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Research Article

FTIR and multivariate analysis of total phenolic content, antioxidant and anti-amylase activities of extracts and milk of *Glycine max* L. and *Phaseolus vulgaris* L.

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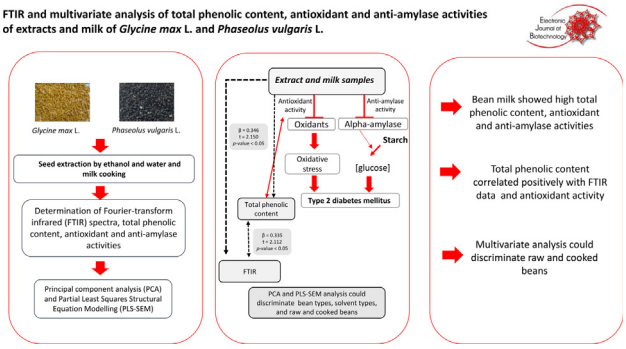
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Alpha-amylase is a digestive enzyme which hydrolyses the glycosidic bonds in polysaccharides into monosaccharides. Inhibition of α-amylase can help to retard carbohydrate digestion in the small intestine and decrease blood glucose level. The effect of solvent extraction and milk processing on total phenolic content (TPC), antioxidant and anti-amylase activities, and Fourier-transform infrared (FTIR) spectra of *Glycine max* L. and *Phaseolus vulgaris* L. were estimated by Principal Component Analysis (PCA) and Partial Least Squares Structural Equation Modelling (PLS-SEM).
Results: The result showed that the aqueous extract of *P. vulgaris* L. had the greatest antioxidant activity and anti-amylase activity, while the ethanol extract of *G. max* L. showed the greatest TPC. Interestingly, the milk of *P. vulgaris* L. seed showed the greatest level of TPC, antioxidant activity and anti-amylase activity. Mixed milk of *G. max* L. and *P. vulgaris* L. (8:2 v/v) showed high anti-amylase activity, while the mixed milk (5:5 v/v) showed high TPC and antioxidant activity. The FTIR result showed five wavenumber ranges associated with functional groups of phenolic compounds. Moreover, the PLS-SEM result revealed a significant positive correlation between TCP and FTIR data ($\beta = 0.335$, $t = 2.112$, $p\text{-value} < 0.05$), and between TCP and antioxidant activity ($\beta = 0.346$, $t = 2.150$, $p\text{-value} < 0.05$).
Conclusions: The result indicated that the solvent types and cooked beans could affect the biological activities and chemical content. The application of chemical content, biological activities, and FTIR technique combined with PLS-SEM and PCA analysis could discriminate bean types, solvent types, and between raw and cooked beans.

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1. Introduction

Diabetes mellitus is a significant threat to global human health and is considered one of the major noncommunicable diseases (NCDs). In 2017, global prevalence and death were 476 million and 1.37 million of both type 1 diabetes and type 2 diabetes, with an increasing trend to 570.9 million and 1.59 million in 2025, respectively [1]. Moreover, it has been reported that globally people with diabetes numbered 436 million in 2019, and this is believed to increase to 700 million in 2045 [2]. Of these, type 2 diabetes accounts for approximately 90% of total diabetics [2], and this has a rising trend which is similar to total diabetes [1]. There are several risk factors associated with type 2 diabetes, such as marriage, low education, genetics, old age, obesity, high systolic blood pressure, low HDL, high triglycerides [3], and modern lifestyles (i.e. eating junk foods, and having low physical activities) [1]. To date, several reports attempt to study methods for the prevention and control of type 2 diabetes, one interesting method is to delay the breakdown of carbohydrate in the human digestive system. Alpha-amylase is a digestive enzyme that hydrolyses glycosidic bonds in polysaccharide into monosaccharides (i.e. glucose, fructose, and maltose) [4]. Thus, inhibition of α-amylase can help to retard carbohydrate digestion in the small intestine and decrease blood glucose level [5]. Interestingly, it has been reported that plants can be a natural source for α-amylase inhibitors. However, the processing of plants as food, beverages and cosmetics may have an effect on chemical contents and biological activities. Therefore, this study focused on seed extracts and milk produced from *Glycine* max L. (soybean) and *Phaseolus vulgaris* L. (black bean).

