



## Original article

Comparative phytochemical profiling of different soybean (*Glycine max* (L.) Merr) genotypes using GC–MS

Salem S. Alghamdi<sup>a</sup>, Muhammad A. Khan<sup>a</sup>, Ehab H. El-Harty<sup>a</sup>, Megahed H. Ammar<sup>b</sup>, Muhammad Farooq<sup>a,c</sup>, Hussein M. Migdadi<sup>a,\*</sup>

<sup>a</sup> Legume Research Group, Plant Production Department, Faculty of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

<sup>b</sup> Rice Research and Training Center, Sakha 33717, Kafri-El-Sheikh, Egypt

<sup>c</sup> Department of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan

## ARTICLE INFO

## Article history:

Received 4 May 2017

Revised 5 October 2017

Accepted 9 October 2017

Available online 12 October 2017

## Keywords:

*Glycine max*

Phytochemicals

GC–MS

Antioxidants

## ABSTRACT

This study aimed to estimate the proximate, phenolic and flavonoids contents and phytochemicals present in seeds of twenty four soybeans (*Glycine max* (L.) Merr) genotypes to explore their nutritional and medicinal values. Crude protein composition ranged between 35.63 and 43.13% in Argentinian and USA (Clark) genotypes, respectively. Total phenolic content varied from 1.15 to 1.77 mg GAE/g, whereas flavonoids varied from 0.68 to 2.13 mg QE/g. The GC–MS analysis resulted identification of 88 compounds categorized into aldehydes (5), ketones (13), alcohols (5), carboxylic acids (7), esters (13), alkanes (2), heterocyclic compounds (19), phenolic compound (9), sugar moiety (7) ether (4) and amide (3), one Alkene and one fatty acid ester. Indonesian genotypes (Ijen and Indo-1) had the highest phenolic compounds than others genotype having antioxidant activities, while the Australian genotype contains the maximum in esters compounds. The major phytochemicals identified in majority of genotypes were Phenol, 2,6-dimethoxy-, 2-Methoxy-4-vinylphenol, 3,5-Dimethoxyacetophenone, 1,2-cyclopentanedione and Hexadecanoic acid, methyl ester. The presence of phytochemicals with strong pharmacological actions like antimicrobial and antioxidants activities could be considered as sources of quality raw materials for food and pharmaceutical industries. This study further set a platform for isolating and understanding the characteristics of each compound for its pharmacological properties.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most widely grown leguminous crops in the world grow in tropical, subtropical, and temperate climates and providing abundant protein and oil for human diet and animal feeding. Its seeds contain more than 36% protein, 30% carbohydrates, and appreciable amounts of dietary fiber, vitamins, and minerals. It also contains about 20% oil, which makes soybean the most important crop for edible oil production (Lim, 2012). Seeds of soybean have been used in Asia and other parts of the world to prepare a variety of fresh, fermented and

dried foods (Probst and Judd, 1973). Soya-based food products such as tofu, soy milk, soy sauce, miso, etc. have been developed for human consumption while the oil extracted soya meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil has multifarious uses in related industries for the production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics (Gupta, 2012). Recently, use of soy oil as biodiesel has opened up another possibility of renewable sources of energy for industrial uses. As a legume crop, soybean is capable of biological nitrogen fixation and is therefore less dependent on synthetic nitrogen fertilizers (Gupta, 2012).

Soybean contains numerous bioactive phytochemicals such as phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids (Luthria et al., 2007; Lee et al., 2008; Gutierrez et al., 2004) and possesses excellent immune-active effects in the human body (Sakai and Kogiso, 2008). The reported pharmacological properties of soy and its phytochemicals include antioxidant, estrogenic, antidiabetic, antihypercholesterolemic, antihyperlipidemic, antiobesity, antihypertensive, anticancer, antimutagenic,

\* Corresponding author.

E-mail address: [hmigdadi@ksu.edu.sa](mailto:hmigdadi@ksu.edu.sa) (H.M. Migdadi).

Peer review under responsibility of King Saud University.



hepatoprotective, antiosteoporotic, antiviral, bifidogenic, anti-inflammatory, immunomodulatory, neuroprotective, wound healing, antimicrobial, goitrogenic anti-skin aging, anti-photoaging activity and the effects of antinutritional factors (Lim, 2012). To a large extent, these pharmacological attributes of soybean are attributable to the presence of isoflavones in soybean (Messina, 2010).

Due to importance of soybean and its products, it is necessary to investigate chemical composition across various genotypes. Therefore, this study was carried out to estimate the active ingredients including total phenolic acid content (TPC), total flavonoid content (TFC), protein content (PC) across 24 genotypes and Phytochemicals in methanolic extracts were evaluated using GC–MS.

## 2. Materials and methods

### 2.1. Plant materials

Twenty four soybean genotypes were introduced from nine countries (USA, Egypt, Syria, China, India, Pakistan, Indonesia, Argentina and Australia). Name and geographical origin of these genotypes are presented in Table 1. Seeds of these genotypes were grown in Dirab Agriculture Research Station, King Saud University, Riyadh, Kingdom of Saudi Arabia (24°25'49.2"N 46°22'12.5"E) on August, 2014.

