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CHEMICAL COMPOSITION OF OKARA (BY-PRODUCT OF SOYMILK)

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ABSTRACT

Okara, the soybean residue from soy milk production, contains nutrients and functional components. The chemical composition of okara (by-product of soy milk) was studied. The proximate analysis was found to be 4.37% moisture , 33.64% crude protein , 21.08% total lipid , 4.67% ash, 18.58% crude fiber, 45.03% total dietary fiber and 22.03% total carbohydrate in okara . The ash contained 1183.0 K, 694.20 Ca , 232.90 Mg , 11.97 Fe and 109.60 Na (mg/100g) in okara respectively. The results showed that phenolic compound contents (mg/100g) were pyrogallol (11.94), oleuropin (31.80) and catechein (4.35) and E- vanilic(75.63) respectively. Our results suggest that could be used as a natural ingredient or supplement for functional food preparation.

Key words: fatty acid, HPLC, minerals, okara ,proximate analysis.

INTRODUCTION

Okara is a byproduct obtained during the processing of soybeans for the production of soy milk. It is yellowish white with a neutral, smooth flavor. It consists of an insoluble fraction obtained from the hydrothermal treatment of the crushed soybean. This residue is generally discarded causing a significant environmental problem because it is susceptible to putrefaction due to its high moisture content.This byproduct still contains many beneficial components, which has attracted the increasing current interest in functional foods

(**Aguado ,2010**). From each ton of processed soybeans around seven tons of soymilk are produced and two tons of okara. The latter contains 85 g.100 g^{-1} moisture (wwb, wet weight basis) (**Grizotto *et al*, 2010**).

The main components of okara are protein (40.0%), crude fiber (16.7%),fat (17.3%), ash (3.7%) and carbohydrate (19.3%). Total dietary fiber of okara is comprised 20.74% of which containing 40.13% soluble dietary fiber. The insoluble dietary fiber of okara was hemicellulose, cellulose and lignin (7.31%). (**Elreffaei *et al*.,2014**). Analysis of okara showed that the okara contained 14.13 mg/g of arginine, 17.32 mg/g of leucine, 11.62 mg/g of valine, and 9.06 - mg/g of isoleucine (**Yang *et al*.,2015**) .

Okara in the past was used as animal feed, but recently has been applied to noodles, baked goods, snacks, pickled side dishes, vegetarian additives, and other food products. In addition, previous researches demonstrated that okara could be useful as a functional ingredient with health-promoting attributes (**Katayama and Wilson ,2008**). The use of okara flour can therefore be used in buns and other bakery products to improve the nutritional quality and help in food security (**Pankuku and Singh,2012**).

The aim of this work was to study the chemical composition of okara.

MATERIALS AND METHODS

1. MATERIALS

Preparation of soybean residue (okara)

The soybean seeds were cleaned and washed by floatation to remove all the foreign

materials. The cleaned beans were blanched in hot water for 25 minutes at 100ºC and soybeans have been allowed to soak in water using 5 litres of water to 1kg of beans and then ground. Soybeans were washed with hot (100°C) water . The slurry obtained was mixed and filtered through a muslin cloth to remove the milk and recover the residue called okara.

Okara, a by-product obtained during the processing of soybeans for the production of soy milk, was obtained from Soybean Factory, Food Technology Research Institute, Agriculture Research Center,

Ministry of Agriculture, Giza, Egypt. Okara was dried in an oven at 50 ±1°C for 24 h and powdered using lab grinder. Then stored at -4°C.

2. METHODS

Chemical composition of the dried okara

Chemical composition of dried okara such as moisture, ash , crude protein , crude fiber, total lipid and total carbohydrate (by difference) were determined according to the methods of **A.O.A.C. (2005**).

Determination of fatty acids

1.Separation of fatty acids

Lipid was saponified with ethanolic KOH (20% w/v) for 24 h at room temperature. The aqueous layer was acidified by HCl (20% w/v), and the liberated fatty acids were extracted with diethyl ether **(A.O.A.C., 2005**).

