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Physicochemical, Nutritional Characteristics and Phenolic Acids Fractionation from Some Cultivars of Faba Bean (*Vicia faba* L.) Seeds

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Abstract: The faba bean seeds of thirteen cultivars were subjected to comparing in physicochemical, nutritional characteristics and phenolic acid fractionation. The cooking quality, functional properties as well as, preparation of burger with 10% and 20% of whole faba bean seeds flour from the four cultivars (having the best cooking quality) and foul medames from the same cultivars were investigated. A comparison of the basic composition of thirteen cultivars of faba bean indicated significant ($P<0.05$) differences among them that could be attributed to the variation in the genotypes, environmental factors, especially temperature, humidity and soil fertility. The results showed that faba bean seeds are good source of nutrients, such as carbohydrates, protein, in addition to phenolics and flavonoids compounds. Phenolic acid fractionation by HPLC analysis showed that faba bean seeds are rich in some phenolic acids, such as Pyrogallol, catechein, vanillic, e-vanillic and salycilic acid. Based on the results obtained from the sensory evaluation, physical properties of supplemented burger, incorporation of whole faba bean seeds flour into beef burger, as good nutritional and functional properties resulted in producing burger with almost sensory attributes besides improving physiochemical properties of the product. The data also showed that there were significant ($P<0.05$) differences between four cultivars and its foul medames samples in the values of Fe, Zn, phosphorus factions, phytic/Zn mole ratio contents. Preparation of foul medames from soaked seeds and cooking a long time was more efficient in decreasing content of phytic acid in addition to improving the bioavailability of Zinc.

Key words: Phenolic Acids · Green Pods · Dry Seeds · Total Phenolics · Phytic Acid · Antioxidant Activity

INTRODUCTION

Legumes have beneficial health implications related to their nutritional properties. They are an excellent source of dietary fiber, choline, lecithin, folate and secondary metabolites such as polyphenols. The faba bean (*Vicia faba* L.) is one of the most important crops of the *Fabaceae* family because of its nutritional importance as seeds eaten green immediately after harvest or they can be harvested at maturity stage after the pods and beans dry out [1, 2].

It is known that the faba bean is widespread in the Mediterranean regions, which is characterized by high protein content and therefore used in animal and human nutrition. Especially in Egypt, where it is considered one of the most consumed crops, it is a popular breakfast food and also used as vegetable green or fresh canned. Therefore, there is need to increase its production by expansion through newly reclaimed areas. The widespread consumption of faba beans may be because they are easy to prepare at home and store as well as their acceptable taste and flavor. Additionally, the crop is an imperative source of income for the farmers in the country [3, 4].

Faba bean seeds contain 20.0-41.0% protein, 51.0-68.0% carbohydrates, 12% fiber, 2.20-2.63 ash and 1.2-4% lipids. Dietary fiber ranges between 15.0 and 30.0%, which depends on the seed variety, hemicellulose being the major component. A large amount of genetic variation in floral biology, seed size and composition has been documented in faba beans. Higher contents of total

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phenolics and flavonoids were found during vegetative and reproductive stages, which also showed a higher antioxidant activity. Moreover, the phenolic acids found in faba beans were caffeic acid, ferulic acid, *p*-coumaric acid and synaptic acid [5, 6].

Burger is one of the most popular meat products used by consumers both locally and internationally. The nutritional quality of the burger varies according to the different raw materials and ingredients used as well as the different processing methods applied. Therefore, regular intake of plant-based foods, such as faba bean, should be considered as partially supplement animal proteins in the diet. So, the processes of replacing meat with other components of plant source have been applied in the food industry to improve quality, health and economic objectives. Therefore, it's important to develop the traits and sensory qualities of meat burgers and reduce the costs of this product [1, 7].

The seeds of faba bean are important sources of minerals such as phosphorus, calcium, iron and zinc. Phytic acid is an antinutritional factor found in faba bean as it is the storage form of phosphorus in most dry legumes seeds, which accounts for 60-90% of total phosphorus. Chemically, 6 groups of phosphoric acid are associated with a myo-inositol alcohol, which has the ability to chelate minerals at six phosphoric acid sites. In addition, it has binding properties associated with protein and starch, leading to a decrease in utilization of these nutrients. However, phytic acid has certain health properties such as reducing the absorption of toxic heavy metals in a diet such as cadmium and lead.The phytic acid content in faba bean ranged from 590.00 to 1180.00 mg/100 g DW [8, 9].