### 2.2. Chemical analysis

#### 2.2.1. Proximate composition

The proximate analyses of soybean samples for crude proteins, moisture, total ash, fat and carbohydrate were carried out in triplicate using the methods described in AOAC, (AOAC, 1990). All the proximate values were reported in g/100 g seed sample. The Kjeldahl method was used to estimate protein content by titration and percentage nitrogen in the food samples were calculated according to (Markham, 1942) equation.

#### 2.2.2. Antioxidants determination

Soybean seeds were ground to a fine powder in a blender. A portion of 1 g of powder was extracted in a capped centrifuge tube with 10 ml 80% methanol. The mixture was shaken at 300 rpm at ambient temperature on an orbital shaker for 3 h. The extracts were then centrifuged by at 3000 rpm for 10 min, and the supernatants were transferred to new tubes. Residues were extracted with 5 ml of the same solvent overnight for the 2nd time. Both extracts were combined and stored at 4 °C in the dark. Extractions were performed in 3 replicates. The amount of total phenolic in extracts was determined with the Folin-Ciocalteu reagent using the calibration curve of gallic acid as standard. The total phenolics were expressed as mg/g gallic acid equivalents (GAE). An aliquot (50 µl) of extract was mixed with 250 µl of Folin-Ciocalteu

reagent and 750 µl of 7.5% sodium carbonate. The volume was made up to 5 ml with water and sample was incubated for two hours. The absorbance was measured at 765 nm against distilled water as blank. Aluminum chloride method was used for flavonoid determination using Quercetin equivalent as standard. An aliquot (250 µl) of extract was mixed 1.25 ml of water and 75 µl 5%NaNO<sub>2</sub> added to the flask. After 6 min, 150 µl 10% AlCl<sub>3</sub> was added to the mixture. At the 5th min, 500 µl 1 M NaOH was added and volume made up to 2.5 ml with distilled water. The absorbance was noted at 410 nm using UV–Visible spectrophotometer.

#### 2.2.3. Gas chromatography–mass spectroscopy

The GC–MS analysis of methanolic extract of 24 genotypes were carried out using a TSQ™ 8000 Evo Triple Quadrupole GC–MS/MS (Thermo Fisher Scientific) equipped with a Elite-5 capillary column (30 nm × 0.25 mm ID × 0.25 µm) and mass detector was operated in electron impact (EI) mode with full scan (50–550 amu). Helium was the carriers gas at a flow rate of 1 ml/min. and the injector was operated at 290 °C and the oven temperature was programmed as follows; 50 °C at 8 °C/min to 200 °C (5 min) at 7 °C/min to 290 °C (10 min). The peaks in the chromatogram were identified on the basis of their mass spectra. Interpretation of mass spectrum of GC–MS was done using the database of National Institute Standard and Technology (NIST). The mass spectrum of phytochemicals was compared with the spectrum of known compounds stored in the NIST library.

### 2.3. Data analysis

The data were analyzed for descriptive statistics (mean, standard deviation, coefficient of variability, minimum and maximum values) and principal component analysis using statistical software Past3 program (Hammer et al., 2001).

## 3. Results and discussion

### 3.1. Proximate analysis

Descriptive values of 24 soybean genotypes for proximate analysis (crude protein, ash fat, carbohydrate, and moisture contents), total phenolic and flavonoid contents are presented in Table 2, and the detailed proximate analysis estimates are shown in supplementary Table S1. Crude protein content values ranged between 35.63 in Argentinian to 43.13% in Clark genotypes, with a mean value of 39.02%. Genotypes recorded higher than 40% crude protein were Clark, Indo-1, Indo-black, Ijen, Romal-1, X 30 and 3803. The results revealed significant differences among genotypes in crude protein content, which reflect the variations in genetic background and/or origin. The higher protein content in these genotypes is coherent with those described previously which ranged from 43

**Table 1**  
Name and geographical origin of the twenty four soybean genotypes.

Entry no.	Genotype name	Source/Origin	Entry no.	Genotype name	Origin
1	Admaril	Pakistan	13	Giza 111	Egypt
2	Romal-1	Pakistan	14	Clark	USA
3	NARC-2	Pakistan	15	3803	Syria
4	Williams 82	USA	15	A-1	Australia
5	X 32	Egypt	17	Ijen	Indonesia
6	Giza 22	Egypt	18	Indo-black	Indonesia
7	Giza 21	Egypt	29	Indo-I	Indonesia
8	X2 L 12	Egypt	20	Indo-II	Indonesia
9	Giza 83	Egypt	21	USA-1	USA
10	Crawford	USA	22	Indian	India
11	Giza 35	Egypt	23	Chinese	China
12	X 30	Egypt	24	Argentinian	Argentina

**Table 2**  
Descriptive statistics of chemical composition in 24 soybean genotypes.

	Crude protein (g/100 g)	Moisture (g/100 g)	Ash (g/100 g)	Total Fat (g/100 g)	Carbohydrate (g/100 g)	Total phenolic content (TPC)	Total flavonoid content (TFC)
N	24	24	24	24	24	24	24
Min	35.63	3.08	4.55	16.92	26.11	1.15	0.68
Max	43.13	5.88	6.28	22.94	33.18	1.77	2.13
Mean	39.02	4.90	5.44	21.16	29.48	1.45	1.24
Stand. dev.	2.09	0.65	0.33	1.41	1.86	0.16	0.36
Coeff. Var.	5.35	13.26	6.11	6.68	6.30	11.58	29.32

