

## Assessment of heavy metals, minerals, alcohol, and fusel oil content of selected fermented plant beverages of Thailand

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### Abstract

Fermented plant beverages (FPB) are produced from cereals, fruits, vegetables and other plant sources. This study aimed to assess the heavy metals, minerals, alcohol, and fusel oil content of seventy FPBs sampled from several parts of Thailand. The results indicated that tested FPBs products were harmless for consumption except some of the products that are made from *Morinda citrifolia* Linn. The iron content of few products was found higher owing to the use of underground water for the production, which can be nullified by controlled consumption of FPBs. About 21 products are not suitable for consumption as per Thai community product standards (TCPS). All the samples were scored based on Australia New Zealand Food Authority standards (ANZFA), TCPS and Thai industrial standards-2089. The outcome of current study has revealed the importance of selection of raw materials for FPBs and also determined the quality of the selected FPB in Thailand.

### Keywords

Fermented plant beverages

Heavy Metals

Minerals

Alcohol

Fusel oil

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### Introduction

Fermented plant beverages (FPB) productions are one of the major small scale plant-based beverage industries in Thailand. FPBs production can be commonly initiated by mixing the raw materials (plant parts, sugar, and water at the ratio of 3:1:10 (w/w/v)) in a container, mostly plastic wares. Fermentation will occur with the help of microbes present in the raw materials that have been used in the preparation. Then the mixture is incubated at room temperature for several months, which depends on the raw materials. *Morinda citrifolia* Linn is used in traditional medicine as predominant raw material for FPB production in Thailand over the years. Even though the chemical constituents of *M. citrifolia* have been determined, the nutritional value of FPB with *M. citrifolia* is yet to be reported. Some reports revealed that raw materials of FPB have an active role in hypertension, ulcers, rheumatism, sore throat, microbial infection, inflammatory, autoimmune and cancer (Wang and Su, 2001; Wang *et al.*, 2002; Kamiya *et al.*, 2004). Red seaweed (*Gracilaria fisheri*), banana (*Musa sapientum* L.), and wild forest noni (*Morinda coreia* Ham) are also commonly used as raw materials for the production of FPBs. FPBs from banana and wild forest noni were reported for their antibacterial

ability against *Pseudomonas aeruginosa*, *Shigella* spp., and *Vibrio parahaemolyticus* (Kantachote and Charernjiratrakul, 2008).

Since these FPB are of plant origin and non-alcoholic, the consumers of Thailand believe that these beverages are healthy (Chaiyasut *et al.*, 2013). The local farmers in Thailand mostly rely on the natural fermentation of the raw materials for the small scale productions of FPB. Moreover, the residing bacteria and chance of contamination are high in these kinds of products. FPBs are frequently reported to be contaminated with yeast cells (Prachyakij *et al.*, 2007). Alcohols are the by-products of yeast fermentation, notably methanol and higher alcohol, alcohol with more than two carbon atoms, or fusel oil, for example, isopropyl alcohol, propyl alcohol, butyl alcohol, isobutyl alcohol and isoamyl alcohol. Non-alcoholic FPB is produced using lactic acid bacteria (LAB) as a starter culture. The fermentation process can be initiated by 10% of starter culture. *Lactobacillus plantarum* and *L. casei* are the typical starter LAB culture for FPB production (Duangjitcharoen *et al.*, 2008).

Quality control and food safety are critical even though the raw material of the product is bioactive against biotic and abiotic hazardous substances. Chemical quality control of FPB is the primary

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concern for consumer safety; in particular, volatile compounds, organic acids, heavy metals, and fusel oils should be critically rationalized during production and quality control. Methanol and ethanol content in the FPB are critical, due to their toxicity and adverse effect on human health. Human skin easily absorbs methanol and isopropanol, and its distribution depends on body water. Peak methanol concentrations occur within 30 to 60 min if exposed to methanol orally (Barceloux *et al.*, 2002). Thus, the current study was employed to investigate the consumer safety with respect to heavy metals, minerals, alcohol, and fusel oil in the selected FPBs produced from different raw materials, sampling from several parts of Thailand. The FPB samples were selected based on the representation of all kind of FPBs commonly used in Thailand at the time of sample collection.

## Materials and Methods

### Sample collection

Fermented plant beverages (FPBs) with different composition and raw materials were collected from a different region of Thailand. Diverse samples were collected from homemade, small-scale, and medium scale FPBs production units. Basic characteristics of the samples were detailed in Table 1.

