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PMFs Analysis of Krachaidum Products by HPLC and GC

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Abstract Polymethoxyflavones (PMFs) are a group of polymethoxylated bioactive flavones with diverse biological activities, including anticancer, anti-inflammatory, antibacterial, and antiviral activities. PMFs are found from various plants such as orange, tangerine, and krachaidum. To establish the simple quantitative analytical methods for PMFs, chromatographic analysis was applied to the selected krachaidum foods because krachaidum contains diverse PMFs compared to other PMF-containing foods. Krachaidum is the rhizome of *Kaempferia parviflora*, and many commercial krachaidum products, such as tea, juice and wine, are commercially available and consumed as health functional foods in Asian countries. Apart from the claimed health promoting benefits, reliable quality assurance and legal guideline for the registration of these products are not available yet. Twelve PMFs were analyzed from the commercial krachaidum foods by GC-FID and HPLC-DAD. No single chromatographic method could not analyze 12 PMFs simultaneously. HPLC-DAD method was found more sensitive to detect PMFs. Based on our analysis data, we proposed 5,7-dimethoxyflaone and 5,7,4'-trimethoxyflavone as index components for the food products.

Keywords GC · HPLC · *Kaempferia parviflora* · krachaidum · polymethoxyflavone · quality assurance

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Introduction

Polymethoxyflavones (PMFs) are structurally related polymethoxylated flavones found in the various plants, including the Citrus fruits (Shiming et al; 2006). PMFs show a broad biological activity, including anti-inflammatory (Middleton et al., 2000; Manthey et al., 2001), anti-carcinogenic (Lopez-Lazaro, 2002; Manthey and Guthrie, 2002), anti-atherogenic (Whitman et al., 2005) and antiviral effects (Li et al., 2007). Diverse structures of PMFs were reported as major bioactive compounds of krachaidum, the rhizome of *Kaempferia parviflora* (Sutthanut et al., 2007; Wongsrikaew et al., 2012). Besides, various krachaidum food products are available in different preparations, such as juice, tea and wine due to the health-promoting effects. Recently, krachaidum was also selected as one of Thailand's royal project products. Although hundreds different products of krachaidum food are available and consumed in many Asian countries, there is no reliable quality assurance of these products. In fact, there is no regulation of food quality and set index components.

PMFs in krachaidum were first analyzed by gas chromatography (GC) (Sutthanut et al., 2007). PMFs composition of the krachaidum extracts was also analyzed by high-performance liquid chromatography (HPLC) (Azuma et al., 2011; Wongsrikaew et al., 2011). Because PMFs are important bioactive flavonoids and more krachaidum products are produced and consumed nowadays, we decide to develop a simple PMFs analysis procedure. Krachaidum extracts also contained diverse PMFs, from dimethoxy- to pentamethoxy-flavones, and which makes krachaidum foods a good analytical system to measure the efficiency of chromatographic separation. PMFs analysis of commercial krachaidum foods can be used to set index components among the analyzable PMFs, and to propose the regulation related to the quality assurance. The results also could provide reference data to evaluate whether the claimed many biological activities of PMFs are really applicable to krachaidum foods.

In this study, we have established the PMFs analysis method with completely characterized 12 standard PMFs and two common chromatographic tools of HPLC and GC. Analytes from krachaidum foods were prepared differently, depending on the

types of 16 krachaidum products.

Materials and Methods

Food Samples. Sixteen krachaidum products were purchased from the markets in Thailand. They include capsules of krachaidum extract (products No. 1 to No. 4), teas (products No. 5 to No. 10), juices (products No. 11 to No. 15), and wine (product No. 16). The images and manufacturers' directions of the krachaidum products used in this study were shown at Supplementary Material.

Chemicals. EtOH (99.5%), chloroform (99%) and acetic acid (99.5%) were purchased from Samchun Chemical Co., Ltd. (Korea) and *N*,*N*-dimethylformamide (99.5%) was purchased from Junsei Chemical Co., Ltd. (Japan). HPLC grade MeOH was purchased from Burdick & Jackson Co. (USA). 5,7-Dimethoxyflavone (PMF-1), 5-hydroxy-7-methoxyflavone (PMF-2), 3,5,7-trimethoxyflavone (PMF-3), 5-hydroxy-3,7-dimethoxyflavone (PMF-4), 5,7,4'-trimethoxyflavone (PMF-5), 5-hydroxy-7,4'-dimethoxyflavone (PMF-6), 3,5,7,4'-tetramethoxyflavone (PMF-7), 5-hydroxy-3,7,4'-trimethoxyflavone (PMF-8), 5,7,3',4'-tetramethoxyflavone (PMF-9), 3,5,7,3',4'-pentamethoxyflavone (PMF-10), 5-hydroxy-3,7,3',4'-tetramethoxyflavone (PMF-11), and 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (PMF-12) were isolated, and purified according to the published method (Wongsrikaew et al., 2011; Chaipech et al., 2012).