G. max L., known as soybean, is one of the major food legumes in the Fabaceae family, which is a native plant in Thailand [6]. *G. max* L. is popularly used as an important protein and lipid source (e.g. cooking oil, protein-rich foods, flavour enhancer and beverages), and has several bioactive agents (i.e. saponins, phytosterols, isoflavones, and phytates) involving risk reduction of several diseases (i.e. cardiovascular diseases, diabetes, cancer, and osteoporosis) [7,8]. Soybean is commonly processed into different products as functional foods such as soy flour, soymilk, sauce, miso, meat, and tofu [9]. Soymilk is widely consumed as a healthy beverage and is frequently consumed in substitute of cow's milk for people with lactose intolerance [10].

P. vulgaris L., also known as black bean, is a major and common bean constituted of high nutrients and phytochemicals (i.e. vitamins, minerals, proteins, carbohydrates, anthocyanins, polyphenols and flavonoids) [11]. It is widely consumed in Thailand and used as a main ingredient in foods. Nowadays, milk from *P. vulgaris* L. (i.e. soybean, red haricot, pinto and yellow kidney beans) are increasingly consumed as an alternative to dairy milk, which are cholesterol free, lactose free, low in fat, and high in nutrients with health-promoting benefits [10,12]. Bean milk is processed from several steps, namely soaking, dehulling, milk extraction and heat treatment, which can have an effect on its nutrients and phytochemical composition [12]. Although food production from soybean is very popular in Thailand, it is insufficient for the demand for human consumption of bean products. Recently, there is an attempt to apply black bean in producing soft tofu instead of soybean [13]. Therefore, it is essential for the development of healthy food products from other beans to respond to costumer's needs. Previously, it has been described that black bean has a high level of proteins with antioxidant and anti-inflammatory activities capable of the diminishment of several metabolic diseases (i.e. diabetes and coronary heart disease) [14]. Similarly, it has been shown that the consumption of black beans with meals can help to decrease postprandial metabolism, oxidative stress, and inflammatory responses in adults with metabolic syndrome [15]. Moreover, the seed coating of black bean has been reported to contain phytochemicals showing health-promoting properties and antiproliferative activities [16].

Nowadays, there are many analytical tools (i.e. gas chromatography/mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC)) which have been developed to understand the composition of biological materials [17,18]. Among these, Fourier-transform infrared (FTIR) spectroscopy is an analytical technique which is capable of fast, cheap, and accurate, analysis using small sample sizes, and without using any reagents [19].

Although there are many reports about using FTIR for the estimation of food elements, there is a little knowledge on the anti-diabetic property and chemical composition of *G. max* L. and *P. vulgaris* L before and after heating processing. Therefore, this study focused on the total phenolic content, antioxidant and anti-amylase activities of extracts and milk from soybean and black bean. Moreover, the FTIR tool was used to identify functional groups of extracts from *G. max* L. and *P. vulgaris* L. Further to this, principal component analysis (PCA) and Partial Least Squares Structural Equation Modelling (PLS-SEM) were applied to analyze the relationship between FTIR spectra, total phenolic content, antioxidant and anti-amylase activities of the extracts and milk products of *G.* max and *P. vulgaris* seeds. This knowledge can help to indicate ideas for the health promotion of raw and cooked beans.

2. Methods and materials

2.1. Chemicals

The 3,5-dinitrosalicylic acid, acarbose and 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) were provided by Sigma. Sodium potassium tartrate, gallic acid and absolute ethanol were provided from Sigma-Aldrich. Folin–Ciocalteu's phenol reagent and α-amylase from porcine pancreas were provided by Merck. Potassium persulfate was purchased from Ajax Finechem.

2.2. Preparation and extraction of sample

G. max L. and *P. vulgaris* L. were purchased from a supermarket in Pathum Thani province of Thailand, and cleaned with water, then dried at 60°C for 48 h. The dried seeds were finely ground by a homogenizer. Each powder sample (10 g) was extracted in 250 ml solvent (ethanol and water) by stirring and incubation at 45°C for 24 h. Then, each extract was sieved by a filter cloth. The sample filtrate was concentrated by an evaporator (IKAa RV10), and adjusted to final a concentration of 100 mg/ml, then kept at −20°C [19]. Each sample was extracted by each solvent in duplicate (n = 4).

For milk preparation, the powder seeds of *G. max* L. and *P. vulgaris* L. were mixed with water at a ratio of 1:6, and boiled with continuous stirring at 85°C for 20 min and left to cool to room temperature. After that, the supernatant was filtered through a filter cloth. Two formulations of milk were prepared by mixing milk of *G. max* L. and *P. vulgaris* L. at a ratio of 8 ml: 2 ml and 5 ml: 5 ml, respectively. Each milk sample was prepared in duplicate (n = 8).

