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NATURAL VARIATION IN FATTY ACID COMPOSITION OF DIVERSE VIETNAMESE RICE GERMPLASM

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SUMMARY

Despite the advantages of rice bran oil in terms of nutrition and healthcare, the knowledge of its fatty acid composition was still limited. A panel of 100 rice varieties generated from the different ecosystems of Vietnam had been used in our study to investigate their fatty acid profile. Using the modern Gas Chromatography-Mass Spectrometry method, five main fatty acids in our rice bran were discovered including myristic (14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids, in which the fatty acids profile were different and specific for each variety. Among the five fatty acids, the essential linoleic fatty acid content was the highest whereas the myristic acid had the lowest content. The genotype G31 (NEP CAM) had been found with the highest linoleic fatty acid content (43.5%) which could be considered a good source of food and potential for breeding. In this study, we also determined the correlation between the five fatty acids (FAs) content in each rice variety and in comparison with different genotypes. A strong positive correlation between palmitic acid content and the ratio of saturated and unsaturated fatty acid ($R = 0.98$) was obtained in this study. The ratio of saturated and unsaturated fatty acid in *Japonica* genotypes is lower than *Indica* ($p < 0.001$), indicating that *Japonica* genotypes have more favorable unsaturated FA. Our study's results may have great importance in contributing to future breeding programs to improve fatty acid components in our Vietnamese rice.

Keywords: Bran oil, fatty acid, GC-MS, Oryza sativa, saturated fatty acid, unsaturated fatty acid

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for the world population, especially in Asia. In Vietnam, from 1989 till now, rice export turnover has unendingly increased, transferring large foreign currency earnings, and creating a significant contribution to the development and renovation of the country. Rough rice comprises a white starchy rice kernel tightly covered by coating bran, enclosed in a tough siliceous hull (Lakkakula *et al*., 2004). Traditionally, white rice has been removed from the husk and bran film. As for brown rice, the husk is separated, and the outer brain membrane remains. Therefore, brown rice seeds are usually brown.

Rice bran is a valuable by-product of the milling process while producing white rice. Rice bran is beneficial from its high antioxidant activity due to phenolic and flavonoid compounds (Sapwarobol *et al*., 2021) as well as rich in nutrition, vitamins and minerals, it contained 12-22% oil, 11-17% protein, 6-14% fiber, 10-15% moisture and 8-17% ash (Oezdestan 2014; Devi *et al*., 2021).

In addition, rice bran oil (RBO) is an excellent source of fatty acids, especially unsaturated fatty acids and essential fatty acids. The fatty acid in RBO was classified into different groups, including 22% saturated, 43% monounsaturated and 35% polyunsaturated fatty acids (Pal, Pratap, 2017). Five major fatty acids had been found in rice bran namely palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) (Cho *et al.*, 2006) in which oleic, linoleic and palmitic fatty acids accounted for more than 90% of the total fatty acid content (Kitta *et al*., 2005; Cho *et al.*, 2006). Each rice variety had different fatty acid content as well as the ratio between saturated and unsaturated fatty acids (SFA/UFA). Oil content in bran is influenced by many factors such as genetics, environmental influences, fertilizer treatment and storage conditions. Because of these reasons, the oil content quite strongly fluctuates (Amissah *et al.*, 2003). The increasing demand for edible oil for the world population and the most recent oil crisis in Europe in 2022 pointed out that the world is in urgent to find the alternative oil. Rice bran oil serves as the perfect oil source for the replacement with a lots of potential advantages.

RBO recently became a more popular edible oil worldwide. Its demand had increased due to its benefits in health protection (Lai *et al*., 2019) such as lowering serum cholesterol levels, preventing cardiovascular diseases, etc. However, in Vietnam, rice milling technology was not fully developed which led to the impurities in the final rice bran after milling including rice husk and sandy soil. In addition, rice bran contained highly active fat oxidizing enzymes, so it often goes rancid quickly after milling. The oil is separated rapidly into free fatty acids under lipase, making it unsuitable for refining and eating (Amarasinghe *et al*., 2009). Therefore, rice bran is currently mainly used as animal feed, causing a massive waste of nutritional ingredients for human health. There are few studies on the extraction of RBO (Nguyen Van Toan & Ngo Thi Thanh Van, 2019; Nguyen Van Khanh *et al*., 2019) despite the fact that Vietnam had a significant genotype source of rice.

Consequently, it is necessary to study the bran oil in an enormous population to have in-depth information. Therefore, in this study, we had enriched the knowledge of the diversity of FA composition in a Vietnamese rice collection containing 100 rice varieties. In perspectives, a genome wide association studies could be performed to find the gene candidates responsible for high bran oil in Vietnamese rice.

