Effect of domestic processing methods of some legumes on phytochemicals content and *in vitro* bioavailability of some minerals

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Abstract: Legumes are valued over the world for their protein content as well as their low glycemic index and are commonly included in Egyptian diets. So, the present study was conducted to determine the effect of common household processes on nutritional value and antioxidant factors as well as *in vitro* bioavailability of iron and zinc. Generally, the domestic processing methods on the legumes under study resulted in increases in protein and crude fiber contents, while total carbohydrates, fat and ash contents were decreased as comparing with the raw materials. The soaking process caused significant decrease in mineral contents of tested legumes, while cooking and germination processes caused increases in Ca, Fe and Zn contents, but caused decreases in Na and K contents as compared to raw materials. Concerning *in vitro* bioavailability percent of Fe and Zn, there were an increment in their contents when the raw materials of legumes subjected to different treatments. The trend observed in the degree to which processing increased the bioavailability percent of Fe and Zn was germination+ cooking > germination > cooking > soaking. From results also, the same trend was observed in total phenols and total flavonoids. The total antioxidant percent increased after germination in all tested legumes relative to soaked ones. Ferulic was the most abundant phenolic compounds present in all studied legumes. Germination caused increase in fractionated phenolic compounds content. According to fractionation of isoflavones, it was clear that genistein was the highest detected component and formononetin was the lowest one in all studied legumes. Treatments of soaking and cooking resulted in a decrement in fractionated isoflavones relative to raw materials. In addition, germination process increased their contents compared to soaked ones. Vitamin B fractions were decreased in all processes except germination treatment. From the present results, it could be concluded that the common household processes for legumes, in particular, germination process could be improved the nutritional value through elevation in the capacity of antioxidant constituents and vitamin B fractions as well as increase *in vitro* bioavailability of minerals.
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Key words: Legumes - Soaking – Germination – Cooking – Antioxidant activity – Fe and Zn Bioavailability – Vitamin B – Isoflavones and Phenols fractions.

1. Introduction:

Legumes represent an important component of human diet in several areas of the world – especially in the developing countries where they complement the lack of proteins from cereals, roots and tubers. Legumes play an important role in the traditional diet in several regions of the world. They are good and economical sources of protein, minerals and B-vitamins (**Messina, 1999**)

Legumes are essential raw material for the modern food industry in the production of protein concentrates, fats and starches as well as functional food ingredients such as protein isolates, protein hydrolysates, dietary fibres, lecithin and isoflavones (**Linden and Lorient, 1994**).

In addition, legumes contain a rich variety of phytochemicals, including phytosterols, natural antioxidants, polyphenols and bioactive carbohydrates (**Amarowicz and Pegg, 2008)**. So, in the developed countries, legumes have an increasing use in dietetic formulations in the treatment and prevention of several diseases. Epidemiological and intervention studies indicated that legume consumption is inversely associated with the risk of coronary heart disease (**Bazzano *et al*., 2001),** tumour risk **(Mathers, 2002),** type II diabetes mellitus **(Villegas *et al*., 2008) and** obesity **(Rizkalla *et al*., 2002),** and results in lower LDL cholesterol and higher HDL cholesterol **(Bazzano *et al*., 2008**).

Nevertheless, legumes contain anti-nutritional factors, such as trypsin inhibitors, phytic acid, a-galactosides and phenolic compounds that can diminish protein digestibility and mineral bioavailability, thus they have to be appropriately treated prior consumption (**Sendberg, 2002**).

Dietary polyphenols show a great diversity of structures, ranging from rather simple molecules (monomers and oligomers) to polymers. Although, polyphenols have been recognized as the most abundant source of anti-oxidants in our diet

(**Thomasset *et al*., 2007)**, but they have the potential to bind positively charged proteins, amino acids and/or multivalent cations or minerals such as iron, zinc and calcium in foods. They thus reduce the bioavailability of essential minerals and a reduction in their content may result in improved absorption of these nutrients (**Gilani *et al*., 2005**). However, the current literature contains substantial evidence that they may exert beneficial effects at low concentrations, protecting against several diseases (**Carbonaro, 2008**). The profiles and quantities of polyphenols and tannins in legumes are affected by processing due to their highly reactive nature, which may affect their anti-oxidant activity and the nutritional value of foods (**Dlamini *et al*., 2009**).

Food processing which including milling, dehulling, soaking, germination, fermentation and cooking save time, energy and fuel as well as yield edible products having a higher nutritional value and lower levels of polyphenols and tannins and antioxidant activities of the beans (**Xu and Chang, 2008a; 2008b and 2009**).Contrary, other studies revealed that germination process of chickpea for 5 days caused increment in total phenolics and antioxidant activity (**Tarzi *et al*., 2012)**

Soaking is an integral part of a number of treatments, such as germination, cooking and fermentation. It consists of hydration of the seeds in water for a few hours. Several studies indicated that soaking can reduce the levels of minerals, phytic acid and proteolytic enzyme inhibitors which can be partly or totally solubilized and eliminated with the discarded soaking solution **(Prodanov, *et al*., 2004).**

Germination is a natural process caused decomposition of high molecular weight polymers and generation of bio-functional substances and improvement of organoleptic qualities due to softening of texture and increase of flavor in grains (**Beal and Mottram, 1993).**

Cooking brings about a number of changes in the physical characteristics and chemical composition of food legumes. Legumes are usually cooked by a boiling process before use. Pressure boiling and steaming can also be used for this purpose. High pressure processing technology may provide high quality such as flavor, color and biological active components food products (**Knorr, 1999**).

The preliminary studies showed that soaking, germination, boiling and steaming processing significantly affected the phenolic components. Limited information is available regarding the content of phytochemicals, with the exception of soybean (**Crozier *et al*., 2009**), in pulses, which constitute a significant source of protein in Egyptian diets.

The present study, therefore, estimated the concentrations of total polyphenols and flavonoids and its fractionations in five legumes which are mainly consumed (faba bean, lentil, white bean, fenugreek and chickpea) and its effects on minerals bioavailability *in vitro* under different domestic processing techniques like, soaking, cooking and germination.