to 45% (Machado et al., 2008). Zarkadas et al. (1993, 1999) have also reported crude protein values in soybean ranging from 33.67% to 42.11%. Moisture content ranged from 3.08% to 5.88% in Giza 83 and Indo-1 respectively with overall mean 4.90% with no significant difference. Ash content in soybean genotypes ranged from 4.55% and 6.28% with average of 5.44%. Romal-1 genotype had the lowest value of ash (4.88%) and Giza 111 genotype had the highest value 6.28%. The values of moisture and ash obtained were lower than that reported by Monteiro et al. (2004). Total fat also varied from 16.92% to 22.94% with a mean value of 21.16%. The genotype 3803 recorded the highest content while the genotype Indo- black contained the lowest. Soybean is considered to be high in fat content compared to other legumes. It was mentioned that soybean has about 47% of its energy value in fat content (Liu, 1997; Messina, 1999). Our total fat values were more or less close to that of (Guillon and Champ, 2002) who reported total fat ranged between 18 and 22 g/100 g in different soybean genotypes. The carbohydrate content ranged from 26.11% in Clark to 33.18% in Argentinian with an average value of 29.48%.

### 3.2. Total phenolic and flavonoid content

Phenolic and flavonoid compounds, as important phytochemicals, are natural antioxidants found in vegetable, fruits and cereal grains. They have multiple biological functions and can play an essential role in defense against cardiovascular disease, cancer and aging (Karimi et al., 2010). Total phenolic and flavonoids contents of seeds from the 24 genotypes of soybean are presented in supplementary Table S1. Total phenolic and flavonoids content of seeds in all genotypes were significantly different from each other. Among all seed extracts investigated, total phenolic content ranged from 1.15 to 1.77 mg GAE/g with mean of 1.45 mg GAE/g (Table 2). Giza 111 genotype contained the lowest phenolic content (1.15 mg/g) while the Romal-1 had the highest value of 1.7 mg/g. The total flavonoid content ranged from 0.68 to 2.13 mg QE/g (Table 2). TPC and TFC of 24 soybean genotype are presented in Table S1. It has been reported that the phenolic content is strongly linked with antioxidant capacity (Zheng and Wang, 2001; Skerget et al., 2005) and can contribute directly to the antioxidative activities (Duh et al., 1999). Applications of phenolic are spreading rapidly in the food industry to improve the nutritional value and quality of food (Aneta et al., 2007).

### 3.3. GC-MS analysis

A large number of phytochemical were identified in the methanolic extract of 24 soybean genotypes using GC-MS analysis. A total of 88 compounds were identified based on peak area, retention time and molecular formula. A large number of bioactive phytochemicals including phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids were also reported previously for soybean (Luthria et al., 2007; Lee et al., 2008; Gutierrez et al., 2004). The first compound identified at retention time of

3.67 min was carbamide, whereas methyl 10 Trans, 12-cis-octadecadienoate was the last compound which with longest retention time (48.53 min) (Table S2). There were wide variation in the compositions of phytochemicals in 24 soybean genotypes and are shown in supplementary Table S3. Table 3 lists some major phytochemicals and their biological activities in different genotypes of soybean. The phytochemicals identified in 24 genotypes were classified into different groups (Fig. 1). The identified 88 compounds were mainly categorized into aldehydes (5), ketones (13), alcohols (5), carboxylic acids (7), esters (13), alkanes (2), heterocyclic compounds (19), phenolic compound (9), sugar moiety (7), ether (4), amide (3), one Alkene and one fatty acid ester.

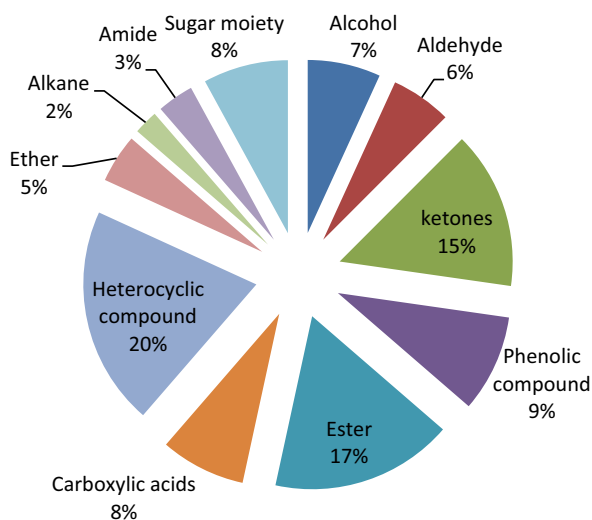
The total ion chromatogram (TIC) of methanol extract of 24 soybean genotypes showing the GC-MS profile of the compounds identified is given (Figs. S1–S24). A typical chromatogram of one soybean genotype is shown in Fig. 2. The GC-MS analyses revealed that the methanolic extract is predominantly composed of heterocyclic compound, ester and phenolic compound. Phenol, 2,6-dimethoxy-, 2-Methoxy-4-vinylphenol, 3,5-Dimethoxyacetophenone, 1,2-cyclopentanedione and Hexadecanoic acid, methyl ester were detected in most of the genotypes. These phytochemicals are responsible for various pharmacological actions like antimicrobial and antioxidants activities (Tapiero et al., 2002). The phytochemicals identified through GC-MS analysis proved to be active in many biological activities as listed in (Table S2). Phytochemicals have been reported to possess potent antioxidant and anti-cancer, anti-carcinogenic, anti-bacterial, anti-viral or anti-inflammatory activities to a greater or lesser extent and play a vital role in plant metabolism (Tapiero et al., 2002; Wei et al., 1999).