### Analysis of heavy metals and minerals

FPBs were assessed for heavy metals and minerals such as potassium (K), calcium (Ca), manganese (Mn), magnesium (Mg), selenium (Se), iron (Fe), zinc (Zn), copper (Cu), arsenic (As), cadmium (Cd) and lead (Pb) content. K, Ca, Mn, Mg, Fe, Zn, and Cu content was estimated by wet-ashing technique followed by atomic absorption spectrometry (AAS) (Model-932 plus, GBC, Germany) according to Association of Analytical Communities (AOAC) Official Method 975.03 (Horwitz 2000). Se content of the FPBs was analyzed by 2, 3-diamine naphthalene (DAN) solution based fluorometric method according to AOAC official method 974.15. The spectrofluorometer (Jasco, model-V-530, No.B167360512) with excitation at 366 nm and emission at 525 nm was used (Horwitz, 2000). Heavy metals like As, Cd and Pb content was estimated by AOAC official method 986.15, and 999.11, respectively (Horwitz, 2000). AAS based determination of As was carried out after generation of metal hydrides by hydride generator.

### Sodium analysis

The sodium (Na) content was determined

by ion selective electrode method as per AOAC official method 976.25. A standard curve of sodium was prepared using sodium chloride (NaCl) at five different concentrations (2, 20, 600, 2000, and 5000 mg/mL). Each concentration of standards was estimated. Briefly, 20 mL of standard solution was mixed with 20 mL of total ionic strength adjustment buffer (TISAB) solution. The voltage (mV) of the solution was determined using Ion Meter (Metrohm, Model-693 VA) equipped with a specific electrode for sodium. Ag/AgCl electrode served as a reference electrode. A standard graph was plotted between the log of concentration (mg/L) and voltage (mV). Samples were analyzed as same as standards and concentration were determined by a linear equation of the standard curve (Horwitz, 2000).

### Ethanol analysis by gas chromatography

A standard curve of ethanol was prepared with an internal standard (isopropanol) of different concentrations. Stock solutions of 2.5% of ethanol (v/v) and 0.5% isopropanol (v/v) was used for standard preparation. Column temperature at 120°C, detector temperature at 200°C and injector temperature at 200°C was used for mixed solution detection by gas chromatography (GC-14B, Shimadzu, Japan). Hydrogen gas and the air was used as flamed gas in flame ionization detector. Helium was used as carrier gas. A standard curve was plotted against concentration and peak area ratio of ethanol/isopropanol. The ethanol content in the sample solutions was analyzed as mentioned above and compared with a linear equation of the standard curve. Ethanol strength was calculated (Equation 1)

$$\text{Alcohol strength} = \frac{\text{Concentration of ethanol in solution} \times \text{RR}}{\text{RF}} \quad (1)$$

Where, RR was calculated from the peak area ratio of ethanol/isopropanol in the sample, and RF was calculated from the peak area ratio of ethanol/isopropanol in standards.

### Methanol and Fusel oil analysis by gas chromatography

A standard curve of acetaldehyde, methanol, n-propanol, ethyl acetate, isobutanol, and amyl alcohol was prepared with an internal standard (n-butanol). 1% (v/v) of each standard compound and 0.5% (v/v) of n-butanol were used as stock solution. Column temperature at 70°C, detector temperature at 200°C and injector temperature at 180°C was used for mixed solution detection by gas chromatography (GC-14B, Shimadzu, Japan). Hydrogen gas and

Table 1. Name, color, packing size, and composition of the tested FPBs (as per the manufacturers instructions).

