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Chemical Composition and Biological Evaluation of Permeated Product (Miso) from Soybean, Barley and Okara

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ABSTRACT

Fermentation process is considering as an important factor in improvement the availability of nutrients. The present investigation are carried out to evaluate the effect of fermented products (miso) prepared from soybean, barley and okara (as soymilk process by products) and its mixtures. Chemical composition, total phenols and antioxidant activity were determined. Also, Biological evaluations of prepared miso samples were studied. Results of chemical composition in all prepared samples showed an observed decrease in protein, fat and carbohydrates, While an increase in ash and fiber were detected. Okara miso sample had the highest level in DPPH radical scavenging activity. While, barley miso sample had the lowest amount of total phenols. Results of biological assay indicated exterminated rats fed on miso samples had an adequate increase in Body weight gain (BWG) in compared to control which group. Moreover, miso samples caused an increase in serum total protein while, there were no significant differences between them. Rats fed on okara miso sample caused a decrease in uric acid. In generates fed on all prepared miso samples caused increase in both of AST and ALT in compared to control group. But, rats fed on all miso samples had increase in serum minerals compared to control one.

Keywords: Fermented products - Miso – Barley – Okara – Liver and kidney functions – Serum minerals contents.

INTRODUCTION

Fermentation process caused to changes in nutritional and chemical which was improve taste, flavor, and functional properties of the products (Nout and Kiers, 2004). Enzymes produced during fermentation affect in protein, fat, and carbohydrates. Fermentation also promotes the synthesis of B group vitamins (Hermana et al., 2001). During fermentation, the fungus produces the enzyme phytase that mobilizes the phytic acid improving bioavailability of minerals especially, iron availability as reported by (Charlotte *et al.,* 2008). Also, the functional properties of soy products are formed as follows: protein is hydrolyzed to amino acids and peptides by proteolytic enzymes, oligosaccharides (stachyose and raffinose) are hydrolyzed to monosaccharides (digestible sugars), phytic acid degraded to inorganic phosphates (Jooyandeh, 2011).

Miso, meaning fermented bean paste, is used as a base for soups or as a flavoring. Varieties of miso are rice miso, barley miso and soybean miso. The production of miso starts with rice, barley, or soybeans that have been soaked, cooked, cooled and inoculated with a mixture of strains of *Aspergillus orzae* and *Aspergillus soyae*. The product is fermented and ripened prior to blending and mashing to form the final product (Liu, 1997).

The soybean plant (*Glycine max*) belongs to the legume family. On average, dry soybean contains roughly 40% protein, 20% oil, 35% carbohydrate and 5% ash (Liu, 2004). Humans can easily digest soy protein products; about 92-100% of soy protein is digestible in humans (Riaz, 1999). Soybeans are high in phytic acid and oxalate substances that can block the uptake of essential minerals namely (calcium, magnesium, iron, copper and zinc) in the intestinal tract. The acceptability of soy bean products may be enhanced by modification or processing methods such as fermentation process (Golbitz, 1995).

Okara was characterized and used as a supplement to enrich dietary fiber in rats. Okara comprised of 43.3%total dietary fiber of which only 55% was soluble, protein (33.4%), fat (19.8%), and ash (3.5%) as said by (Préstamo *et al*., 2007).

In Indonesia, many kinds of Oncom, a traditional unsalted food fermented from pressed peanut cake or Okara (the solid waste of soybean milk production) by Neurospora intermedia, have been developed for consumption (Sastraatmadja *et al.*, 2002).

The most abundant cereal grain in the world is barley, consisting of 49-66% starch, 14-28 % dietary fiber and 9-22% crude protein, varies for different varieties (Kuswanto and Rahayu, 2004). The preparation of many indigenous or traditional fermented foods remains today a household art for the improvement of nutritional value of the food through fermentation (Oscarsson *et al*., 1996). Fermentation barley was improved minerals availability by reducing the phytate content (Charlotte et al., 2008).

The present study aimed to investigate the chemical composition of miso product by from soybean, barley and okara (byproduct of soymilk process), and evaluation of changes during storage period. Moreover, evaluation the effect of fed rats on soybean, barley and okara miso samlpes on body weight gain, liver and kidney functions and serum mineral contents, to assess the potential of soybean, barley and okara miso as a health-promoting effect.

MATERIALS AND METHODS

Materials: *Aspergillus oryzae* was obtained from the laboratory of Food Science and Technology Institute, Korea. *Aspergillus oryzae* was maintained and sub-cultured on malt agar medium and kept at 4^{o}C until use.

Soybean and okara were obtained from Food Technology Research Institute, Agriculture Research Center, Egypt. Barely was obtained from the Institute of Crops Field, Agriculture Research Centre, Giza, Egypt.

Casein, vitamins, minerals, cellulose, and choline were purchased from El-Gomhoria Company, Cairo, Egypt. Starch and corn oil were purchased from local market, Giza, Egypt.