The results obtained showed five compounds belongs to aldehyde group namely Methoxy-propanal, Benzeneacetaldehyde, 3,4-dimethylbenzaldehyde, p-Hydroxyphenylglyoxal and Propanal, 2-(benzoyloxy)-, Benzeneacetaldehyde, were detected in 10 genotypes (Table S2). 3-Methoxy-propanal was found in two genotypes (Admaril and Williams 82). 3,4-dimethylbenzaldehyde was detected in three genotypes (Indo- black, Indo- I and Indo -II) whereas p-Hydroxyphenylglyoxal and Propanal, 2-benzoyloxy, were present only in one genotype (Giza 35 and X30 respectively). The genotype Williams 82 recorded the highest number of aldehyde compounds (2). Aldehydes are reported to possess powerful antimicrobial activity because of their highly electronegative arrangement of an aldehyde group conjugated to a carbon to carbon double bond (Moleyar and Narasimham, 1986), suggesting an increase in electronegativity increases the antibacterial activities in those genotypes (Kurita et al., 1979; Kurita et al., 1981). Such electronegative compounds react with vital nitrogen components, e.g. proteins and nucleic acids and consequently inhibit microorganisms.

A total of 13 compounds related to ketone were identified. They included 1,2-propanone, 1-(dimethylamino)-, 1,2-cyclopentanedione, 6-Oxa-bicyclo[3.1.0]hexan-3-one, Dihydroxyacetone, 2-Pyrrolidinone, 1-methyl, 2,4,6-Cycloheptatrien-1-one, 4-methyl-, 3,5-Dimethoxyacetophenone, 2,4-Dihydroxy-2,5-dimethyl-3(2

**Table 3**  
List of important phytochemicals identified in the methanolic seed extract of soybean genotypes by GC–MS.

Compound	Other names	Nature	Activity	RT	MW
22 2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-,(2S-cis)-	Edulan II	Heterocyclic compound		7.58	192
27 1,2-cyclopentanedione		Ketone	Antioxidant	7.98	98
28 Pyran-4-Carboxylic acid, 4-(4-methoxyphenyl)-tetrahydro-		Heterocyclic compound		8.02	236
34 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one		Ketone		9.74	144
36 2H-Pyran-2,6(3H)-dione	Glutaconic anhydride	Heterocyclic compound		10.75	112
39 2-Pyrrolidinone, 1-methyl	M-Pyrol	Ketone		11.86	99
42 2,5-Dimethyl-4-hydroxy-3(2H)-furanone		Ketone		12.28	128
44 Phenol, 2-methoxy-		Phenolic compound	Antimicrobial, Antioxidant, Anti inflammatory, Analgesic	13.83	124
49 4H-Pyran-4-one,3-hydroxy-2-methyl-	Maltol	<u>Heterocyclic compound</u>	Flavor enhancer	14.78	126
50 5-hepten-3-one, 5-methyl-		<u>Ketone compound</u>		15.09	126
52 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one		Heterocyclic compound	Antimicrobial, anti-inflammatory	16.61	144
57 Phenol, 4-ethenyl-, acetate	4-Vinylphenyl acetate	Phenolic compound	Antimicrobial, Antioxidant, Anti inflammatory,	19.29	162
60 Benzofuran, 2,3-dihydro	Coumaran	Heterocyclic compound	Anthelmintic, Anti-inflammatory, Anti-diarrhoeal	20.16	120
62 Benzeneacetaldehyde, 3-methyl	m-tolualdehyde	Aldehyde	Antimicrobial	20.34	120
61 1,2-Benzenediol,3-methoxy-	Pyrocatechol, 3-methoxy	Phenolic compound	Antioxidant	21.01	140
64 2-Methoxy-4-vinylphenol	Phenol, 4-ethenyl-2-methoxy-	Phenolic compound	Antimicrobial, Antioxidant, Anti inflammatory,	23.35	150
68 Phenol, 2,6-dimethoxy-	Pyrogallol 1,3-dimethyl ether	Phenolic compound	Antimicrobial, Antioxidant, Anti inflammatory,	24.99	154
70 Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-	Butylated Hydroxytoluene	Phenolic compound	Antimicrobial,Antioxidant, Anti inflammatory, Analgesic	30.99	220
71 Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenol, 2,4-di-tert-butyl-	Phenolic compound	Antimicrobial,Antioxidant, Anti inflammatory,	31.19	206
72 5-tert-Butyl-1,2,3-benzenetriol	5-tert-Butylpyrogallol	Phenolic compound	Antioxidant,antiseptic antibacterial, antidermatitic fungicide, pesticide	31.91	182
76 3,5-Dimethoxyacetophenone		Ketone	Antioxidant	33.65	180
85 Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	Ester	Antioxidant, Flavor, Hypcholesterolemic, Nematicide	46.13	270



**Fig. 1.** Pie Diagram showing the percentage of phytochemical groups identified in 24 soybean genotypes.

H)-furan-3-one,2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine, Butyrolactone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 5-hepten-3-one, 5-methyl-, were detected. The genotype 3803 and Indo-11 recorded the highest number of ketonic compounds which is eight (8) in

number followed by Giza 35 and USA-1 genotypes having six (6) ketonic compounds each. Ketones might be formed by beta-oxidation of fatty acids, which generated a few important flavor compounds (Yu et al., 2008).