Type of Sample	Color	Ingredient
130 type fermented fruit juice	Clear yellow	90% MFJ, 10% H
Bio extract juice	Turbid red to brown	95% MFJ, 5% S
Bio fermented juice	Brown with dark dregs	70% M, 10% IGB, 10% wild grape, 5% Banana, 5% H
BG and <i>Morinda</i> herb juice	Turbid Red to brown	70% BGJ, 20% MJ, 7% H, 3% SCJ
BG bio extract	Yellow to Dark	90% BGJ, 5% H, 5% BS salt
(C) BG herb juice	Turbid red to brown	90% BGJ, 5% Nut grass Bael juice, 5% H
BG herb juice	Turbid Red to brown	70% BGJ, 10% IGBJ, 10% <i>Chebolic myrobalan</i> , 10% H
BG herb juice	Dark red to brown	90% BGJ, 10% SCJ
BGJ (L)	Turbid Red to brown with purple dregs	80% BGJ, 15% MJ, 5% SCJ
BG, H, BS juice	Turbid dark brown with purple dregs	75% BGJ, 17% MJ, 3% H, 5% SCJ
Cha-tu-pa-la ti-ka herb juice	Brown with brown dregs	32% <i>C. myrobalan</i> , 32% <i>Terminalia bellirica</i> , 32% IGBJ, 4% SCJ
(C, F) MFJ	Turbid brown	100% (F)MFJ
(C, F) MFJ	Turbid red to brown	70% MFJ, 30% H
(C) MJ	Red brown	10% H, 80% MJ, 10% IGBJ and other fruits Juice
(C) MJ	Turbid red to brown	80% MJ (L), 5% IGBJ (L), 5% other fruits juice (L), 10% H
(C) MJ (enzyme)	Brown	94% MJ, 3% SCJ, 3% H.
<i>Curcuma xanthorrhiza</i> Roxb. herb juice	Clear yellow to brown	60% <i>C. xanthorrhiza</i> Roxb., 10% H, 30% other herbs juice
(F) BJ	Turbid yellow	60% BJ, 5% S, 35% MFJ
(F) BJ	Clear yellow	90% BJ, 10% H
(F) BGJ	Red to brown	70% BGJ, 10% S, 20% MFJ
(F) Cabbage juice	Light brown	90% Cabbage juice, 5% H, 5% S
(F) Enzyme bio extract	Yellow to Brown	90% MFJ, 5% S, 5% H
(F) Herbal juice	Dark red to brown	65% MJ, 10% nut grass, 5% wild finger root, 5% Thai grape juice, 5% Garlic juice, 5% S, 5% <i>C. xanthorrhiza</i> Roxb.
(F) IGBJ	Turbid yellow	90% IGBJ, 10% S
(F) Fruit Juice (L)	Dark brown	90% MFJ, 10% SCJ
(F) Longan juice	Turbid yellow	70% Longan juice, 5% S, 25% MFJ
(F) MFJ	Red to brown	60% IGBJ, 10% Lemon juice, 25% Longan juice (L), 5% S
(F)MFJ	Turbid yellow	90% mixed juice, 5% S, 5% H
(F) MJ	Red	93% MJ, 7% SCJ
(F) MJ, BJ, H	Red to brown	80% MJ, 10% BJ, 10% H
(F) <i>Morinda</i> , BJ	Red to brown	80% MJ, 10% BJ, 10% S
(F) <i>Morinda</i> herb juice	Dark red to brown	90% MJ, 10% S,
(F) MJ	Red to brown	95% MJ, 5% SCJ
(F) MJ	Red to brown	90% MJ, 10% SCJ
(F) MJ	Red to brown	92% MJ, 8% SCJ
(F) MJ	Dark red to brown	90% MJ, 10% SCJ
(F) MJ	Clear brown	90% MJ, 5% S, 5% H
(F) MJ	Turbid yellow	90% MJ, 10% SCJ
(F) MJ	Turbid yellow	90% MJ, 5% S, 5% H
(F) MJ	Dark brown	90% MJ, 10% SC
(F) MJ	Clear yellow	90% MJ, 10% H
(F) MJ	Yellow to Brown	90% MJ, 5% H, 5% S
(F) MJ	Yellow	90% MJ, 10% H,
(F) MJ	Red to brown	89% MJ, 0.95% citric acid, 0.05% aspartam natural coloring and flavoring, 10% S
(F) MJ	Dark brown	90% MJ, 10% H
(F) MJ(LAB as starter)	Yellow to brown	93% MJ, 7% SCJ
(F) PFJ	Yellow	92% PFJ, 8% H
(F) PFJ	Dark brown	90% PFJ, 10% H
(F) Mixed MJ	Dark brown	80% MJ, 10% H, 10% MFJ
Mixed Fermented MJ	Turbid yellow	90% MJ. 5% S. 5% H
Mixed fruit enzyme	Yellow to Brown	92% MFJ, 8% S
Mixed fruit enzyme	Turbid yellow	60% MFJ, 30% MJ, 10% S
Mixed fruit enzyme juice	Clear yellow	92% MJ, 8% H
Mixed herb juice	Red to brown	92% Mixed herb juice, 8% S
<i>Morinda</i> and IGB mixed juice	Turbid Red brown	80% MJ, 8% H, 5% SCJ, 5% IGBJ, 2% Other fruit Juice
<i>Morinda</i> Bio Extract	Turbid Red brown	80% MJ, 10% H, 10% BS
<i>Morinda</i> herb extraction	Brown	50% MJ, 20% IGBJ, 20% Apple Juice, 10% H
(C) <i>Morinda</i> herb extraction	Turbid light to brown	75% MJ (L), 5% IGBJ (L), 20% H
<i>Morinda</i> herb juice	Dark red to brown	90% MJ, 5% S, 5% H
MJ	Dark brown	92% MJ, 8% SCJ
MJ	Red to brown	92% Ripe MJ, 8% BS
MJ	Dark brown	85% MJ, 9% H, 5% blueberry juice, 1% iodine salt
MJ (L)	Dark red brown	NA
MJ (L)	Dark red brown	95% MJ, 5% SCJ,
(P, F) Herb juice	Dark red to brown	60% MJ, 5% S, 5% H, 30% MFJ
(P) <i>Morinda</i> herb juice with H	Turbid dark red to brown	60% (F) MJ, 20% MJ (L), 20% H
Premium fruit enzyme	Turbid dark brown	90% MFJ, 10% SCJ
(F) Sour fruits juice	Clear red to brown	90% MFJ, 10% SCJ
(P, F) Sour MJ	Dark brown	90% MJ, 9% H, 1% iodine salt
(F) Sweet fruits juice	Clear red to brown	80% MFJ, 20% SC