Iron and zinc are considered to be macro elements of human nutrition, as a deficiency in their diet is leading to major health problems worldwide. The faba bean seeds are an important source of Iron and Zinc where the amount of iron ranges from 3.0 to 11.30 and the amount of zinc ranges from 1.1 to 4.4 mg/100g DW. The low bioavailability of minerals (calcium, iron, zinc.. etc.) is related to phytochemicals such as phytic acid and polyphenols which consider anti-nutritional factors. Cooking generally inactivates heat sensitive factors such as trypsin and chymotrypsin inhibitors and volatile compounds. In addition, many manufacturing processes such as soaking, fermentation and germination reduce the amount of anti-nutrients, which improves the availability of iron and zinc. The decrease anti-nutrients during the soaking process, for example, is attributed to the leaching into soaking water as a result of the concentration gradient [10-12].

The aim of this investigation was to determine the gross chemical composition, total phenolics, flavonoids, phenolic acids content of thirteen faba bean (*Vicia faba* L.) cultivars: Assiut 9, Assiut 11, Assiut 12, Assiut 13, Assiut 99, Assiut 115, Assiut 143, Assiut 215, Assiut 85/37, Assiut 95/2, Assiut 104/2, Romi 3 and Sakha 1, as well as, preparation of burger with 10% and 20% of whole faba bean seeds flour from four cultivars (having the best cooking quality namely: Assiut 215, Assiut 85/37, Assiut 104/2, Romi 3) and foul medames from the same cultivars were investigated.

MATERIALS AND METHODS

Materials: Thirteen faba beans (*Vicia faba* L.) cultivars: Assiut 9, Assiut 11, Assiut 12, Assiut 13, Assiut 99, Assiut 115, Assiut 143, Assiut 215, Assiut 85/37, Assiut 95/2, Assiut 104/2, Romi 3 and Sakha 1 were grown under Assiut governorate conditions at the Experimental Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt, during season 2018.

Sample Preparation: The seeds were manually removed from ripe fruit, dried at 40°C, selected and milled in laboratory mill to obtain whole flour, which stored at 4°C in glass containers until analysis.

Methods

Physical Characteristics of Faba Bean Seeds: Dried faba bean seeds were analyzed for bulk density, swelling capacity, swelling coefficient, hydration coefficient and cook-ability. Each of physical character was determined according to their specified method [13, 14].

Proximate Composition Analysis: Chemical composition which including moisture, ash, protein and crude fiber of whole faba bean seeds flour was carried out using the standard procedures of the Association of Official Analytical Chemists [15]. The total carbohydrate was calculated by difference according to Pellet and Sossy [16]. The means were reported from triplicate determinations for each sample. The caloric value (energy) determined according to Wilson *et al.* [17] as follow:

$$\text{Energy (Kcal/100g)} = (\text{protein content}\times 4) + (\text{fat content}\times 9) + (\text{carbohydrate content} \times 4).$$

The contents of Fe and Zn in the some studied samples were determined by ICAP6200 (ICP-OES) Inductively Coupled Plasma Emission Spectrometry [18]. Total phosphorus content was determined by spectrophotometer [19] after wet ashing following method described in AOAC [15]. Total phosphorus (TP) = Phytate phosphorus (Pp) + Inorganic phosphorus (Ip).

Determination of Total Phenolics and Total Favonoids: The total phenolics content of samples was determined according to a colorimetric method using modified Folin-Ciocâlteu [20]. The extracts of samples were dissolved in 80% methanol and further dilution was made to be similar of the gallic acid standard curve reading limits. The total phenolic content in the extracts were oxidized by Folin-Ciocâlteu reagent (120 µl) and after 5 min, 340 µl of Na_2CO_3 was added for neutralization. The samples were kept for 90 min in a dark place and after that the absorbance at 750 nm was reading. The contents of total phenolics were calculated as milligram of gallic acid equivalents/100 g sample (mg GAE/ 100 g sample).

The flavonoids determination was done using aluminium chloride colorimetric assay, as described by Marinova *et al*. [21]. The flavonoids in the samples (n = 3) was extracted by homogenizing 2.00 g of the sample in 50 mL distilled water. The mixture was transferred into a rotary shaker for 12 h to ensure full extraction of flavonoids. After shaking, the mixture was filtered to obtain extract which made up to 50 mL. Immediately, 1 ml of sample extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/ L) was added to test tubes containing 4 ml of redistilled water. A 0.3 ml of 5% $NaNO_2$ was added to this mixture. 0.3 ml 10% $AlCl_3$ was added after 5 min, then 2 ml 1M NaOH was added immediately and the total volume was made up to 10 ml by adding redistilled water. After mixing the solution thoroughly the absorbance of both blank and standard was read at 510 nm using UV-Visible spectrophotometer Model UV 1601 version 2.40 (Shimadzu). The mg catechin equivalents (mg catechin/100g sample D.W) were expressed as total flavonoids content.