2.Preparation of fatty acids methyl ester

Fatty acids of standards and samples were converted to methyl esters using ethereal solution of diazomethane. Fatty acids were dissolved in 0.5 ml anhydrous diethyl ether, and methylated by dropwise addition of diazomethane solution until the yellow color persisted (**Vogel, 1975**). The mixture was then left at room temperature for 15 min and the solvent was evaporated on a water bath maintained at 60°C. Finally the fatty acid methyl esters were dissolved in pure chloroform and aliquots of this solution were subjected to GLC analysis.

3. Fractionation of okara fatty acids by GLC method

The methyl esters of okara acids , and standard compounds were analyzed by using HP 6890 GC capillary column gas liquid chromatography with a dual flame ionization were carried out on (30 m X 0.32 mm X 0.25μm)DB-225 capillary column, stationary phase (50% cyan propyl phenyl +50% dimethyl polysiloxane). Column temperature: initial temperature was 150°C, the temperature was programmed by increasing the temperature from 150-170°C at the rate of 10°C /min , then increased from 170-192°C at the rate of 5◦C/min, holding for five min and then increased from 192-220°C during 10min , holding three min . The injector and detector temperature were 230°C and 250°C, respectively. Carrier gas : Hydrogen flow rate 40ml/min, and air flow rate was 450 ml/min. Peak identification was established by comparing the retention times obtained with standard

methyl esters. The area under the chromatographic peak were measured with electronic integrator (**A.O.A.C., 2005**).

Determination of amino acids

Acid hydrolysis was carried out according to the method of **Block *et al* .,(1958**). The dried grinded sample (100mg) was hydrolyzed with 6N. HCl (10ml) in a sealed tube at 110 °C in an oven for 24 hours. The excess of HCl was then freed from 1 ml. hydrolyzed under vacuum of 80 °C with occasionally addition of distilled water, then evaporated to dryness . The HCl free residue was dissolved in exact (2ml) of loading buffer (6.2M.,pH2.2).

Chromatographic analysis of phenolic compounds of okara by HPLC

Phenolic compounds were determined by HPLC according to the method of **Goupy *et al.* (1999)** as follows: 5 g or 0.1g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min (HERMLE Z 206 A, Germany) and the supernatant was filtered through a 0.2 μm Millipore then 1-3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with autosamplling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35oC. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Hewllet Packared software.

Chromatographic analysis of flavonoid of okara by HPLC

The flavonoid compounds of okara powder, fractionated and identified by HPLC according to the method of Mattila et al. (2000) as mentioned before at the same condition except that of detector which set at 330 nm.

Determination of minerals content.

Minerals contents for okara were determined according to **A.O.A.C. (2005**) using Atomic Absorption Spectrophotometer (Perkin – Elmer, Model 3300, USA).

Statistical analysis

Data were subjected to the convenient statistical analysis methods. Where, mean and standard error was calculated. Data were analyzed using two way-classifications ANOVA as described by **Snedecore and Chochran (1980**) followed by Duncans multiple comparison tests to find the statistical significant difference between the ten treated groups. Mean separation was done according to the Least Significant Differences (L.S.D 5%) Duncans multiple range tests according to **Waller and Duncan (1969).**

RESULTS AND DISCUSSION

Chemical composition of okara.

Proximate analysis

Data in **Table (1)** showed the proximate analysis of okara. The moisture content was found to be 4.37%. This result is nearly similar to those of **Grizotto *et al.* (2010**) who found it 6.51% in okara. The protein content was found to be 33.64%. This result is slightly lower than that of **Elreffaei *et al* (2014**) who found 40.0% protein and was nearly similar to that found by **Pr´estamo *et al.* (2007**) who found it 33.4% in okara.

Okara contained ash (4.67%) , fat (21.08%), crud fiber (18.58%) and carbohydrate (22.03%). Total dietary fiber of okara was 45.03% . **Grizotto *et al*., 2010** reported that okara contains 35% protein, 17% lipid and 17 to 21 % fiber which is comparable to our results.

Table 1. Proximate analysis of okara (%dry weight).