MFJ =Mixed fruit juice; H = Honey; IGBJ = Indian goose berry juice; SC = Sugar cane; BG = Black galingale; BGJ = Black galingale juice; MJ = *Morinda* juice; L = Leavening; Banana juice = BJ; PFJ = Passion fruit juice; C = Concentrated; F = Fermented; P = Pasteurized; BS = Brown sugar; S = Sugar; NA = Not Available

the air were used as flamed gas in flame ionization detector. Helium was used as carrier gas. A standard curve was plotted against concentration and peak area ratio of standard/ n-butanol. All the samples were filtered, and 10 mL of samples were mixed with 1 mL of 0.5% (v/v) n-butanol and made up to 100 mL using 10% (v/v) ethanol. Samples were analyzed as detailed above compared with a linear equation of the standard curve.

### Statistical analysis

All the experiments were performed in triplicates. Results were represented as a range of minimum and maximum concentration of analyzed metals and minerals in FPBS. Alcohol and fusel oil measurements were represented as the number of samples with a respective concentration of components for clear interpretation of results.

## Results and Discussion

Metals and minerals like selenium (Se), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) was assessed and compared with recommended dietary allowances (RDA), respectively. These fundamental elements are vital for several biological functions (Vallee, 1952). Selenium is one of the essential trace elements, absorbed in the form of the inorganic complex from food (Finley, 2006). Selenium content is rich in low molecular weight proteins and vitamins, especially vitamin A, C and E (Navarro-Alarcon and Cabrera-Vique, 2008). Selenium deficiency leads to the increased risk of neoplastic (gastric carcinoma, pulmonary carcinoma, colonic carcinoma, and prostate carcinoma), cardiovascular and nervous system related diseases. Selenium is also vital for proper functioning of defense system (Pieczynska and Grajeta, 2015). Excess intake of selenium causes selenosis with symptoms that include hair loss, a garlic odor on the breath, gastrointestinal disorders, fatigue, sloughing of nails, irritability, and neurological damage. Cirrhosis of the liver, pulmonary edema and death can also be recorded in extreme cases. About 0.203-0.491 mg of selenium per kg of FPBs was recorded in this study, and the results indicated that consumption of 60 g of FPBs provides 0.012-0.029 mg of selenium (Figure 1A). Selenium content was in an approved level as per ANZFA.

Iron deficiency-prone to cause blood loss and impairment in blood clotting mechanism. Especially, it affects women during their reproductive years. Defects in iron absorption, frequent blood