2. Material and methods:

Materials:

Legumes; Faba bean; *vicia faba* L., White bean; *phaseolus vulgaris*, Chickpea; *Cicer arietinum* L., Lentil; *lens culimaris* and fenugreek seeds; *trigonella foenum-graecum* L. were grown during the 2013 season, and obtained from the Crops Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt

Chemicals:

Pepsin, pancreatin, lipase and cetylpyridinium bromide, and all other used chemicals were of analytical reagent grade. Folin-Ciocalteu phenol reagent (2N), quercetin dihydrate (2,3,4- dihydroxyphenyl), standards of phenolic acids, isoflavones and vitamins B were purchased from Sigma-Aldrich Chemicals Co. (St Louis, MO, USA). Bile extracts from Win Lab Laboratory chemicals reagents (Mumbai, India). Sodium carbonat (99.8%), and HPLC solvents were from Fisher Scientific (Fair Lawn, NJ, USA).

Preparation of samples:

Soaking: Legumes seeds were soaked in tap water for 12 hours in ratio 1:5 w/v with added 5ml/L acetic acid (5%). At the end of soaking period, the soaked water was discarded and rinsed twice in tap water. The amount of soaked seed were divided into two parts, one part was subjected to germination process and other part was subjected to cooking process.

Germination: the soaked seeds were allowed to germinate under a wet muslin cloth for 48 hours.Part of germinated seeds was subjected to analysis and the other part was subjected to cooking process.

Cooking: The soaked seeds and the germinated samples were subjected to cooking treatment by boiling in a covered stainless-steel pot and cooked on a moderate flame with water retention. For all cooking treatments, the minimum cooking time to reach a similar tenderness for an adequate palatability and taste according to the Egyptian eating habits.The all treated seeds were dried at 45±5 °C under vacuum and ground in a Laboratory mill to obtain fine flour and kept at -18 °C until analysis.

Methods:

Moisture, protein, total fat, crude fiber and ash contents were determined according to **A.O.A.C. (2000).** The carbohydrates [Nitrogen free extract

(NFE)] were calculated by difference. Approximate calorific value products were calculated using the appropriate factor (1g of protein X 4 + 1g of carbohydrates X 4 + 1g of fat X 9) as described by **Lawrence (1965).** Total minerals content were determined according to the method outlined in **A.O.A.C (2000)** by using the Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer. Total phenols in legumes were determined by folin-Ciocalteau's reagent according to **Arnous *et al.*, (2001).** Total flavonoids content was determined using aluminum chloride method according to **Chang *et al*. (2002).** Antioxidant activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method as described by **Brand-Williams *et al.* (1995).** The bioavailability of iron and zinc was determined by *in vitro* digestion method as described by **Kiers *et al.* (2000).** Fractionation of phenolic compounds and isoflavones after hydrolysis and vitamins B were conducted using high performance liquid chromatography (HPLC). Which equipped with a variable wave length detector (Agilant, Germany) 1100, auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C_{18} reverse phase (BDS 5µm, Labio, Czech Republic) packed stainless-steel column (4×250 mm, i.d.). To determine phenolic compounds and isoflavones after hydrolysis, samples were prepared according to the method described by **Jakopič *et al.* (2009).** The chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by **Schieber *et al.* (2001).** All chromatograms were plotted at 280 nm to estimated phenolic acids and at 254 nm for isoflavones. Vitamin B fractions were determined according to the method described by **Kall (2008).**

Statistical analysis:

Statistical analyses were carried out by SPSS19 program. Data were expressed as means ± SEM and the statistical analysis was performed using one-way analysis of variance followed by Duncan's tests as according to **(Snedecor and Cochran, 1989**).

Results and Discussion

Effect of domestic processing methods of legumes on chemical composition

The results of proximate analyses of different legumes were recorded in table (1). The data showed that protein content was reduced significantly in all treatments in comparing with raw materials. Also, it is obvious that in comparison with soaking treatment, protein content was reduced in soaking plus cooking, while increased by germination and germination plus cooking. Fat content was decreased significantly in all treatments relative to the raw materials and the same trend was observed as comparison of soaking treatment with other treatments (soaking plus cooking, germination and germination plus cooking). Ash content decreased in all treatments as compared with raw materials. Treatments after soaking such as cooking and germination had a significant decreasing in ash contents especially in germination plus cooking process. Respect to crude fiber content, there was an increment in all treatments than raw materials, whereas germination and germination plus cooking treatments had the highest values of crude fiber content after soaking. NFE as carbohydrates content was increased when raw materials of different legumes subjected to soaking, cooking and germination. However, germination and germination plus cooking caused a significant reduction in NFE content relative to soaking treatment. Energy content was high in raw materials in comparison with other treatments. The results can be arranged in descending order such as soaking > soaking plus cooking > germination > germination plus soaking. The results are in agreement with **Hefnawy (2011)** who reported that cooking by boiling reduced protein% and fat % in lentil seeds in comparison with raw materials. **Khalil *et al*. (2007)** found that effect of sprouting showed a rise in protein and a decrease in NFE contents in chickpea cultivars. This apparent increase in the total protein was observed with the time of germination may be attributed to oxidation and consumption of the other water-soluble classes (like sugars and minerals) in the germination process (**Mostafa and Rahma, 1987).** Accordingly, **D′souza (2013)** found that ash content was decreased with soaking, germination and cooking might be due to the leaching out of both macro and micro elements into the soaking water. While crud fiberwas significantly increased by cooking treatment, this increase could have been due to protein fiber complex formed after possible chemical modification induced by the soaking and cooking of dry seeds (**Bressani, 1993).** The increase in crude fiber during germination was reported to be mostly due to changes in the polysaccharides found in the cell wall such as cellulose, glucose and mannose, suggesting that the changes were due to an increase in the cellular structure of the plant during germination (**Rumiyati *et al*., 2012).** Concerning to the decrement of carbohydrates and fat contents during germination may be due to starch hydrolysis to oligosaccharides and monosaccharides that required for energy production for various metabolic processes during germination. Also, fat used for energy production **(Kaushik *et al*., 2010)**.