Determination of Phenolic Acids: To analysis of phenolic acids the HPLC apparatus consisting of Merck-Hitachi L-7455 diode array detector (DAD) and pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System was used. The separation was performed on a Li ChroCART® 125-3 Purospher ®RP-18 (5 µm) Merck column. An 80% acetonitrile in 4.5% formic acid (reagent A) and 2.5% acetic acid (reagent B) were used as an eluent. The temperature of column oven was set to 30°C. The flow rate was 1 ml/min. The concentration of reagent A was stepwise increased to reach 15% after 7min, 20% after 15 min and 100% after 16 min. To stabilize the column after 10 min of elution the concentration of reagent A was reduced to 0%. During analysis the solvent were degassed in Merck degasser. Data logging were monitored at wavelength 280 nm. Retention times and spectra were compared to those of pure standards [22].

Antioxidant Activities Assays: Total reduction activity by Fe^{3+}- Fe^{2+} transformation: The reducing activity of samples was determined by the method of Oyaizu [23]. With recording the absorbance at 700 nm after incubation the capacity of samples to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined. A great reduction capability was done when absorbance of the reaction mixture was increased.

Hydrogen Peroxide Scavenging Activity: The hydrogen peroxide scavenging ability of samples was determined by the method of Ruch *et al.* [24]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Sample extract, at the 30 µg ml-1 concentration in 3.4 ml of phosphate buffer, was added to an H_2O_2 solution (0.6 ml, 40 mM). Against blank solution containing the phosphate buffer without H_2O_2 the absorbance value of the reaction mixture was recorded at 230 nm.

Processing of Burger with 10% and 20% of Whole Faba Bean Seeds Flour: According to the formula presented in Table (1) control beef burger was prepared. The supplemented burger were prepared using the same formula except for replacing the beef meat with 10% and 20% whole faba bean seeds flour each of the four cultivars: Assiut 215, Assiut 85/37, Assiut 104/2 and Romi 3 which were had a good cooking quality. The ingredients of each formulated burger were homogenized in Braun Cutter Machine, then from the homogenized meat mixture and processed into burger of about 100 g weight, 9 cm diameter and 1.3 cm in thickness [25].

Preparation of Beef Burger Samples: The beef burger samples under study were cooked, then cooking loss, cooking yield, shrinkage were determined and calculated according to the following equations as described by AMSA [25]:

Cooking loss = [(weight of raw sample - weight of cooked sample) ÷ weight of raw sample] × 100.

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight}}{\text{Raw weight}} \times 100$$

$$\text{Shrinkage (\%)} = \frac{\text{(Raw thickness-Cooked thickness) + (Raw diameter- Cooked diameter)}}{\text{Raw thickness + Raw diameter}} \times 100$$

Table 1: Formulation of burgers containing whole faba bean seeds flour

Ingredients (g)	Control	10%	20%
Beef meat	76.00	68.40	60.80
Whole faba bean seeds flour	--	7.60	15.20
Onion	10.00	10.00	10.00
Starch	10.00	10.00	10.00
Garlic	1.00	1.00	1.00
Salt	2.00	2.00	2.00
Spices	1.00	1.00	1.00

Sensory Evaluation of Burger Samples: Burger samples in pouches coded with different numbers were presented to the judges from the staff of Food science and Technology department, Faculty of Agriculture, Assiut University, who were asked by assigning a score for color (10), odor (10), tenderness (10), taste (10), texture (10) appearance (10) and overall acceptability (10) of cooked samples to rate each sensory attribute according to the method described by AMSA [25].

Preparation of Foul Medames: Faba bean seeds of four cultivars Assiut 215, Assiut 85/37, Assiut 104/2 and Romi 3 which were had a good cooking quality soaked in water 1:3 (w/v) for 9 hours at room temperature (20±4°C). After soaking the seeds were rinsed in water and the soaking solution was drained off. Soaked seeds were cooked with water on a quiet hot plate until the seeds became soft as felt between fingers, so the foul medames was obtained. The cooked seeds without water were dried and milled then stored at 4°C until analyzed.

Determination of Phytic Acid: The phytic acid was determined by the method described by Kent-Jones and Amos [26], in which the phytic acid is precipitated with known iron content of iron-III solution and the phytate phosphorus content was measure as a decrease in iron in the supernatant. Based on the empirical formula $C_6 P_6 O_{24} H_{18}$, the amount of phytate phosphorous multiplying by a factor of 3.55 to obtained estimated phytate [27].

Phytic Acid to Zinc Mole Ratio: The phytic acid to zinc mole ratio was calculated according to IZiNCG, [28] as illustrated in the equation as follows:

$$\frac{\text{Phytic acid (mg/100g) / 660}}{\text{Zinc (mg/100g) /65.4}}$$

Statistical Analysis: The collected data were analyzed by analysis of variance (ANOVA) Procedures. Differences between means were compared by LSD at 5% level of significance [29].