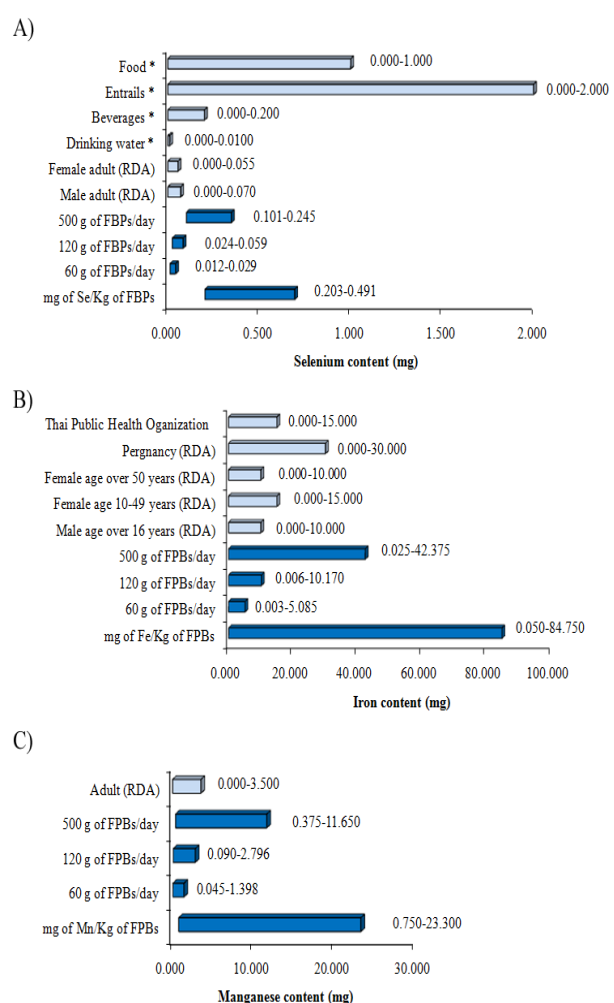


Figure 1. The content of selenium (A), iron (B), and manganese (C) in the tested FPBs and acceptable level (RDA) of respective elements for human consumption. All the elements were detected under the standards of RDA. \* indicates the acceptable values. Results were represented as a range of minimum and maximum concentration of detected values.

transfusions, heavy doses of dietary iron and some rare metabolic disorders leads to iron overload or toxicity. About 0.05-84.70 mg of iron content per kg of FPBs was recorded in this study, and the results indicated that consumption of 60 g of FPBs provides 0.003-5.085 mg of iron (Figure 1B). Thai Public Health Organization announcement (2001) revealed that the allowable iron content in drinking water is 15 mg/L and RDA of iron content differs based on the consumer age, gender, pregnancy status. The results showed that the iron content in some of the FPBs is higher than the standard quantity allowed for consumption. Hyper ironic content can rapidly lead to transferrin saturation, ensuing free iron circulation in the serum, which is directly noxious to organs. An in-depth interview with the manufacturers, we found that iron was from underground water that was used in the FPBs. Underground water contains rich



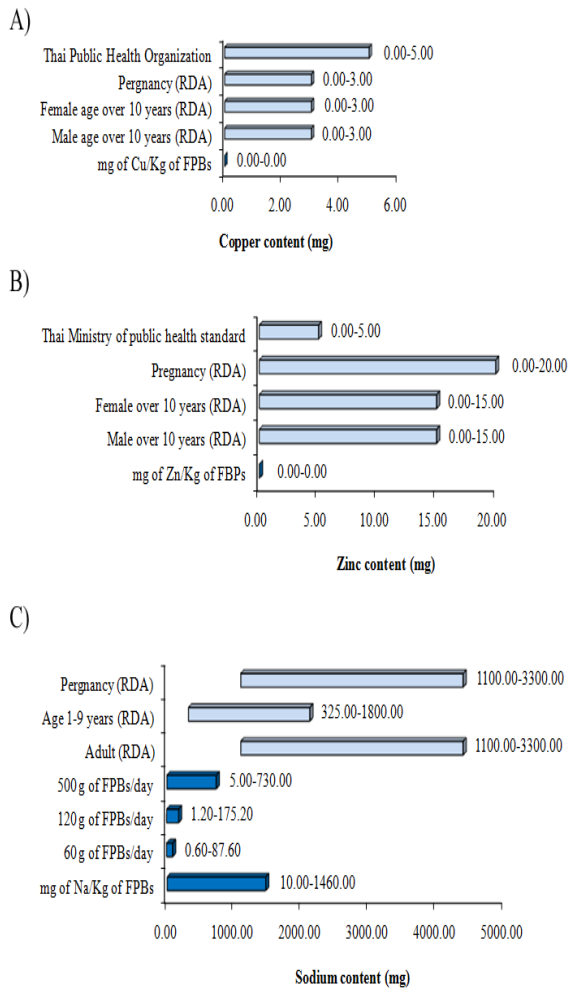


Figure 2. The content of copper (A), zinc (B), and sodium (C) in the tested FFBs and its acceptable level (RDA) of respective elements for human consumption. All the elements were detected under the standards of RDA. The copper and zinc content was not detected in any of the samples tested in this study.\* indicates the acceptable values. Results were represented as a range of minimum and maximum concentration of the detected values

ferrous ion at the place of manufacturing unit (data not shown). Therefore, unrefined underground water is not recommended to use in FFBs production.