Chemicals: Folin-Ciocalteu phenol reagent (2N), and 1,1- diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Kits used to determine serum glucose, total protein, uric acid, urea, creatinine, ALT, AST and minerals were punched from Gamma-Tread Company, Cairo, Egypt.

Methods

Fungal inoculum: Flasks containing 100g of wheat bran, soaked in distilled water for 1hr. Then, incubated with5 discs of agar cut from the margin of actively growing colony of fungus (*Aspergillus oryzae*) and stored from 2-3 days at 25^{o}C.

Koji preparation: soybean, barley and okara koji were prepared as first step in solid state fermentation. Soybean, barely and okara koji were prepared by the following which procedures described by Chiou *et al*. (1997). The soybean, barely and okara for each type were soaked in (Type of water) for 4 h, them into steam-cooker for 40 min, after that the samples were cooled and incubated with *Aspergillus oryzae* which maintained on wheat bran at ratio of (10: 1, w/w). The soybean, barely and okara for each type were covered with two layers of cheese-cloth and incubated in a bamboo tray at room temperature (26-28^{o}C) for 3 days. The koji was mixed as necessary when the temperature reached 35^{o}C and kept in a 3 cm layer to facilitate aeration and heat dissipation. The prepared koji was stored at 4^{o}C for one week until used. This koji is used as a starter for the production of miso (Kitamoto, 2002).

Soybean and barley preparation: Soybeans and barley were washed and soaked for 12 hrs in tap water at room temperature (26 - 28^{o}C). After soaking, the drained beans were steam-cooked in an autoclave for 20 min. Cooked samples were kept till used to prepared miso. Okara was sterilized in an autoclave for 20 min and kept till used to prepared miso.

Preparation of Miso: it was made from cooked soybean, barley and okara, to prepared 100% for each type. Then, the samples were mixed with different ratio as shown in Table (1)**.** Each type of mixed with salt and stored in glass jar a year till used in experimental biology study.

Table 1. Ingredients of miso (w/w)

Samples	% of ingredients		
	Barely	Soy bean	Okara
Barley(B)	100	--	--
Soy(S)	--	100	--
Okara(O)	--	--	100
S:B(1:1)	50	50	--
B:O(1:1)	50	--	50
B:O(2:1)	66.66	--	33.34
B:O(1:2)	33.34	--	66.66

Barley(B) Soy (S) Okara (O)Soy:barley (S:B)'barley:Okara (B:O)

Biological experiment:

One hundred and eighty adult Sprague-Dawely rats (160g±5 Male and 150g±5 Female) were purchased. Rats were housed in well cages under hygienic condition (22 ± 2°C and 40-60 Relative humidity) at Animal House in Ophthalmology Research Institute Giza, Egypt, and all groups were fed on basal diet according to Reeves *et al,* (1993) for one week as adaptation period. After that the rats were randomly divided into 30 groups (15 male and 15 female each group contained 6 rats) according to the following diet scheme (Table 2).

Table 2. The schematic of the experimental diet.

Items	Basal diet	Miso ratio						
		Barley	Soy	Okara	B:S (1:1)	B:O (1:1)	B:O (2:1)	B:O (1:2)
Control	100	--	--	--	--	--	--	--
5% of miso samples								
G1	95	5	--	--	--	--	--	--
G2	95	--	5	--	--	--	--	--
G3	95	--	--	5	--	--	--	--
G4	95	--	--	--	5	--	--	--
G5	95	--	--	--	--	5	--	--
G6	95	--	--	--	--	--	5	--
G7	95	--	--	--	--	--	--	5
10% of miso samples								
G1	90	10	--	--	--	--	--	--
G2	90	--	10	--	--	--	--	--
G3	90	--	--	10	--	--	--	--
G4	90	--	--	--	10	--	--	--
G5	90	--	--	--	--	10	--	--
G6	90	--	--	--	--	--	10	--
G7	90	--	--	--	--	--	--	10

Barley (B) Soy (S) Okara (O)Soy: barley (S:B)' barley: Okara (B:O)

G1: fed on basal diet + 5% barley miso G2: : fed on basal diet + 5% soy miso G3: : fed on basal diet + 5% okara miso G4: fed on basal diet + 5% (barley + soy) miso G5: : fed on basal diet + 5% barley +okaro (1:1) miso G6: : fed on basal diet + 5% barley +okaro (2:1) miso G7: : fed on basal diet + 5% barley +okaro (1:2) miso

Blood samples were collected from eye plexuses of rats. Them cold in refrigerator for 15 min and centrifuged at 3000 rpm for 15 min to separate the serum. Serum was carefully separated and transferred into dry clean eppendorf tubes and kept frozen at -18°C till analysis. Liver and kidney were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed in saline, then dried using filter paper and weighted.