RESULTS AND DISCUSSION

Physical Characteristics and Functional Properties of Faba Bean Seeds: The physical properties which express cooking quality were related to acceptability of the cooked product, its nutritive value and the method of processing it. As shown in Table 2 the analysis of variance showed that genotype variation exhibited a significant ($P<0.05$) effect on seed density, swelling capacity, swelling coefficient, hydration coefficient and cook-ability (Table 2). The seed density, the swelling capacity of bean seeds was ranged from 0.63 to 0.70 (g/mL) and from 50.62% to 59.73%, respectively, in the studied cultivars (Table 2). The swelling coefficient, hydration coefficient and cook-ability (g/100g) were significantly ($P<0.05$) higher in Assiut 215, Romi 3, Assiut 104/2 with values 254.37%, 206.79% and 27.92%, respectively, as compared with other studied cultivars. The differences in the above mentioned properties may be due to agricultural, environmental conditions, climatic factors which affects the physical properties, including the cook-ability, as both consumers and producers prefer seeds of faba beans that have a high hydration and swelling coefficients, which in turn leads to the production of a larger quantity of the product, this is consistent with findings of Elsheikh and Ahmed [30].

Water, oil absorption capacities are essential functions of protein, oil in food functionality, as they influence emulsion and other properties which improves the mouth feel of foods [31]. These WAC and OAC of faba bean seeds samples are shown in Table 2.

Table 2: Cooking quality and functional properties of thirteen cultivars of faba bean seeds

Sample	Weight (g) 100 seeds	Volume (ml) 100 seeds	Seed density (g/ml)	Swelling capacity (%)	Swelling coefficient (%)	Hydration coefficient (%)	Cook-ability (%)	Functional properties (%)* WAC	OAC
Assiut 9	64.71	98	0.66	59.73	238.51	194.23	20.76	234.44	141.30
Assiut 11	67.54	104	0.65	53.42	232.95	190.11	21.42	239.43	174.02
Assiut 12	61.93	90	0.69	50.62	206.36	192.70	19.73	270.15	132.50
Assiut 13	84.42	131	0.64	53.07	221.18	182.68	25.28	230.55	194.80
Assiut 99	97.06	149	0.65	53.91	203.79	169.20	22.34	264.91	141.43
Assiut 115	68.82	98	0.70	53.23	247.10	189.97	24.64	223.27	181.40
Assiut 143	87.91	129	0.68	51.53	238.28	189.93	23.15	207.30	171.35
Assiut 215	68.86	104	0.66	52.13	254.37	196.24	24.47	300.72	131.18
Assiut 85/37	67.65	99	0.68	54.96	248.08	198.77	22.44	255.70	189.01
Assiut 95/2	74.40	118	0.63	52.70	222.88	185.28	22.86	259.76	172.35
Assiut 104/2	71.24	109	0.65	59.70	241.61	203.81	27.92	294.57	177.10
Romi 3	77.28	120	0.64	55.40	240.45	206.79	26.42	251.60	172.69
Sakha 1	76.22	119	0.64	58.76	223.95	192.31	19.41	278.02	193.20
L.S.D 0.05	1.03	1.13	0.01	0.08	1.52	1.86	0.051	3.56	2.14

*Results calculated on dry weight basis (g/100g)

Water absorption capacity (WAC) of the thirteen seeds flours samples ranged between 207.30 and 300.72%. Assiut 215 sample showed significantly ($P<0.05$) the highest water absorption (300.72%) as compared with other samples. The OAC was found in high content reached 194.80% in seeds flour of Assiut 13, while the lowest value of such content was recorded in seeds flour of Assiut 215 (131.18%). There was no significance ($P<0.05$) difference in oil absorption capacity of Assiut 9 (141.30), Assiut 99 (141.43); Assiut 12 (132.50), Assiut 215 (131.18); Assiut 95/2 (172.35) and Romi 3 (172.69 g/ 100gm dry weight) seed flours, respectively. The WAC, OAC value of thirteen cultivars of faba bean under study is comparable to those of many beans [32], so faba bean flour may be used in food preparations, such as soups, doughnuts, baked goods, sausages, meat replacers and extenders.