MATERIALS AND METHODS

Rice varieties and field experiments

A core Vietnamese rice collection, which contained 100 rice varieties, originated from different regions of Vietnam were provided by the Plant Resource (supplemented Table 1). One gram of selected paddy of each rice variety had been dried at 50 - 60^{o}C for 36 h so that the hulls were easily removed together with the low-quality kernels. Rice was grinned by a pestle and mortar until it became powder which was able to sieve through an 840-µm-sieve. One hundred fifty mg of rice bran powder of each varieties were stored in a 2 mL glass vial at -20^{o}C until the rice oil extraction experiment was carried out.

Bran oil extraction and methylation

Oil content was extracted using method of E.G.Bligh and W.J. Dyer (1959), in which 2 mL of chloroform/methanol (1:2) was added to the vial containing 150 mg of rice from each variety prior to being shaken every 15 minutes for 1 h. A total of 0.67 mL of chloroform and MilliQ water were added to the mixture and followed by an immediate shake for 1 minute after each addition. The mixture was then centrifuged at 3000 rpm for 5 minutes to collect the lower part solution, using a two-glass pipette system while the solvent was evaporated using nitrogen gas. After that, the FA was esterified for GC analysis by dissolving in 500 µL of 14 % v/v BF3-Methanol (14 mL Borontrifluoride and 86 mL Methanol) (Sigma-Aldrich) to be methylated (Metcalfe *et al.*, 1966). The solution was incubated for 1 h at

80°C in a water bath with additional shake every 5 minutes. The solution was cooled down at room temperature and 2 mL of n-hexane was added with a gently shake for 30 seconds prior to the addition of 2 mL of saturated NaCl (3.6 g in 10 mL distilled water) to the mixture for washing. The mixture was then centrifuged for 3 minutes at 1300 rpm, and the lower part containing NaCl was removed. This washing step was repeated twice. Then, the mixture was evaporated using nitrogen gas until 1 mL of the mixture was remained. This 1 mL mixture was continue to be filtered by using a 0.22 µm menbrane and kept for chromatography.

Fatty acid profile analysis by Gas Chromatography-Mass Spectrometry (GC–MS)

One µL of extracted FA sample in n-hexane eluent was used to determine the fatty acid profile of each rice varieties using GC/MS on a Thermo Scientific GC/MS Trace 1310 Series with a capillary column (30 m × 0.25 mm × 0.25 µm, Thermo Scientific, USA). The total run time of a thermo cycle was 28 minutes which first initiated with 110ºC for 2 min, then increases up to 200ºC at 10ºC/min before subsequently ramping up to 250ºC at a rate of 4ºC/min, followed by 20ºC/min to 280ºC and held for 3 minutes. The FAs profiles were analyzed using MS in which the percentage amounts of each identified FA methyl esters were expressed as % in comparison to the total FA peak areas.

Analysis of fatty acid content

The percentage of different FA was calculated as follows:

$$Percentage\ of\ each\ FA = \frac{Peak\ area\ of\ FA}{Total\ area\ of\ FAs} \times 100\%$$

The ratio of saturated fatty acid and unsaturated fatty acid was calculated by:

$$\frac{SFA}{UFA} = \frac{(C16{:}0) + (C18{:}0)}{(C18{:}1) + (C18{:}2) + (C18{:}3)}$$

The correlation between the average of fatty acids in 100 Vietnamese rice accessions was analyzed using Graphpad Prism v.8.3.

The average content of five main fatty acids and SFA/UFA ratio in our two rice subgroups including *Indica* and *Japonica* was calculated by calculating the mean of each fatty acid and SFA/UFA ratio in 62 varieties in *Indica* subgroups and 38 varieties in *Japonica* subgroups. The dispersion of average values was presented by the value of standard deviation.

Statistic Analysis

Statistically significant differences between groups at a 95% confidence level were determined by one way ANOVA in the form of a mean and standard deviation in GraphPad Prism version 8.3.

RESULTS

Diversity of fatty acid composition in Vietnamese rice accessions

The FA composition of 100 Vietnamese rice varieties was analyzed. The Gas Chromatography spectrum of one rice accession was presented in Figure 1 supplemented. The main FA composition, average content, the minimum and maximum content for each FA in 100 Vietnamese rice collection were presented in Table 1 The results showed that palmitic, linoleic and oleic together dominated with 97% of total FAs, the saturated FAs including myristic acid and stearic acid only accounted for approximately 3%.

Among the five concerning FAs, linoleic acid content was the highest. The amount of this essential polyunsaturated acid were lowest and highest in G101 and G37 rice accessions, respectively. In contrast, myristic, a saturated FA, had the lowest content among the five FAs. G125 and G202 rice accessions had the minimum and maximum contents of this FA. These results suggested that in our rice collection, the content of beneficial FAs outweighed the other FAs.

Table 1. The average content, minumum and maximum values of five investigated fatty acids.