Effect of domestic processing methods of legumes on minerals content

Data in table (2) demonstrated that soaking process caused significant decrease in mineral contents of legumes under study, while the germination process caused increases in Mg and Ca contents and decreases in Na and K content as compared to raw materials. Also, the results showed that cooking after soaking (12hrs.) had significant decreases in minerals, expect Ca which showed an increase relative to raw materials. Also, cooking after germination tended to reduce Mg, Na and K; but an increase in Ca content respect to raw ones. The results are in the line with **Kaushik *et al.* (2010)** who found that the cooking process caused decrements in Mg and K contents, but Ca content was increased in soybean. Another study of **Hefnawy (2011)** reported that, content of minerals were decreased in lentil after soaking and cooking as a result of the minerals leached into water. Moreover, **Lee and Karunanithy (1990)** explained the high losses in K and the increment of Ca content in soybean by germination may be attributed to binding the divalent minerals to protein and formation of phytate-cation-protein complex. Also, these increment may be due to the presence of divalent minerals such as Ca and Mg in tap water. As noticed in table (2), the raw materials in different legumes under study had the highest values for total iron and total zinc. The soaking process decreased significantly the total iron and total zinc as compared to raw materials. The treatments such as cooking, germination and germination plus cooking caused a pronounced increment in values of total iron and zinc compared to soaking process only, but it remained lower than those in raw materials. The trend observed was consistent to the results of **Hooda and Jood (2003)** who suggested that soaking of fenugreek resulted in a decrease in total Ca, Fe and Zn but germination caused an increase in its contents relative to the raw material. **Sharma (2006)** reported that in mung beans, cowpeas, chickpeas and lentils there were increases in Mn, Zn and Fe contents upon ordinary and pressure cooking. The present finding disagreed with the study of **Kaushik *et al.* (2010)** who demonstrated that the cooking treatments of soybean led to a significantly decrease in total Fe and Zn. This may be due to the water used in cooking discarded in contrast of the present study the cooking water was retained with cooked grains.

Table (1): Effect of domestic processing methods of legumes on chemical composition (g/100g DM)

Items	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	NFE (%)	Energy (Kcal/100g)
Chickpea:						
Raw	24.51±0.50^{a}	5.13±0.15^{a}	3.90±0.01^{a}	4.24±002^{d}	62.88±0.44^{d}	396±0.60^{a}
Soaking	18.56±0.26^{c}	4.90±0.06^{a}	3.75±0.02^{b}	4.30±0.01^{d}	68.79±0.30^{b}	394±0.33^{b}
Soaking plus cooking	17.33±0.06^{d}	4.40±0.05^{b}	3.60±0.01^{c}	4.66±0.05^{c}	70.00±0.07^{a}	389±0.56^{c}
Germination	23.88±0.05^{a}	3.98±0.07^{c}	3.44±00.02^{d}	5.28±0.04^{b}	63.09±0.04^{d}	384±0.59^{d}
Germination plus cooking	22.26±0.08^{b}	3.74±0.02^{c}	3.23±0.06^{e}	5.60±005^{a}	64.49±0.16^{c}	381±0.17^{e}
Fenugreek seeds:						
Raw	29.33±0.81^{a}	6.80±0.05^{a}	3.85±0.03^{a}	6.55±0.03^{d}	53.83±0.77^{c}	394±0.32^{a}
Soaking	20.80±0.12^{d}	6.41±0.03^{b}	3.75±0.02^{b}	6.78±0.01^{d}	62.46±0.13^{a}	391±0.24^{b}
Soaking plus cooking	19.66±0.08^{d}	6.21±0.01^{c}	3.64±0.02^{c}	7.22±0.02^{c}	63.25±0.09^{a}	388±0.10^{c}
Germination	24.83±0.12^{b}	5.75±0.03^{d}	3.54±0.o1^{d}	10.11±0.11^{b}	55.55±0.13^{b}	373±0.34^{d}
Germination plus cooking	23.28±.17^{c}	5.39±0.02^{e}	3.48±0.01^{e}	10.90±0.12^{a}	56.57±0.14^{b}	368±0.46^{e}
Lentil:						
Raw	30.89±0.99^{a}	0.95±0.075^{a}	4.55±0.03^{a}	4.14±0.10^{e}	60.19±1.10^{b}	373±0.65^{a}
Soaking	26.96±0.29^{c}	0.76±0.008^{b}	4.25±0.02^{b}	4.52±0.01^{d}	63.85±0.31^{a}	370±0.09^{b}
Soaking plus cooking	25.10±0.20^{d}	0.72±0.006^{b}	4.01±0.02^{c}	4.97±0.04^{c}	65.18±0.19^{a}	368±0.08^{c}
Germination	28.84±0.36^{ab}	0.68±0.003^{b}	3.88±0.02^{d}	7.01±0.05^{b}	59.21±0.40^{b}	358±0.32^{d}
Germination plus cooking	28.40±0.05^{b}	0.66±0.005^{b}	3.82±0.02^{d}	7.41±0.04^{a}	58.97±0.09^{b}	355±0.15^{e}
Faba bean:						
Raw	26.61±1.45^{a}	2.38±0.01^{a}	4.11±0.08^{a}	7.09±0.09^{e}	60.56±1.49^{b}	370±0.35^{a}
Soaking	18.59±0.21^{c}	2.25±0.02^{b}	3.97±0.04^{a}	7.32±0.01^{d}	68.27±0.23^{a}	368±0.27^{b}
Soaking plus cooking	17.76±0.09^{c}	1.95±0.02^{c}	3.76±0.03^{b}	7.55±0.02^{c}	68.96±0.14^{a}	364±0.33^{c}
Germination	23.50±0.06^{b}	1.84±0.03^{d}	3.56±0.03^{c}	8.58±0.04^{b}	62.10±0.16^{b}	359±0.19^{d}
Germination plus cooking	22.85±0.03^{b}	1.62±0.01^{e}	3.35±0.06^{d}	8.75±0.02^{a}	62.65±0.03^{b}	357±0.11^{e}
White bean:						
Raw	32.05±1.53^{a}	1.43±0.008^{a}	4.91±0.02^{a}	6.23±0.08^{d}	56.09±1.44^{b}	366±0.30^{a}
Soaking	28.83±0.33^{c}	1.27±0. .014^{b}	4.70±0.05^{b}	6.48±00.2^{c}	59.16±0.31^{a}	363±0.22^{b}
Soaking plus cooking	27.21±0.11^{c}	1.00±0.057^{c}	4.43±0.08^{c}	6.59±0.02^{c}	60.76±0.08^{a}	361±0.70^{c}
Germination	31.51±0.41^{ab}	0.85±0.008^{d}	4.25±0.02^{d}	8.04±0.06^{b}	54.88±0.43^{b}	353±0.39^{d}
Germination plus cooking	29.20±0.36^{bc}	0.75±0.005^{e}	4.18±0.02^{d}	8.34±0.02^{a}	56.79±0.37^{b}	351±0.17^{e}

