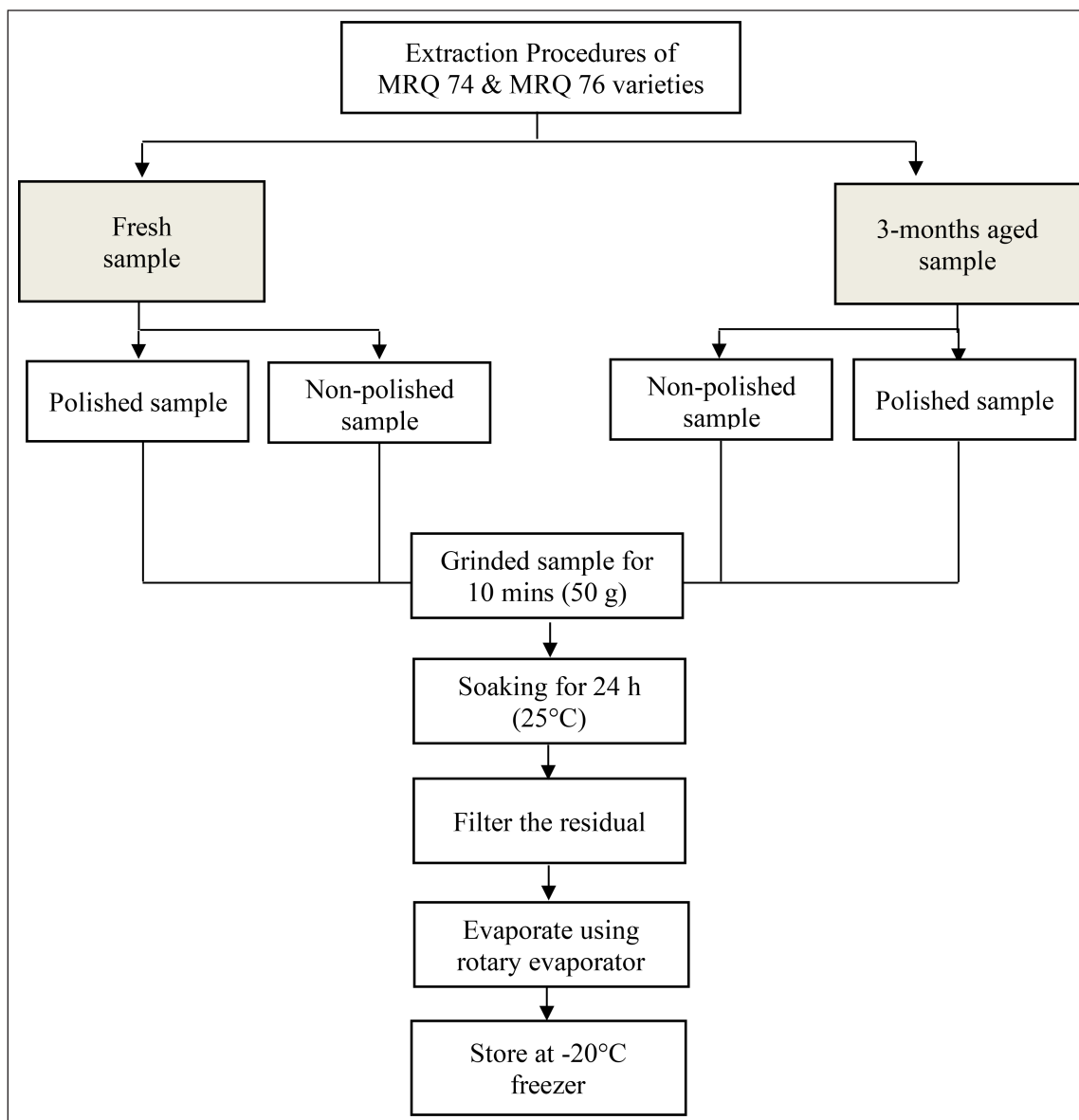
Chemicals/reagents (Catalogue no./product code)	Manufacturer/Supplier
TPC	
Folin Ciocalteu phenol reagent (HC28583501)	Merck, Germany
Gallic acid, certified reference material (91215-100MG)	Supelco, Pennsylvania, USA
Sodium carbonate anhydrous PRS (C0748)	Hamburg, Germany
Total flavonoid content	
Sodium nitrate anhydrous (6552-20)	R&M AG, Switzerland
(+)-Catechin, pharmaceutical secondary standard, certified reference material (PHR1963-50MG)	Supelco, Pennsylvania, USA
Aluminium chloride anhydrous powder sublimed for synthesis (801081.0500)	Merck, Germany
Sodium hydroxide pellets (106498.1000)	Merck, Germany
FRAP assay kit (ab234626)	Abcam, China
General reagent	
Methanol, absolute 100% (HPLC grade) (102952.1000)	Merck, Germany
Distilled water	

of 2 mM were prepared with the range concentration of 0–20 μ l (nmoles). A stock solution of 1 mg/ml was prepared by dissolving 1 mg of sample with 1 ml of 100% methanol (HPLC grade). Then,

10 μ l of extract samples were added into a 96-well plate and mixed thoroughly with 190 μ l of Reaction Mix solution. The samples then were incubated for 60 minutes at 37°C. The absorbance was



Appendix 1. The fragrant rice variety of MRQ 74 and MRQ 76; (a) 74 FP (MRQ74 fresh polished), (b) 74 FUP (MRQ74 fresh unpolished), (c) 76 FP (MRQ76 fresh polished), (d) 76 FUP (MRQ76 fresh unpolished), (e) 74 3P (MRQ74 aged polished), (f) 74 3UP (MRQ74 aged unpolished), (g) 76 3P (MRQ76 aged polished), (h) 76 3UP (MRQ76 aged unpolished).