Finally somatic index was calculated as follows:

Somatic index= (weight of organ/body weight) ×100

Chemical composition analysis:

Moisture, protein, total fat, crude fiber and ash contents were determined according to the (AOAC, 2000). Carbohydrate was determined by the difference. Approximate calorific value products were calculated using the appropriate factors described by (Lawrence 1965). Bold total phenols in all samples were determined by folin-ciocalteau's reagent as described by (Arnous *et al*, 2001). Antioxidant activity % was determined using1 ,1 – diphenyl – 2 – picryl - hydrazyl (DPPH) method as reported by (Brand-Williams, *et al,* 1995).

Biochemical assay: Serum glucose was determined by the method of (Tietz, 1986). Serum uric acid, urea and creatinine determined by the methods of (Tietz, 1990), (Patton and Crouch, 1977) and (Bonsens and Taussky, 1984). Serum AST, ALT and total protein were assayed according to (Reitman and Frankel, 1957 and Henry, 1964).

Minerals in serum: Serum total calcium and phosphorus were estimated calorimetrically by the method given by Weatherburn *et al.* (1982). Serum magnesium was estimated according to the method of Bohuon *et al*. (1962). Serum sodium and potassium contents determined calorimetrically by using methods of (Güder *et al*., 1982 and Tietz, 1976).

Statistical analysis: The statistical analysis was confidant using SPSS, program PC Statistical Software (version 19.0 SPSS Inc. Chicago, USP). Data were analyzed by one way analysis of variance (ANOVA). The least significant differences (LSD) test at ≤ 0.05.

RESULTS AND DISCUSSION

Chemical composition of miso:

Results in Table (3) showed that the chemical composition for different prepared miso samples. Results showed an observed increasing in moisture, ash and crude fiber. While, it showed decrease in protein, fat, carbohydrates and energy. Prepared miso from okara (100%) have the highest decreasing in protein content being (23.82%) prolonged a storage period for 12 month. While, the lowest decreasing was detected in miso prepared product from 100% barley (4.26 %). Changes in fat were observed during a storage ranged between (8.94%) to (32.31%) in miso product contained barley combine with soybean at the ratio of (1:1) and for miso product with barley Combine okara (2:1). Miso soybean had the maximum decrease in carbohydrates (30.86%), followed by miso product prepared from (1:1) barley combine with soybean being (29.79%). Meanwhile, the decreasing of energy was ranged from 16.29 to 23.62 %. During preparation of its koji culture (i.e., solid starter culture), *A. oryzae* secretes a large variety of enzymes namely amylases and proteases when grown on steamed rice (for rice-miso), soybean (for soybean-miso), or barley (for barley-miso). These enzymes are essential for efficient in maceration and degradation of the essential ingredients (Oike *et al.,* 1984). These results are in the line with (Matsuo and Takeuchi, 2003) who reported that soybean miso was a higher increase in soy bean miso than okara miso in chemical composition.

Table 3. Chemical composition of different prepared samples of miso (g/100g)

Treatments	Moisture (g/100g)		Protein (g/100g)		Fat (g/100g)		Ash (g/100g)		Fiber (g/100g)		Carb. (g/100g)		Energy (Kcal)	
	Zero time	A year later	Zero time	A year later	Zero time	A year later	Zero time	A year later	Zero time	A year later	Zero time	A year later	Zero time	A year later
Barley	35.74^{a}	40.40^{a}	12.22^{c}	11.70^{c}	7.67^{e}	5.75^{f}	15.34^{bc}	17.23^{c}	3.08^{b}	3.37^{c}	25.95^{a}	21.55^{a}	221^{e}	185^{d}
Soybean	31.41^{d}	38.51^{b}	19.87^{a}	17.80^{a}	18.01^{a}	14.52^{ab}	11.67^{d}	15.26^{d}	2.07^{d}	2.20^{e}	16.95^{e}	11.72^{c}	309^{a}	249^{a}
Okara	33.49^{bc}	40.05^{ab}	16.67^{b}	12.70^{b}	16.77^{b}	13.42^{c}	11.18^{d}	12.58^{e}	2.55^{c}	3.36^{c}	19.31^{c}	17.89^{b}	295^{b}	243^{a}
Barley:Soybean (1:1)	33.51^{bc}	39.07^{ab}	12.82^{c}	10.49^{c}	15.32^{c}	13.95^{a}	17.75^{a}	21.90^{a}	2.14^{d}	2.28^{e}	18.46^{cd}	12.96^{c}	263^{c}	219^{b}
Barley: Okara (1:1)	31.15^{cd}	39.63^{ab}	15.92^{b}	13.12^{b}	13.62^{d}	10.82^{d}	15.76^{bc}	17.38^{c}	2.08^{d}	2.58^{d}	19.47^{c}	16.47^{b}	264^{c}	216^{b}
Barley: Okara (2:1)	32.56^{cd}	38.40^{b}	10.83^{d}	10.01^{c}	13.71^{d}	9.32^{e}	16.33^{b}	19.73^{b}	4.80^{a}	5.11^{a}	21.76^{b}	17.42^{b}	254^{d}	194^{c}
Barley: Okara (1:2)	35.23^{ab}	40.80^{a}	13.17^{c}	11.18^{c}	15.96^{c}	13.69^{bc}	14.94^{c}	18.13^{c}	3.17^{b}	3.86^{b}	17.54^{de}	12.33^{c}	266^{c}	217^{b}