Chemical Composition, Total Phenolics, Total Flavonoids and Antioxidant Activities Assays Contents of Thirteen Cultivars of Faba Bean Seeds: The chemical composition of thirteen faba bean seeds cultivars differed significantly ($P<0.05$) in all parameters (Table 3). Moisture content of the cultivars was ranged from 8.10 to 9.65%, while ash content ranged from 2.29 to 5.15 g/100g D.W. The oil content of samples under study was found in a small quantity ranging from 1.40 to 2.29 g/100g D.W. The protein content (g/100g D.W) of the genotypes under study was ranged from 17.30 for Sakha 1 to 29.50 for Assiut 85/37. The variation in crude protein contents of studied cultivars considerably depending on the genotypes. Data in Table 3 showed that Assiut 11 had the highest level of total carbohydrates (66.38 g/100g D.W) among the other cultivars, while the caloric value content was highest in Assiut 115 genotype (362.44kcal/100g D.W). The lowest levels of total carbohydrates, caloric value was found in Assiut 143 cultivar with contents 52.23 g/100g D.W and 325.93 kcal/100g D.W, respectively. Similar values for moisture, ash, protein and total carbohydrates was reported previously for many faba bean genotypes, as well as, the lower fat content (1.40-2.29 g/100g D.W) of the thirteen faba bean genotypes under study comparing to the other chemical parameters was agreement with many previous studies recorded fat content within the range of 0.70-2.00 g /100g D.W for various faba beans genotypes. The fiber content of genotypes in the current study was higher with range of 7.77 to 19.09 g/100g D.W. g kg^{-1}, which is similar to those reported previously for various faba bean genotypes and demonstrated the expected beneficial effect of as a result of eating faba beans to improve the food digestibility, so reducing the risk of many intestinal diseases [33, 34]. A comparison of the basic composition of thirteen cultivars of faba bean as shown in Table 3 indicated significant differences among them that could be attributed to the variation in the genotypes, environmental factors, especially temperature, humidity, soil fertility, insects, diseases that may affect nitrogen absorption and thus affect the quality of the resulting faba bean seeds.

Data listed Table 3 showed that significant ($P<0.05$) differences were recorded in total phenolics, total flavonoids and antioxidant activities assays (total reduction activity, H_2O_2 scavenging activity) in most tested cultivars. The samples extracts showed a clear different contents of total phenolics (mg GAE/100g D.W),

Table 3: Chemical composition*, total phenolics (mg GAE/100g D.W), total flavonoids (mg catechin/100g D.W) and Antioxidant activities assays (mg/100g D.W) contents of thirteen cultivars of faba bean seeds

										Antioxidant activities assays	
Sample	Moisture	Ash*	Oil*	Protein*	Crude fiber*	Total carbohydrates*	The caloric value (Kcal/100g)*	Total phenolics	Total flavonoids	Total reduction activity[1]	H_2O_2 scavenging activity[2]
Assiut 9	8.40	3.97	1.72	23.90	13.14	57.27	340.16	467.03	22.27	19.95	33.89
Assiut 11	9.43	4.16	1.40	19.20	8.86	66.38	354.92	570.82	19.47	20.31	35.07
Assiut 12	9.20	3.78	1.68	24.70	9.92	59.92	353.60	424.30	22.08	19.47	32.22
Assiut 13	9.32	3.93	1.89	27.60	9.36	57.22	356.29	418.19	21.36	19.17	31.26
Assiut 99	8.24	3.61	2.28	21.70	8.99	63.42	361.00	525.30	19.56	20.06	34.22
Assiut 115	8.47	4.22	2.08	26.70	7.77	59.23	362.44	674.31	17.28	20.76	35.36
Assiut 143	8.10	2.29	2.29	24.10	19.09	52.23	325.93	738.71	16.13	22.48	42.51
Assiut 215	8.78	4.11	1.72	21.50	12.39	60.28	342.60	705.69	17.09	22.11	41.22
Assiut 85/37	9.65	3.56	2.04	29.50	8.60	56.30	361.56	763.13	16.05	23.88	47.22
Assiut 95/2	9.02	4.45	1.63	22.10	10.36	61.46	348.91	430.40	22.01	19.85	33.53
Assiut 104/2	8.62	3.52	1.56	25.20	8.30	61.42	360.52	622.71	16.85	21.06	35.07
Romi 3	8.96	3.40	1.76	20.20	9.17	65.47	358.52	741.76	15.28	23.36	42.51
Sakha 1	8.68	5.15	1.85	17.30	10.48	65.22	346.73	644.08	17.03	21.09	37.67
L.S.D 0.05	0.04	0.06	0.03	0.12	0.43	0.91	1.37	5.47	0.05	0.17	0.21

*Results calculated on dry weight basis (g/100g)

with a range from 418.19 to 763.13. Assiut 85/37 showed the highest content of phenolics, with 763.13, followed by Romi 3, with 741.76. The lowest phenolic content was obtained from Assiut 13 with 418.19 mg GAE/100g D.W. The amount of flavonoids varied in faba bean genotypes from 15.28 to 22.27 mg catechin equivalents/100 gm dry weight in Romi 3 and Assiut 9, respectively; also the content of total flavonoids in the extract of the rest genotypes was found in a moderately contents. The antioxidant activities of faba bean seeds extracts have been determined using total reduction activity and H_2O_2 scavenging activity methods. The Total reduction activity of studied samples was ranged from 19.17 for Assiut 13 to 23.88 reducing ferric ions/100 gm dry weight for Assiut 85/37. The H_2O_2 scavenging activity in methanolic extract of 13 samples of faba bean was determined. The range of H_2O_2 scavenging activity values was between 31.26 in Assiut 13 and 47.22 H_2O_2 molecules/100 gm dry weight in Assiut 85/37. From the above mentioned results it could be say that the various studied genotypes of faba beans were had reasonable amounts of phenolic and flavonoid substances, as well as a reasonable antioxidant activities, which in turn confirms the importance of faba beans as a good natural antioxidant. Consequently, these vital compounds have an important and major role in health applications [35].