Each value in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Table (2): Effect of domestic processing methods of legumes on minerals content (mg/100g DM)

Items	Mg	Na	K	Ca	Fe	Zn
Chickpea:						
Raw	224.84±1.55^{b}	68.16±0.84^{a}	919.09±1.11^{a}	154.85±1.35^{c}	10.78±0.04^{a}	6.17±0.05^{a}
Soaking	186.00±0.97^{c}	53.43±1.39^{b}	830.60±0.98^{b}	144.85±1.15^{d}	9.36±0.40^{c}	3.89±0.35^{d}
Soaking plus cooking	115.87±0.30^{d}	41.60±1.33^{d}	592.80±1.21^{e}	162.44±1.20^{b}	9.53 ±0.21 ^{bc}	4.12±0.11^{c}
Germination	250.90±1.32^{a}	47.33±0.89^{c}	792.09±0.91^{c}	168.30±0.96^{a}	10.33±0.07^{ab}	4.81±0.05^{c}
Germination plus cooking	185.87±0.94^{c}	43.39±1.52^{d}	773.10±1.70^{d}	170.63±0.89^{a}	9.64±0.34^{bc}	5.62±0.20^{b}
Fenugreek seeds:						
Raw	164.44±0.79^{a}	114.22±2.05^{a}	973.89±0.73^{a}	186.92±0.77^{c}	22.33±1.20^{a}	3.59±0.02^{a}
Soaking	147.05±0.79^{c}	97.60±0.62^{d}	877.00±0.79^{b}	174.12±1.14^{d}	10.93±1.02^{c}	2.25±0.03^{c}
Soaking plus cooking	122.58±0.84^{d}	92.60±0.52^{e}	717.14±0.32^{e}	203.64±1.04^{b}	14.47±0.45^{b}	2.56±0.16^{b}
Germination	165.37±1.30^{a}	105.43±0.47^{b}	815.50±0.43^{c}	210.28±0.88^{a}	11.99±0.52^{c}	2.65±0.09^{b}
Germination plus cooking	161.07±0.80^{b}	101.00±0.58^{c}	786.11±0.87^{d}	210.53±1.27^{a}	12.55±0.32^{c}	2.70±0.19^{b}
Lentil:						
Raw	145.80±1.24^{b}	87.45±1.25^{a}	963.65±1.79^{a}	77.82±0.85^{c}	21.57±0.58^{a}	4.70±0.10^{a}
Soaking	122.74±1.13^{d}	81.97±0.77^{b}	868.94±1.03^{b}	55.85±1.19^{d}	10.56±0.18^{c}	4.26±0.14^{b}
Soaking plus cooking	117.48±0.99^{e}	74.25±0.66^{c}	657.59±1.05^{c}	86.90±0.79^{b}	11.45±0.63^{c}	4.29±0.15^{b}
Germination	152.65±1.17^{a}	72.71±1.07^{c}	621.13±1.35^{d}	91.30±1.23^{a}	12.89±0.03^{b}	4.49±0.09^{ab}
Germination plus cooking	130.62±1.05^{c}	71.55±1.10^{c}	615.41±0.91^{e}	93.94±0.77^{a}	11.53±0.20^{c}	4.36±0.08^{ab}
Faba bean:						
Raw	153.16±1.35^{b}	122.78±1.63^{a}	328.53±2.49^{a}	95.15±2.01^{c}	7.91±0.32^{a}	4.44±0.18^{a}
Soaking	143.65±0.70^{c}	115.26±1.39^{b}	297.90±1.64^{b}	85.26±1.30^{d}	4.53±0.23^{d}	3.03±0.33^{c}
Soaking plus cooking	124.03±0.83^{d}	108.92±2.58^{c}	163.87±2.66^{e}	99.26±0.53^{c}	5.65±0.18^{c}	3.50±0.32^{bc}
Germination	166.48±1.75^{a}	103.13±1.67^{d}	287.11±1.57^{c}	127.29±2.32^{b}	6.44±0.27^{b}	3.68±0.09^{bc}
Germination plus cooking	150.46±1.06^{b}	99.61±1.69^{e}	277.27±0.95^{d}	133.88±0.77^{a}	7.21±0.14^{b}	4.00±0.25^{b}
White bean:						
Raw	155.61±0.57^{ab}	38.71±1.19^{a}	1293.49±1.89^{a}	155.50±0.82^{c}	12.55±0.27^{a}	4.86±0.11^{a}
Soaking	143.92±1.09^{c}	31.98±0.82^{b}	1142.37±1.19^{b}	147.19±0.84^{d}	7.98±0.34^{c}	3.38±0.19^{d}
Soaking plus cooking	140.48±1.01^{d}	29.55±0.74^{b}	1093.34±1.47^{e}	174.91±0.73^{b}	8.63±0.09^{c}	3.85±0.22^{cd}
Germination	158.37±0.89^{a}	24.11±0.96^{c}	1132.77±1.08^{c}	177.29±1.20^{b}	9.35±0.9^{b}	4.19±0.24^{c}
Germination plus cooking	154.10±0.85^{b}	22.12±0.89^{c}	1124.55±0.89^{d}	182.60±1.07^{a}	8.62±0.03^{c}	4.61±0.11^{b}

Each value in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Effect of domestic processing methods of legumes on *in vitro* bioavailability of iron and zinc

As mentioned in fig (1), the bioavailability percent of Fe and Zn *in vitro* were increased when the row materials of legumes underwent to different treatments. The trend observed in the degree to which processing increased the bioavailability percent of Fe and Zn was germination+ cooking > germination > cooking > soaking. The data showed that the values of bioavailability percent of Fe and Zn ranged between 17.92% - 37.90% and 28.36% - 41.28% in chickpea; 8.04% - 25.31% and 11.70% - 23.77% in fenugreek; 11.30% -34.66% and 29.79%-45.87% in lentil; 12.90%-34.67% and 24.10%-36.50% in faba bean and 10.37% - 27.45% and 25.10% - 38.39% in white bean, respectively.