Manganese is associated with amino acid, lipid, and carbohydrate metabolism and bone development, found in different enzymes, like superoxide dismutase, glutamine synthetase, arginase, and activates several transferases, hydrolases, and carboxylases. Macroglobulins and albumin are the major manganese transporters (Fraga, 2005). An excess amount of manganese is toxic to brain health and cause Parkinson-type syndrome (Aschner, 2000). About 0.75-23.30 mg of manganese per kg of FFBs was recorded in this study, and the results indicated that consumption of 60 g of FFBs provides 0.045-1.398 mg of manganese (Figure 1C). RDA approved the level of manganese intake by an adult was 3.5 mg.

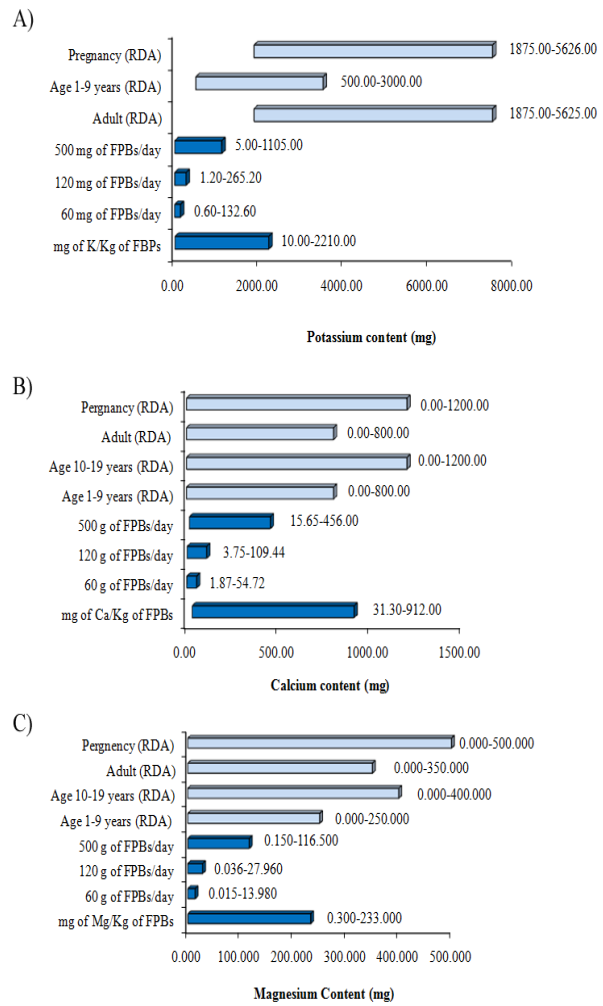


Figure 3. The content of potassium (A), calcium (B), and magnesium (C), in the tested FFBs and acceptable level (RDA) of respective elements for human consumption. All the elements were detected under the standards of RDA. Results were represented as a range of minimum and maximum concentration of detected values

Therefore, the results suggested that consumption of less than 120 g of FFBs per day will provide approved level of manganese. Excess consumption of FFBs leads to hyper manganese content.

Meats (particularly internal organs), sea foods, nuts, and whole grains and seeds are the main sources of dietary copper and zinc (Gibson, 1994; Chan *et al.*, 1998). Copper act as a prosthetic group of several enzymes and is crucial for the bone marrow and nervous system. Copper deficiency leads to anemia (Dunlap *et al.*, 1974), cytopenia (Weihl and Lopate, 2006) and myelopathy (Schleper and Stuerenburg, 2001). Zinc deficiency leads to defects in the gastrointestinal tract, central nervous, immune, and also reproductive systems. Copper, zinc (Figure 2A, B), arsenic, cadmium and lead content (data not shown) was not detected in the FFBs used in the current study.