Appendix 2. Flowchart of the extraction procedure of MRQ 74 and MRQ 76 new fragrant rice varieties.

measured and recorded at 594 nm on a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Massachusetts).

Determination of bioactive compounds in MRQ74 and MRQ76 extracts using liquid chromatography mass spectrometry (LCMS)

Preparation of MRQ74 and MRQ76 extracts

A stock solution of 1 mg/ml was prepared by dissolving 1 mg of sample with 1 ml of 100% methanol HPLC grade. Then, each of the stock solutions was filtered through a 0.45 µm PTFE hydrophobic syringe filter into an LCMS vial prior used for profiling.

Chromatography and mass spectrometry of metabolite profiling

The method introduced by the Integrative Pharmacogenomics Institute, Universiti Teknologi Mara,

Selangor Darul Ehsan was used in this study. The screening of bioactive compounds was performed using an Liquid chromatography/mass spectrometry- quadrupole time-of-flight (LC/MS-QTOF) system comprised of an Agilent 1200 liquid chromatography system (Agilent-1200, Agilent Technologies Inc, Santa-Clara), equipped with a binary pump, a vacuum degasser unit, an auto sampler and 6,520 quadrupole time of flight mass spectrometers with an electrospray ionization source. The column used was Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (2.1 × 100 mm), 1.8 µm. The chromatographic separation was performed at 40°C with (A) 0.1% formic acid in distilled water and (B) 0.1% formic acid in acetonitrile for positive mode. The gradient elution program was 0.00–18.00 minutes, 5%–95% (A); 18–23 minutes; 95% (B); 23.01 minutes; 5% (C). The total run time is 30 minutes. The LC condition was re-equilibrated for 2 minutes before starting the new injection. The sample injection volume was set at 2 µl

and the flow rate of the mobile phase was set at 0.25 ml/minute. The mass spectrometer was operated in positive electrospray ionization mode with optimum gas temperature at 325°C, gas now at 11 l/minute, and nebulizer at 35 psi, respectively.

Data analysis

The data reported was explored using descriptive and inferential statistical analysis. The analysis of variance compared the treatment and control groups adjusting for the baseline value as a covariate was performed. The IBM SPSS, version 20.0 software (IBM Corp. Armonk, NY) is used for statistical analysis. All tests are two-sided and $p < 0.05$ is considered statistically significant. While, LCMS analysis (MS data (.d)) used Agilent Mass Hunter Qualitative Analysis B.05.00 software (Agilent Technologies Inc, Santa Clara). The chromatographic profiles were analyzed based on the accurate mass data identified and the predicted compounds were annotated using metabolite and chemical entity database database.

RESULTS AND DISCUSSION

TPC and TFC

Table 2 shows the TPC and TFC results for the extraction of MRQ74 and MRQ76 with different samples. The calibration curve of gallic acid was plotted with $y = 0.0089x + 0.0244$ and $R^2 = 0.9889$, while the calibration curve of catechin was plotted with $y = 0.0014x + 0.0024$ and $R^2 = 0.9968$. Based on the findings, the 76 FUP sample gives the highest TPC with a value of 261.35 ± 0.01 mg GAE/100 g, followed by the 74 FUP sample with 162.47 ± 0.01 mg GAE/100 g, and 74 3UP sample with 142.25 ± 0.01 mg GAE/100 g. While the 74 FP sample had the lowest TPC with the value of 88.31 ± 0.03 mg GAE/100 g, among all extraction samples. Overall, the TPC values differed significantly ($p < 0.05$) among different samples of MRQ74 and MRQ76.

For TFC, it was observed that the 76 FP sample gives the highest TFC with the value of 1715.00 ± 0.01 mg CE/100 g, followed by 74 3UP sample with 1640.00 ± 0.01 mg CE/100 g, and 76 3UP sample with 1609.05 ± 0.01 mg CE/100 g. Yet, 74 FUP samples had the lowest TFC with the value of 1411.43 ± 0.05 mg CE/100 g, among all extraction samples. There is no significant difference of TFC values among different samples of MRQ74 and MRQ76 ($p > 0.05$).

According to the findings, polished rice grains had the lowest TPC due to the total removal of the rice bran which consequently leads to a higher carbohydrate content (Appendix 3), demonstrating this effect visibly. Tian *et al.* [11] found that brown rice had the highest concentration of total extractable phenolics, followed by white rice, which is consistent with the findings of this investigation. According to Goufo and Trindade [12], rice phenolics are composed of 12%–28% hydroxybenzoic acids and 61%–89% hydroxycinnamic acids. However, when compared to processed white rice, the quantity of phenolic esters such as hydroxycinnamate sucrose esters in germinated brown rice was reduced by 70% [11], resulting in a drop in total extractable phenolics content.