Analytes Preparation. Krachaidum capsule products (No. 1–4) and tea bag products (No. 5–10) were weighed by 1.0 g and extracted by EtOH (20 mL) for 24 h at room temperature. The EtOH solution was filtered and dried under reduced pressure for further analysis. Krachaidum tea products in the form of tea bag (No. 5–7) were brewed for 2 min in 200 mL of hot water (75°C). The brewed tea (10 mL) then was lyophilized for further analysis. Tea samples were also prepared from the products in the form of lyophilized granules (No. 8–10) according to the manufacturers' direction. Recommended amounts of lyophilized krachaidum tea products (No. 8–10) were dissolved in hot water (200 mL, 75°C) and each sample (10 mL) was filtered before lyophilization. The residue was dissolved in MeOH for HPLC and chloroform for GC analysis. All liquid products (No. 11–16) (2 mL) were taken for filtration with syringe filters (0.45 μm) and the filtrates were directly analyzed by chromatography.

GC and HPLC analysis. For GC analysis, Agilent 7890A GC-FID system with a HP-5 column (30 m×320 μm×0.25 μm) was used. N_2 was used as a carrier gas (2 mL/min), the temperature of injector, oven, and detector were 280, 245 and 300°C, respectively. Sample (1.0 μL) was injected in a split (10:1) mode. One mg of residue obtained from EtOH extraction (No. 1–8) was dissolved in 5 mL of chloroform. For product No. 9 and 10, 9 mg and 1 mg of EtOH-extracted residues were dissolved in 1 mL of chloroform, respectively, and filtered for GC analysis. For the brewed tea (No. 5–8) 1 mg of each lyophilized residue was dissolved in 1 mL of chloroform and analyzed for GC. Product No. 9 and 10 contained less PMFs and 150 mg and 100 mg of each lyophilized residue were dissolved in 1 mL of chloroform. All chloroform solutions were filtered through the syringe filter and injected (1.0 μL) to GC. For liquid products No. 11 to 16, 2 mL of samples were filtered through syringe filter and the filtrates were directly injected (1.0 μL) to GC. For the GC calibration curves, standard compounds of PMF-1–PMF-8, and PMF-11 were prepared at 7 different concentrations (0.0031, 0.0063, 0.0125, 0.025, 0.05, 0.1, 0.2 mM) in DMF. Standard solution of PMF-12 was prepared at 6 different concentrations (0.0063, 0.0125, 0.025, 0.05, 0.1, 0.2 mM) in DMF. The linear equations for the standard curves were shown at the Supplementary Material.

For HPLC analysis, Finnigan Surveyor Plus HPLC with thermo PDA Plus detector, equipped with a C18 Hypersil GOLD™ column (4.6×100 nm; 5 μm; Thermo Scientific, USA) was used. One mg of each EtOH extraction residue from product No. 1, 2, 5, 6, and 8 was dissolved in 5 mL of MeOH, and 1 mg of each EtOH extraction residue from product No. 3, 4, and 7 was dissolved in 10 mL of MeOH. For product No. 9 and 10, 9 mg and 1 mg of EtOH extraction residues were dissolved in 1 mL of MeOH, respectively. For the brewed tea (No. 5 to No. 8) 1 mg of each lyophilized residue was dissolved in 1 mL of MeOH. For product No. 9 and 10, 150 mg and 100 mg of each lyophilized residue, respectively, were dissolved in 1 mL of MeOH. All the MeOH solutions were filtered through the syringe filter and the filtrate was injected to HPLC. For liquid products No. 11 to 16, 2 mL of the samples were filtered by the syringe filters and the filtrates were directly analyzed. The injection volume for HPLC analysis was 10 μL and the flow was 0.8 mL/min. The mobile phase was comprised of 1% acetic acid in deionized water (solvent A) and MeOH (solvent B). For the gradient elution, Solution B was start at 55%, increased to 65% in 20 min, to 70% in 25 min, and finally to 90% in 30 min. For the HPLC calibration curves of 12 PMFs, standard compounds at five or six different concentrations were prepared in MeOH; PMF-1 (0.0125, 0.025, 0.05, 0.1, 0.2 mM), PMF-2 (0.0030, 0.0060, 0.0120, 0.0230, 0.0470 mM), PMF-3 (0.0097, 0.0194, 0.0291, 0.0388, 0.0485 mM), PMF-4 (0.0031, 0.0063, 0.0125, 0.025, 0.05, 0.1 mM), PMF-5 (0.0036, 0.0087, 0.0205, 0.0465, 0.0940 mM), PMF-6 (0.0105, 0.0210, 0.0420, 0.0840 mM), PMF-7 (0.0025, 0.005, 0.01, 0.02, 0.04, 0.08 mM), PMF-8 (0.0115, 0.0230, 0.0460, 0.0920 mM), PMF-9 (0.0005, 0.0018, 0.0040, 0.0088, 0.0186 mM), PMF-10 (0.0019, 0.0218, 0.0435, 0.0870 mM), PMF-11 (0.0008, 0.0020, 0.0043, 0.0088, 0.0186 mM), and PMF-12 (0.0125, 0.025, 0.05, 0.1 mM). The linear equations for the standard curves were shown at the Supplementary Material.