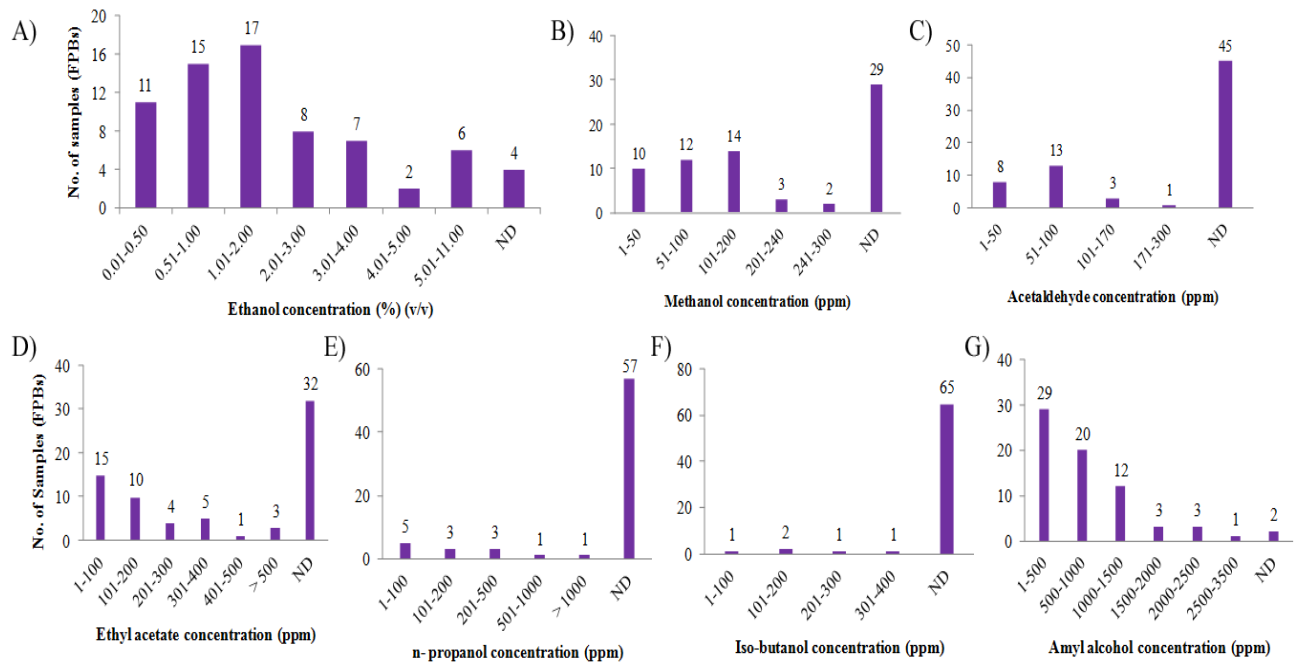


Figure 4. The content of ethanol (A), methanol (B), acetaldehyde (C), ethyl acetate (D), n-propanol (E), isobutanol (F), and amyl alcohol (G) in the tested FPBs. Numbers on each bar represent the number of samples at a particular group. Alcohol and fusel oil measurements were represented as the number of samples with a respective concentration of the corresponding components for clear interpretation of results.

A balance of both Na: K and Mg: Ca ratios at both cellular and system levels is necessary to maintain the healthy blood pressure (Rosanoff, 2005). Deficiency of  $K^+$  is connected to  $Na^+$  and  $K^+$  pumps in skeletal muscle.  $Mg^{2+}$  alteration also affect the  $Na^+$ ,  $K^+$  pump concentration (Dorup, 1996). Electrolyte toxicity is very rare and lead to delirium or acute confusion state and death. The results showed that the content of sodium and potassium were ranging from 10-1460 and 10-2210 mg/kg of FPBs, respectively. The consumption of 60 g of FPBs provides 0.6-87.6 mg of sodium and 0.6-132.6 mg of potassium (Figure 2C, 3A). Calcium and magnesium content of the FPBs were found as, ranging from 31.30-912.0 and 0.30-233.0 mg/kg of FPBs, respectively. The consumption of 60 g of FPBs provides 1.878-54.720 mg of calcium and 0.015-13.980 mg of magnesium (Figure 3B, C).

The metabolic pathways of alcohols were clearly described by Kraut and Kurtz (2008). Ethanol poisoning is cruel to human health, for example, slurred speech, ataxia, coma, respiratory depression, and death (Adinoff et al., 1998). The threat of methanol intoxication might be augmented with low hepatic tetrahydrofolate concentrations, which affects the formate metabolism, thereby causing visual abnormalities (Haviv et al., 1998; Barceloux et al., 2002). According to Thai community product standard (TCPS 481/2004), the permissible level of methanol and ethanol in FPBs is 240 mg/L and 3% (v/v), respectively. Food standard differs based