The present results are confirmed by **Hooda and Jood (2003)** who demonstrated that soaking and germination improved the availability of Ca, Fe and Zn in fenugreek as compared with raw. The explanation contributed to phytic acid in plant foods forms complexes with essential dietary minerals such as Ca, Fe, Zn and Mg makes them biologically unavailable for absorption. The phytase activity increased on germination causing catabolism of phytic acid. Phytases, or myo-inositol hexaphosphate phosphohydrolases, are enzymes that hydrolyze myo-inositol 1,2,3,4, 5, 6,-hexakis (dihydrogen phosphate) to myo-inositol and inorganic phosphate and thereby increasing the *in vitro* availability of divalent minerals **(Jood and Kapoor, 1997)**. Another study is in agreement with the current finding ,they suggested that all the processing subjected to mungbean (soaking, germination, cooking, fermentation and dehulling) resulted in an increase in iron bioavailability *in vitro*; the maximum bioavailability was in germinated cooked mungbean, followed by fermented cooked mungbean and germinated raw mungbean **(Barakoti and Bains, 2007).**

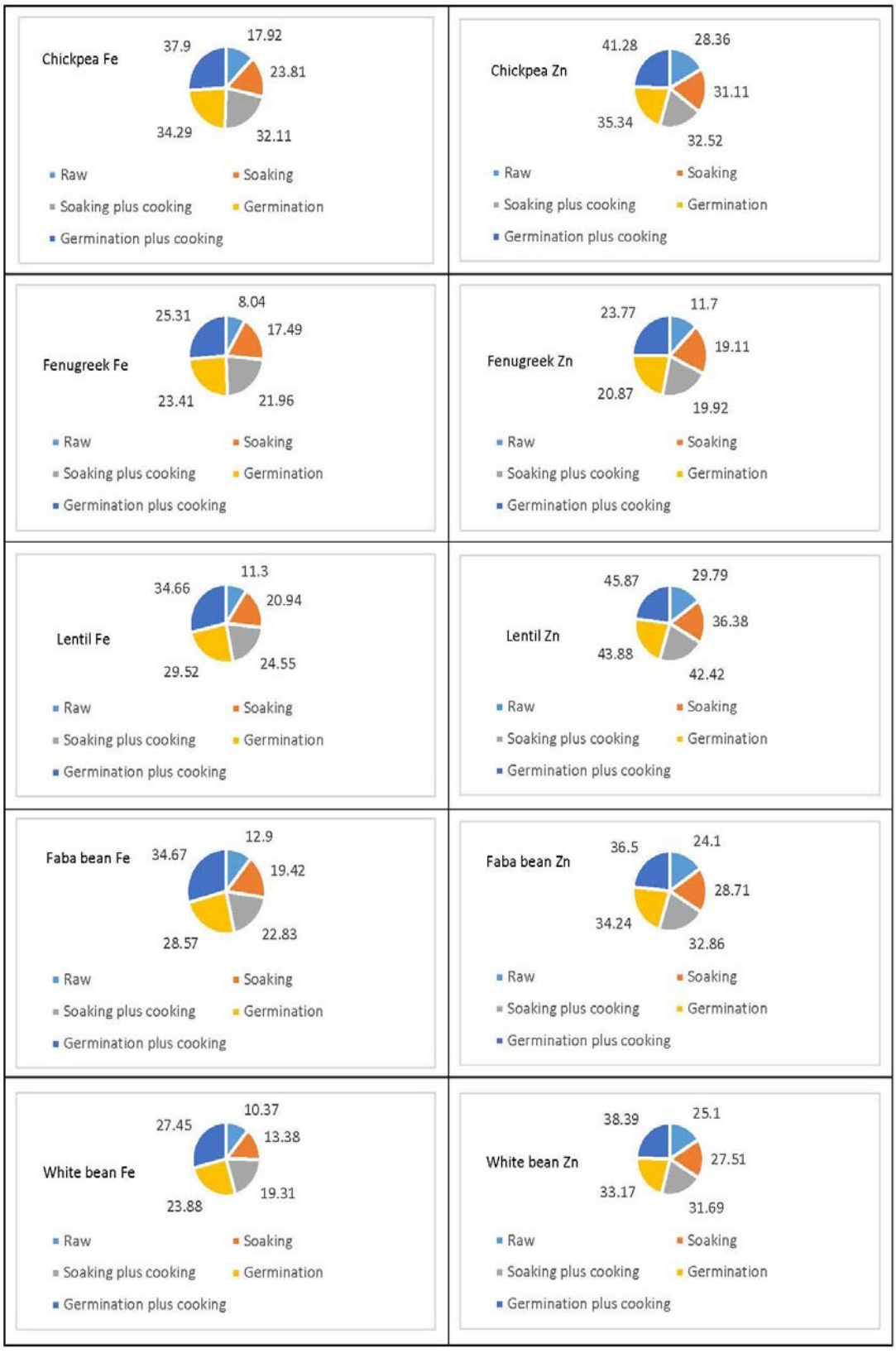

Fig (1): Bioavailability % of Fe and Zn.

Effect of domestic processing methods of legumes on total phenols, total flavonoids and total antioxidants

Table (3) illustrates the content of total phenols, total flavonoids and total antioxidants in legumes under investigation after different process like soaking, cooking after soaking, germination and cooking after germination. It was cleared that soaking of these legumes caused decreases in total phenols and total flavonoids content. The decrease ratios relative to raw materials are 17.60%, 27.94%, 26.99%, 30.39 % and 25.33% in total phenols while, in flavonoids content, are 23.61%, 19.61%, 26.03 %, 24.55% and 34.83% in chickpea, fenugreek and lentil, faba bean and white bean, respectively. These results are in accordance with those of **Saharan *et al.* (2002)** who explained that this reduction may be a result of leaching into the soak water or binding of phenols with other organic substances such as carbohydrate or protein. Also, the same trend of decrease was found in case of cooking investigated legumes after soaking. These results are in agreement with findings of **Duhan *et al.* (2000)** who found that the decrease in phenolic compounds as a result of cooking could be due to thermal degradation, as well as breakdown of phenols.