**** each value in a column followed by the same letter is not significantly different at ($p \geq 0.05$)**

Total phenols and antioxidant activity:

Generally, the results in fig (1) showed that, all miso samples had a high level of antioxidant activity and total phenols. The lowest level of total phenols was found in barley miso being (53.66 mgGA/100g). While, the highest level of total phenols was found in soybean miso (124.53mgGA/100g). The scavenging activity using DPPH for miso samples were ranged from 82.51% to 95.09%. The results in fig_1 indicated also that the highest level of scavenging activity using DPPH was found in Soybean miso, which is known to occur because of the production of daidzein, genistein and melanoidins during fermentation as reported by (Takasak *et al*., 2010 and Wu *et al.,* 2015).

Effect of miso diet on body weight gain (BWG) and organ somatic index:

In the beginning of experiment, there was no significant differences in rats weight between groups, While, there were high significant differences in the final body weight between groups (p≥0.05). Generally, the rats in control group had highest increase in BWG. The male rats fed on miso (barley (B): okara (O), 1:1) in 5 and 10 % showed the lowest increasing of BWG (7.33 and 10.33g,) respectively. While, the female rats fed on 5% miso (B: O, 1:2) and 10% miso (100% soybean) showed the lowest increasing of BWG (8.67 and 9g, respectively). On other hand, the results showed that, liver somatic and kidney somatic index and absolute organs weight were lower in male rats fed on okara miso (100%) compared to control as shown in table (4). While, kidney somatic index were higher in female rats fed on miso samples compared to control. And, liver somatic index were lower in female rats fed on miso contained soy or okara compared to control. There are significant differ as less in liver and kidney weight between groups (p≥0.05). These results are agreed with Préstamo *et al*. (2007) has shown that, the rats fed on basal diet contained (10%) okara had lower BWG than the rats fed on basal diet (100%). According, Wu *et al*. (2015) who reported, in the *in vivo* experiments, mice were fed for 5 weeks with a high fat diet (HFD) supplemented with (10%) adzuki bean miso, (10%) black bean miso or (10%) soybean miso had a decreased in BWG compared to control group. Also, the rats were

decrease in liver weight. Orihashi (2012) who reported that, prevented the cows of fatty liver disease by feeding on diet contained fermented miso for 6 weeks. The hepatoprotective effect of miso may be depend on the fermentation time and vary with experimental spices and feeding period.

Table 4. Effect of fed on prepared miso sample on body weight gain (BWG) on experimental ratio for