on the agency and country, for example, ANZFA, Australia New Zealand Food Authority, authorize the presence of 8 g/L of methanol in spirit beverages (Chaiyasut et al., 2013). In this study, many FPBs samples displayed high content of ethanol with reference to Thai public health announcement-214, 2000. The permissible level of ethanol in food beverages is not more than 0.5%, but only 15 tested samples (11+4) fall under the regulation (Figure 4A). About 3-11% of ethanol was recorded in 15 (7+2+6) samples, equals to alcoholic beverages. The composition of the samples revealed that mixed fruits and the fermented *Morinda* juice are accounted for a high content of ethanol. Excess intake of ethanol leads to high blood alcohol concentration, increases the osmolality, and the accumulation of metabolites can cause high anion gap metabolic acidosis, acute renal failure, or unexplained neurologic disease (Kraut and Kurtz, 2008). The possible reason for the elevated amount of ethanol is due to the microbial contaminant during production, which facilitates the production of ethanol in FPBs. The methanol content (Figure 4B) of FPBs suggested that all the products are as per the TCPS (not more than 240 ppm) except two products. Merely five products have crossed 200 ppm level of methanol. The FPBs produced from *Morinda* accounts for a high content of methanol, and two of them have crossed the TCPS limit. Only one product made from *Morinda* displayed the over limit of acetaldehyde (not more than 170 ppm) in this

study, whereas rest of the products were safe in this aspect (Figure 4C).

The acid content of the food, especially for FPB, is necessary to prevent the bacterial contamination and growth during the production process and storage (Foegeding and Busta, 1991). The reduction in the pH suppresses the growth of pathogenic anaerobic bacteria like *Clostridium botulinum*, the causative agent of Botulism. Fusel oils are the by-product of alcohol fermentation. The fusel oils are a fusion of several alcohols, mostly amyl alcohol. There is no detailed study on fusel oil content in FPBs. Moreover, *Suncus murinus* based study revealed that the fusel oil in whiskey had no impact or role on the alcohol induced emetic responses (Hori *et al.*, 2003). Fusel oil drinks affect the physio-psychic nature of healthy human beings, and it is also evidenced by more conflicting electroencephalography (EEG) curve among the volunteers (Nagy *et al.*, 1978). Acceptable level of fusel oil in fermented liquor is  $\leq 5,500$  ppm, as per the TCPS (TCPS 481/2004). The fusel oil content of FPBs was assessed by quantifying the ethyl acetate, n-propanol, isobutanol, and amyl alcohol concentration (Figure 4). Almost all the samples follow the non-lethal level of fusel oil content except some samples. Amyl alcohol content was almost found in all the tested FPBs but mostly not found at higher concentration. Fusel oil was not detected in the majority of the samples except amyl alcohol. Very least found component is isobutanol, which was detected only in 5 samples. The origin of fusel oil in FPBs is, of course, due to the alcohol fermentation during processing. The small scale and homemade products were not undergone post-fermentation processes to remove excess or unfavorable contents.

Overall, the results suggested that none of the tested FPBs contain an excess amount of metals and minerals except  $\text{Fe}^{2+}$ .  $\text{Fe}^{2+}$  amount is also acceptable if the level of consumption volume is up to  $\sim 180$  g of FPBs (Figure 1B). As per TCPS regulations for methanol content, about 21 products were not appropriate for consumption. Based on Thai industrial standards regulations, about 1 and 2 FPB products were unsuitable for consumption with respect to acetaldehyde and fusel oil content. According to ANZFA, tested FPBs were tabulated as safe (67 products with less than 8% of methanol/ethanol) and risk (3 products with more than 8% of methanol/ethanol) drinks. Chaiyasut *et al.*, (2013) have studied the factors that contribute to methanol production in FPBs containing *M. citrifolia*. Raw material size, the sterilization process, pectin methylesterase (PME) of raw material and soluble pectin at various fermentation times were studied. Knowing the

factors that affect the formation of alcohol during the fermentation will be useful for the assessment of consumer safety and quality of FPBs.

## Conclusions

The current study concludes that the tested FPBs of Thailand region are safer for human consumption except for some of the products that are made from *Morinda* as a major ingredient. Regarding minerals, iron content was higher in some products, but the quantity of the daily consumption influences the lethal dosage of the iron in FPBs. Consumption of 30 ml of FPB per day is the recommended serving size. Based on this, all the products tested were safe at 30 ml quantity. The outcome of current work was from representative products only. Thus, the further detailed study of every product is necessary before the approval of the human consumption. Moreover, since the products are from the fermentation process, assessment of microbiological safety also plays a vital role in product approval.

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