2.3. Antioxidant activity

The ABTS assay was used to detect antioxidant activity based on the mechanism of scavenging $ABTS^{\bullet+}$ radicals, which was prepared from 7 mM ABTS (10 ml) and 140 mM potassium persulfate (179 µl) in dark conditions at room temperature for 16 h. Briefly, the diluted $ABTS^{\bullet+}$ (3.9 µl) with an absorbance of 0.700 ± 0.050 at 734 nm was reacted with each sample extract (20 µl) in the dark at room temperature for 6 min [19]. All of the samples were performed for three times (n = 36). Percent inhibition of the $ABTS^{\bullet+}$ scavenging activity was measured using **Equation 1**.

$$ABTS^{\cdot+}\ \text{scavenging activity}(\%) = ((A_{ABTS} - A_{sample})/A_{ABTS}) \times 100 \quad \text{Equation1}$$

where A_{ABTS} was the absorbance of the diluted $ABTS^{\bullet+}$ solution and A_{sample} was the absorbance of each extract reacted with the diluted $ABTS^{\bullet+}$ solution.

The percent inhibition of $ABTS^{\bullet+}$ scavenging activity of each sample at a 1:2 serial dilution was used to generate a simple linear graph for estimating 50% effective concentration (EC_{50}), which was effective concentration for 50% $ABTS^{\bullet+}$ scavenging activity.

2.4. Total phenolic content

Total phenolic content was spectrophotometrically measured by the Folin–Ciocalteu test. Approximately, 300 µL of each crude extract was combined and gently mixed with 1.5 ml of Folin–Ciocalteu reagent, and incubated for 5 min at a room temperature. After that, 1.2 ml of sodium carbonate (7.5% w/v) was gently mixed and allowed to stand for 30 min at the room temperature [19]. The absorbance of the reaction was detected using a spectrophotometer (Model T60UV) at 765 nm. All of the samples were performed for two times (n = 24). Total phenolic content was calculated from a calibration curve of gallic acid (0–1 mg/mL) and expressed in gallic acid equivalents per g extract.

2.5. Anti-amylase activity

Anti-amylase activity was measured by a colorimetric method. Each reaction was performed by mixing each extract (100 µl) with 1.5 units/ml alpha-amylase (100 µl), and allowed to stand at 37°C for 10 min. About 100 ml of color reagent solution (96 mM 3,5-dinitrosalicylic acid (20 ml), 5.3 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and distilled water (12 ml)) were then added and allowed to stand at 85°C for 15 min [19]. Distilled water (900 µl) was added, and its absorbance was measured at 540 nm. All of the samples were performed for two times (n = 24). Acarbose (0–1 mg/ml) was selected as a positive control. The percentage of anti-amylase activity was calculated using **Equation 2**.

$$\%\text{Anti} - \text{amylase activity} = [1 - (A_{sample} - A_{sample\_blank})/(A_{water} - A_{water\_blank})] \times 100 \quad \text{Equation2}$$

A_{sample_blank} and A_{sample} represented the absorbance of each sample without amylase enzyme and with the enzyme, respectively.

A_{water_blank} and A_{water} represented the absorbance of distilled water without amylase enzyme and with amylase enzyme, respectively.

The EC_{50}, which was the effective concentration for 50% anti-amylase inhibition, was calculated from the above description.

2.6. Fourier-transform infrared spectra (FTIR)

Fourier-transform infrared spectrophotometry (FTIR) was performed to identify functional groups of the active components in plant samples. Each extract was loaded on plate in FTIR spectroscope (PerkinElmer spectrum IR version 10.6.0) and collected across the wavenumber range of 550 to 4,000 cm^{-1} and at a resolution of 4 cm^{-1}. The FTIR spectra were measured in duplicate. Functional groups predicted in aqueous and ethanol extracts of *G. max* L. and *P. vulgaris* L., and their milk samples were compared with previous reports [20,21,22,23].

2.7. Statistical analysis

A descriptive analysis was used to explain total phenolic content, antioxidant activity and anti-amylase activity. A one-way analysis of variance (ANOVA) was used to detect differentiation of total phenolic content, antioxidant activity and anti-amylase activity among mean from different sample groups. The significant value was performed at *p-value* < 0.05. The statistical analyses were carried out by PSPP program version 0.10.5 (the Free Software Foundation) [24]. Principal component analysis (PCA) was carried out by the paleontological statistic program version 3.16 [25]. The Partial Least Squares Structural Equation Modelling (PLS-SEM) implemented in Smart PLS version 3 was performed at 10,000 bootstraps [26].