Data in table (3) also explained total antioxidant in legumes under investigation. It could be noticed that there are reductions comparing to raw legumes in total antioxidants concentration due to soaking and cooking after soaking. It might due to leakage of antioxidant components in soaking water. This is in agreement with **Xu and Chang (2008c)** who revealed that the decrease in the overall antioxidant properties of soaked and cooked cool season legumes could be related to several types of factors including, oxidative reaction, leaching of water-soluble antioxidant compositions and solid losses during treatments. The last factor may describe the reduction in cooked samples.

Germination is a method that can modify the presence of nutrients and anti-nutrients in legume seeds. It is still a household practice during the preparation of traditional Egyptian foods such as nabet soup. In the current study according to the phenols content, **Table (3)** showed that germination resulted in increase in phenols content with ratios 18.51%, 9.19%, 22.24%, 22.40% and 6.60% relative to soaked materials in chickpea, fenugreek, lentil, faba bean and white bean, respectively. Our results are consistent with the findings of **Dueñas *et al.* (2009)** who indicated that germination caused an increase in total phenols in lupin seeds. Furthermore, **Sokrab *et al*. (2012)** mentioned that the increment in phenols could be a result of solubilization of condensed tannin when the seeds were soaked in water and migration of phenols to the outer layer as a result of germination as indicated by the browning of the germinated seeds.

In terms of total flavonoids, germination also caused increase in its contents respect to soaking process. According to total antioxidants, it could be seen that germination resulted in increase in total antioxidants relative to soaked legumes. These results are in line with the findings of **Tarzi *et al.* (2012)** who found that germination led to significant increase in antioxidant activity of chickpea seeds. Inaddition, **Lopez-Amoros *et al.* (2006)** studied the effects of varying germination conditions for beans, lentils and peas on bioactive compounds and expressed that peas and beans undergo a significant increase in antioxidant activity after germination, whereas lentils show a decrease after these process. The greatest therapeutic effect of phenolic compounds, such as antioxidant activity, depends on their structure, including the number of hydroxyl groups and their position. In general the flavonoid compounds present a stronger antioxidant activity than non-flavonoids and the combined forms, such as glycosides, have lower activity than free forms **(Jovanovic *et al.*,1998).**

Effect of domestic processing methods of legumes phenolic compounds fractions

The identification of phenolic compounds in tested legumes after hydrolysis were recorded in table (4). Compositional analysis of phenolic compounds by HPLC revealed a wide variation in the distribution of them among investigated legumes. Twelve phenolic compounds were detected after hydrolysis (ferulic, pyrogallol, protocatechuic, catechin, syringic, epicatechin, vanillic, gallic, caffeic, chlorogenic, ellagic and coumarin). It was notable that ferulic was the most abundant phenolic compounds present in all studied legumes. Besides ferulic, pyrogallol, protocatechuic, catechin and syringic and epicatechin were other major phenolic compounds detected. Whereas vanillic, gallic, caffeic, chlorogenic, ellagic and coumarin were minor. It was clear that soaking led to decrease in the content of detected phenolic compounds as compare to raw legumes. On the other hand germination caused increase in their contents relative to soaked legumes . These results are in accordance with those of **Sreerama *et al.* (2010)** who reported that ferulic acid was the most abundant phenolic compounds present in seed coat, cotyledon and embryonic axe fractions of chickpea. Majority of phenolic compounds in seeds were stored as soluble conjugate or insoluble forms. Hence, increase of compounds in germinated materials may due to mobilization of stored phenolics by active enzymes such as polyphenol oxidase during germination and also the release of bounded form of

free phenols by the cellular constituents **(Vadivel *et al.*, 2012)**. Therefore, the different phenolic content of the various legumes exhibited different levels contribution for the antioxidant activities. Material with higher total phenolic compounds exhibited higher antioxidant ability because phenols contain hydroxy which could provide more phenolic hydroxyl and hydrogen atoms with higher amount of total phenols , thus their scavenging effect on free radicals were stronger. (**Zhao *et al.*, 2014)**.

Effect of domestic processing methods of legumes isoflavone fractions

The isoflavone contents (mg/100g) in tested legumes are presented in **Table (5)**. As shown in this table, isoflavones components were detected as genistein, daidzein and formononetin. It was clear that genistein was the highest detected competent in all studied legumes. it ranged from 5.96 mg/100g in raw chickpea to 16.56 mg/100g in raw lentil. on the other hand, formononetin was the lowest detected competent in legumes under investigation. These results are consistent with the findings of **Konar *et al.* (2012)** who found that genistin was the main isoflavone present in green, red, and yellow split lentils as well as in haricot beans. Differences between the isoflavone contents of different kinds of the same legume and between different legumes of the same kinds may due to several factors, such as origin, harvesting time, environmental conditions which may cause this variability between different kinds of legumes.

From the same table it could noticed that there are various decreases in detected isoflavones content according to soaking comparing with raw materials. The highest decreasing ratio occurred in soaked faba bean (35.17%) relative to raw material. On the other hand, the lowest decreasing ratio placed in case of chickpea (2.52%). The present data are in agreement with **Kao *et al.* (2004)** who reported that during soaking of soybean, isoflavones (about 12 to 57%) are lost in the water. The same authors explained that leaching of isoflavones to the soaking water depend on time and temperature and raises by increasing temperature and time.

Table (3): Effect of domestic processing methods of legumes on total phenol, total flavonoids and total antioxidant