Items	5%								10%							
	Control	Barley	Soybean	Okara	B:S (1:1)	B:O (1:1)	B:O (2:1)	B:O (1:2)	Control	Barley	Soybean	Okara	B:S (1:1)	B:O (1:1)	B:O (2:1)	B:O (1:2)
Male																
Initial weight(g)	157.00^{a}	160.00^{a}	163.33^{a}	160.00^{a}	160.00^{a}	165.33^{a}	157.00^{a}	164.00^{a}	160.30^{a}	163.00^{a}	163.30^{a}	167.00^{a}	163.00^{a}	160.00^{a}	164.67^{a}	162.70^{a}
Final weight (g)	188.33^{a}	170.00^{c}	176.33^{c}	171.32^{b}	167.66^{d}	172.66^{c}	184.66^{ab}	182.70^{b}	188.33^{a}	174.00^{d}	179.00^{ab}	182.66^{abc}	182.70^{abc}	170.33^{cd}	180.00^{bc}	183.00^{abc}
BWG (g)	31.33	10	13	11.32	7.66	7.33	27.66	18.70	28.03	11	15.70	15.66	18.70	10.33	15.33	20.30
Kidney	0.91^{ab}	0.85^{ab}	1.00^{a}	0.60^{b}	0.90^{ab}	0.95^{a}	0.82^{ab}	0.90^{ab}	0.91^{a}	0.86^{a}	0.93^{a}	0.92^{a}	0.81^{a}	0.95^{a}	0.92^{a}	0.91^{a}
Kidney relative	0.48^{ab}	0.50^{ab}	0.57^{a}	0.35^{b}	0.54^{a}	0.55^{a}	0.44^{ab}	0.49^{ab}	0.48^{ab}	0.49^{ab}	0.52^{ab}	0.50^{ab}	0.44^{b}	0.56^{a}	0.51^{ab}	0.50^{ab}
Liver	4.46^{ab}	3.66^{cd}	4.86^{a}	3.23^{d}	4.16^{abc}	4.46^{ab}	4.10^{bc}	4.66^{ab}	4.46^{a}	4.06^{ab}	4.20^{ab}	3.40^{b}	4.16^{ab}	4.43^{a}	4.06^{ab}	4.46^{a}
Liver relative	2.37^{abc}	2.15^{cd}	2.76^{a}	1.89^{d}	2.48^{abc}	2.58^{ab}	2.22^{bc}	2.55^{ab}	2.37^{a}	2.33^{a}	2.35^{ab}	1.86^{b}	2.28^{ab}	2.60^{a}	2.26^{ab}	2.44^{a}
Female																
Initial (g)	152.66^{a}	150.00^{a}	150.66^{a}	152.66^{a}	145.33^{a}	150.00^{a}	146.00^{a}	147.33^{a}	152.66^{a}	150.66^{a}	154.00^{a}	150.00^{a}	148.66^{a}	151.66^{a}	147.33^{a}	150.66^{a}
Final (g)	170.00^{a}	166.00^{b}	166.00^{b}	166.00^{b}	156.00^{c}	167.33^{b}	165.00^{b}	156.00^{c}	170.00^{a}	162.00^{b}	163.00^{b}	163.00^{b}	163.33^{b}	164.00^{ab}	163.33^{b}	162.33^{b}
BWG (g)	17.34	16	15.34	13.34	10.67	17.33	19	8.67	17.34	11.34	9	13	14.67	12.34	16	11.67
Kidney	0.94^{b}	1.13^{ab}	1.20^{a}	1.00^{ab}	0.90^{b}	0.98^{ab}	1.00^{ab}	1.00^{ab}	0.94^{c}	1.20^{a}	1.06^{abc}	0.98^{bc}	1.10^{abc}	1.06^{abc}	1.13^{ab}	0.98^{bc}
Kidney relative	0.55^{c}	0.68^{a}	0.72^{a}	0.60^{a}	0.58^{a}	0.59^{a}	0.61^{a}	0.64^{a}	0.55^{c}	0.74^{a}	0.65^{abc}	0.60^{bc}	0.67^{abc}	0.65^{abc}	0.68^{ab}	0.60^{bc}
Liver	5.80^{a}	6.30^{a}	5.20^{c}	5.13^{c}	4.70^{d}	5.20^{c}	5.00^{cd}	4.00^{e}	5.80^{a}	4.90^{b}	4.20^{c}	4.90^{b}	5.86^{a}	5.80^{a}	5.80^{a}	5.80^{a}
Liver relative	3.41^{a}	3.80^{a}	3.13^{bc}	3.09^{bc}	3.01^{c}	3.11^{bc}	3.03^{bc}	2.56^{d}	3.41^{a}	3.02^{b}	2.58^{c}	3.01^{b}	3.59^{a}	3.54^{a}	5.80^{a}	3.57^{a}

Each value in a raw followed by the same letter is not significantly different at ($p \geq 0.05$)
BWG: Body Weight Gain
Barley (B) Soy (S) Okara (O)Soy: barley (S:B)' barley: Okara (B:O)

Effect of fed on miso diets on glucose, liver and kidney functions:

Serum glucose levels in different rat groups were illustrated in Table (5). The results indicated that the serum glucose in rats which fed on 10% of miso was lower than those of other fed on 5% of miso samples at end of experiment. Also, the rats which fed on 10% of miso from barley, soybean, okara and their mixture decreasing the level of serum as glucose in compared with control one. This obtained within those obtained by result Maeda *et al.* (2015) who found that, the rats fed on Hokurikukona 243 which produced by high pressure treatment (HPT) after soaking in unsalted rice *koji miso* had lower blood glucose level than those in control group.

Table 5. Effect of fed on prepared miso samples on blood glucose, liver and kidney functions