Previously, Melissa *et al.* [13] investigated the concentration of TPC and antioxidant activity of rice grains with light brown, red, and black pericarp colors. Results revealed significant differences in the concentrations of TPC and antioxidants among varieties with higher values obtained for grains with red and black colors, while light color with lowest values. Summpunn *et al.* [14] had assessed five different Thai non-glutinous varieties along with each sample; white rice, brown rice, germinated brown rice, and rice grass for antioxidant components. The results observed that TPC was found in all rice varieties, in the range of 16.4–31.4 mg GAE/100 g (dw), with the highest value in rice grass and germinated brown rice, indicating that milling to generate white rice had an adverse effect on those components.

In recent decades, researchers have focused on the phenolic and flavonoid content of plant-based materials due to their importance for human health and nutrition [15]. Phenolic chemicals, which include a wide spectrum of structurally varied molecules such as flavonoids, have been shown to have strong antioxidant and anti-inflammatory properties, making them promising research targets [16].

Antioxidant capacity (FRAP)

The result observed in Table 2 shows FRAP antioxidant assay, also known as ferric ion reducing antioxidant power that measures the antioxidant capacity of foods, beverages, and nutritional supplements containing polyphenols. In this study,

Table 2. TPC, total flavonoid content (TFC) and antioxidant FRAP assay of MRQ74 and MRQ76 variety extracts.

Samples	MRQ74				MRQ76				F-statistics (df)	p-value
	FUP	FP	3UP	3P	FUP	FP	3UP	3P		
TPC (mg GAE/100 g)	162.47 ± 0.01	88.31 ± 0.03	142.25 ± 0.01	128.24 ± 0.01	261.35 ± 0.01	136.63 ± 0.01	122.40 ± 0.01	104.79 ± 0.01	24.44 (7)	0.01*
TFC (mg CE/100 g)	1411.43 ± 0.05	1418.57 ± 0.06	1640.00 ± 0.01	1570.95 ± 0.01	1229.29 ± 0.08	1715.00 ± 0.01	1609.05 ± 0.01	1544.76 ± 0.01	0.65 (7)	0.71
FRAP activity (mM Ferrous equivalent)	0.24 ± 0.01	0.23 ± 0.01	0.26 ± 0.01	0.36 ± 0.01	0.23 ± 0.01	0.37 ± 0.01	0.26 ± 0.01	0.36 ± 0.01	0.75 (7)	0.64

Data represent the mean ± SD of three independent experiments.

*One-Way ANOVA test showed a significant difference, $p < 0.001$.

Appendix 3. The composition of MRQ74 and MRQ76 rice varieties (100 g) with different samples.

Samples Nutrient parameters (g/100 g)	MRQ74				MRQ76				F-statistics (df)	p-value
	3UP	3P	FUP	FP	3UP	3P	FUP	FP		
Moisture	13.93	13.69	11.73	11.80	12.63	12.38	11.16	11.40	197.265 (7)	<0.001*
Ash	1.23	0.53	1.24	0.44	1.36	0.49	1.28	0.49	382.043 (7)	<0.001*
Protein	8.90	8.19	7.97	7.16	8.27	7.48	8.67	7.84	381.036 (7)	<0.001*
Carbohydrate	72.00	76.14	75.61	79.55	73.08	77.33	75.34	79.06	861.554 (7)	<0.001*
Fat	3.95	1.46	3.45	1.05	4.66	2.31	3.55	1.20	291.526 (7)	<0.001*
Energy (kcal/100 g)	359.14	350.42	365.38	356.30	367.34	360.07	367.97	358.41	467.379 (7)	<0.001*

*One-Way ANOVA test showed a significant difference, $p < 0.001$.

76 FP samples give the highest antioxidant capacity with the value of 0.37 ± 0.01 mM Ferrous equivalent, followed by 74 3P and 76 3P samples which give similar values of 0.36 ± 0.01 mM Ferrous equivalent, among all extraction samples. Consequently, 74 FP and 76 FUP samples give the lowest antioxidant capacity with values of 0.23 ± 0.01 mM Ferrous equivalent. It was observed that there is no significant difference of FRAP antioxidant assay values among different samples of MRQ74 and MRQ76.

According to Abuajah *et al.* [17], antioxidants are classes of substances that counteract reactive oxygen species and free radicals in cells. In the scientific community, antioxidant activity in food and drink has emerged as one of the most intriguing characteristics. These antioxidants shield the body against harm brought on by free radicals, which have been linked to the development of numerous chronic illnesses, such as cardiovascular disorders, aging, heart disease, anemia, cancer, and inflammation [18].