Statistical analyses. All measurements, including analyte preparations, were done at least in triplicate. The data were processed to produce averages and standard deviations by Microsoft Excel.

Table 1 PMFs contents of the krachaidum products

Product	Extraction yield (%)[a]	Total PMFs in the product (%)[b]		Total PMFs per serving (mg)		Serving size
		GC	HPLC	GC	HPLC	
No. 1	6.40	4.35±0.09	4.30±0.05	34.84	34.43	2 capsule before meal, 1cap=0.4g
No. 2	7.60	5.43±0.22	5.42±0.53	32.58	32.55	2 capsule times before meal, 1 cap=0.3g
No. 3	7.10	6.71±0.14	6.09±0.08	67.10	60.90	1 capsule times after meal, 1 cap=0.5g
No. 4	7.43	6.96±0.21	6.42±0.17	69.57	64.20	1 capsule times after meal, 1 cap=0.5g
No. 5	1.10	4.82±0.13	4.79±0.42	24.08	23.95	1 tea bag=0.5g
No. 6	3.88	1.33±0.04	1.30±0.03	47.88	46.80	1 tea bag=3.6g
No. 7	1.18	1.98±0.20	1.97±0.03	19.80	19.70	1 tea bag=1 g
No. 8	1.24	4.63±0.11	4.60±0.16	27.78	27.60	1/2 tea spoon=0.6g
No. 9	15.36	$3.20\pm0.05\times10^{-2}$	$3.20\pm0.28\times10^{-2}$	1.15	1.15	3 tea spoon=3.6g
No. 10	26.68	$2.88\pm0.01\times10^{-2}$	$2.11\pm0.09\times10^{-2}$	1.04	0.72	3 tea spoon=3.6g
No. 11	na[c]	$6.33\pm0.22\times10^{-3}$	$5.39\pm0.15\times10^{-3}$	9.49	8.09	1 bottle=150 mL
No. 12	na	$2.29\pm0.13\times10^{-3}$	-[d]	3.44	-	1 bottle=150 mL
No. 13	na	$2.13\pm0.03\times10^{-3}$	$2.05\pm0.03\times10^{-3}$	0.64	0.61	30 mL (1 bottle=750 mL)
No. 14	na	$5.93\pm0.09\times10^{-3}$	$4.03\pm0.09\times10^{-3}$	1.78	1.21	30 mL (1 bottle=750 mL)
No. 15	na	$9.27\pm0.16\times10^{-3}$	$9.30\pm0.27\times10^{-3}$	2.78	2.79	30 mL (1 bottle=750 mL)
No. 16	na	$3.31\pm0.02\times10^{-3}$	$3.06\pm0.06\times10^{-3}$	0.99	0.92	30 mL (1 bottle=750 mL)

[a]Based on the residue from solid products (wt%). [b]Obtained from chromatographic analysis of each PMF after addition; product No. 1–4 (%PMFs (mg) in 1 g of sample), product No. 5–10 (%PMFs (mg) (200 mL tea)), and product No. 11–16 (wt/vol). [c]na=not applicable. [d]not detected (below the detection limit).

Results

Analytes preparation. Since krachaidum foods are available in many different formulas, analytes for chromatographic analysis were prepared differently. Solid capsules were extracted by EtOH due to the expected low solubility of PMFs in water. Analytes from tea products available as pouches and formulated granules were prepared in two ways, tea brewing with hot water and EtOH extraction, to compare extraction efficiency of PMFs. Residues obtained from EtOH and water extractions were redissolved in MeOH or chloroform for the chromatographic analysis. Analytes from liquid foods were not prepared separately, they were directly injected to chromatography.