Items	5%								10%							
	Control	Barley	Soybean	Okara	B:S (1:1)	B:O (1:1)	B:O (2:1)	B:O (1:2)	Control	Barley	Soybean	Okara	B:S (1:1)	B:O (1:1)	B:O (2:1)	B:O (1:2)
Male																
Glucose (mg/dl)	92.72^{c}	109.23^{a}	75.03^{e}	90.84^{cd}	84.96^{d}	100.93^{b}	109.35^{a}	86.87^{cd}	92.72^{a}	83.73^{c}	71.23^{d}	71.81^{d}	85.41^{bc}	82.06^{c}	90.39^{a}	89.31^{ab}
T. Protein (g/dl)	6.95^{a}	7.36^{a}	8.81^{a}	8.06^{a}	6.45^{a}	7.65^{a}	7.69^{a}	7.26^{a}	6.95^{a}	7.37^{a}	7.29^{a}	7.83^{a}	7.27^{a}	7.69^{a}	7.19^{a}	7.76^{a}
ALT (U/L)	2.23^{d}	4.87^{c}	7.71^{a}	2.45^{d}	7.87^{a}	2.67^{cd}	6.29^{b}	2.09^{d}	2.23^{e}	6.56^{c}	9.44^{ab}	8.85^{b}	10.64^{a}	3.86^{d}	10.74^{a}	4.74^{d}
AST (U/L)	18.55^{d}	47.67^{a}	44.97^{ab}	47.88^{a}	41.39^{b}	47.75^{b}	27.79^{c}	27.51^{c}	18.55^{d}	40.12^{b}	50.49^{a}	35.07^{bc}	42.59^{b}	17.29^{e}	30.88^{cd}	24.84^{de}
Creatinine (mg/dl)	0.50^{a}	0.63^{a}	0.50^{a}	0.46^{a}	0.51^{a}	0.51^{a}	0.54^{a}	0.42^{a}	0.50^{c}	0.65^{a}	0.65^{a}	0.64^{a}	0.63^{ab}	0.55^{b}	0.50^{c}	0.65^{a}
Uric acid (mg/dl)	1.00^{cd}	1.00^{cd}	0.81^{d}	1.59^{b}	1.09^{c}	1.98^{a}	1.62^{b}	0.83^{d}	1.00^{c}	0.95^{c}	1.27^{b}	1.65^{a}	1.84^{a}	1.61^{a}	1.66^{a}	0.97^{c}
Urea (mg/dl)	32.41^{ab}	35.46^{ab}	34.50^{ab}	37.43^{a}	29.48^{b}	32.54^{ab}	35.99^{ab}	37.89^{a}	32.41^{e}	34.04^{de}	52.96^{a}	49.95^{bc}	39.85^{cd}	37.81^{cde}	40.56^{cd}	49.02^{ab}
Female																
Glucose (mg/dl)	83.96^{b}	85.87^{b}	101.08^{a}	87.02^{b}	103.56^{a}	105.54^{a}	102.13^{a}	105.58^{a}	83.96^{b}	76.16^{c}	74.32^{c}	75.83^{c}	96.44^{a}	73.66^{c}	97.13^{a}	83.58^{b}
T.Protein (g/dl)	6.40^{a}	6.80^{a}	7.41^{a}	6.79^{a}	7.25^{a}	7.69^{a}	7.40^{a}	7.12^{a}	6.40^{a}	7.23^{a}	7.25^{a}	6.92^{a}	6.69^{a}	7.53^{a}	7.21^{a}	7.26^{a}
ALT (U/L)	11.25^{a}	10.96^{a}	10.58^{a}	8.64^{ab}	7.12^{b}	11.04^{a}	7.93^{b}	7.40^{b}	11.25^{a}	7.35^{c}	6.51^{c}	11.86^{a}	9.48^{b}	6.09^{c}	5.48^{c}	6.44^{c}
AST (U/L)	28.01^{d}	41.37^{ab}	33.20^{cd}	42.45^{ab}	38.17^{bc}	45.60^{a}	28.34^{d}	32.37^{cd}	28.01^{c}	42.86^{a}	25.30^{cd}	36.36^{b}	40.72^{a}	44.24^{a}	21.85^{d}	24.31^{cd}
Creatinine (mg/dl)	0.45^{ab}	0.49^{a}	0.49^{a}	0.52^{a}	0.37^{b}	0.43^{ab}	0.42^{ab}	0.45^{ab}	0.45^{c}	0.47^{c}	0.57^{b}	0.64^{a}	0.57^{b}	0.65^{a}	0.58^{b}	0.52^{ab}
Uric acid (mg/dl)	0.95^{b}	1.33^{ab}	1.43^{a}	0.96^{b}	1.10^{ab}	1.35^{a}	1.32^{ab}	1.22^{ab}	0.95^{e}	1.70^{a}	1.52^{b}	0.99^{e}	1.59^{b}	1.09^{d}	1.17^{d}	1.36^{c}
Urea (mg/dl)	34.17^{a}	38.13^{a}	37.03^{a}	39.02^{a}	32.95^{a}	32.83^{a}	33.30^{a}	34.48^{a}	34.17^{c}	45.67^{ab}	43.36^{b}	43.28^{b}	34.21^{c}	47.96^{a}	43.32^{b}	47.79^{a}

*** Each value in a raw followed by the same letter is not significantly different at ($p \geq 0.05$)**
Barley (B) Soy (S) Okara (O)Soy: barley (S:B)' barley: Okara (B:O)

Results in Table (5) showed also, that kidney functions namely serum (creatinine, urea and uric acid) in different groups. Feeding rats by 5% different of miso had a similar result in creatinine as well as control one. While, feeding rats on 10% of miso increase cereatinine compared with rats in control group. Rats fed on basal diet with barley miso (5%) had a similar result in uric acid as rats in control group. While, rats fed on barley miso (10%) were decrease in serum uric acid. Also, the miso product from barley and okara at the ratio of (1:2) decreased uric acid in both 5% and 10%. Meanwhile, the female rats which fed on miso at (5 and 10%) were higher that those female rats in control group. Concerning to serum urea, the rats in control groups had the lowest levels of urea in compared with other groups which fed on miso with (5 and 10%) of diet expect the rats in group fed on miso product from barley and soy at the ratio of (1:1) were clearly decreased. These obtained results are disagree with Préstamo *et al.* (2007) who stated that, there were no significant differences in serum uric acid in rats fed on basal diet and rats which fed on basal diet contained (10%) okara.