Chemical composition	Okara %
Moisture	4.37±0.31
Ash	4.67±0.09
Protein	33.64±0.38
Total lipid	21.08±0.23
Crude fiber	18.58±0.08
Total dietary fiber	45.03±0.19
Total carbohydrate	22.03±0.72

Fatty acid contents of okara

Fatty acid contents of okara are shown in **Table (2)** .The results showed that linoleic represents the major content (54.77%) followed by oleic (22.20%) and linolenic (5.49%) respectively. These results indicate that okara has high concentration of essential fatty acids. Okara contains saturated acids (10.84% palmitic ,4.63% stearic , 0.36% arashidic and 0.31% behenic acide) and unsaturated acids (5.49 % linolenic, 54.77% linoleic , 22.20 % oleic and 1.21% vaccinic acid). These results are in agreement with **Penalvo *et al* (2004)** who found that the fatty acid contents are 10.7% palmitic acid ,3.56% stearic acid , 19.6% oleic acid, 55.1% linoleic acid , 7.67% linolenic acid, 1.49% vaccinic acid ,0.32% arachidic acid and 0.36% behenic acid .

Table (2) : Fatty acid contents in okara.

Fatty Acid	Okara%
Palmitic acid (C16:0)	10.84
Stearic acid (C18:0)	4.63
Oleic acid (C18:1) ω9	22.20
Vaccinic acid (C18:1) ω7	1.21
Linoleic acid (C 18:2) ω6	54.77
Linolenic acid (C 18:3) ω3	5.49
Arachidic acid (C20:0)	0.36
Gadolic acid(C 20:1) ω9	0.19
Behenic acid (C 22:0)	0.31

Amino acid contents of okara

Essential amino acid contents of okara are shown in **Table (3).** These results indicate that okara has high concentration of essential amino acids.It contains leucine which represents the major content (9.10%) followed by lysine (6.78%). There are methionine (0.46%) , valine (5.77%), isoleucine (4.38%) ,hisitadine (2.82%) and phenylalanine (3.37%). This result is nearly similar to those of **Yang *et al*. (2015)** who found okara contains leucine (8.2%) , lysine (7.1%) , methionine (0.6%) , valine (5.5%) , isoleucine (4.3%) ,hisitadine (3.2%) and phenylalanine (5.4%). Threonine content was found to be

1.40%. This result is lower than that of **Yang *et al.* (2015**) who found 4.8% threonine.

Table(3) : Chemical composition of essential amino acid of okara

Amino Acid	Okara%
Leucine (Leu)	9.10
Lysine (Lys)	6.78
Threonine (Thr)	1.40
Methionine (Met)	0.46
Valine (Val)	5.77
Isoleucine (Ile)	4.38
Hisitadine (His)	2.82
Phenylalanine (Phe)	3.37

Non-essential amino acid contents of okara are shown in Table (4). The results show glutamic acid represents the major content (18.15%) followed by aspartic acid (11.68%). Okara also contains serine (3.44%) , proline (0.16%) , glycine (9.19%) , arginine (4.42%) , tyrosine(2.50%) and alanine(6.96%). **Li *et al.,*(2013)** reported that okara contains 11.7 % aspartic acid ,5.0% serine and 19.5% glutamic acid which is comparable to our results. Glycine and alanine contents were found to be 9.19% and 6.96% respectively. This result is higher than that of **Yang *et al.* (2015**) who found glycine and alanine contents were 5.2% and 4.9% respectively.

Table(4) : Chemical composition of non- essential amino acid of Okara

Amino Acid	Okara%
Aspartic (Asp)	11.68
Serine (Ser)	3.44
Glutamic (Glu)	18.15
Proline (Pro)	0.16
Glycine (Gly)	9.19
Ammonia (Amm)	9.43
Arginine (Arg)	4.42
Tyrosine (Tyr)	2.50
Alanine (Ala)	6.96

Phenolic compounds of okara.