Volatile compounds are also formed through fatty acid metabolism, producing alcohols, acids, and esters. Most alcohols are derived from bioremediation of unsaturated fatty acids, and are also a prerequisite for the formation of long-chain esters. Many alcoholic compounds like 4-methyl-2-haptanol, 1-undecanol alcohol, 1,2,3-Propanetriol, Isosorbide (D-Glucitol, 1,4,3,6-dianhydro) and 1,3-Dioxolane-4-methanol (Glycerol formal) were detected among the tested genotypes (Table S3). 4-methyl-2-haptanol was detected in one genotype (Giza 35) while 1,2,3-Propanetriol was present in nine genotype and Isosorbide was appeared in three genotype (Table S3). The genotype clark recorded the three (3) alcoholic compounds which is highest than others genotypes. Alcohols are known to possess antibacterial against vegetative cells. Glycerol and its derivatives are reported to have bacterial inhibiting effect (Saegeman et al., 2008).

The following seven carboxylic acids namely acetic acid, Acetic acid, 2,2-[oxybis(2,1-ethanedioxy)]bis, 2-Pyridinecarboxylic acid (also called Picolinic acid), Butanoic acid, 4-hydroxy-, Propanedioic acid, Propyl-(also called 2-Propylmalonic acid), Benzoic Acid, Butanoic acid, 4,4-dithiobis[2-amino-,[S-(R\*,R\*)]] were detected (Table S3). Acetic acid was recorded in seven genotypes. 2-Pyridinecarboxylic acid was recorded in five genotypes. Butanoic acid, 4-hydroxy- was recorded in three genotypes while Benzoic Acid was present in one genotype (Table S3). The maximum num-

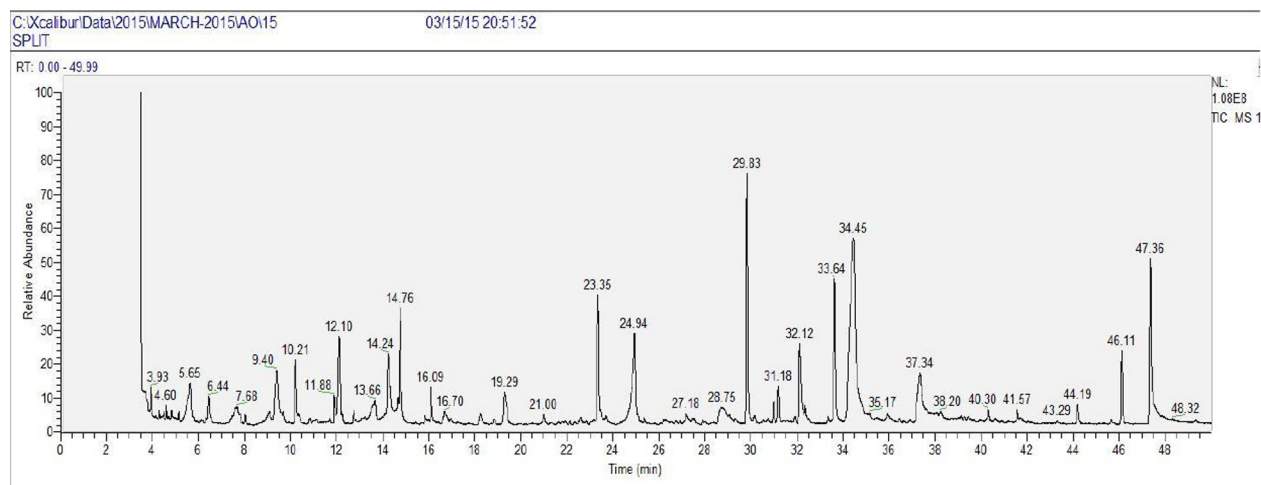


Fig. 2. A typical GC–MS profile of seeds of soybean genotype.

bers of carboxylic acids compounds were found in genotypes X30, Giza 35, Chinese and Argentinian. Each one of these contained two (2) carboxylic compounds.

A total of 13 esters were identified. The Butyrolactone, Acetic acid, 2-(dimethylamino)ethyl ester, Formic acid, 3-methylbut-2-yl ester, pentanoic acid, 2-isopropoxyphenyl ester, Phthalic acid, hex-3-yl-isobutyl ester, Hexadecanoic acid, methyl ester, 5,8,11-heptadecatrienoic acid methyl ester, Phthalic acid, butyl undecyl ester, 1,2-benzenedicarboxylic acid, dibutyl ester, methyl 10-trans, 12-cis-octadecadienoate, 9,12-Octadecadienoic acid(Z,Z)-methyl ester, Benzoic acid, 4-ethoxy-, ethyl ester, and pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl, ester were detected. The genotype A-1 contained maximum 6 esters compounds followed by others genotypes (Romal-1, Giza 83, Clark, 3803 and Argentinian) having five (5) esters compounds. Hexadecanoic acid ethyl ester shows antioxidant, hypocholesterolemic, and nematocidal activities (Priya and Vijaylakshmi, 2011).