Antioxidants can be divided into two categories: synthetic and natural. Furthermore, antioxidants can be classified as endogenous or exogenous based on their source, enzymatic or non-enzymatic based on their activities, or water- or lipid-soluble depending on their solubility [19,20]. Natural antioxidants are compounds found in foods that prevent processes such as disruption, sourness, and color change. Natural antioxidants are mostly derived from plants, such as rice, and their activity varies depending on plant species, diversity, extraction and/or processing techniques, and growth conditions [13, 21].

Phytochemical characterization by LCMS-QTOF

Liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) is an analytical technique that advantageously combines quadrupole technologies with a time-of-flight mass analyzer [22]. Widely, mass spectrometry (MS) applications have become a widely accepted technique across numerous fields of biological and pharmaceutical research including metabolite identification, peptide analysis, drug discovery, and food applications industry [23–25]. Based on Figure 1, the total ion chromatograms (TICs) of each MRQ74 and MRQ76 extract in the positive mode are being shown.

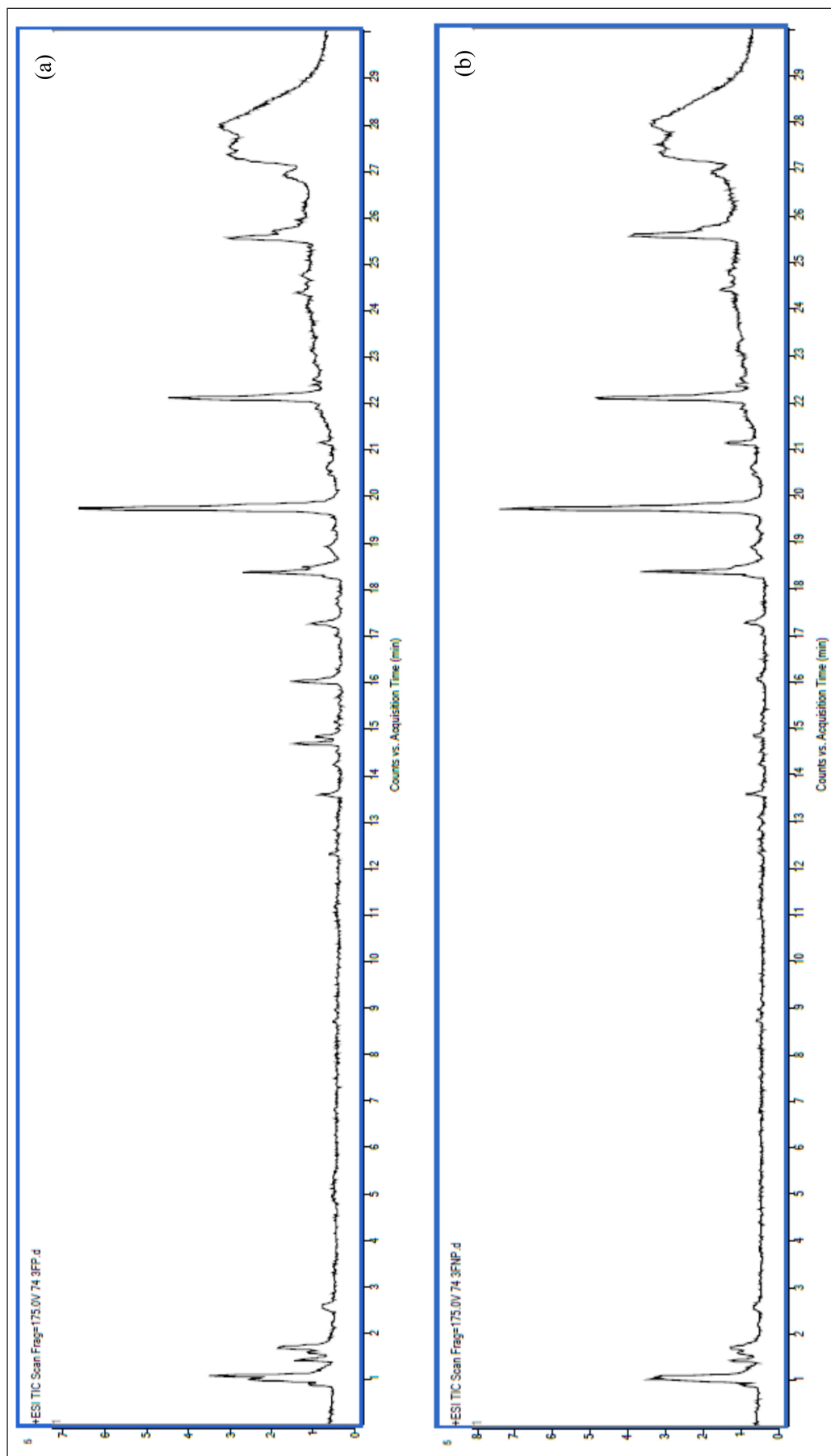
It can be observed that the patterns of chromatographic profiles among the MRQ74 and MRQ76 sample varieties were slightly different. There are more than

10 peaks in each sample that were detected and a total of 48 compounds were identified based on the LC/MS-QTOF data, following the confirmation by comparison to reported data, library search, and authenticity compounds (score Db from 90% to 100%) (Tables 3 and 4). Therefore, the identified chemical components in the samples were suggested as small organic acids, amino acids, vitamins, flavonoid derivatives, and phenolic acids.