Items	Total phenols (mg/100g)	Total Flavonoids (mg/100g)	Total Antioxidant(%)
Chickpea:			
Raw	5794.63±2.48^{a}	553.68±3.37^{a}	72.80±1.44 ^{c}
Soaking	4774.35±1.76^{d}	422.94±0.97^{d}	65.06±1.10^{d}
Soaking plus cooking	4544.06±8.90^{e}	400.57±0.91^{e}	54.85±0.11^{e}
Germination	5658.23±5.08^{b}	483.68±4.42^{b}	86.73±1.40^{a}
Germination plus cooking	5443.38±1.90^{c}	442.77±1.56^{c}	83.11±0.65^{b}
Fenugreek seeds:			
Raw	5614.36±2.93^{a}	657.78±5.27^{a}	90.69±1.15^{a}
Soaking	4045.43±2.26^{d}	528.77±2.94^{d}	90.54±0.06^{a}
Soaking plus cooking	3634.09±5.62^{e}	505.00±1.56^{e}	88.73±0.14^{b}
Germination	4417.53±9.65^{b}	561.00±0.97^{b}	91.12±0.22^{a}
Germination plus cooking	4223.09±4.33^{c}	553.50±1.23^{c}	90.61±0.31^{a}
Lentil:			
Raw	6038.93±3.00^{a}	554.34±2.45^{a}	81.65±1.13^{b}
Soaking	4409.00±2.40^{c}	410.00±3.72^{d}	79.26±0.24^{c}
Soaking plus cooking	3605.31±2.58^{d}	375.08±2.41^{e}	76.52±0.46^{d}
Germination	5389.64±6.49^{b}	484.68±1.40^{b}	92.14±1.84^{a}
Germination plus cooking	3545.31±1.50^{e}	458.97±4.54^{c}	78.26±0.37^{c}
Faba bean:			
Raw	3365.34±5.760^{a}	639.67±2.94^{a}	95.16±0.79^{a}
Soaking	2342.60±12.95^{d}	482.63±2.83^{d}	89.50±0.77^{b}
Soaking plus cooking	2114.38±11.78^{e}	424.57±1.14^{e}	85.94±1.70^{c}
Germination	2867.55±23.30^{b}	560.38±2.76^{b}	94.57±1.20^{a}
Germination plus cooking	2458.33±3.040^{c}	515.23±5.77^{c}	91.56±0.21^{b}
White bean:			
Raw	3880.58±3.94^{a}	485.27±2.93^{a}	60.47 ± 0.07^{b}
Soaking	2897.46±1.41^{d}	316.21±1.67^{d}	50.76 ± 0.42^{c}
Soaking plus cooking	2674.46 ±1.99^{e}	296.20±1.92^{e}	36.45 ± 0.20^{d}
Germination	3088.88± 7.27^{b}	449.97±2.57^{b}	77.89 ± 1.41^{a}
Germination plus cooking	2900.86±1.24^{c}	410.00±1.01^{c}	76.25 ± 0.27^{a}

Each value in a column followed by the same letter are not significantly different at ($p \leq 0.05$).

Table (4): Effect of domestic processing methods of legumes on phenolic compound fractions (mg/100 DM)

Items	Ferulic	Pyrogallol	Protocatechuic	Catechin	Syringic	Epicatechin	Vanillic	Gallic	Caffeic	Chlorogenic	Ellagic	Coumarin
Chickpea:												
Raw	478	380	237	161	151	75	25	35	47	59	22	28
Soaking	356	214	213	145	132	56	21	21	40	50	18	21
Soaking plus cooking	300	131	175	120	105	46	14	12	22	43	12	18
Germination	384	374	225	154	148	65	23	28	45	55	24	24
Germination *plus cooking	254	292	215	132	127	41	18	19	31	52	20	19
Fenugreek seeds:												
Raw	367	276	207	184	185	76	21	40	38	66	19	43
Soaking	227	244	164	162	161	64	16	28	31	57	14	37
Soaking plus cooking	208	191	111	151	140	52	11	23	27	47	10	29
Germination	335	264	187	167	164	72	18	37	44	61	17	42
Germination plus cooking	285	235	152	127	139	46	14	25	29	55	16	31
Lentil:												
Raw	427	328	191	207	170	98	54	29	51	48	39	30
Soaking	339	300	155	180	154	86	48	23	42	38	29	23
Soaking plus cooking	221	264	146	132	144	72	43	18	30	30	25	20
Germination	380	315	172	200	158	88	50	37	47	43	32	26
Germination plus cooking	320	271	154	117	151	74	42	24	35	34	30	22
Faba bean:												
Raw	399	277	259	175	154	84	54	50	62	77	45	35
Soaking	342	225	229	165	136	71	46	46	53	71	39	31
Soaking plus cooking	314	204	213	152	124	61	38	38	51	64	30	24
Germination	357	246	234	174	150	74	50	54	65	79	42	37
Germination plus cooking	205	212	216	157	134	68	37	42	58	64	38	28
White bean:												
Raw	389	291	227	157	171	106	81	60	55	57	25	45
Soaking	214	261	186	126	155	88	65	52	51	49	18	41
Soaking plus cooking	184	230	134	119	145	72	46	41	43	36	16	34
Germination	275	278	216	155	163	99	78	64	60	50	22	46
Germination plus cooking	227	234	196	137	141	85	54	48	52	42	15	37

Table (5): Effect of domestic processing methods of legumes on isoflavones fraction (mg/100g DM)

Items	Genistein	Daidzein	Formononetin
Chickpea:			
Raw	5.96	2.95	1.78
Soaking	5.81	1.61	0.59
Soaking plus cooking	5.74	1.46	0.40
Germination	9.18	5.02	ND
Germination plus cooking	7.68	3.52	ND
Fenugreek seeds:			
Raw	7.23	5.09	0.77
Soaking	6.66	3.62	0.38
Soaking plus cooking	5.71	1.52	0.03
Germination	16.35	9.85	1.02
Germination plus cooking	13.40	7.22	0.35
Lentil:			
Raw	16.56	3.78	1.33
Soaking	12.21	3.70	1.10
Soaking plus cooking	7.85	2.21	0.94
Germination	22.33	5.84	1.75
Germination plus cooking	20.79	5.77	1.47
Faba bean:			
Raw	16.32	5.75	3.74
Soaking	10.58	3.91	3.29
Soaking plus cooking	9.54	3.46	3.23
Germination	23.20	5.64	ND
Germination plus cooking	17.10	4.47	ND
White bean:			
Raw	7.92	1.04	0.19
Soaking	6.85	0.82	0.13
Soaking plus cooking	6.04	0.47	0.08
Germination	11.23	1.42	0.38
Germination plus cooking	9.08	1.10	0.24

Results in table (5) also indicate that there are increment in fractionated isoflavones content in germinated studied legumes. Genistein showed a highest increase in all germinated studied legumes. Germination caused increase in genistein content relative to its soaked contents with 58.0%, 145.50%, 82.88%, 119.28 and 63.94% in chickpea, fenugreek, lentil, faba bean and white bean, respectively. In addition, daidzein content raised to 5.02, 9.85, 5.84, 5.64 and 1.42 mg/100g in chickpea, fenugreek, lentil, faba bean and white bean, respectively compared with its content in respective soaked materials. However, formononetin is the lowest detected isoflavones component. It also was undetectable in chickpea and faba bean. Our results are in the same line with the findings of **Pei-Yin and Hsi-Mei (2006)** who reported that isoflavones were significantly changed after germination and influenced by the varieties and stages of germination. Furthermore, the aglycones, daidzein, genistein, and glycitein, significantly increased after one day of germination. At the beginning of germination, not only aglycones releasing from glucosides through the catalysis of activated glucosidase in seeds but also isoflavones produced through the biosynthesis in the malonate and phenylpropanoid pathways **(Hahlbrock and Scheel, 1989).** Germinated legumes may be a source of phytoestrogens as isoflavones. There are many biological activities associated with the isoflavones, including a reduction in osteoporosis, cardiovascular disease and prevention of cancer and for the treatment of menopause symptoms **(Cassidy *et al.*, 2006)**.