Regarding phenolic compound, a total of 9 compounds were detected. 1,2-Benzenediol,3-methoxy-, 5-tert-Butyl-1,2,3-benzenetriol, Phenol, 4-ethenyl-, acetate, 2-Methoxy-4-vinylphenol, Phenol, 2,6-dimethoxy-, Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-, Phenol, 2,4-bis(1,1-dimethylethyl)-, and phenol, 2-methoxy. The distribution of these compounds among genotypes indicated that the genotypes Ijen and Indo-1 contained the highest number of phenolic compounds which is five while the genotypes NARC-2, Giza 35, Clark, Indo-11 and USA-1 contained the four (4) phenolic compounds each. The plant phenolics compounds have great interest to human due to their antioxidative and possible anticarcinogenic activities. The dietary phenolics are considered anti-carcinogens as they are antioxidants, but there is no clear evidence supporting this supposition (Yang et al., 2001). Phenolic may inhibit carcinogenesis by influencing the molecular events in initiation, promotion, and progression stages. Isoflavones and lignans from soybean may affect tumor formation by mediating estrogen-related activities. They also modulate the growth of benign and malignant prostatic epithelial cells *in vitro* (Hedlund et al., 2003).

The following sugar moiety, L-Galactose, 6-deoxy-, 3,4-O-Isopropylidene-d-galactose, 3-O-methyl-d-glucose,a-Methyl-D-mannopyranoside, a-D-Galactopyranoside, methyl were detected among soybean studied genotypes. The relatively remarkable amounts of heterocyclic compounds were identified in 24 genotypes including the important compound like 2,6-diisopropyl-naphthalene, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one,4H-Pyran-4-one,3-hydroxy-2-methyl-, Pyrazine, ethyl-, Oxirane, 2-ethyl-2-methyl, 1H-indazole, 4,5, 6, 7-

tetrahydro, N-aminomorpholine, and Benzofuran, 2,3-dihydro. The distribution of these compounds in 24 genotypes is shown in Table S3. The genotype X30 have four sugars compounds while genotypes Indo-1, Indo-11 and USA-1 have three sugars compounds each. Benzofurans are known to possess anti-oxidant, anti-inflammatory and antimicrobial effect (Kamal et al., 2011).

Taken together, some of the detected compounds are reported to be potential therapeutic agents. Moreover, antimicrobial, antioxidant and anti-inflammatory activities were demonstrated by most compounds in this study indicating that different compounds can display similar activity and this could be due to presence of similar functional groups (Table S2). The presence of various antioxidant and anti-inflammatory compounds may be the reason for the presence of antioxidant properties of soybean methanolic seed extract.

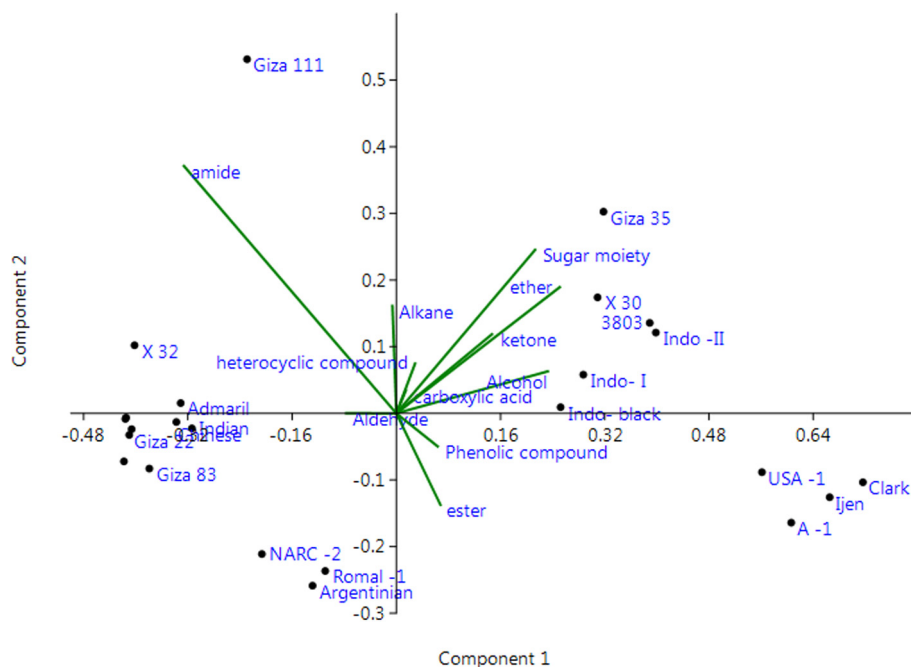
### 3.4. Principal component analysis

The principal component analysis identified three important components. These components analysis explained 78.64% of total variations among genotypes (Table 4 and Fig. 3). For each main component, greater positive coefficients were considered as a significant factor. In the first component which described 59.65% of total variation, phytochemical classes of ether, alcohol, sugar moiety ketone and phenolic compounds compound had high coefficients. These classes have a positive significant loading for the first component and the genotypes showed most variability based

Table 4

Eigen values and proportion of the variance explained for the three principal components of the 24 soybean genotypes based on phytochemical components.