Extraction yields and PMFs contents of krachaidum products, and total PMFs contents per serving were shown at Table 1. All the capsules (product No. 1–4) resulted in about 7% EtOH extraction yields. Tea bags (product No. 5–7) and a lyophilized granule (product No. 8) yielded 1–4% of extraction residues from the brewed tea. However, high extraction yields of 15.36 and 26.68% were observed from the other two granules, product No. 9 and 10, respectively, which is probably due to high contents of sugar in the products. Tea products were also extracted with EtOH, to compare PMFs extraction efficiency (Table 2). EtOH extraction yields were higher than the tea brewing in case of products No. 5–8, but lower in products No. 9 and 10 because of the reason mentioned above. Among products No. 5–8, total PMFs contents of brewed tea was much smaller than those of EtOH extracts as expected (Table 2). Therefore, tea brewing appeared not so efficient for PMFs extraction. In case of products No. 9 and 10, PMFs contents in EtOH extracts were not higher than those in tea brewing, probably due to the complications from the high contents of sugar.

Table 2 Krachaidum tea products analysis by EtOH extraction

Product	Extraction yield (%)[a]	Total PMFs in the product (%)[b]	
		GC	HPLC
No. 5	6.47	5.48±0.12	5.45±0.95
No. 6	4.60	3.99±0.04	3.99±0.14
No. 7	8.24	7.78±0.23	7.24±0.13
No. 8	7.17	4.92±0.14	4.92±0.12
No. 9	1.54	$1.45\pm0.03\times10^{-2}$	$1.31\pm0.06\times10^{-2}$
No. 10	2.82	$1.82\pm0.04\times10^{-3}$	$1.27\pm0.04\times10^{-3}$

[a]Based on the residue from solid products (wt%). [b]Obtained from chromatographic analysis of each PMF after addition; product No. 5–10 (%PMFs (mg) in 1 g of sample) .

Liquid products of juices and wine were found to contain more additives, such as other plant extracts and sugars, and less krachaidum extract. They were directly injected to chromatography after filtration, and PMFs contents were obtained.

Chromatographic analysis. PMFs were able to be easily detected by FID and DAD, although GC analysis requires relatively high column temperature. The GC-FID chromatogram of the chloroform analyte prepared from product 5 tea brewing were shown at Fig. 1. HP-5 column could not resolve PMF-9 and PMF-10 under various temperature gradient conditions and an isothermal GC conditions were optimized for PMFs analysis. GC analytes were dissolved in chloroform to suppress solvent peak. One μL of analyte was injected to GC injector and an 10:1 split-mode was adopted, while 10 μL of analyte were injected to HPLC. The same analyte in MeOH was used for the HPLC-DAD analysis and the resulting chromatogram were shown in Fig. 2.

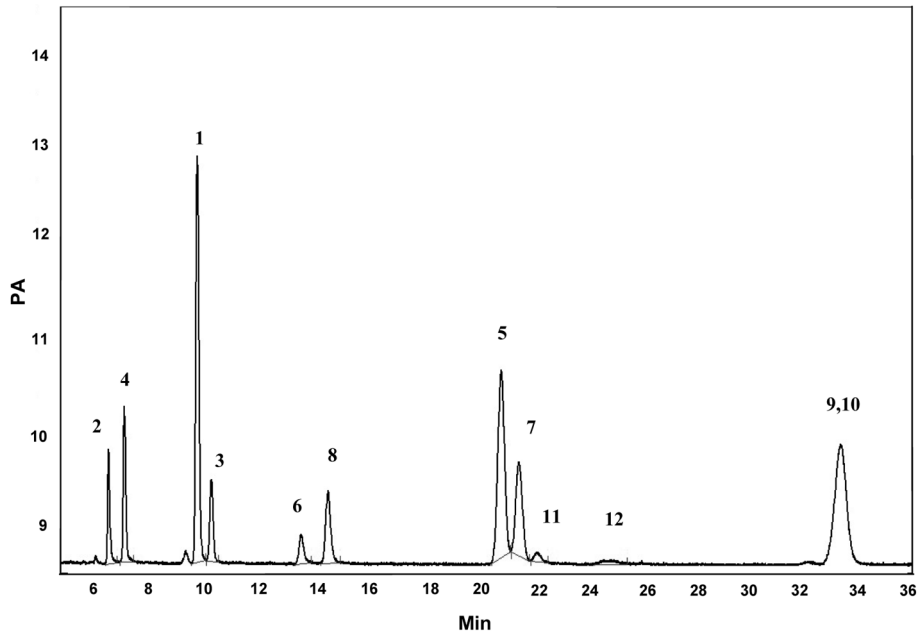

Fig. 1 GC chromatogram of krachaidum product No. 5 (tea bag). The numbers at the peaks represent PMF number.