3. Result and discussion

In this study, the result showed that total phenolic content was found the highest in ethanol extract of *G. max* L. (1.10 ± 0.07 mg gallic acid/ mg extract), followed by ethanol extract of *P. vulgaris* L. (0.92 ± 0.05 mg gallic acid/mg extract), aqueous extract of *P. vulgaris* L., and aqueous extract of *G. max* L., respectively. The antioxidant activity was found the highest in aqueous extract of *P. vulgaris* L. ($1/EC_{50}$ = 0.0097 ± 0.0029), followed by ethanol extract of *P. vulgaris* L. ($1/EC_{50}$ = 0.0094 ± 0.0019), ethanol extract of *G. max* L. ($1/EC_{50}$ = 0.0035 ± 0.0006), and aqueous extract of *G. max* L. ($1/EC_{50}$ = 0.0026 ± 0.0005), respectively. The anti-amylase activity was found aqueous extract of *P. vulgaris* L. ($1/EC_{50}$ = 0.0389 ± 0.0065), followed by ethanol extract of *P. vulgaris* L. ($1/EC_{50}$ = 0.0263 ± 0.0100), ethanol extract of *G. max* L. ($1/EC_{50}$ = 0.0149 ± 0.0062), and aqueous extract of *G. max* L. ($1/EC_{50}$ = 0.0114 ± 0.0042), respectively (Table 1). Corresponding with the previous report, beans with dark seed coats (i.e. kidney beans) had higher total phenolic content and antioxidant activity in comparison with those with pale-colored seed coats e.g. soybeans [27].

For milk from *G. max* L. and *P. vulgaris* L., total phenolic content was found the highest value in the milk from *P. vulgaris* L. (1.37 ± 0.26 mg gallic/mg dried weight), followed by the mixed milk formula 2 (0.91 ± 0.03 mg gallic/mg dried weight), the milk from *G. max* L. (0.92 ± 0.06 mg gallic/mg dried weight), and the mixed milk formula 1 (0.85 ± 0.08 mg gallic/mg dried weight) (Table 1). In previous studies, it has been reported that bean milk had decreased phytochemicals and fibers, while it had greater protein digestibility [12]. Moreover, *P. vulgaris* L. extracted with water–ethanol 50% cosolvent showed the best value of antioxidant activity and tyrosinase inhibitory activity [28]. Similarly, flavonoids from *P. vulgaris* seed coats have been isolated and provide antioxidant activity [29].

Table 1
Total phenolic content, antioxidant activity and anti-amylase activity of aqueous and ethanol extracts of *G. max* L. and *P. Vulgaris* L.

Samples	Types of samples	Total phenolic content (mg gallic acid/mg extract)*	Antioxidant activity		Anti-amylase activity	
			EC_{50} (mg/ml)	$1/EC_{50}$	EC_{50} (mg/ml)	$1/EC_{50}$
G. max L.	Ethanol	1.10 ± 0.07	293.83 ± 55.95	0.0035 ± 0.0006	74.62 ± 24.21	0.0149 ± 0.0062
	Aqueous	0.45 ± 0.10	388.78 ± 64.99	0.0026 ± 0.0005	96.57 ± 37.23	0.0114 ± 0.0042
	Milk	0.92 ± 0.06	159.40 ± 46.88	0.0067 ± 0.0023	116.62 ± 60.83	0.0102 ± 0.0048
P. vulgaris L.	Ethanol	0.92 ± 0.05	110.17 ± 22.24	0.0094 ± 0.0019	42.31 ± 14.98	0.0263 ± 0.0100
	Aqueous	0.79 ± 0.20	110.19 ± 33.02	0.0097 ± 0.0029	26.16 ± 3.75	0.0389 ± 0.0065
	Milk	1.37 ± 0.26	61.13 ± 9.55	0.0167 ± 0.0026	50.15 ± 5.97	0.0202 ± 0.0025
Formula 1 (8:2)	Mixed milk	0.85 ± 0.08	84.22 ± 39.38	0.0142 ± 0.0066	68.55 ± 14.80	0.0151 ± 0.0032
Formula 2 (5:5)		0.91 ± 0.03	68.20 ± 12.37	0.0151 ± 0.0029	105.41 ± 14.53	0.0096 ± 0.0014
P-value		0.000[a]		0.000[b]		0.000[c]
P-value		0.001[d]		0.001[e]		0.023[f]

