

EFFECT OF FUNGAL PHYTASE ON DEGRADATION OF THE PHYTIC ACID DURING FALAFEL PREPARATION

Ziena, H.M. S.

Department of Food Science and Technology, Faculty of Agriculture, Damanhour Branch, Alexandria University, Damanhour, 22516, Egypt.

ABSTRACT

The present study aimed to investigate the enzymatic degradation of phytic acid in faba bean cotyledons to be used in Falafel preparation. Phytase was used in soaking water at a rate of 10 g / kg cotyledons. The volume of soaking water was previously adjusted to be equal to the hydration coefficient of cotyledons in order to overcome the effect of leaching out phenomenon during soaking for 12 hr / 20°C. Moreover, such an effect was investigated for fermentation step (180 min/37°C) followed by frying of Falafel patties.

Data indicated that phytic acid contents (and phytate phosphorus) were declined by 6% and 23% (untreated sample) and by 17 % and 65 % (phytase - treated sample) after soaking and after soaking followed by fermentation , respectively. The inorganic phosphorus increased sharply while the organic phosphorus other than phytate phosphorus seemed to be unaffected. The dialyzed portion of phytate on which phytase mainly acted on was 60% in raw cotyledons. Frying process had a slight effect on the aforementioned constituents . The *in vitro* protein digestibility increased significantly specially for phytase- treated samples . The sensorical properties were not deteriorated due to addition of fungal phytase . The natural fermentation significantly affected the flavour character and overall acceptability . The fermentation of Falafel paste up to 120 min didn't deteriorate the quality of Falafel . Addition of such a commercial preparation of phytase during the processing of Falafel should be taken into consideration . It may overcome the risk of the phytic acid as an antinutritional factor and thereby increase the bioavailability of phosphorus .

keywords : Fungal phytase, phytate degradation, phosphorus compounds, Faba bean cotyledons, soaking, fermentation, Falafel preparation, frying, organolyptic properties.

INTRODUCTION

Falafel (Taamia) is one of the most popular foods consumed by the majority of the population in Egypt (Ziena *et al*, 1988). The main constituent of Falafel is cotyledons of faba bean (*Vicia faba* L.) with variable amounts of onion , garlic and some vegetables such as Egyptian leek , herbs and parsley . The whole mixture is finely ground prior to forming into patties which are finally deep fried in cotton seed oil (Rizk *et al* , 1986 ; Youssef *et al* . 1986) .

Faba bean , in common with other grain legumes , represents an excellent source of phosphours and other nutritionally essential minerals . However , a considerable portion of the total phosphorus content is known to be present in the form of phytates in faba beans (Griffiths and Thomas , 1981) . Phytic acid is strongly negatively charged over most of the pH scale , suggesting a tremendous potential for complexing positively charged molecules such as cations and positively charged proteins with the formation

of complexes that may be insoluble or otherwise unavailable under physiologic conditions (Cheryan, 1980; Serraino and Thompson, 1984) and therefore, reduces the bioavailability of protein and minerals in diets (Eskin and Wiebe, 1983; Juliano *et al*, 1991; Ayet *et al*, 1997 and Almaná, 2000). As well, phytates could inhibit enzymes such as pepsin, α -amylases and trypsin (O'Dell and de Boland, 1976 and Hincks and Stanley, 1987).

Phytates are generally considered to be heat-stable with only a small fraction being destroyed during heat processing (Deshpande and Cheryan, 1984 and Ziena *et al*, 1992, a), and decreased slightly on soaking of dry legumes mainly due to leaching out process (Tabekhia and Luh, 1980; El-Shimi, 1980; Ologhobo and Fatuga, 1984; Hamza and Youssef, 1988; Khan *et al*, 1988 and Vijayakumari *et al*, 1995, 1996 and 1998).