In Table 4, the results showed that 76 FP samples identified more compounds by LC/MS-QTOF, followed by 74 3P, 74 3UP, and 74 FP samples. While 76 FUP samples identified the lowest compounds by LC/MS-QTOF. In terms of rice varieties, MRQ74 extracts displayed more compounds than that of MRQ76 extracts. As the highest detected fragment ion [M-H]⁻ at m/z at 496.3412 in 74 FUP extract, this compound had been identified as 23-Acetoxyoladulcidine which belongs to the class of spirostanes and its derivatives compound (Table 3). 23-Acetoxyoladulcidine is a steroidal alkaloid isolated from natural herbs that exhibit anti-proliferation, anti-bacterial, anti-viral, insecticidal, and anti-metastatic effects on various types of cancers [26].

Meanwhile, it can be observed that fragment ion [M-H]⁻ at m/z at 393.2974 and 416.3729 were identified as docosanedioic acid and tetracosanedioic acid compounds, types of carboxylic acids. In this study, these two compounds were present and/or detected in almost each sample of MRQ74 and MRQ76 extract varieties. Docosanedioic acid is an alpha, omega-dicarboxylic acid that possesses some antioxidant activity such as anti-fungal and anti-bacterial activities [27]. Tetracosanedioic acid is a very long-chain fatty acid also known as adipic acid has been shown to have antioxidant activity with anti-inflammatory, anti-fungal, anti-viral, and anti-cancer properties [28].

Oleamide, a fatty acid having the chemical structure C18 H35 NO, is a derivative of oleic acid that was present/or found in 74 3UP, 74 FUP, and 76 FUP samples. Oleamide (cis-9-octadecenamide) is commonly used for a variety of pharmacological effects, including pain relief, anti-atherogenic properties, vasodilation, sedation, lipid metabolism modification, and gap junction inhibition [29,30]. Oleamide is also an endogenous bioactive signaling molecule that operates on a variety of cell types, resulting in sleep induction effects that could attributed to changes in cannabinergic CB-1 receptors in synapse [31]. Aside from