Fatty acids	Mean value[a]	Minimum (%)	Rice varieties	Maximum (%)	Rice varieties
Myristic Acid	1.10 ± 0.49	0.229659	G125 (So crioong)	2.62	G202 (Nong to)
Palmitic Acid	31.3 ± 3.56	23.68345	G98 (Ngoi tia)	42.08	G172 (Vn-95/20)
Linoleic Acid	34.15 ± 3.05	27.38649	G101 (Dieo kbin)	43.51	G37 (Nep cam)
Oleic Acid	31.82 ± 3.84	24.12926	G125 (Nep nuong)	41.85	G130 (Lua gia bo)
Stearic Acid	1.64 ± 0.47	0.805964	G145 (Ba ria)	2.97	G93 (Po le po lau xa)

[a]: the content of each fatty acid is expressed as a percentage of total fatty acid content.

Frequency distribution of five FA in a Vietnamese rice collection

The distribution of FA content in the Vietnamese rice collection was presented in Figure 1. The concentration of Myristic acid (C14:0) ranged from 0.2 to 2.6% with the major proportion varied between 1.2 to 1.7%, accounted for approximately 37%. Palmitic acid contents ranged from 23.7 to 42.1% in the 100 rice samples evaluated. The Linoleic acid (C18:2) content ranged from 27.4 to 43.5%. Oleic acid (Omega-9 or C18:1) varied greatly, ranging from 24.1 to 41.8%. Stearic acids (C18:0), ranging from 0.8 to 2.97%, account for the lowest content among the five FAs.

The correlation between five fatty acids

The correlation between five FAs was analyzed further in Table 2. The results in Table 2 showed a strong positive correlation between palmitic acid content and the SFA/UFA ratio (R = 0.98) while the correlation between palmitic acid and oleic acid content was relatively high negative (R = - 0.7). The correlation implied that the more palmitic acid in the rice varieties, the higher SFA/UFA and the lower content of oleic could be obtained. Other FAs performed only a small correlation.

The distribution of SFA/UFA ratio in Vietnamese rice collection

Using SFA/FA ratio to determine the value of bran oil in Figure 2, 100 rice varieties from the Vietnamese rice collection showed a wide range of ratio varied from 0.35 to 0.82 with the major proportion less than 0.5. The results suggested that the amount of unsaturated fat in the Vietnamese rice varieties was more than the amount of the saturated ones which could become potentially valuable.