	PC 1	PC 2	PC 3
Eigen values	0.17	0.03	0.02
Percent of variance	59.65	10.63	8.36
Cumulative percentage	59.65	70.28	78.64
Alcohol	0.42	0.11	-0.12
Aldehyde	-0.14	0.00	0.24
Alkane	-0.01	0.29	0.75
Amide	-0.59	0.67	-0.28
Sugar moiety	0.39	0.44	-0.06
Carboxylic acid	0.03	0.06	0.31
Ester	0.12	-0.25	-0.26
Ether	0.45	0.34	0.02
Heterocyclic compound	0.05	0.14	-0.16
Ketone	0.27	0.21	-0.18
Phenolic compound	0.11	-0.09	0.24



**Fig. 3.** Two-dimensional biplot ordination of 24 soybean genotypes on principal component axes according to 11 phytochemical classes.

on this component can be selected for these classes. Genotypes Ijen, Clark, A-1, USA-1, Indo-II, 3803, X 30, Giza 35, Indo-black and Indo-I showed the most variability according to these components. The second component illustrated 10.63% of the total variance and classes with higher scores were amide, sugar moiety, ether, alkane, ketone and carboxylic acid. The genotypes showed most variability based on this component were Giza 111, Giza 35, X 30, X 32, Indo-II and 3803. The third component includes Alkane, Aldehyde, Carboxylic acid and Phenolic compound. The genotypes showed most variability based on this component were Giza 35, X 32. According to these results we can concluded that the most divergent genotypes were Giza 35, X 30, Indo -II and genotype 3803 these genotypes showed positive loading in at least two out of the three components. Utilizing PCA effectively reduces the number of variables needed to classify cultivars and permitted soybean researchers to more easily develop significant relationships between important soybean characteristics. Shin et al. (2012) have classified soybean cultivars using (PCA) of the fatty acid data. The four significant principal components yielded together 81.49% of the total variance, where PC1 mainly contributed to by oleic, linoleic, and gondoic acids, PC2 by stearic, linolenic and arachidic acids, PC3 by behenic and lignoceric acids, and PC4 by palmitic acid. The plots generated between PC1-PC2 and PC3-PC4 segregated soybean cultivars based on fatty acid composition. Moreover PCA has been used effectively to define relationships that exist in fatty acid characterization studies of food lipids due to its ability to manage and interpret large data sets (Brodnjak-Voncina et al., 2005). Although soybean oil has been included in some chemometric studies comparing vegetable oils, soybean cultivars have yet to be extensively classified using multivariate techniques (Zagonel et al., 2004; Brandao et al., 2012).

#### 4. Conclusion

The results demonstrate that soybean genotypes contain variable patterns of total proteins flavonoids, phenolic and various bioactive volatile compounds. On the basis of the mass spectrometry analysis, the studied soybean genotypes are a rich source of bioactive

compounds and moreover the most of the obtained compounds have noticeable antioxidant properties. Phenol, 2,6-dimethoxy-, 2-Methoxy-4-vinylphenol, 3,5-Dimethoxyacetophenone, 1,2-cyclopentanedione, Hexadecanoic acid methyl ester and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one were found in majority of genotypes. The research indicated that majority of soybean genotype are a source of bioactive compounds with antioxidant, anti-inflammatory, antimicrobial and other functions. However, the genotypes Ijen and Indo-1 contributed more phenolic compound than others genotype. Genotype A-1 was contained the maximum compound in esters compounds. From ketone compounds, the genotypes 3803 and Indo-11 contribute maximum compounds. In heterocyclic compound, Giza 111 contribute maximum compounds. Some genotypes may have therapeutic potential and may serve as a potential source in drug development as a health supplement. This study also further set a platform for isolating and understanding the characteristics of each compound for its pharmacological properties.

#### Acknowledgments

The authors extend their appreciation to the International Scientific Partnership Program (ISPP) at King Saud University for funding this research work through ISPP# 0085.

#### Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.sjbs.2017.10.014>.

#### References

- Aneta, W., Jan, O., Renate, C., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* 105, 940–949.
- AOAC, 1990. *Official Methods of Analysis of the AOAC*. AOAC International, Washington DC, USA.
- Brandao, L.F., Braga, J.W., Suarez, P.A., 2012. Determination of vegetable oils and fats adulterants in diesel oil by high performance liquid chromatography and multivariate methods. *J Chromatogr.* 1225, 150–157.