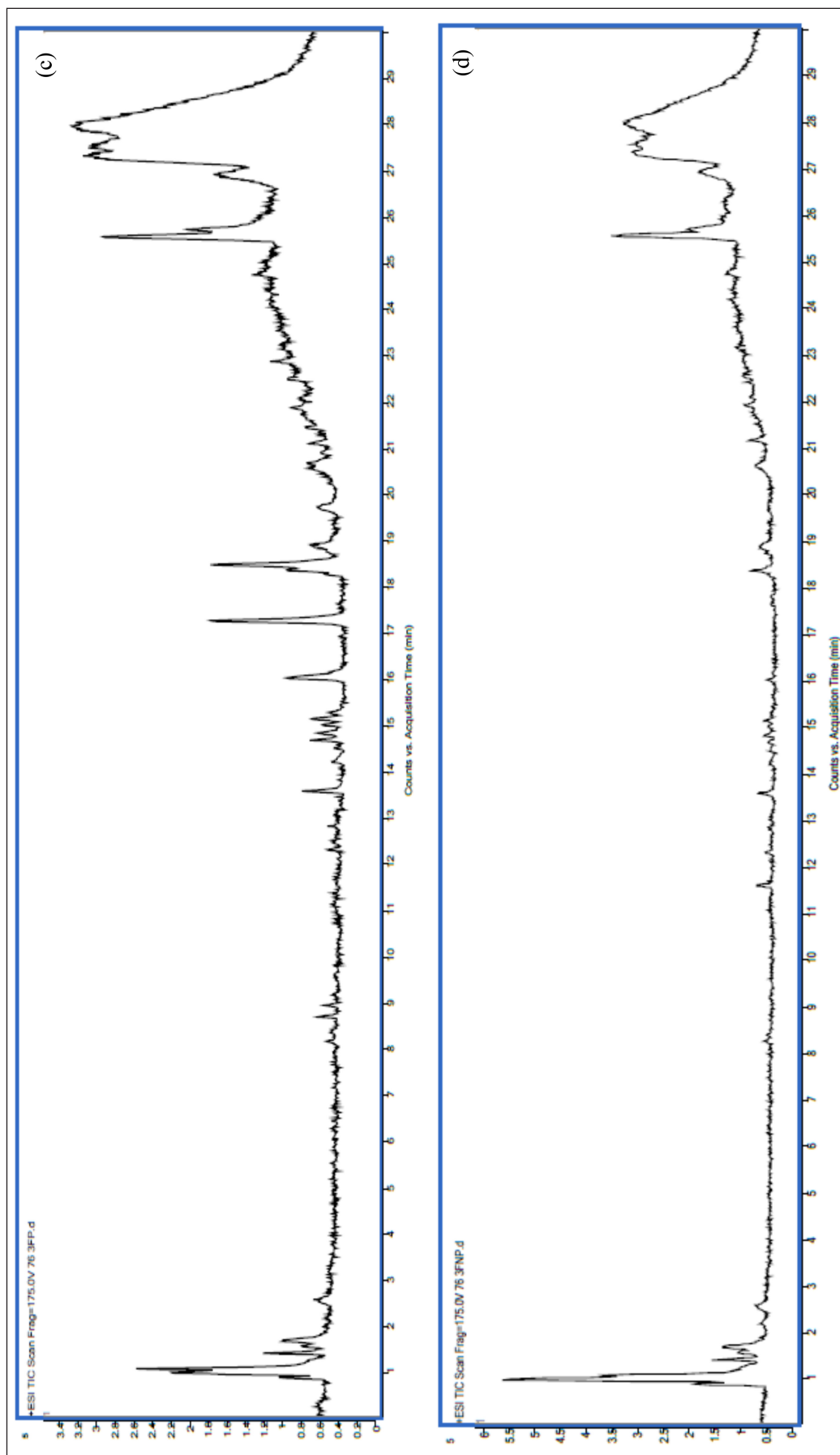
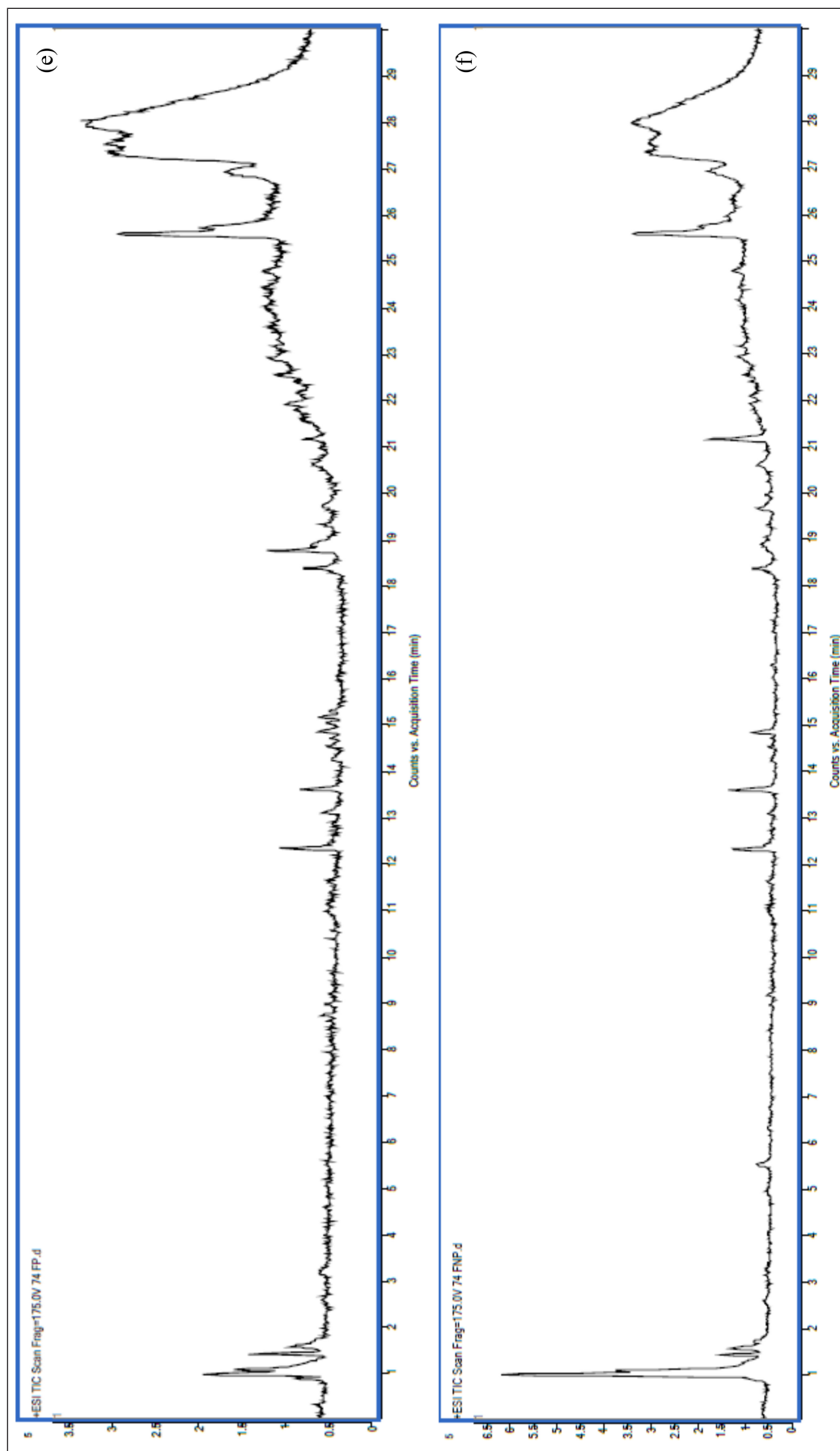


Figure 1. Continued.