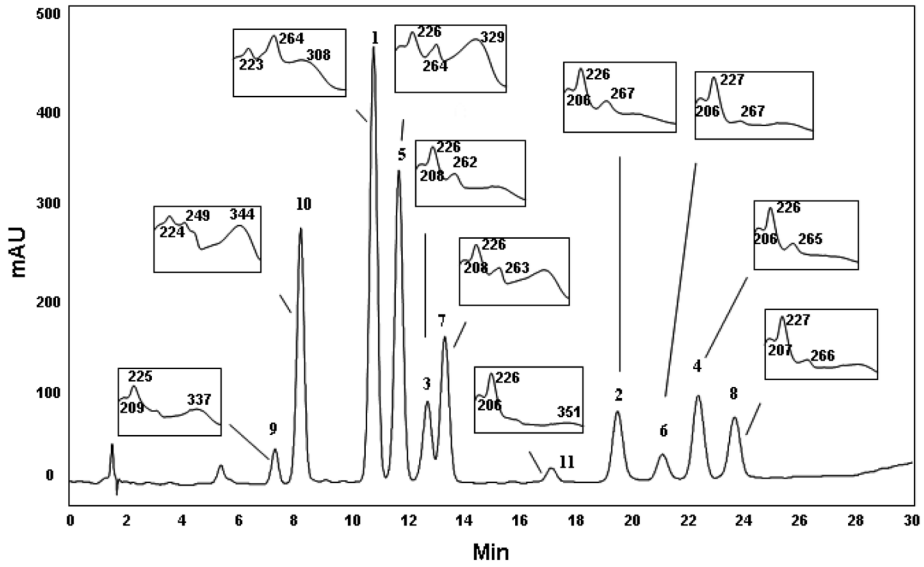

Fig. 2 HPLC chromatogram (265 nm) of krachaidum product No.5 (tea bag). The insets represent UV spectrum of each PMF. The numbers at the peaks represent PMF number.

C18 Hypersil GOLD^{TM} column-equipped HPLC analysis was able to resolve 12 PMFs, including 4'-hydroxy-5,7-dimethoxyflavone at 5.5 min which was not quantitated.

PMFs analysis by GC and HPLC practically resulted in the same results (Table 1), although GC analyses resulted in slightly higher PMFs contents than HPLC analyses generally. Certain PMFs were analyzed by only one analytical method. For examples, PMF-9 and PMF-10 could not be separated on the GC chromatogram (Fig. 1). Besides, PMF-12 was eluted between PMF-3 and PMF-7 on HPLC and the reliable integration values could not be obtained. Total PMFs contents were obtained by addition of each PMF analyzed (Table 1). Overall, capsule products (No. 1–4) of which analytes were prepared by EtOH extraction were found to contain high contents of PMFs (4.35–6.96% by GC and 4.30–6.42% by HPLC). Very wide range of PMFs contents (0.03–4.82% by GC and 0.02–4.79% by HPLC) were measured from analytes prepared from the brewed tea products (No. 5–10). Four tea products, No. 5–8, contained relatively high percentage of PMFs (1.33–4.82% by GC and 1.30–4.79% by HPLC). However, the other two, No. 9 and 10, contained very low percentage, about 0.03%, of PMFs. All the krachaidum juices and wine (No. 11–16) were found to contain less than 0.01% PMFs. When the analyzed

PMFs contents were converted to total PMFs weight per serving size, all capsules and some teas (No. 5–8) were found to provide 20–70 mg per serving. Two lyophilized tea products No. 9 and 10, only provided about 1 mg per serving. PMFs uptake from drinks, including wine, was found to provide less than 10 mg PMFs per serving. When the tea products No. 5–10 were analyzed by EtOH extraction, total PMFs contents in the products were found higher than tea extracts, except products No. 9 and 10 (Table 2). This is probably due to the fact that PMFs are more soluble in EtOH than hot water.

GC analysis provided contents of 10 PMFs, except PMF-9 and PMF-10, and the results are shown at Table 3. When the contents of individual PMF compounds analyzed by GC were compared, PMF-1, PMF-3, PMF-5, and PMF-7 were abundant and detected from all products. Among these PMFs, PMF-1, 5,7-dimethoxyflavone, was most abundant and eluted in shortest retention time. HPLC analysis provided contents of 11 PMFs, except PMF-12 (Table 4). Except product No. 12 juice, which does not contains any significant amounts of PMFs, PMF-1, PMF-3, PMF-5, PMF-7, and PMF-10 were analyzed from all products. Among these five PMFs, PMF-1, PMF-5, and PMF-10 were well separated from other peaks, most abundant, and eluted in short retention times.