- Brodnjak-Voncina, D., Kodba, Z.C., Novic, M., 2005. Multivariate data analysis in classification of vegetable oils characterized by the content of fatty acids. *Chemometr. Intell. Lab Syst.* 75, 31–43.
- Duh, P.D., Tu, Y.Y., Yen, G.C., 1999. Antioxidative activity of water extracts of Harnj Jur (*Chrysanthemum morifolium*). *Food Sci. Technol.* 32, 269–277.
- Guillon, F., Champ, M.M.J., 2002. Carbohydrate fractions of legumes, uses in human nutrition and potential for health. *Br. J. Nutr.* 8, 293–306.
- Gupta, S.K., 2012. *Technological Innovations in Major World Oil Crops*. Springer, New York, USA.
- Gutierrez, E., Wang, T., Fehr, W.R., 2004. Quantification of sphingolipids in soybeans. *J. Am. Oil Chem. Soc.* 81, 737–742.
- Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. PAST, Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 1–9.
- Hedlund, T.E., Johannes, W.U., Miller, G.J., 2003. Soy isoflavonoid equol modulates the growth of benign and malignant prostatic epithelial cells in vitro. *Prostate* 54, 68–78.
- Kamal, M., Shakya, A.K., Jawid, T., 2011. Benzofurans: A new profile of biological activities. *IJMPS*. 1, 1–15.
- Karimi, E., Oskoueian, E., Hendra, R., Jaafar, H.Z.E., 2010. Evaluation of *Crocus sativus* L. Stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules* 15, 6244–6256.
- Kurita, N., Miyaji, M., Kurane, R., Takahara, Y., 1981. Antifungal activity of components of essential oils. *Agric. Biol. Chem.* 45, 945–952.
- Kurita, N., Miyaji, M., Kurane, R., 1979. Antifungal activity and molecular orbital energies of aldehyde compounds from oils of higher plants. *Agric. Biol. Chem.* 43, 2365–2371.
- Lee, S.J., Kim, J.J., Moon, H.I., Ahn, J.K., Chun, S.C., Jung, W.S., Lee, O.K., Chung, I.M., 2008. Analysis of isoflavones and phenolic compounds in Korean soybean *Glycine max* (L.) seeds of different seed weights. *J. Agric. Food Chem.* 56, 2751–2758.
- Lim, T.K., 2012. *Edible Medicinal and Non-Medicinal Plants*, vol 2, Fruits, Springer Science, Business Media B.V.
- Liu, K., 1997. Chemistry and nutritional value of soybean components. In: Liu, K., (Ed.), *Soybeans, Chemistry, Technology and Utilization*. Chapman & Hall, New York, USA, pp. 25–113.
- Luthria, D.L., Biswas, R., Natarajan, S., 2007. Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. *Food Chem.* 105, 325–333.
- Machado, F.P.P., Queróz, J.H., Oliveira, M.G.A., Piovesan, N.D., Peluzio, M.C.G., Costa, N.M.B., et al., 2008. Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. *Food Chem.* 107, 649–655.
- Markham, R., 1942. Distillation apparatus suitable for mikrokjeldahl analysis. *Biochem. J.* 36, 970–1791.
- Messina, M., 2010. Soybean isoflavone exposure does not have feminizing effects on men, a critical examination of the clinical evidence. *Fertil Steril.* 93, 2095–2104.
- Messina, M., 1999. Legumes and soybeans, overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* 70, 439–450.
- Moleyar, V., Narasimham, P., 1986. Antifungal activity of some essential oil components. *Food Microbiol.* 3, 331–336.
- Monteiro, M.R.P., Costa, N.M.B., Oliveira, M.G.A., Pires, C.V., Moreira, M.A., 2004. Qualidade protéica de linhagens de soja com ausência do inibidor de tripsina kunitz e das isoenzimas lipoxigenases. *Rev Nutr.* 17, 195–205.
- Priya, K., Vijaylakshmi, V.K., 2011. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. *New York Sci J.* 4, 16–20.
- Probst, H., Judd, R.W., 1973. Origin, US history and development, and world distribution. In: Caldwell, B.E. (Ed.), *Soybean, Improvement, Production, and Uses*. Amer. Soc. Agronomy, Madison, USA, pp. 1–15.
- Saegeman, V.S.M., Ectors, N.L., Lismont, D., Verduykt, B., Verhaegen, J., 2008. Short and long term bacterial inhibiting effect of high concentration of glycerol used in preservation of skin allografts. *Burns*. 34, 205–211.
- Sakai, T., Kogiso, M., 2008. Soy isoflavones and immunity. *J. Med. Invest.* 55, 167–173.
- Shin, E., Hwang, C.E., Lee, B.W., Kim, H.T., Ko, J.M., Baek, I.Y., Lee, Y., Choi, J., Cho, E.J., Seo, W.T., Cho, K.M., 2012. Chemometric approach to fatty acid profiles in soybean cultivars by principal component analysis (PCA). *Prev. Nutr. Food Sci.* 17, 184–191.
- Skerget, M., Kotnik, P., Hadolin, M., Hras, A.R., Simoncic, M., Knez, Z., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* 89, 191–198.
- Tapiero, H., Tew, K.D., Nguyen, B.G., Mathé, G., 2002. Polyphenols, do they play a role in the prevention of human pathologies? *Biomed. Pharmacother.* 56, 200–207.
- Wei, Y., Liu, Z.Q., Liu, Z.L., 1999. Antioxidant effect of coumarin derivatives on free radical initiated and photosensitized peroxidation of linoleic acid in micelles. *J. Chem. Soc. Perkin Trans. 2*, 969–974.
- Yang, C.S., Landau, J.M., Huang, M.T., Newmark, H.L., 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Ann. Rev. Nutr.*, 381–406.
- Yu, A.N., Sun, B.G., Tian, D.T., Qu, W.Y., 2008. Analysis of volatile compounds in traditional smoke-cured bacon (CSCB) with different fiber coatings using SPME. *Food Chem.* 110, 233–238.
- Zagonel, G.F., Peralta-Zamora, P., Ramos, L.P., 2004. Multivariate monitoring of soybean oil ethanolsis by FTIR. *Talanta* 63, 1021–1025.
- Zarkadas, C.G., Voldeng, H.D., Yu, Z.R., Choi, V., 1999. Assessment of the protein quality of nine northern adapted yellow and brown seed coated soybean cultivars by amino acid analysis. *J. Agric. Food Chem.* 47, 5009–5018.
- Zarkadas, C.G., Yu, Z.R., Voldeng, H.D., 1993. Minero-Amador, A. Assessment of the protein quality of a new high-protein soybean cultivar by amino acid analysis. *J. Agric. Food Chem.* 41, 616–623.
- Zheng, W., Wang, S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 49, 5165–5170.