The results in the same table observed that the liver functions for rats groups fed on miso at the ratio of (5 and 10%) of diet. Addition of miso caused an observed increase for total protein for both of 5 and 10% in compared with control group. There are no significant differences between groups in serum protein. This result in agree not with Préstamo *et al.* (2007) who said that, there were no significant differences in serum total protein in rats fed on basal diet and rats fed on basal diet contained (10%) okara. Rats male in control group have lowest level of ALT and AST. Rats' female had changes between groups' more than male rats. Serum Aline Amino Transferees (ALT) were the highest level for female rats in control group compared with groups fed on miso both 5 and 10% of diet. The female rats fed on miso 5% of diet were higher than female rats in control group for Asparte Amino Transferee (AST). Moreover, the female rats fed on (10%) miso product by 100% soybean and miso of barley and okora were lower than female rats in control group. These results are in the lines with Abd El-Star (2007) who found that, the female rats fed on miso were higher than control in liver functions (ALT and AST) and serum total protein.

Effect of miso diets on serum mineral contents:

Results in Table (6) showed that some serum minerals for experimental rats which fed on miso samples at the ratio of (5 and 10%). Addition of miso by 5 and 10% caused an increase in serum minerals for Na, Mg and Ca compared with control group. for male and female rats. Meanwhile, the K in serum was decrease with the addition of 5% miso to basal diet group in male rats. Although addition of barley miso by 10% of diet decreased in K serum. Soybean miso sample or miso the other mixture with barley and okara increased the amount of serum K for male rats. Concerning to female rats, diet contained 5% miso product barley combine soybean or okara decreased clearly in serum K and diet contained 10% miso increased in serum K.

Table 6. Effect of fed on miso on serum mineral contents (mg/dl)

	5%								10%							
Mineral	**Control**	**Barley**	**Soybean**	**Okara**	**B:S (1:1)**	**B:O (1:1)**	**B:O (2:1)**	**B:O (1:2)**	**Control**	**Barley**	**Soybean**	**Okara**	**B:S (1:1)**	**B:O (1:1)**	**B:O (2:1)**	**B:O (1:2)**
Male																
Na	19.86^{a}	20.04^{a}	19.44^{a}	19.39^{a}	20.52^{a}	20.26^{a}	19.82^{a}	19.55^{a}	19.86^{a}	20.00^{a}	20.50^{a}	20.43^{a}	20.63^{a}	20.66^{a}	20.26^{a}	20.27^{a}
K	386.60^{a}	385.40^{a}	367.20^{c}	377.60^{b}	335.40^{e}	389.60^{a}	385.00^{a}	340.2^{d}	386.60^{b}	361.6^{c}	375.60^{c}	415.8^{a}	393.4^{b}	389.6^{b}	414.00^{a}	360.6^{c}
Mg	3.30^{d}	3.82^{c}	4.68^{b}	4.72^{b}	5.33^{a}	5.50^{a}	4.57^{b}	4.79^{b}	3.30^{c}	4.99^{ab}	4.43^{bc}	5.01^{ab}	6.04^{a}	5.75^{ab}	5.63^{ab}	5.42^{ab}
Ca	6.54^{c}	6.58^{c}	7.96^{a}	7.00^{bc}	7.89^{ab}	7.41^{abc}	6.86^{c}	7.01^{bc}	6.54^{c}	8.14^{ab}	8.36^{ab}	7.30^{bc}	8.67^{a}	7.22^{bc}	7.22^{bc}	7.72^{ab}
P	6.22^{a}	6.24^{a}	6.33^{a}	5.71^{a}	5.82^{b}	6.37^{a}	5.73^{a}	5.83^{a}	6.22^{ab}	6.52^{a}	6.54^{a}	6.63^{a}	6.83^{a}	6.70^{a}	5.93^{b}	6.16^{a}
Female																
Na	19.48^{a}	20.07^{a}	19.79^{a}	20.40^{a}	19.43^{a}	18.92^{a}	19.90^{a}	19.95^{a}	19.48^{a}	20.60^{a}	20.20^{a}	20.80^{a}	20.32^{a}	20.62^{a}	20.50^{a}	20.26^{a}
K	403.93^{c}	436.40^{a}	411.60^{c}	438.53^{a}	401.87^{c}	430.40^{a}	392.33^{d}	387.33^{d}	403.93^{e}	449.47^{a}	431.20^{c}	441.00^{bc}	400.60^{e}	448.33^{ab}	417.30^{d}	398.77^{e}
Mg	4.24^{b}	5.80^{ab}	4.38^{b}	5.94^{ab}	4.29^{b}	6.90^{a}	6.04^{ab}	6.48^{ab}	4.24^{c}	6.04^{abc}	6.64^{ab}	6.18^{ab}	5.28^{bc}	7.55^{a}	7.29^{a}	7.43^{a}
Ca	6.90^{ab}	7.94^{ab}	7.24^{ab}	8.52^{a}	8.91^{a}	8.30^{a}	6.79^{ab}	5.70^{b}	6.90^{ab}	7.89^{ab}	6.26^{b}	8.73^{a}	8.43^{ab}	8.73^{a}	7.13^{ab}	7.82^{ab}
P	6.79^{a}	7.28^{a}	6.37^{a}	5.76^{a}	6.24^{a}	5.88^{a}	5.12^{a}	5.47^{a}	6.79^{a}	7.29^{a}	6.81^{a}	6.83^{a}	6.94^{a}	6.90^{a}	7.02^{a}	7.12^{a}