Fractions of Phenolic Acids of Faba Bean Seeds: The composition of polyphenolic acids in faba bean seeds samples are presented in Table (4). From these results it is clear that beans samples contained many acids with different contents. Pyrogallol, catechein, vanillic, e-vanillic and salycilic acid are presented in reasonable amounts in all the studied samples. The results of HPLC have identified also, catechol, protocatchuic, epicatechin, chlorogenic, p-OH-benzoic, caffeine, caffeic, P-coumaric, alpha-coumaric, ferulic, iso-ferulic, ellagic, coumarin, benzoic and cinnamic acids as phenolic constituents of beans samples.

It is clear that from the Table 4, the Assiut 85/37 sample was the highest among studied cultivars which have the largest contents (µg acid / 100 g D.W) of phenolic acids: protocatchuic (448.82), epicatachin (272.67), P-OH-benzoic (475.30), caffeine (748.12), P-coumaric (248.22), ferulic (315.22), e-vanillic (1880.39), ellagic (761.45), alpha-coumaric (142.68), 3, 4, 5-methoxy-cinnamic (94.88) and cinnamic acid (25.45). In another hand, the Assiut 95/2, Assiut 104/2, Assiut 115 cultivars also contained high amounts of some phenolic acids when compared to other beans genotypes. In general, the content of phenolic acids presented in faba bean samples is influenced by variation in genetic factors, soil fertility, any agricultural factors such as temperature and humidity. The importance of these phenolic acids is known to act as an antioxidant, as vital compounds that help in maintaining human health and this has been discussed previously [35].

Sensory Evaluation and Physical Properties of Supplemented Beef Burgers: Sensory attributes of processed tested burger are represented in Table (5) and Fig. (1). Form results presented in Table (5), it confirmed

Table 4: Fractions of Phenolic compounds of faba bean seeds*

Phenolic compounds	Assiut 9	Assiut 11	Assiut 12	Assiut 13	Assiut 99	Assiut 115	Assiut 143	Assiut 215	Assiut 85/37	Assiut 95/2	Assiut 104/2	Romi 3	Sakha 1
Pyrogallol	522.12	478.36	535.09	366.25	530.56	166.20	456.23	510.35	1357.56	4123.03	1559.32	624.82	1082.47
Gallic	28.69	42.25	20.51	32.12	9.80	15.30	32.28	31.76	66.38	253.15	62.78	13.71	39.66
4-Amino-benzoic	9.21	21.03	5.32	15.10	23.30	18.42	22.32	19.58	26.30	431.99	15.54	10.86	32.78
Protocatchuic	91.10	86.21	72.35	58.61	339.82	281.36	310.52	182.49	448.82	108.19	283.35	62.44	275.90
Catechein	1020.23	1321.62	1104.25	1625.01	2223.28	2821.57	1452.36	1921.93	1659.11	1142.68	1899.42	2639.50	2562.15
Catechol	201.23	185.02	217.82	265.01	88.63	39.60	25.10	61.95	106.93	41.62	403.62	409.83	392.20
Chlorogenic	98.06	130.28	80.78	151.09	148.96	138.60	179.26	172.50	213.45	114.56	354.41	269.53	218.25
Epicatachin	22.36	42.12	17.69	51.71	62.01	67.98	120.56	132.56	272.67	26.11	128.02	98.30	89.67
P-OH-benzoic	187.45	157.98	130.11	128.10	333.03	216.85	168.69	119.49	475.30	95.96	470.13	416.21	364.54
Caffeine	153.21	105.30	78.92	69.57	124.33	59.62	87.25	321.79	748.12	99.64	283.10	122.83	106.08
Caffeic	81.39	76.09	34.38	40.21	44.37	37.46	32.30	113.97	117.05	86.09	115.61	101.96	217.23
Vanillic	576.30	625.14	520.62	712.08	865.67	2714.54	1054.20	709.48	741.53	358.40	503.31	589.35	1264.42
P-coumaric	59.21	29.03	33.32	48.23	62.34	66.62	56.28	87.56	248.22	44.74	54.78	27.86	26.94
Ferulic	143.98	163.91	196.49	208.20	109.94	149.32	160.13	238.85	315.22	118.77	69.51	151.15	181.13
Iso-ferulic	49.01	59.28	100.47	77.49	51.98	31.98	48.05	36.93	88.58	37.71	38.50	67.72	20.67
e-vanillic	1365.20	1479.30	1061.45	1297.60	1491.42	509.31	798.01	605.30	1880.39	289.49	1518.44	1064.22	573.16
Ellagic	198.71	175.90	472.75	356.30	446.70	82.50	96.54	175.10	761.45	228.36	587.14	123.71	142.84
Alpha-coumaric	28.09	20.07	25.62	31.20	62.03	28.32	29.20	77.38	142.68	32.48	42.85	41.37	14.42
Benzoic	97.40	100.94	263.45	76.30	1114.25	94.58	102.35	393.10	1171.99	156.56	1449.82	282.25	163.56
3, 4, 5-methoxy-cinnamic	38.57	55.01	46.08	62.01	57.37	19.01	28.10	23.20	94.88	22.38	53.59	13.06	27.23
Coumarin	174.90	147.68	170.62	159.76	134.73	28.93	41.02	37.27	202.13	60.04	211.89	5.97	40.43
Salycilic	1262.20	1197.35	1836.07	1351.06	1144.88	847.40	1123.20	204.83	736.19	194.50	1454.40	46.41	168.69
Cinnamic	8.05	12.46	9.20	20.70	6.53	4.65	4.01	7.54	25.45	3.96	11.89	1.49	1.37