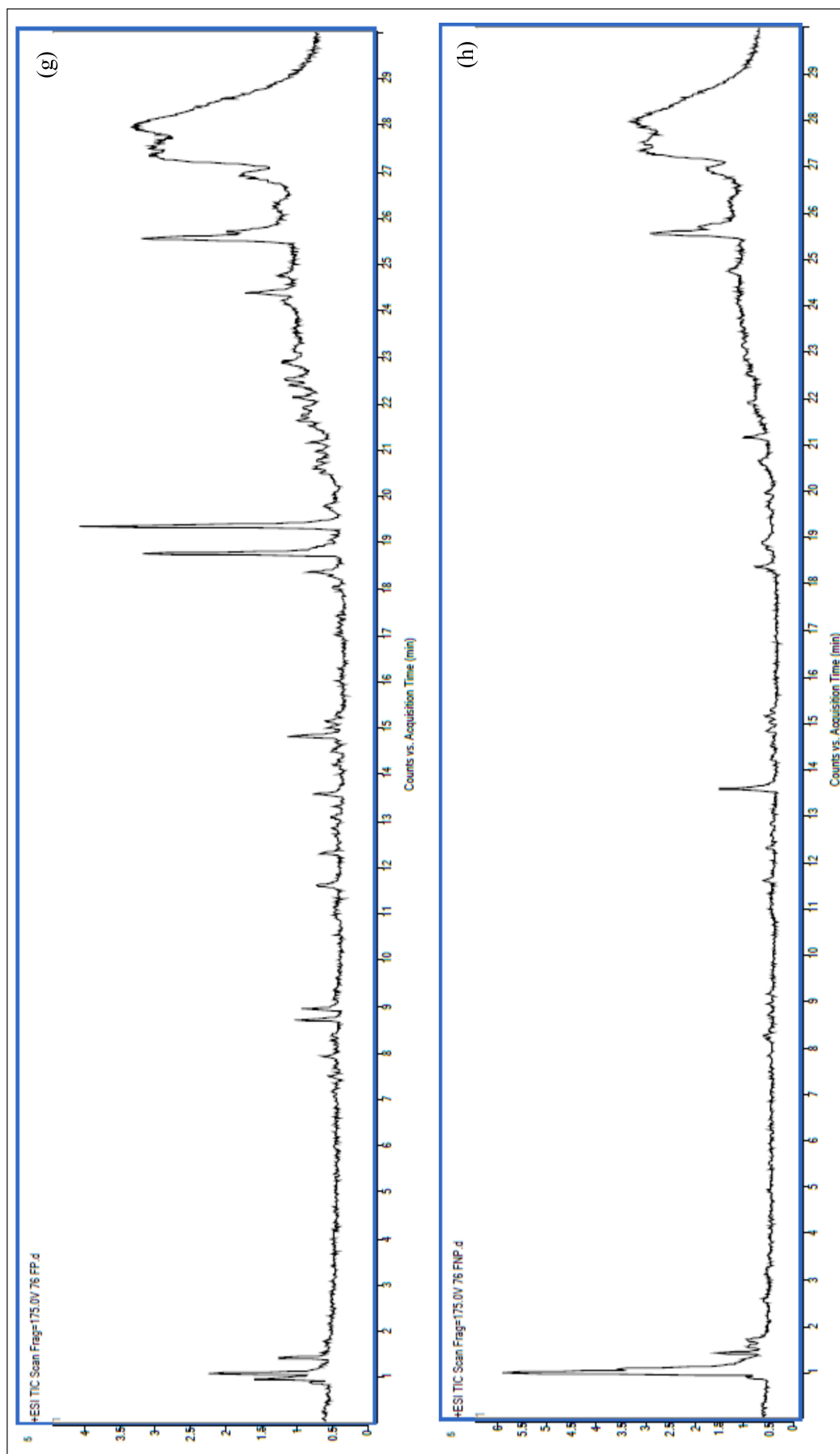


Figure 1. LC/MS-QTOF chromatograms in positive electrospray ionization from the analysis of the MRQ74 and MRQ76 rice extracts; (a) 74 3P, (b) 74 3UP, (c) 76 3P, (d) 76 3UP, (e) 74 FP, (f) 74 FUP, (g) 76 FP, (h) 76 FUP.

Table 3. Identification of main compounds in MRQ74 rice extracts with retention times.