The hydrolysis of phytate is catalysed by the enzyme phytase (myoinositol hexa phosphate phosphohydrolase E.C. 3.1.3.8 to inositol and free orthophosphate (Eskin and Wiebe, 1983). It is the lack of this endogenous enzyme system that renders phytic acid phosphorus generally unavailable to humans (Cheryan, 1980). However, plant phytases are found in the seeds of most cereals and legumes (Chen and Pan, 1977). Processing prior to heating as well as germination and/or fermentation of legumes may increase phytase activity and therefore favor minerals bioavailability and proteins (Chitra *et al*, 1996; Cuadrado *et al*, 1996; Silva and Trugo, 1996; Agte and Joshi, 1997 and Sharma and Khetarpaul, 1997). However, it appears that the treatment with phytase is an effective mean of lowering phytate content in food systems (Serraino and Thompson, 1984 and Harland and Narula, 1999).

Application of fungal phytase (EC.3.1.3.8) in the food industry was reviewed by Zyta (1992). Microbial phytase to liberate minerals from the phytate complex and thereby improved availability was reported by Saxen (1990). It was obvious that addition of wheat phytase to uncooked oatmeal increased iron solubility from 4 to 11 and in precooked to 18%, while endogenous phytase of uncooked oatmeal had less effect on phytate digestion and iron solubility (Sandberg and Svanbrg, 1991). In accordance, addition of *A. niger* phytase to the doughs during breadmaking exhibited more pronounced degradation of phytate than endogenous flour phytase did (Tuerk and Sandberg, 1992). Meanwhile, addition of microbial phytase to phytate-rich diets based on soy protein isolate was found to improve the availability of zinc in growing rats (Rinbach and Pallauf, 1993). It was found that addition of microbial phytase to the meal containing phytate increased iron adsorption from 14.3 ± 2.6 to 26.1 ± 3.8 % (Sandberg *et al*, 1996). On the other hand, five strains of lactic acid bacteria isolated from sour doughs were found to degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour (Lopez *et al*, 2000). According to Ferdrikson *et al*. (2001) almost complete degradation of inositol hexa, penta, tetra and triphosphates was achieved by incubation of the pea protein solution with exogenous phytase for one hour. Studies of Porres *et al*. (2001) revealed that 85% of phytic acid in whole wheat flour during breadmaking was degraded when microbial phytase in combination with citric acid was applied. Such a combination enhanced total iron dializability 15 folds.

The effect of fungal phytase during the preparation of Falafel paste (i.e. soaking and short fermentation) on phytic acid content, phosphorus forms, the *in vitro* protein digestibility and sensory evaluation was the aim of the present study.

MATERIALS AND METHODS

Materials :

A sample of faba bean cotyledons (season 2001) and other ingredients were purchased from the local market in Damanhour, Egypt. The fungal phytase (Natuphos), 1000 FTU, a venture product of Gist-Brocades, The Netherlands, and BASF, Germany, which was produced from *Aspergillus niger* was used in the present study. One unit of phytase activity is defined as the quantity of enzyme that liberate 1 μ mole of inorganic phosphorus per min from 5.1 mM sodium phytate at pH 5.5 at 37°C.

Methods :

Preparation of Falafel :

The method adapted for Falafel preparation is that used by Falafel processors (Fig. 1). Two experiments were carried out (i.e. without and with addition of fungal phytase). To the enzyme- treated sample, the fungal phytase was added to soaking water at 10 g / kg dry cotyledons as recommended by Zhang *et al* (1999). The volume of soaking water was previously adjusted to be equal the hydration coefficient of the cotyledons to avoid leaching out effect. The effect of adding phytase was examined after each step of the present study (i.e. after 12 hr soaking, after fermentation for 30,60,90,120,150 & 180 min / 37 °C and after the all treatments mentioned previously followed by frying at 175°C for 6 min). In case of untreated Falafel the same technique was used as mentioned previously except that the addition of phytase was omitted.