*Calculated as µg acid / 100 gm dry weight

Table 5: Sensory Evaluation and physical properties of supplemented beef Burgers

		Sensory evaluation								Physical properties		
Sample		Color (10)	Odor (10)	Tenderness (10)	Taste (10)	Texture (10)	Appearance (10)	Overall acceptability (10)	Total score (70)	Cooking loss (%)	Shrinkage (%)	Cooking yield (%)
Control	A	8.39	9.10	8.30	8.56	8.95	8.56	8.45	60.31	38.03	20.95	62.00
Assiut 85/37	B	8.49	8.05	8.05	8.15	8.10	8.13	8.05	57.02	35.67	16.52	64.33
	C	8.45	7.95	7.38	8.10	7.58	7.95	7.95	55.36	25.38	15.24	74.62
Assiut 215	B	8.45	8.38	7.95	8.38	8.63	8.30	8.38	58.47	35.21	18.05	64.79
	C	8.59	7.56	7.49	7.78	7.80	7.68	7.78	54.68	24.66	15.24	75.35
Assiut 104/2	B	8.10	7.86	8.05	8.10	7.88	8.10	8.10	56.19	36.24	16.95	63.76
	C	8.63	7.48	7.20	7.66	7.58	7.58	7.66	53.79	24.49	14.48	75.51
Romi 3	B	8.78	7.95	8.10	8.30	8.49	8.20	8.30	58.12	35.35	18.90	64.65
	C	8.49	7.60	7.30	7.38	7.58	7.30	7.38	53.03	24.85	14.29	75.15
L.S.D 0.05	0.04	0.03	0.04	0.02	0.06	0.04	0.05	0.26	0.08	0.14	0.05	

A = Control burger. B = Burger supplemented with 10% of whole faba bean seeds flour. C = Burger supplemented with 20% whole faba bean seeds flour.

that all the burger samples and control blends possessed the best color which has an important role in consumer acceptance and sensory of products as a vital quality attribute of foods [36]. The odor, tenderness values for supplemented burgers ranging 8.05- 8.38, 8.05 -8.10 comparing with 9.10 and 8.30 for control, respectively. On the other hand, among supplemented burger samples Assiut 215 burger 10% had significantly ($P<0.05$) the highest values of taste (8.38), texture (8.63), appearance (8.30) and overall acceptability (8.38), followed by Romi 3 burger 10% in the same attributes. The total score which expresses consumer acceptability was significantly ($P<0.05$) highest for burgers containing 10% of Assiut 215, Romi 3 whole flour, with values 58.47 and 58.12, respectively, as compared with 60.31 for control (100% beef meat).

Table (5) shows the changes in burger after cooking which expressed as physical properties. The frying by oil method, has led to a decrease in the weight of the burger and consequently decreasing in cooking loss which they were ranging from 35.21 - 36.24% for supplemented burger 10%; 24.49- 25.38% for supplemented burger 20%, comparing with 38.03 for control. The changes in diameter, thickness of the burger after cooking has led to decrease in the shrinkage value which they were ranging from 16.52-18.90% for supplemented burger 10%; 14.29-15.24% for supplemented burger 20%, comparing with 20.95 for control. Consequently, the decreasing in cooking loss, shrinkage values increasing the cooking quality values where it was found in quantities of 63.76 to 75.51 for supplemented burgers samples, compared to 62.00 for control. This loss because of meat and meat products lose

Fig. 1: Showing control (100% beef meat), 10% and 20% supplemented burger with whole faba bean seeds flour

Table 6: Fe, Zn, total phosphorus (TP), phytate phosphorus (Pp), phytic acid contents (mg/100g D.W) and phytic/Zn mole ratio of four cultivars of faba bean seeds