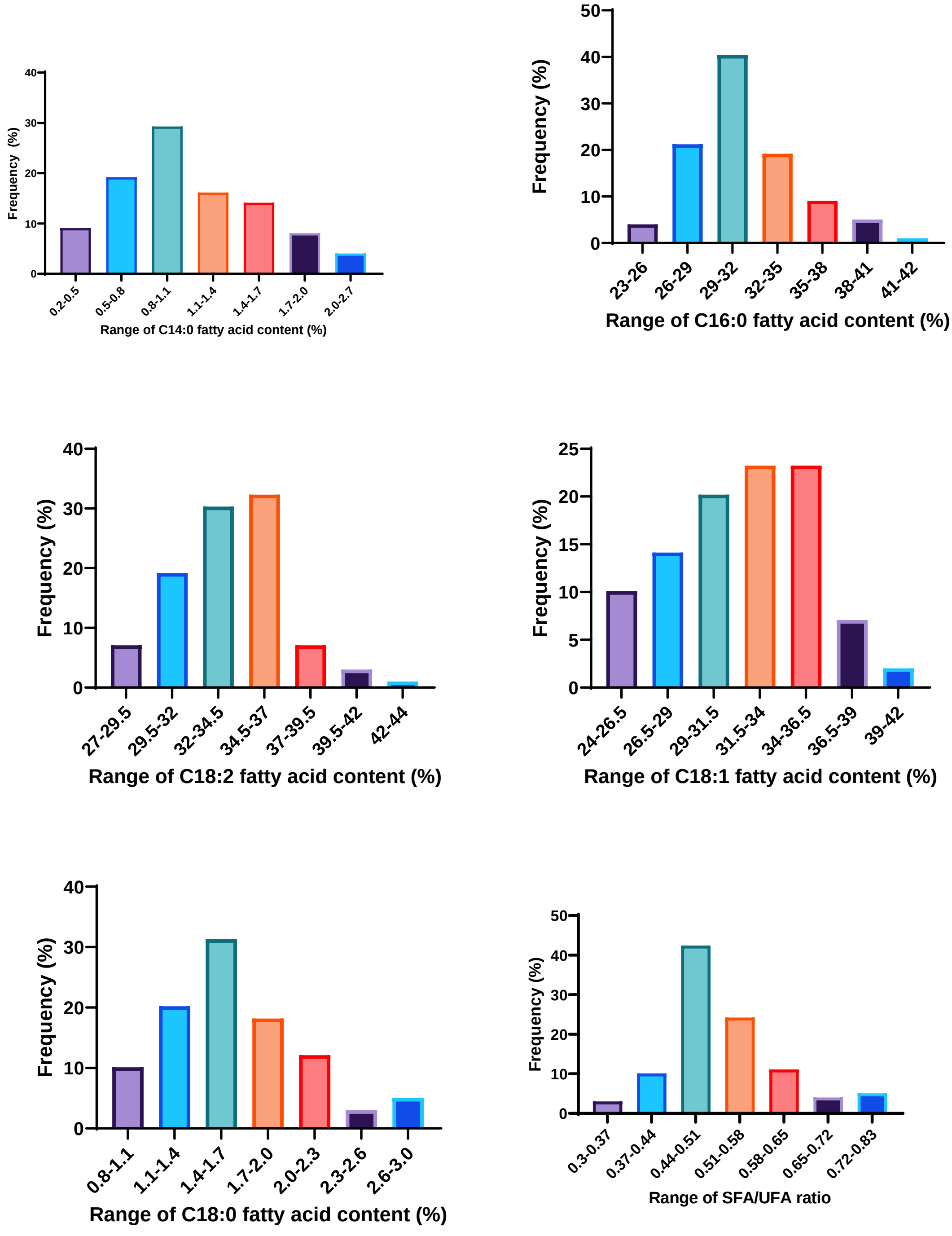

Figure 1. Frequency distribution of five FAs (%) and SFA/UFA ratio in a collection of 100 Vietnamese rice.

Table 2. Correlation between fatty acids content in 100 rice varieties in the Vietnamese rice collection. The correlation between fatty acid content was analyzed using excel. The numbers closer to +1 or -1 indicate the strong correlation between FAs. The numbers far away from -1 or +1 indicate loser correlation between FAs.

	C14:0	C16:0	C18:2	C18:1	C18:0	SFA/UFA
C14:0	1.00					
C16:0	0.10	1.00				
C18:2	-0.18	-0.31	1.00			
C18:1	-0.08	-0.70	-0.44	1.00		
C18:0	-0.06	0.07	-0.3	0.06	1.00	
SFA/UFA	0.21	0.98	-0.36	-0.67	0.18	1.00

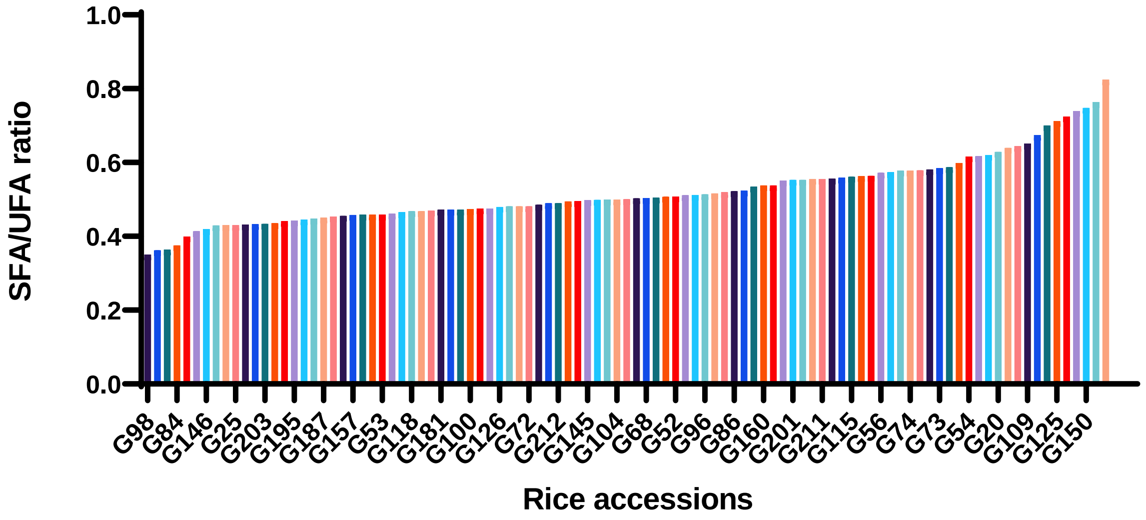

Figure 2. The ratio of saturated fatty acid to the unsaturated fatty acid content in100 rice varieties Each colum with different colours indicate each ratio in each rice variety.

Table 3. The mean values of five fatty acid content and the SFA/UFA ratio in *Indica* and Japonica subgroups.

Type	n	Myristic Acid	Palmitic Acid	Linoleic Acid	Oleic Acid	Stearic Acid	SFA/UFA ratio
India	62	1.09 ± 0.5	32.44 ± 3.86***	34.04 ± 3.17	30.65 ± 3.87***	1.64 ± 0.49	0.55 ± 0.01***
Japonica	38	1.12 ± 0.49	29.54 ± 2.28	34.07 ± 2.65	33.6 ± 3.12	1.62 ± 0.47	0.48 ± 0.05

(***) indicates significant difference determined by one-way ANOVA analysis using Graphpad Prism software, p<0.001.