Compounds	Molecular formula	Retention times (minutes)	[M-H] ⁻ (m/z)	Main Fragment Ions (m/z)
74 3P				
Aminocaproic acid	C6 H13 N O2	1.7080	132.1020	131.0947
4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	C22 H26 O6	13.594	387.1802	386.1734
3Z,6Z,9Z,12Z,15Z-Tricosapentaene	C23 H38	16.029	332.3315	314.2978
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.555	338.3420	337.3348
3α,12α-Dihydroxy-5β-cholesterol-8(14)-en-24-oic Acid	C24 H38 O4	25.586	413.2662	390.2771
CAY10621	C26 H45 N O4	25.587	436.3419	435.3351
Docosanedioic acid	C22 H42 O4	25.725	393.2974	370.3083
74 3UP				
4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	C22 H26 O6	13.597	387.1796	386.1725
Emmotin A	C16 H22 O4	18.365	301.1410	278.1518
3-Butylidene-7-hydroxyphthalide	C12 H12 O3	18.365	205.0857	204.0787
Oleamide	C18 H35 N O	21.129	282.2788	281.2716
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.570	338.3418	337.3345
Docosanedioic acid	C22 H42 O4	25.753	371.3148	370.3077
Tetracosanedioic acid	C24 H46 O4	25.758	416.3729	398.3390
74 FP				
C16 Sphinganine	C16 H35 N O2	12.340	274.2733	273.2660
16Z-octadecenoic acid	C18 H34 O2	18.771	283.2637	282.2565
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.586	338.3417	337.3344
3α,12α-Dihydroxy-5β-cholesterol-8(14)-en-24-oic Acid	C24 H38 O4	25.624	413.2660	390.2769
CAY10621	C26 H45 N O4	25.628	436.3417	435.3348
Docosanedioic acid	C22 H42 O4	25.762	393.2979	370.3086
Tetracosanedioic acid	C24 H46 O4	25.764	416.3737	398.3394
74 FUP				
2-Amino-3-methyl-1-butanol	C5 H13 N O	0.9990	104.1068	103.0995
C16 Sphinganine	C16 H35 N O2	12.326	274.2738	273.2665
23-Acetoxysoladulcidine	C29 H47 N O4	16.297	496.3412	473.3520
Oleamide	C18 H35 N O	21.165	282.2794	281.2721
Docosanedioic acid	C22 H42 O4	25.761	393.2977	370.3085

Table 4. Identification of main compounds in MRQ76 rice extracts with retention times.

Compounds	Molecular formula	Retention times (minutes)	[M-H] ⁻ (m/z)	Main fragment ions (m/z)
76 3P				
3Z,6Z,9Z,12Z,15Z-Tricosapentaene	C23 H38	16.032	332.3303	314.2962
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.571	338.3426	337.3353
3α,12α-Dihydroxy-5β-cholesterol-8(14)-en-24-oic Acid	C24 H38 O4	25.614	413.2663	390.2773
CAY10621	C26 H45 N O4	25.615	436.3422	435.3354
Docosanedioic acid	C22 H42 O4	25.753	393.2971	370.3080
76 3UP				
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.566	338.3416	337.3343
3α,12α-Dihydroxy-5β-cholesterol-8(14)-en-24-oic Acid	C24 H38 O4	25.598	413.2667	390.2777
Docosanedioic acid	C22 H42 O4	25.735	393.2979	370.3088
Tetracosanedioic acid	C24 H46 O4	25.738	416.3733	398.3395
76 FP				

Continued

Compounds	Molecular formula	Retention times (minutes)	[M-H] ⁻ (m/z)	Main fragment ions (m/z)
Prosopinine	C16 H33 N O3	14.829	288.2539	287.2467
Bacterioplanetetrol cyclitol	C41 H73 N O8	17.963	708.5392	707.5327
14-hydroxy stearic acid	C18 H36 O3	18.770	323.2563	300.2672
16Z-octadecenoic acid	C18 H34 O2	18.772	283.2638	282.2565
cis-9,10-Epoxystearic acid	C18 H34 O3	19.361	299.2583	298.2511
6E,9E-octadecadienoic acid	C18 H32 O2	19.361	281.2477	280.2404
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.560	338.3425	337.3353
3 α ,12 α -Dihydroxy-5 β -chol-8(14)-en-24-oic Acid	C24 H38 O4	25.595	413.2663	390.2769
Docosanedioic acid	C22 H42 O4	25.731	393.2982	370.3089
76 FUP				
Oleamide	C18 H35 N O	21.168	282.2790	281.2718
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.549	338.3422	337.3349
3 α ,12 α -Dihydroxy-5 β -chol-8(14)-en-24-oic Acid	C24 H38 O4	25.582	413.2664	390.2774
Docosanedioic acid	C22 H42 O4	25.718	393.2978	370.3088

that, prosopinine is a naturally occurring alkaloid that is only present and/or found in 76 FP samples with the fragment ion [M-H]⁻ at m/z 288.2539.

CONCLUSION

In conclusion, this study examined the TPC, TFC, antioxidant content, and LC-MS chemical profiling of polished and unpolished MRQ74 and MRQ76 samples. Sample 76 FUP has the highest TPC value of 261.35 ± 0.01 mg GAE/100 g, while sample 74 FP has the lowest at 88.31 ± 0.03 mg GAE/100 g. Sample 76 FP had the highest TFC value (1715.00 ± 0.01 mg CE/100 g), whereas sample 74 FUP had the lowest at 1411.43 ± 0.05 mg CE/100 g. All samples showed antioxidant activity, with the 76 FP sample having the highest value (0.37 ± 0.01 mM Ferrous equivalent). The chemical components found in each sample, according to the LC/MS-QTOF data, were minor organic acids, amino acids, vitamins, flavonoid derivatives, and phenolic acids. The chemicals present and/or detected included 23-Acetoxysoladulcidine, docosanedioic acid, tetracosanedioic acid, oleamide, prosopinine, and others. Thus, the findings suggested that MRQ74 and MRQ76 rice varieties should be considered superior choices for good health advantages when consumed.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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