Sample	Fe	Zn	TP	Pp	Phytic acid	Phytic/ Zn mole ratio
Assiut 215	8.32	5.32	613.85	296.63	1053.04	19.60
Assiut 85/37	7.62	5.11	607.66	297.89	1057.51	20.49
Assiut 104/2	8.04	6.14	565.31	288.61	1024.57	16.52
Romi 3	7.18	5.36	617.65	298.33	1059.08	19.56
L.S.D 0.05	0.23	0.21	0.09	0.12	0.16	0.71

Table 7: Fe, Zn, total phosphorus (TP), phytate phosphorus (Pp), phytic acid contents (mg/100g D.W) and phytic/Zn mole ratio of foul medames

Foul medames sample	Fe	Zn	TP	Pp	Phytic acid	Phytic/ Zn mole ratio
Assiut 215	6.32	3.08	142.25	76.38	271.15	8.72
Assiut 85/37	6.84	3.62	153.63	84.09	298.52	8.16
Assiut 104/2	7.01	4.23	122.82	68.56	243.39	5.70
Romi 3	6.22	3.72	143.93	76.95	273.17	7.27
L.S.D 0.05	0.68	0.18	0.11	0.10	0.07	0.25

Fig. 2: Showing the raw, soaked and cooked (foul medames) faba bean seeds of cultivar Assiut 215

a variable parentage of the weight during cooking, as a result of partial replacement of meat with whole faba bean seeds flour, it was due to loss in weight, decrease in the amount of the proteins and some tissues of meat, causing the shrinkage of the burgers [37].

Fe, Zn, Phosphorus Fractions Contents, Phytic/Zn Mole Ratio of Four Cultivars of Faba Bean Seeds and its Foul Medames: The Fe, Zn, phosphorus factions, phytic/Zn mole ratio contents in four faba bean cultivars and its foul medames are illustrated in Tables 6, 7. From these data, there were significant ($P<0.05$) differences between cultivars as well as, foul medames samples in the values of all contents. The study showed that there was a noticeable amount of Fe and Zn remains in medames samples. Fe, Zn contents (mg/100g DW) was decreased significantly ($P<0.05$) in the medames samples ranging: 6.22-7.01; 3.08-0.23 when compared with values:

7.18-8.32; 5.11-6.14 for raw seeds before soaking and cooking, respectively. This might be due to leaching of some elements during the treatments used for preparing the foul medames. The values of Tp (total phosphorus), Pp (phytate phosphorus) (mg/100g) ranged from 565.31 to 617.65, from 288.61 to 298.33 in seeds cultivars when compared with values 122.82 to153.63 and 68.56 to 84.09 for the foul medames samples, respectively. The phytic acid content of faba bean cultivars under study ranged from 1024.57 to 1059.08mg/100g DW. Romi 3 cultivar has the highest value, while Assiut 104/2 has the lowest value (Table 6). The phytic acid content in the foul medames samples decreased significantly with percentages from 71.77% to 76.25% due to the nature of the cooking preparation which the seeds soaked first then cooked. The phytic content in foul medames was ranged from 243.39 for Assiut 104/2 to 298.52 g/100g DW for Assiut 85/37 (Table 7).

Preparation of foul medames as shown in Fig. 2 (cultivar Assiut 215 for example) from soaked seeds and cooking a long time was more efficient in decreasing content of phytic acid. As the bioavailability of zinc was determined by molar ratio, the phytic acid /Zn mole ratio contents ranged from 16.52 to 20.49% in seeds cultivars when compared with values 5.70 to 8.72 for the foul medames samples. Phytic/Zn molar ratio was associated with zinc absorbtion capacity. Phytic acid to zinc mole ratio >15, 5-15 and <5 is equal to zinc bioavailability as low (10-15), moderate (30-35%) and high (50-55%), respectively, for a special food according to WHO [38]. Therefore, zinc bioavailability in raw faba bean seeds is low (10-15%), while foul medames samples have moderate zinc bioavailability (30-35%). During processing (soaking or cooking) the phytic acid was reduced significantly as a result of endogenous and exogenous enzymes formation, as well as, improving the bioavailability of Zinc [10, 11].

CONCLUSION

Results of the present study indicated that the thirteen cultivars of faba bean are rich in most nutritional components, such as protein, total carbohydrates, phenolic acids, good source of phenolic compounds, flavonoids and have antioxidant activities which possess potential health benefits. Besides, the genotypes of faba beans seeds can be used in various food applications due to their good cooking properties. The incorporation of whole faba bean seeds flour in processing beef burger with 10, 20% percent from meat weight which used in formulation of burger resulted in cooked burger with good almost sensory properties in addition to improving physical properties of the product. Preparation of foul medames from soaked seeds and cooking a long time was more efficient in decreasing content of phytate phosphorus and consequently phytic acid. During processing (soaking or cooking) the phytic acid was reduced significantly as a result of endogenous and exogenous enzymes formation, as well as, improving the bioavailability of Zinc.

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