Fatty acid content in rice subgroups

Rice was claasified into two main subgroups including long-grain rice *Indica* and round-grain rice *Japonica*. Table 3 showed the FAs compositions of *Indica* (n = 62) and *Japonica* (n = 38) genotypes in which there were statistically significant differences in terms of C16:0 ($P < 0.001$), C18:1 ($P < 0.001$), and SFA/UFA ratio ($P < 0.001$). In contrast, no significant difference was notified in the C14:0, C18:0 and C18:2 FAs between them ($P > 0.05$). The palmitic acid content and SFA/UFA ratio were significantly higher in *Indica* genotypes than in *Japonica* genotypes especially this ratio suggested that *Japonica* genotypes have more favourable unsaturated FA than the *Indica* genotypes.

Linoleic acid, oleic acid and palmitic acid content were found abundant for both *Indica* and *Japonica* of Vietnamese varieties.

DISCUSSION

The current study investigates the FA composition in a large Vietnamese rice collection which originated from different ecosystems in Vietnam. Since Vietnam has the big advantage of producing rice as well as RBO, which demands greatly to fully exploit the high potential of RBO in food application in Vietnam. However, in Vietnam, there are only few studies which investigated the effect of RBO on the biomass of *Lactobacillus acidophilus* A1, *Bifidobacterium bifidum* A2 (Hoang Van Tuan *et al*., 2013), or the capacity of RBO to reduce the risk of atherosclerosis in post-menopausal Vietnamese women (Bui Thi Nhung *et al*., 2016), or the effects of Vietnamese rice bran oil as vegetable shortening substitution on the physical and sensory of baked cookies (Nguyen Van Toan, Ngo Thi Thanh Van, 2019). The study on the RBO in a large collection of Vietnamese rice has never been conducted.

Among five main FAs, 97% of total FAs was the palmitic, linoleic and oleic. The same results were obtained in other studies (Hwang *et al*., 2002; Cho *et al.*, 2006; Yoshida *et al*., 2011, 2014; Yang *et al.*, 2018). Other study also indicated that FA composition of rice grains was noticeably high compared to oats, maize, wheat, barley and other cereals (Thomas *et al*., 2015). Moreover, rice grains have lower levels of saturated FAs than other staple food or cereals. All of these features make rice serve as a healthy and better choice for human health (Eshak *et al*., 2011).

Among the five concerning FAs, linoleic acid was accounted for the highest content. This resutl was very interesting because linoleic acid is the main Omega-6-FA, having multiple cardiovascular or mental disease prevention functions. Our results differed from some other studies where they found that oleic acid accounted for the highest amount among FAs (Yoshida *et al.*, 2011, 2014). We also obtained high content of monounsaturated fatty acids, especially oleic acid, which is preferable as cooking oil because it could improve the stability and nutrition value of rice (Galanakis, 2020).

We obtained the strong correlation between palmitic acid content and the SFA/UFA ratio with R = 0.98. The same observation was also obtained by Gofman *et al.,* (2003) where they obtained the high ratio of SFA/UFA to the palmitic acid content ($r^2 = 0.97$) (Goffman *et al*., 2003).

Moreover, most ratios of SFA/UFA in our rice collection were less than 0.5, which means that there was more unsaturated fat than the saturated ones. This result shows the high potential value of our Vietnamese rice collection. Approximately 70% of UFA was also obtained in the study conducted in Japan using three different coloured Japanese rice cultivars: black, red and green (Yoshida *et al*., 2014). The modification of each FA content and the ratio of SFA/FA have been emphasized to reach the wish of humans. For this purpose, the disruption of the *OsFAD2-1* gene by CRISPR/Cas9-mediated in rice was perform resultedin increasing the content of oleic acid, which is an unsaturated FA, more than twice that of the wild type (Abe *et al*., 2018).

The significant differences in content of C16:0 ($P < 0.001$), C18:1 ($P < 0.001$), and SFA/UFA ratio ($P <0.001$) was obtained in *Indica* and *Japonica* genotypes. The C16:0 content and SFA/UFA ratio were significantly higher in *Indica* genotypes than in *Japonica* genotypes. Similar results were obtained by Goffman and his colleagues, who analyzed the FA composition in 204 rice varieties in America (Goffman *et al*., 2003). In some other studies, the FA composition was used to distinguish the *Indica* and *Japonica* subgroups (Taira, Chang

1986; Taira, 1989). We also found the high amounts of unsaturated fatty acids in both *Indica* and *Japonica* genotypes, especially essential fatty acids, successfully proved the nutritional values of the Vietnamese varieties' rice bran oil.

Lastly, we obtained the normal distribution of each FA among our six investigation. The wide range of FA distribution was also found in various rice population world-wide (Goffman *et al.*, 2003; Yoon *et al.*, 2012; Sahu *et al.*, 2019).