Discussion

Various biological activities, such as anti-inflammatory effect (Sae-Wong et al., 2011), xanthine oxidase inhibition (Nakao et al., 2011), and steroid 5-α reductase inhibition (Kim and Han, 2013), are still being updated with the individual PMFs. Krachaidum is known to contain structurally diverse PMFs, and 14 PMFs have been identified from krachaidum until now (Chaipech et al., 2012). To find a simple PMFs analysis methods, analyte preparation from krachaidum foods, followed by GC and HPLC analyses of 12 PMFs, has been carried out in this report. Even though PMFs in the local wine products have been analyzed once (Vichitphan et al., 2007), PMFs analysis of the various forms of krachaidum products has never been reported. We wanted to analyze and compare PMFs contents in foods by two common chromatographic techniques. By doing this, we are expected to provide simple and reliable analyte preparation methods, sensitivity of each chromatographic tools, reasonable quality control criteria, and index components of PMF-containing foods.

Following the first GC analysis of krachaidum dichloromethane extracts, PMFs analysis by HPLC has conventionally been used, and it is estimated that 9–12 PMFs can simultaneously be analyzed from krachaidum extracts so far (Azuma et al, 2011; Shimada et al., 2011). Recently, HPLC-MS analysis was employed, and PMF-10 and its metabolite were analyzed by HPLC-MS for pharmacokinetic study (Chen et al., 2011). Electrochemical detector was also adopted for HPLC analysis of the 5-hydroxy-PMFs (Dong et al., 2010).

PMFs shows an excellent solubility in nonpolar organic solvents, and are also extracted by polar organic solvents, such as chloroform and MeOH, at the low concentration. Although water is not a good solvent for PMFs, hot water could extract PMFs during the process of tea brewing. Therefore, PMFs analytes preparation was achieved by a few different ways. Capsules were extracted by EtOH and the residue was used for analyte preparation. Tea is consumed by brewing and hot water extraction was adopted for analyte preparation. Liquid products of juices and wine were directly analyzed after filtration. Quantitation of PMFs in the chromatographic analysis was achieved by calibration curves. At the optimized conditions, GC could not determine the quantity of PMF-9 and PMF-10. Likewise, HPLC could not quantitate PMF-12. Therefore, it was obvious that quantitative PMFs analysis including PMF-9 and PMF-10 should be carried out by HPLC and the one including PMF-12 should be carried out by GC. All PMFs in the analytes were analyzed to the ìM (ppm) order of concentrations on GC and to the sub-μM (ppb) order of concentrations on HPLC.

Accurate measurement of total PMFs contents in krachaidum foods is very difficult even the values are obtained from the addition of each analyzed PMF because all the PMFs cannot be detected by a single chromatographic analysis as mentioned above. However, minimum total PMFs contents calculated from GC or HPLC analysis can be provided. Therefore, the numbers shown in Table 1 should be interpreted in this rationale. Estimated dietary flavonoid intake is known to reach 50–800 mg/day/kg (Pietta, 2000), or even higher if dietary supplements are consumed. Additional consumption of krachaidum foods does not seem to alter this number significantly.

From the study, 5,7-dimethoxyflavone (PMF-1), 5,7,4'-trimethoxyflavone (PMF-5), and 3,5,7,3',4'-pentamethoxyflavone (PMF-10) are found most abundant in krachaidum foods. These PMFs could exist in a few μM concentration in a body after uptake of krachaidum capsules and teas. Interestingly, the demthylated PMF-1, chrysin, in normal Rainbow trout hepatocytes was reported to exhibit cell toxicity by inhibition of DNA synthesis at the very low (<2 μM) concentrations (Tsuji and Walle, 2008). PMF-5 was found to exhibit anti-plasmodial activity against *Plasmodium falciparum* and antifungal activity against *Candida albicans* with IC_{50} values of 3.70 and 39.71 μg/mL, respectively (Yenjai et al., 2004). Therefore, these biological activities can potentially be beneficial to human after considering the uptake to the body.

This research reported a simple and efficient analyte preparation procedure from krachaidum foods for GC and HPLC analyses. The procedure can be applied to the PMFs analysis of any PMF-containing food products. Composition analysis data suggested 5,7-dimethoxyflavone (PMF-1), 5,7,4'-trimethoxyflavone (PMF-5), and 3,5,7,3',4'-pentamethoxyflavone (PMF-10) as index components, and these three PMFs can be used to set the guideline for the regulation and registration of krachaidum foods. The study also provided insights whether uptake of krachaidum foods actually increased the physiological concentration of PMFs to the level that exhibiting the specific biological activity.