Note: [a,b,c] Differentiation of total phenolic content, antioxidant and anti-amylase activities was significantly found among 8 sample groups namely *G. max* L. ethanol extracts, *P. vulgaris* L. ethanol extracts, *G. max* L. aqueous extract, *P. vulgaris* aqueous extract, *G.* max milk, *P. vulgaris* milk, mixed milk formula 1 (*G.* max milk: *P. vulgaris* milk in ratio of 8:2) and mixed milk formula 2 (*G.* max milk: *P. vulgaris* milk in ratio of 5:5) at *P-value* < 0.05.
[d,e,f] Differentiation of total phenolic content, antioxidant and anti-amylase activities was significantly found among 3 sample groups namely ethanol extracts, aqueous extract, and milk at *P-value* < 0.05.
*Total phenolic content of milk and mixed milk was expressed as mg gallic/mg dried weight.

Additionally, it has been reported that 80% methanol extract of *G. max* L. consists of aldehydes, ketones, alcohols, carboxylic acids, esters, alkanes, heterocyclic compounds, phenolic compound, sugar moiety, ether, amide, alkene and fatty acid ester [30]. It has been reported that phenolic-rich extracts of soybean show α-amylase and α-glucosidase inhibitory activity involving against type 2 diabetes [31]. Interestingly, it has been reported that the cooking of seeds of *P. vulgaris* L. did not have a negative effect on its antioxidant activity [32]. However, it has been reported that phenolics, tannin and phytate were decreased in beans after cooking [33]. This is consistent with previous studies in which raw black and red kidney bean showed the greatest antioxidant activity which decreased after cooking [34]. The reduction of antioxidant activity and total phenolic content can be influenced by cooking procedures (e.g. heat from boiling in water) [35]. In general, cooking of beans by heating can decrease antioxidant activity, which correlates with a decreasing content of phenolics and tannins [36]. However, soaking and roasting can influence phenolic, flavonoid and antioxidant contents in dry beans. For example, soaking and cooking of dry bean can increase tannin, catechin and polyphenol content, while phenolics increase after toasting, and the antioxidant activity of dark-colored beans increases after roasting [27].

The total number of FTIR peaks was classified into 8 wavenumber ranges. Of these, there were 5 wavenumber ranges of FTIR peaks namely 3009–3347, 1634–1744, 1540–1550, 1377–1407, and 1048–1160 cm^{-1} corresponding with functional groups of phenolic compounds (O—H and N—H stretch, N—H bending vibrations, C═O bending vibrations, aromaticity, primary or secondary O—H bending (in-plane), phenol or tertiary alcohol (O—H bend and C—O stretching vibrations) shown in Table 2. For a comparison of the FTIR spectra of the extracts and their milk mixture, differentiation of the FTIR patterns among ethanol extracts, aqueous extracts and milk samples was found (Fig. 1). From the FTIR spectra, it indicated that the ethanol extracts of *G. max* L. and *P. vulgaris* L. showed high amounts of FTIR peaks, which corresponded to the presence of high total phenolic content in the extracts.

For PLS-SEM analysis, path coefficients (standardized beta, β) were calculated from the partial least squares structural equation to reveal relative values between independent and dependent variables. The result showed that a positive relationship was significantly found between total phenolic content and FTIR spectra (β = 0.335, t = 2.112, *p-value* < 0.05), and between total phenolic content and antioxidant activity (β = 0.346, t = 2.150, *p-value* < 0.05). Moreover, a negative relationship was significantly found between anti-amylase activity and FTIR spectra (β = -0.469, t = 4.057, *p-value* < 0.05). However, a relationship was insignificantly found between antioxidant activity and FTIR spectra (β = 0.147, t = 0.651, *p-value* > 0.05), between total phenolic content and anti-amylase activity (β = 0.065, t = 0.392, *p-value* > 0.05), and between anti-amylase activity and antioxidant activity (β = 0.157, t = 1.025, *p-value* > 0.05) (Fig. 2). The current findings implied that some phenolic compounds in the samples can act as antioxidants. In corresponding with the earlier report, some phenolic compounds (i.e. ferulic acid, caffeic acid, sinapic acid and *p*-coumaric acid) in beans reveal antioxidant activity [37,38,39,40].

Table 2
Functional groups predicted in aqueous and ethanol extracts of *G. max* L. and *P. vulgaris* L., and their milk samples.