CONCLUSION

Our study was the first study investigating the FA content of in a big Vietnamese rice collection. The results showed a wide range distribution of all investigated FAs with a high amount of unsaturated fatty acids including linoleic acid (C18:2) and oleic acid (C18:1) which could be promising for nutritional value of our rice bran oil. The SFA/UFA ratio in *Indica* genotypes were significantly higher than the *Japonica* subgroups, which suggests more favourable unsaturated FA in *Japonica* rice genotypes than the *Indica* genotypes. More studies with a larger scale of rice varieties are needed to be done in order to generate more definitive and accurate evidence.

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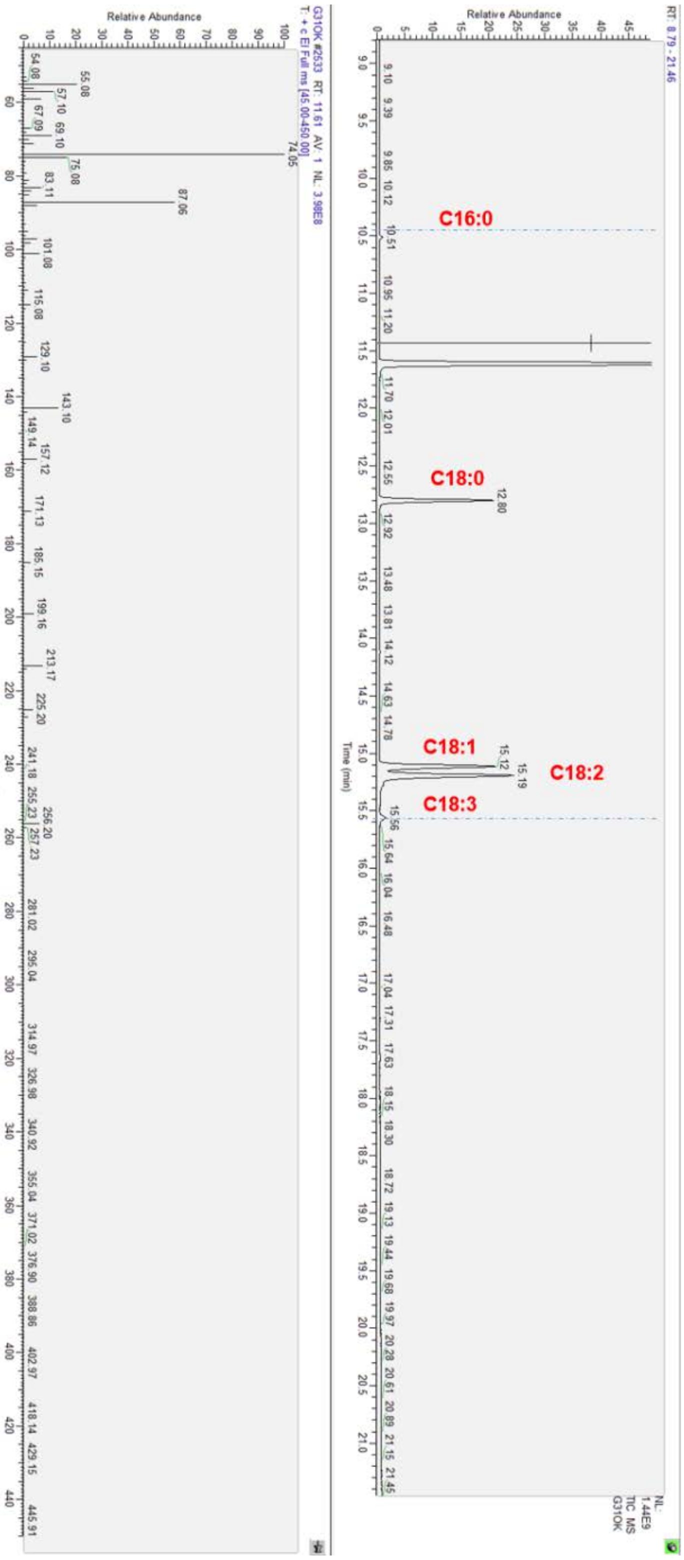

Suppemented figure 1. The Gas Chromatography spectrum of G31 accession (Nep Cam).