Phenolic compounds (mg/100g) of okara are shown in **Table (5).** It is clear that the E-vanilic acid is the major component which it was found to be 75.63 followed by oleuropin 31.80, benzoic acid 17.86, ellagic acid 13.50, pyrogallol 11.94 , coumarin 11.56 , protocatchoic acid 7.50 , salycilic acid 7.14 , p-coumaric acid 4.70 , catachein 4.35 , iso-ferulic acid 2.26 , vanillic acid 1.43 and small amount of gallic acid, 4-amino-benzoic acid , chlorogenic acid , catechol, ferulic acid and other compounds.

Table (5). Phenolic compounds of okara

Phenolic compound	mg/100g okara
Pyrogallol	11.94
Gallic acid	0.11
4-amino-benzoic	0.21
Protocatchoic acid	7.50
Catechein	4.35
Catechol	0.53
Chlorogenic	0.21
Epicatechein	0.23
Oleuropin	31.80
Caffeic acid	0.52
Vanillic	1.43
P-Coumaric	4.70
Ferulic	0.90
Iso- Ferulic	2.26
E- Vanilic	75.63
Ellagic	13.50
Benzoic	17.86
3,4,5-Methoxy-cinnamic	0.40
Coumarin	11.56
Salycilic	7.14
Cinnamic	0.22
Caffeine	0.30
Alpha-coumaric	0.30

The results of phenolic compounds are lower than **Naeem *et al.*(2015)** who revealed that caffeic acid , salycillic acid , cinnamic acid and protocatchoic acid were 1.9 ,19.6 , 2.8 and 23.3 mg/100g

respectively, but higher than ellagic acid (6.5 mg/100g) and benzoic acid (9.6mg/100g).

Flavonoid compounds of okara.

Flavonoid compound contents (mg/100g) of okara are shown in **Table (6).** The results showed that the major compound in okara is hespirdin (3.78 mg/100g) followed by hespirtin (1.30 mg/100g).

Table (6): Flavonoid compound contents in okara.

Flavonoid compound	mg/100g okara
Naringin	0.02
Hespirdin	3.78
Rutin	0.33
Rosmarinic	0.05
Quercetrin	0.28
Quercetine	0.27
Hespirtin	1.30
Kaempferol	0.44
Apigenin	0.16

Mineral content of okara

Table (7) showed the ash mineral content of okara (mg/100g).It is clear that potassium represents the high content of okara (1183.0mg/100g) , followed by calcium (694.20mg/100g). Magnesium, sodium and iron were 232.90 , 109.60 and 11.97 mg/100g respectively.

Table 7. Mineral contents in *okara.*

Element	mg/100g
K	1183.0
Ca	694.20
Mg	232.90
Fe	11.97
Na	109.60

Vong and Liu (2016) found that okara contained high potassium (1350 mg /100g) , calcium (428 mg/100g) , magnesium (165mg/100g) , sodium (96 mg /100g) and iron (11 mg/100g).

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التركيب الكيميائى للاوكارا (مخلف من انتاج لبن الصويا)

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قسم الكيمياء الحيويه-كليه الزراعه-جامعه القاهره**

تم دراسه التركيب الكيميائي للاوكارا (مخلف من انتاج لبن الصويا) حيث كانت نسبه الرطوبه 4.3%والبروتين الخام 33.64%و الدهون الكليه 21.08% و الرماد4.67% والالياف الخام 18.58% و الكربوهيدرات الكليه 22.03 % .كما وجد ان الرماد يحتوي علي 1183 بوتاسيوم و694.2 كالسيوم و232.9 ماغنسيوم و109.6 صوديوم محسوبة (مجم / 100جم) على التوالي . كما اظهرت النتائج احتواء الاوكارا على احماض دهنية اساسية واحماض امينية اساسية و مركبات فينولية مثل بيروجالول 11.94 مجم/ 100جم ، اوليروبين 31.80 مجم/ 100جم و كاتيشين 4.35 مجم/ 100جم . هذه النتائج توضح امكانية ادخال الاوكارا والاستفادة منها في التغذية الصحية والوظيفية.

الكلمات الداله: اوكارا, التحليل الكيميائى ،معادن ،فينولات.