Order number of wavenumber ranges	Wavenumber ranges of FTIR peaks in samples (cm^{-1})	Function groups
1	3009–3347	O—H and N—H stretch
2	2853–2928	CH_2 and CH_3 stretching vibrations
3	1634–1744	N—H bending vibrations, C═O bending vibrations
4	1540–1550	Aromaticity
5	1399–1457	CH_3 lipids/proteins and COO^- of amino acids
6	1377–1407	Primary or secondary O—H bending (in-plane), and phenol or tertiary alcohol (O—H bend)
7	1048–1160	C—O stretching vibrations
8	721–997	C—H bending vibrations

However, it has been reported that some phytochemicals could act as both antioxidant and amylase inhibitors. In general, white bean (*P. vulgaris* L.) contains alpha-amylase inhibitors which can help to decrease the rate of carbohydrate absorption leading to lower blood glucose level and weight loss [41]. Although bioactive compounds which act as both antioxidant and amylase inhibitors have not been reported in any beans, it has been reported in other plants that a new isoflavonoid glycoside or known as iridin A from *Iris germanica* rhizomes shows both α-amylase inhibitory and antioxidant activities [42]. Moreover, FTIR data showed a positive correlation with total phenolic content. Therefore, FTIR data could be applied as a chemical fingerprint in detecting the phenolic compounds of beans.

Interestingly, the principal component analysis (PCA) of chemical content and biological activities demonstrated that the first principal component (PC1) described 51.54% of overall variation

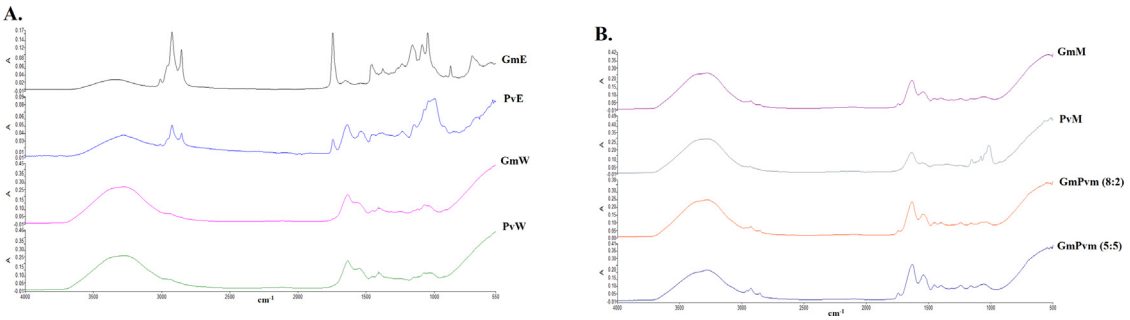

Fig. 1. Example of FTIR spectra of aqueous and ethanol extracts (A) and milk samples (B) of *G. max* L. and *P. vulgaris* L. GmE = ethanol extract of *G. max* L., GmW = aqueous extract of *G. max* L., PvE = ethanol extract of *P. vulgaris* L., PvW = aqueous extract of *P. vulgaris* L., GmM = milk from *G.* max seeds, PvM = milk from *P. vulgaris* L., GmPvm (8:2) = mixed milk from *G. max* L. and *P. vulgaris* L. in ratio of 8:2 ml, GmPvm (5:5) = mixed milk from *G. max* L. and *P. vulgaris* L. in ratio of 5:5 ml.