Supplemented table 1. List of 100 Vietnamese rice varieties

ID	Name	PRC No	Province	Zone	Type	Eco	DArT P	GBS	GBS P
G100	KHAU QUAI DANG 2	6969	TUYEN QUANG	NE	T	UP	J	y	J1
G101	DIEO KBIN	7295	DAK LAK	SCC	T	UP	J	y	J1
G102	TZO KOH DANG 2	7303	THUA THIEN-HUE	NCC	T	u	I	y	Im
G103	CU PUA DANG 1	7304	THUA THIEN-HUE	NCC	T	u	J	y	Jm
G104	CU PUA DANG 2	7305	THUA THIEN-HUE	NCC	T	u	I	y	I6
G109	MANH GIE	7349	QUANG BINH	NCC	T	UP	I	y	Im
G11	TAM TRON HAI DUONG	219	HAI DUONG	RRD	T	u	I	y	I4
G110	RAN TRANG	7823	BINH THUAN	SE	T	UP	I	y	Im
G111	NEP RAY	7824	BINH THUAN	SE	T	UP	I	y	Im
G113	NANG THIET	7827	VUNG TAU	SE	T	IR	I	y	I2
G115	KOI LOI	7910	HA NOI	RRD	T	IR	I	y	I1
G117	KHAO SANG	7930	QUANG TRI	NCC	T	UP	J	y	J1
G118	L03	9175	HA NOI	RRD	I	IR	I	n	
G12	TAM CAO VINH PHUC	226	VINH PHUC	RRD	T	IR	I	y	I4
G125	NEP NUONG	9356	QUANG NINH	NE	T	u	I	y	Im
G126	KHAU DAM DOI	9466	NGHE AN	NCC	T	UP	J	y	J1
G130	LUA DA BO	9509	KHANH HOA	SCC	T	UP	J	y	J3
G134	PADAI CALOC	9530	KHANH HOA	SCC	T	UP	J	y	J3
G138	NANG QUAT	9563	BEN TRE	MRD	T	RL	I	y	Im
G14	TAM NHO BAC NINH	318	BAC NINH	RRD	T	IR	I	y	I4
G141	LUA NANG NIEU CHUM	9573	BEN TRE	MRD	T	RL	I	y	I2
G145	BA RIA	9580	BEN TRE	MRD	T	RL	J	y	J1
G146	NANG LOAN HAT TRON	9584	BEN TRE	MRD	T	RL	I	y	I2
G150	NEP DIA PHUONG	9595	BEN TRE	MRD	T	IR	I	y	I2
G152	LOC SOM	9871	BAC GIANG	NE	T	IR	J	y	J1
G153	TE NUONG	9874	SON LA	NW	T	UP	I	y	I3
G154	NEP THOM	9878	HA TAY	RRD	T	IR	J	y	J2
G157	SO CRIOONG	9984	SEKONG	CH	T	u	J	y	Jm
G160	JASMINE	12059	AN GIANG	MRD	I	UP	I	y	I1
G161	BN1	12066	AN GIANG	MRD	I	IR	I	y	Im
G169	JASMINE 95	12102	KIEN GIANG	MRD	I	IR	I	y	I1
G17	NEP GA GAY HAI DUONG	394	HAI DUONG	RRD	T	IR	I	y	Im
G171	NEP THAI	12104	KIEN GIANG	MRD	T	IR	I	y	I1
G172	VND 95-20	12105	KIEN GIANG	MRD	I	IR	I	y	I1
G179	BLAO PU LAU	12581	HOA BINH	NW	T	u	J	y	J1
G180	CA DUNG HAT	12637	HA NOI	RRD	T	IR	I	y	I2