In terms of cooking after both soaking or germination, it could found in **Table (5)** that cooking led to decrease isoflavones content after soaking as well as after germination in all examined legumes. It could be due to thermal degradation. In addition **Rochfort *et al.* (2011)** found that cooking process reduce isoflavones content in 13 varieties of pulse including field pea, chickpea, and lentil.

Table (6): Effect of domestic processing methods of legumes on vitamins B fractions (mg/100g DM)

Items	Niacin B3	Thiamine B1	Pyridoxine B6	Folic acid	Riboflavin B2
Chickpea:					
Raw	0.463	1.072	7.871	1.199	0.285
Soaking	0.391	0.964	6.867	0.935	0.264
Soaking plus cooking	0.341	0.854	6.179	0.847	0.255
Germination	0.632	1.465	8.803	2.321	0.327
Germination plus cooking	0.591	1.244	7.222	1.565	0.308
Fenugreek seeds:					
Raw	0.513	2.862	2.683	1.403	0.440
Soaking	0.452	2.479	2.264	1.175	0.378
Soaking plus cooking	0.366	2.173	1.971	1.047	0.245
Germination	0.733	3.405	4.139	2.329	0.725
Germination plus cooking	0.678	3.147	4.072	2.127	0.6.97
Lentil:					
Raw	0.219	2.675	4.147	2.794	0.335
Soaking	0.189	2.052	3.834	2.172	0.293
Soaking plus cooking	0.148	1.682	2.922	2.010	0.226
Germination	0.310	3.683	5.641	4.268	0.584
Germination plus cooking	0.278	2.986	4.721	4.047	0.479
Faba bean:					
Raw	0.305	3.547	3.348	1.525	0.369
Soaking	0.271	3.292	2.621	1.386	0.279
Soaking plus cooking	0.249	3.069	2.511	1.216	0.233
Germination	0.489	5.564	4.657	1.788	0.531
Germination plus cooking	0.352	4.934	3.885	1.681	0.477
White bean:					
Raw	0.655	1.754	1.873	1.881	0.423
Soaking	0.602	1.549	1.694	1.578	0.391
Soaking plus cooking	0.523	1.278	1.396	1.334	0.314
Germination	0.898	2.822	2.828	1.968	0.663
Germination plus cooking	0.769	2.107	2.535	1.752	0.611

Effect of domestic processing methods of legumes on vitamin B fractions:

Vitamins B fraction contents in our study are shown in **Table (6)**. It was noticeable that soaking led to decrease in all vitamins B fraction contents comparing with raw materials. In case of chickpea the reduction ratios are 15.55%, 10.07%, 12.75%, 22.02% and 7.37% in niacin (B3), thiamine (B1), pyridoxine (B6), folic acid and riboflavin (B2), respectively. Furthermore, the reduction ratios in fenugreek seeds were 11.89%, 13.38%, 15.6%, 16.25%, 14.09%; in lentil were 13.69%, 23.28%,

7.54%, 22.26%, 12.54%; in faba bean were 11.14%, 7.19%, 21.71%, 9.11%, 24.39% and in white bean were 8.09%, 11.68%, 9.55%, 16.10%, 7.57%, respectively.

The present results of our study are in agreement with those published by **Prodanov *et al* . (2004)** who detected that soaking faba beans produced losses in thiamine and riboflavin. On the other hand, the same authors reported that soaking caused no changes in niacin content. They explained the reason of vitamin losses after soaking by multiple effect of several factors. Such as, the vitamin removal with the drained liquid after soaking, the time taken for legume hydration and the forms of vitamin which it exist in the plants. For example, thiamine exist as thiamin, mono, di and tri-phosphates; riboflavin can exist as, flavin-mononucleotide (FMN) and flavin-adenine dinucleotide (FAD). In case of germination, **Table (6)** also shows that there are increase in contents of vitamin B fractions in tested legumes relative to raw ones due to germination. The increasing ratios chickpea are 36.50 %, 36.66%, 11.84%, 93.57% and 14.73% in niacin (B3), thiamine (B1), pyridoxine (B6), folic acid and riboflavin (B2), respectively. Furthermore, these increasing ratios in fenugreek seeds were 42.88%, 18.97%, 54.26%, 66.0%, 64.77%; in lentil were 41.55%, 37.68%, 36.02%, 52.75%, 74.32%; in faba bean were 60.32%, 56.86%, 39.09%, 17.24%, 43.90% and in white bean were 37.09%, 60.88%, 50.98%, 4.62%, 56.73%, respectively. These findings are concord with those obtained by **Satya *et al.* (2010)** who described that germination process caused increment in vitamin B content due to their biosynthesis during germination. It also increase crude protein, ascorbic acid and improve the protein digestibility. It was notable according to **Table (6)** that cooking process recorded reduction in the contents of vitamin B fractions in cooked after soaking samples. Also, the same reduction trend was found in case samples which cooked after germination. These results are in accordance with those of **Uherova *et al.* (1993)** who illustrated that conventional cooking caused a high loss of thiamin, riboflavin and ascorbic acid in vegetables, but microwave cooking and autoclaving improved the retention of these vitamins compared to boiling.

In conclusion, this study indicated that common household processes for legumes, in particular, germination process could be improved the nutritional value through increasing in protein content, the capacity of antioxidant constituents and vitamin B fractions as well as *in vitro* bioavailability of minerals. Also, germination treatment considered as a cheap bio- process, therefore it is recommend that increasing its utilization in food products besides as ingredient in normal food preparation. Hence, it is necessary to further explore the effect of studied legumes *in vivo* examination in the future.

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