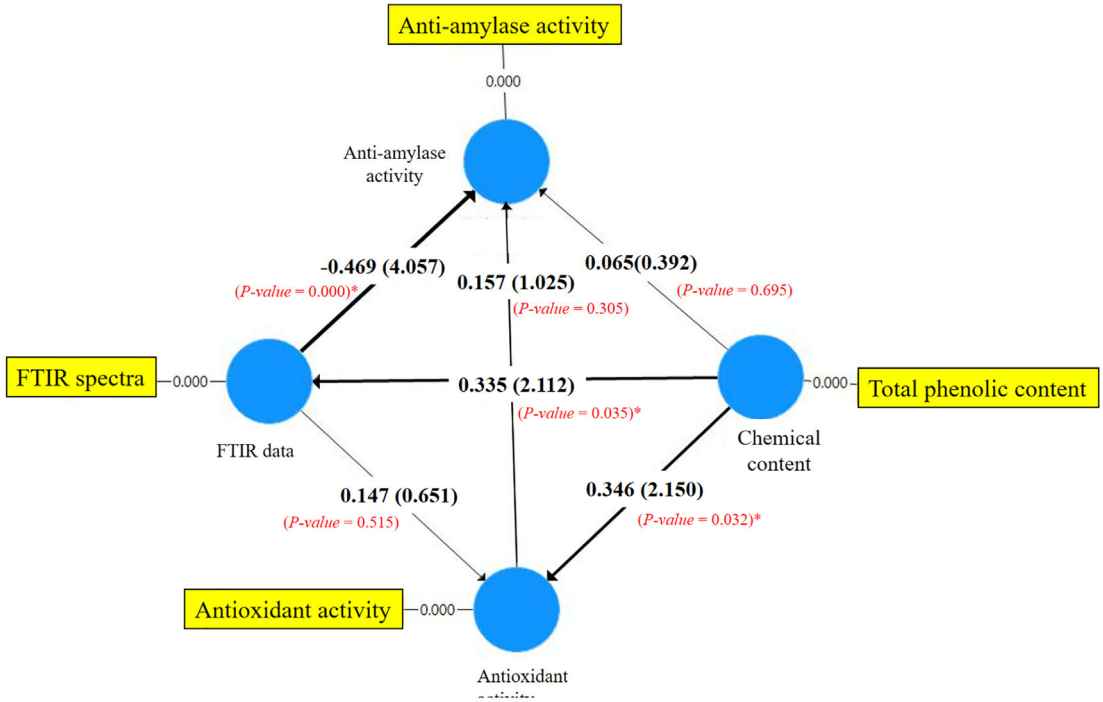

Fig. 2. Path coefficients and T-values calculated from the partial least squares structural equation by using 10,000 bootstrapping (Smart PLS version 3). The arrows showed relative values between total phenolic content and FTIR spectra, between antioxidant activity and anti-amylase activity, between antioxidant activity and FTIR spectra, between total phenolic content and antioxidant activity, between total phenolic content and anti-amylase activity, and between anti-amylase activity and FTIR spectra, respectively. A *p-value* less than 0.05 was a significant level, indicated by asterisk (*).

that consisted of total phenolic content and antioxidant activity, while the second principal component (PC2) described 32.46% of overall variation that consisted of anti-amylase activity (Fig. 3A). Moreover, it indicated that the aqueous extract of *P. vulgaris* L. had the greatest antioxidant activity and anti-amylase activity, while ethanol extract of *G. max* L., milk and mixed milk from *P. vulgaris* L. and *G. max* L. had high total phenolic content.

Moreover, the PCA analysis based on FTIR data could discriminate that the PC1 explained 45.17% of overall variation that consisted of GmM, GmW, PvW and PvE samples, while the PC2 explained 30.95% of overall variation that consisted of GmE, PvM, GmPvm(8:2) and GmPvm (5:5) samples (Fig. 3B). The result showed that the mixed milk samples were near several wavenumber ranges (numbers 1–8, described in Table 2) on positive direction of PC2, followed by ethanol extract and milk from *G. max* L. (Fig. 3B). It indicated that the mixed milk samples, ethanol extract and milk from *G. max* L. showed the highest number of FTIR peaks corresponding with the FTIR spectra shown in Fig. 1A and Fig. 1B.

Moreover, the ethanol extract of *G. max* L. and *P. vulgaris* L. consisted of major peaks at wavenumber ranges of 3009–3347, 2853–2928, 1634–1744, and 721–997 cm^{-1}, the aqueous extract of *G. max* L. and *P. vulgaris* L. consisted of major peaks at wavenumber ranges of 3009–3347, 1540–1550, 1377–1407 and 1048–1160 cm^{-1}. For milk samples, major peaks were found at wavenumber ranges of 3009–3347, 1634–1744, 1377–1407, 1048–1160 and 721–997 cm^{-1}, while mixed milk samples showed major peaks were found at all 8 wavenumber ranges (Fig. 3B).

This study indicated that application of the determination of chemical content and biological activities, FTIR technique combined with PCA analysis was used successfully to discriminate beans from different solvents and bean types. In corresponding