ID	Name	PRC No	Province	Zone	Type	Eco	DArT P	GBS	GBS P
G181	BLAU PLAN PIENG	12970	SON LA	NW	T	UP	J	y	I6
G186	KHAU NO	13309	SON LA	NW	T	UP	I	y	I6
G187	KHAU DUONG PHUONG	13320	SON LA	NW	T	UP	J	y	J1
G189	KHAU NAM RINH	13362	DIEN BIEN	NW	T	UP	I	y	I3
G190	PLE PHMA CHUA	13363	DIEN BIEN	NW	T	UP	I	y	I6
G192	KHAU BAO THAI	13423	DIEN BIEN	NW	T	RL	I	y	Im
G193	BLE PE XA	13424	DIEN BIEN	NW	T	UP	J	y	J1
G195	BLE BDE	13426	DIEN BIEN	NW	T	UP	J	y	J1
G20	TE LE HOA BINH	553	HOA BINH	NW	T	u	I	y	I4
G201	CHA XU PHU LU	13435	LAI CHAU	NW	T	UP	I	y	Im
G202	NONG TO	13442	LAI CHAU	NW	T	UP	J	y	J1
G203	PLAU CA BANH	14212	DIEN BIEN	NW	T	UP	J	y	J1
G205	BLE BLAU CHO	14251	SON LA	NW	T	UP	I	y	I3
G208	KHAU BOONG LAM	14279	SON LA	NW	T	RL	I	y	Im
G21	GIE TRANG HOA BINH	614	HOA BINH	NW	T	u	I	y	I4
G210	KHAU LECH	14408	LAO CAI	NE	T	UP	J	y	J1
G211	PLAU NGOANG PLAC	14587	LAO CAI	NE	T	UP	m	y	Im
G212	PLAU BULAT	14589	LAO CAI	NE	T	UP	J	y	J1
G219	KHAU LA LANH	14792	SON LA	NW	T	RL	I	y	Im
G22	TRUNG TRANG TUYN QUANG	760	TUYEN QUANG	NE	T	u	I	y	I4
G220	PLE LA	T5300	LAI CHAU	NW	T	UP	J	y	J1
G221	KHAU MAC CO	T5455	LAI CHAU	NW	T	UP	J	y	J1
G25	NEP VANG ONG LAC SON HB	1058	HOA BINH	NW	T	u	J	y	J2
G26	KHAU CAI NOI	1325	TAY BAC	NW	T	u	J	y	J1
G29	NEP CON	1427	BAC THAI	NE	T	UP	I	n	
G295	CTO VIND IET6155	u	u	u	T	u	I	n	
G299	BLAO SINH SAI	4806	HOA BINH	NW	T	UP	J	y	J1
G31	NANG CHI	1629	CAN THO	MRD	T	u	I	y	I2
G37	NEP CAM	1845	HA GIANG	NE	T	RL	I	y	Im
G38	NEP NUONG	1849	HA GIANG	NE	T	UP	J	y	J1
G4	NHONG DO HAI DUONG	135	HAI DUONG	RRD	T	u	I	y	I4
G45	NEP CUC	2367	NINH BINH	RRD	T	RL	J	y	J4
G48	LUA NGOI	2371	NAM DINH	RRD	T	MG	J	y	J4
G49	DT10	2395	u	u	I	u	I	y	I1
G5	NHONG TRANG HAI PHONG	149	HAIPHONG	RRD	T	u	I	y	I4
G50	LUA NEP BA THANG DANG 1	3323	QUANG NAM	SCC	T	UP	J	y	J2
G51	BA TRANG HUONG	3332	QUANG NAM	SCC	T	UP	I	y	I5

ID	Name	PRC No	Province	Zone	Type	Eco	DArT P	GBS	GBS P
G52	BA TRANG HUONG	3334	QUANG NAM	SCC	T	UP	I	y	I5
G53	LUA CAN DO	3351	u	u	T	u	I	y	I6
G54	LUA LOC DO	3360	QUANG NAM	SCC	T	RL	I	y	I6
G56	LUA MAN	3363	QUANG NAM	SCC	T	RL	I	y	I5
G6	SOM GIAI HUNG YEN	170	u	u	T	u	I	y	I4
G67	LUA TRI DO DANG 2	3485	BINH DINH	SCC	T	RL	I	y	Im
G68	NEP 3 THANG	3487	BINH DINH	SCC	T	IR	m	y	m
G72	LUA CANG DANG 1	3494	BINH DINH	SCC	T	RL	I	y	Im
G73	LUA CANG DANG 1	3495	BINH DINH	SCC	T	RL	I	y	I6
G74	NEP QUA CO RAU DANG 2	3497	BINH DINH	SCC	T	RL	I	y	I6
G77	CANG KIEN DANG 1	3506	BINH DINH	SCC	T	RL	I	y	I6
G78	CANG KIEN DANG 2	3507	BINH DINH	SCC	T	RL	I	y	I6
G79	LUA DA DANG 2	3508	BINH DINH	SCC	T	RL	I	y	I6
G8	CHON TU 502 HOC VIEN	175	u	u	T	u	I	y	I4
G83	NEP VANG	3522	QUANG NGAI	SCC	T	UP	J	y	Jm
G84	BA CHO KTE	3525	BINH DINH	SCC	T	u	J	y	J3
G86	TAN NGAN	3588	YEN BAI	NE	T	u	J	y	J2
G91	BLAO CO KEN	4815	HOA BINH	NW	T	UP	J	y	J1
G92	BLAO CO CAM	4820	HOA BINH	NW	T	UP	J	y	J1
G93	PO LE PO LAU XA	5034	NGHE AN	NCC	T	UP	I	y	I5
G94	LUA DO	5111	THUA THIEN-HUE	NCC	T	UP	I	y	I5
G95	LUA CHAM	5127	NAM DINH	RRD	T	RL	I	y	I4
G96	CHIEM RONG	6191	NAM DINH	RRD	T	IR	I	y	I5
G98	NGOI TIA	6203	NAM DINH	RRD	T	RL	J	y	J4
G99	LUA CHAM BIEN	6234	NINH BINH	RRD	T	RL	I	y	I4

u = unknown; na = not analyzed; Zone: NE = Northeast; NW = Northwest; RRD = Red River Delta; NCC = North Central Coast; SSC = South Central Coast; CH = Central Highands; SE = Southeast; MRD = Mekong River Delta; Type: T = traditional, I = improved; Ecosystem: IR = irrigated, RL = rainfed lowland; MG = mangrove; UP = upland; DArT P: population assignments based on Structure results using the DArT markers.