Table 3 GC analysis of PMFs (mg/g or mg/mL) in krachaidum products

Compound	No.1 (mg)	No.2 (mg)	No.3 (mg)	No.4 (mg)	No.5 (mg)	No.6 (mg)	No.7 (mg)	No.8 (mg)	No.9 (mg)	No.10 (mg)	No.11 (mg)	No.12 (mg)	No.13 (mg)	No.14 (mg)	No.15 (mg)	No.16 (mg)
1	11.40 ±0.13	12.75 ±0.76	9.5 6±0.17	14.42 ±0.73	6.26±0.07	19.15 ±0.73	3.98±0.89	7.06±0.11	3.69±0.06 $\times10^{-1}$	1.95±0.02 $\times10^{-1}$	4.27±0.06	1.43±0.09	1.35±0.01 $\times10^{-1}$	5.06±0.07 $\times10^{-1}$	8.50±0.14 $\times10^{-1}$	2.23±0.01 $\times10^{-1}$
2	2.22±0.06	1.86±0.16	4.60 ±0.07	3.60 ±0.14	0.90±0.01	1.95±0.04	1.00±0.03	1.05±0.09	0.04±0.08 $\times10^{-2}$	-	-	-	-	-	0.97±0.02 $\times10^{-1}$	-
3	3.73±0.14	4.09±0.01	3.91±0.05	5.36±0.24	2.03±0.02	2.42±0.06	2.69±0.09	2.84±0.12	8.73±0.25 $\times10^{-2}$	1.38±0.00 $\times10^{-1}$	0.95±0.06	0.48±0.02	9.76±0.11 $\times10^{-2}$	2.32±0.04 $\times10^{-1}$	3.64±0.06 $\times10^{-1}$	1.07±0.04 $\times10^{-1}$
4	4.95±0.17	4.22±0.06	6.60±0.05	5.31±0.19	2.79±0.20	3.94±0.10	2.63±0.11	2.97±0.07	0.13±0.09 $\times10^{-2}$	-	-	-	-	-	1.56±0.03 $\times10^{-1}$	-
5	8.28±0.12	11.71 ±0.19	11.92 ±0.33	10.11 ±0.29	5.27±0.15	6.75±0.12	3.19±0.57	5.84±0.02	2.52±0.01 $\times10^{-1}$	4.44±0.01 $\times10^{-1}$	2.24±0.09	1.12±0.08	2.41±0.05 $\times10^{-1}$	6.52±0.06 $\times10^{-1}$	6.92±0.17 $\times10^{-1}$	4.31±0.01 $\times10^{-1}$
6	2.21±0.10	1.91±0.08	4.14±0.25	3.89±0.03	0.83±0.06	2.36±0.01	0.69±0.02	0.85±0.03	0.04±0.06 $\times10^{-2}$	-	-	-	-	-	0.76±0.00 $\times10^{-1}$	-
7	5.75±0.08	6.16±0.12	8.65±0.32	8.11±0.30	2.25±0.02	3.78±0.05	2.66±0.10	3.41±0.03	9.33±0.12 $\times10^{-2}$	2.60±0.01 $\times10^{-1}$	1.02±0.09	0.42±0.01	1.67±0.03 $\times10^{-2}$	3.89±0.12 $\times10^{-1}$	4.04±0.02 $\times10^{-1}$	2.31±0.02 $\times10^{-1}$
8	2.29±0.03	4.20±0.09	7.52±0.02	6.72±0.14	1.98±0.02	2.01±0.05	1.02±0.14	1.78±0.20	0.05±0.14 $\times10^{-2}$	-	-	-	-	-	6.04±0.09 $\times10^{-2}$	-
11	1.32±0.01	3.14±0.02	4.46±0.02	4.58±0.02	0.95±0.02	2.64±0.02	0.45±0.03	1.00±0.01	-	-	-	-	-	-	-	-
12	1.40±0.05	4.26±0.72	5.76±0.11	7.48±0.02	0.83±0.05	2.93±0.08	1.47±0.06	1.01±0.01	0.09±0.3 $\times10^{-2}$	-	1.01±0.03	-	-	-	8.12±0.38 $\times10^{-2}$	-