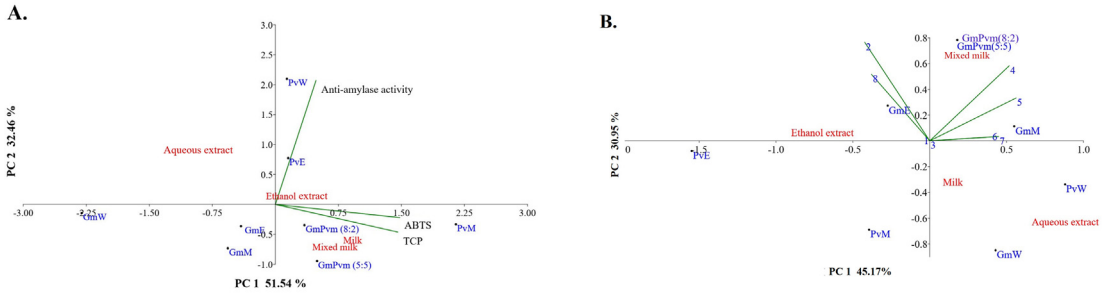

Fig. 3. Principal component analysis (PCA) of total phenolic content, antioxidant activity and anti-amylase activity (A), and FTIR spectra (B) of aqueous and ethanol extracts of soybean (*G.* max) and black bean (*P. vulgaris* L.). Numbers 1–8 were order of wavenumber ranges shown in Table 2. GmE = ethanol extract of *G. max* L., GmW = aqueous extract of *G. max* L., PvE = ethanol extract of *P. vulgaris* L., PvW = aqueous extract of *P. vulgaris* L., GmM = milk from *G.* max seeds, PvM = milk from *P. vulgaris* L., GmPvm (8:2) = mixed milk from *G. max* L. and *P. vulgaris* L. in ratio of 8:2 ml, GmPvm (5:5) = mixed milk from *G. max* L. and *P. vulgaris* L. in ratio of 5:5 ml. For order number of wavenumber ranges, 1 = 3009.8–3347.29 cm^{-1}, 2 = 2853.36–2928.16 cm^{-1}, 3 = 1634.55–1744.66 cm^{-1}, 4 = 1540.56–1550.72 cm^{-1}, 5 = 1399.95–1457.77 cm^{-1}, 6 = 1377.9–1407.33 cm^{-1}, 7 = 1048.68–1160.53 cm^{-1} and 8 = 721.74–997.99 cm^{-1}.

with previous studies, it has been reported that the FTIR technique with PCA analysis can be used to differentiate plant extracts and products [19].

Moreover, an interesting result was found in this study; although milk of *G.* max and *P. vulgaris* seeds was obtained by heat processing at 85°C, it was still in existence of total phenolic content, antioxidant activity and anti-amylase activity at high levels, especially milk of *P. vulgaris* seeds. Moreover, mixing milk of *G. max* L. and *P. vulgaris* L. with equal ratio showed high antioxidant activity, while it showed an effect on lower anti-amylase activity in comparison with those of milk mixed with a higher amount of *G.* max milk. This implies that the seed coat color of *P. vulgaris* L. may have a positive effect on antioxidant activity. It has been reported that seed extract of *P. vulgaris* L. consists of major phenolic compounds namely 3-hydroxybenzoic acid and p-coumaric acid which involve the presence of a strong antioxidant activity and amylase activity [43]. Moreover, solvent extracts from *G. max* L. consist of 37 flavonoids (i.e. hydroxygenistein, epicatechin, procyanidin B2 and cyanidin-3-O-glucoside), which relate with the presence of antioxidant activity [44].

However, some non-phenolic compounds in *G.* max milk may help to improve anti-amylase activity involving the prevention of chronic diseases, such as bioactive peptides from heating processing [45,46]. Therefore, the fresh seeds in this study were found to be functional foods when cooked before consumption.

4. Conclusions

In current study, the highest antioxidant activity and anti-amylase activity were found in the aqueous extract of *P. vulgaris* L., while the highest total phenolic content was found in ethanol extract of *G. max* L. Interestingly, milk samples of *G.* max and *P. vulgaris* seeds were still in possession of total phenolic content, antioxidant activity and anti-amylase activity at high levels. Positive correlation was significantly found between total phenolic content and FTIR spectra, and between total phenolic content and antioxidant activity. In particular, five wavenumber ranges of FTIR peaks associated with functional groups of phenolic compounds (O—H and N—H stretch, N—H bending vibrations, C═O bending vibrations, aromaticity, primary or secondary O—H bending (in-plane), phenol or tertiary alcohol (O—H bend and C—O stretching vibrations). Moreover, a specific wavenumber range of FTIR peaks was found in the extracts of *G. max* L. and milk samples which contained *G. max* L. In the present study, it indicated that the cooked beans can be useful for health promotion. The application of chemical content and biological activities, FTIR technique combined with PCA analysis was used successfully to differentiate between bean types, between solvent types and between raw and cooked beans.

Author contributions

- Study conception and design: S Thummajitsakul; P Paensanit; T Saeieo; J Sirirat; K Silprasit.
- Data collection: S Thummajitsakul; P Paensanit; T Saeieo; J Sirirat; K Silprasit.
- Analysis and interpretation of results: S Thummajitsakul; K Silprasit.
- Draft manuscript preparation: S Thummajitsakul.
- Revision of the results and approval of the final version of the manuscript: S Thummajitsakul.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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