*** Each value in a raw followed by the same letter is not significantly different at ($p \geq 0.05$)**
Barley (B) Soy (S) Okara (O)Soy: barley (S:B)' barley: Okara (B:O)

Concerning to results of serum phosphorus (P), indicated that the male rats which fed on 5 % miso product prepared from okara (100%), barley and soybean at the ratio of (1:1), barley and okara (2:1) and barley and okara (1:2) had decrease in P in compared with male rats in control group. While, the male rats fed on 10% miso samples which product prepared from barley with okara (2:1 and 1:2) were lower than those male rats in control group for serum P. Female rats fed on 5% miso which product prepared contained from okara (100%) and barley mixed with soybean or okara lowering the level of serum P in compared with female rats in control group.

CONCLUSION

The results concluded that, different prepared samples of miso had a high nutritive value besides its biological functions. But it could not be consummated

more than 5% only. Moreover, prepared miso samples with were rich in antioxidant components such as polyphenols. Combination between barley, soybean and okara in different may be miso products useful as on adequate to increase weight gain. Also, miso (as fermented product) improved the serum protein for both male and female. Barley miso samples were decrease serum uric acid in male rats. While, okara miso had decrease in serum uric acid in female rats. Finally, this study concluded that, it could be use miso as (flavor ingredient) for the preparation of other foods.

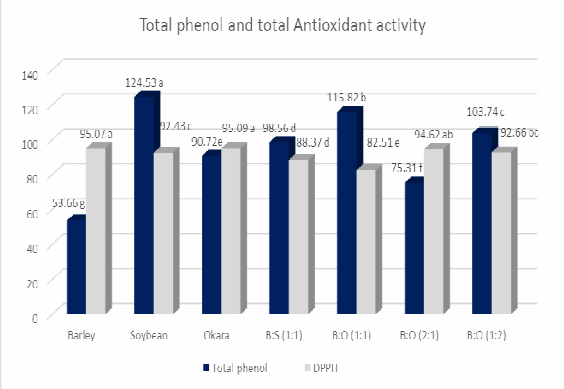

Fig 1. Total phenol and Antioxidant activity (DPPH %) in different miso samples.

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التركيب الكيميائي والتقييم البيولوجي للمنتج المتخمر (ميزو) الناتج من فول الصويا والشعير وأوكارا

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تعتبر عملية التخمير عاملا مهما في تحسين خواص الأغذية. وتهدف هذه الدراسة الى تقييم (الميزو كمنتج متخمر) من فول الصويا والشعير وأوكارا (مخلف ناتج أثناء تصنيع لبن الصويا) وخليط بينهما. كما تم تقدير التركيب الكيميائي، والفينولات الكلية والنشاط المضاد للأكسدة. أيضا تم التقييم البيولوجي لعينات الميزو المصنعة لمدة ٨ أسابيع. وأظهرت النتائج التركيب الكيميائي في جميع العينات المحتوية علي الميزو انخفاض في البروتين والدهون والكربوهيدرات. بينما زاد محتوي الرماد والألياف. عينات الميزو المصنع من الأوكارا أعلى في نشاط مضادات الأكسدة(%). بينما عينات الميزو المصنع من الشعير كان أقل محتوي من الفينولات الكلية. وكانت نتائج التقييم البيولوجي زيادة الوزن في الفئران المغذاة علي وجبة محتوية علي ميزو أقل من زيادة وزن الجسم من الفئران مجموعة الحاكمة. وعلاوة على ذلك التغذية علي وجبات محتوية علي ميزو أدي الي زيادة بروتين الكلي في الدم وليس هناك فروق معنوية بين المجموعات. وجد نقص في حمض اليوريك بالسيرم في المجاميع المغذاة علي وجبات محتوية علي الميزو المصنع من الأوكارا . ولكن، تسببت جميع العينات ميزو زيادة في وظائف الكبد مقارنة بالمجموعة الحاكمة. وقد زادت الفئران التي تغذت على جميع عينات ميزو في الأملاح المعدنية مقارنة بالمجموعة الحاكمة.