Table 4 HPLC analysis of PMFs (mg/g or mg/mL) in krachaidum products

Compound	No.1 (mg)	No.2 (mg)	No.3 (mg)	No.4 (mg)	No.5 (mg)	No.6 (mg)	No.7 (mg)	No.8 (mg)	No.9 (mg)	No.10 (mg)	No.11 (mg)	No.12 (mg)	No.13 (mg)	No.14 (mg)	No.15 (mg)	No.16 (mg)
1	11.08 ±0.08	11.61 ±0.45	14.24 ±0.07	26.41 ±0.31	6.47±0.45	12.98 ±0.36	7.69±0.07	5.76±0.33	2.44±0.19 $\times10^{-1}$	3.49±0.02 $\times10^{-1}$	1.98±0.07	-	2.27±0.02 $\times10^{-1}$	3.12±0.05 $\times10^{-1}$	7.02±0.17 $\times10^{-1}$	2.50±0.80 $\times10^{-1}$
2	2.12±0.07	1.45±0.30	1.94±0.04	1.51±0.07	0.99±0.08	2.11±0.07	0.51±0.02	1.13±0.03	3.26±0.39 $\times10^{-2}$	2.92±0.35 $\times10^{-2}$	-	-	-	-	0.42±0.00 $\times10^{-1}$	-
3	3.05±0.01	3.00±0.60	2.46±0.07	4.42±0.15	1.86±0.13	2.00±0.05	1.37±0.02	1.70±0.05	6.72±0.61 $\times10^{-2}$	8.17±0.51 $\times10^{-2}$	0.58±0.02	-	7.21±0.06 $\times10^{-2}$	0.88±0.05 $\times10^{-1}$	1.64±0.02 $\times10^{-1}$	0.88±0.05 $\times10^{-1}$
4	4.31±0.06	2.78±0.36	2.24±0.05	2.26±0.19	0.88±0.13	0.52±0.02	0.23±0.01	1.09±0.02	0.45±0.18 $\times10^{-2}$	-	-	-	-	-	0.38±0.03 $\times10^{-1}$	-
5	8.27±0.11	11.34 ±0.46	12.85 ±0.11	11.23 ±0.13	5.60±0.75	9.11±0.01	3.21±0.05	5.44±0.19	2.30±0.23 $\times10^{-1}$	1.10±0.02 $\times10^{-1}$	0.70±0.01	-	6.95±0.20 $\times10^{-2}$	1.14±0.06 $\times10^{-1}$	6.03±0.14 $\times10^{-1}$	1.11±0.13 $\times10^{-1}$
6	2.20±0.04	1.44±0.12	1.19±0.06	0.88±0.04	0.53±0.05	0.79±0.02	0.19±0.11	0.63±0.01	1.97±0.28 $\times10^{-2}$	-	-	-	-	-	0.26±0.00 $\times10^{-1}$	-
7	5.07±0.06	5.28±0.86	5.78±0.12	4.74±0.20	3.15±0.22	3.67±0.03	1.39±0.02	2.79±0.05	1.15±0.08 $\times10^{-1}$	7.99±0.29 $\times10^{-2}$	0.51±0.02	-	7.13±0.28 $\times10^{-2}$	0.92±0.04 $\times10^{-1}$	2.77±0.05 $\times10^{-1}$	0.97±0.02 $\times10^{-1}$
8	2.11±0.04	3.09±0.34	3.56±0.02	2.42±0.21	1.32±0.09	1.79±0.02	0.52±0.05	1.46±0.03	4.40±0.39 $\times10^{-2}$	3.80±0.21 $\times10^{-2}$	-	-	-	-	0.49±0.14 $\times10^{-1}$	-
9	0.66±0.01	0.82±0.04	0.77±0.05	0.43±0.01	0.58±0.06	0.65±0.01	0.25±0.02	0.44±0.01	2.44±0.22 $\times10^{-2}$	0.91±0.05 $\times10^{-2}$	0.23±0.00	-	-	0.33±0.02 $\times10^{-1}$	0.56±0.02 $\times10^{-1}$	0.32±0.00 $\times10^{-1}$
10	2.86±0.05	11.30 ±1.56	14.05 ±0.33	8.78±0.21	8.28±0.62	12.41 ±0.30	3.66±0.04	6.24±0.19	3.41±0.27 $\times10^{-1}$	6.38±1.62 $\times10^{-2}$	4.09±0.10	-	1.74±0.01 $\times10^{-1}$	5.71±0.06 $\times10^{-1}$	8.00±0.23 $\times10^{-1}$	3.25±0.44 $\times10^{-1}$
11	1.30±0.01	2.14±0.30	1.77±0.15	1.14±0.05	0.80±0.06	0.84±0.01	0.70±0.40	0.93±0.02	3.07±0.39×10 $^{-2}$	-	-	-	-	-	0.36±0.21× 10^{-1}	-

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