Soaked cotyledons, fermented pasts belong to different time periods and Falafel samples were dried at 45°C for 24 h, ground to fine powder passing through 40 mesh sieve and then stored in air- tight Kilner jars at -20 °C prior to analysis as outlined by Srivastava & Khokhar (1996). Dried Falafel samples were defatted with hexane by soxhlet extraction for 16 hr following Brooks & Morr (1984), prior to grinding to fine powder. All analysis were carried out in triplicates. Samples were analyzed for moisture and protein contents using the standard methods of the AOAC (1980). The phytate content was determined by a procedure based on the ferric hydroxide precipitation method described by Wheeler and Ferrel (1971). Finally, the iron content of ferric phytate was measured according the AOAC method (1980) using α - α dipyridyl reagent. The ration of iron to phosphorus in ferric phytate was assumed to be 4 : 6 to calculate phytate phosphorus content and the later value was converted into phytate by assuming it contained 28.2 % phosphorus (Brooks & Morr, 1984). For determenation of total phosphorus, 1 g sample was digested with 20 ml of a mixture of sulphuric acid and perchloric acid (10 : 1), while water soluble inorganic phosphorus was

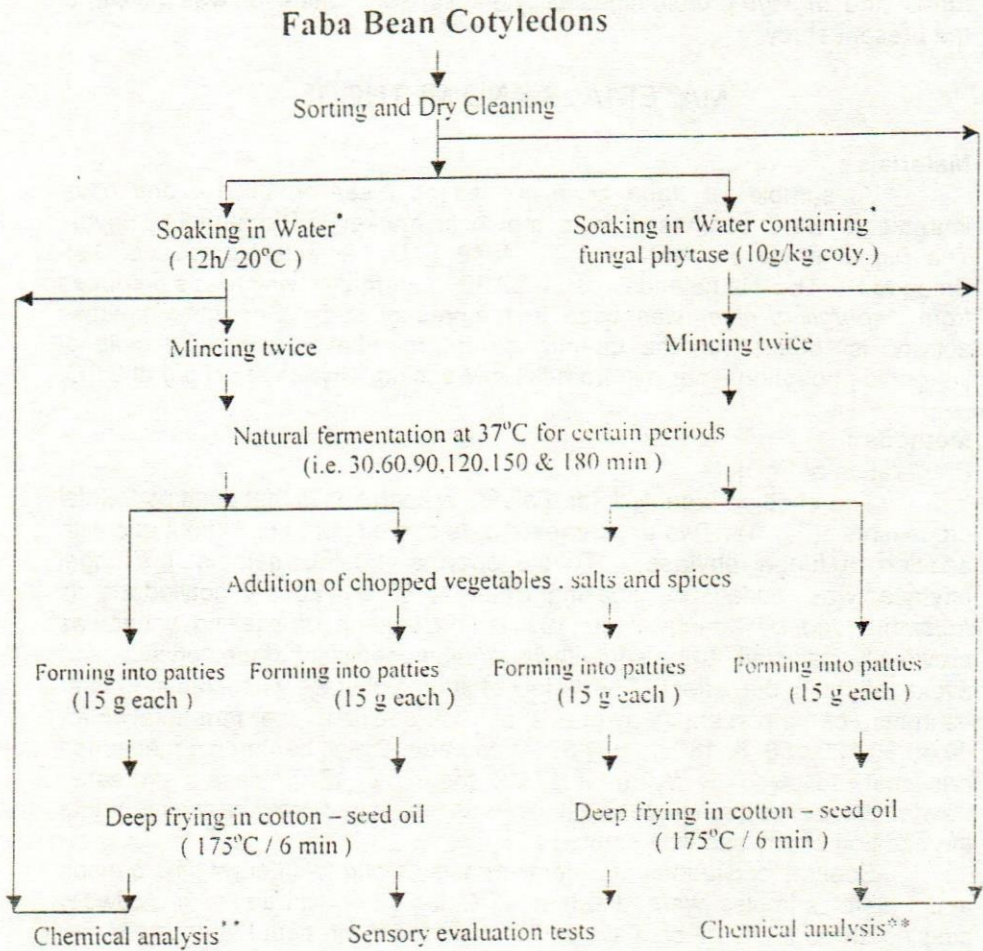


Fig. 1 : Flow chart for preparation of Falafel .

* A preliminary experiment was done to determine the hydration coefficient of faba bean cotyledons (Ziena 1989), then the soaking process in the main study was carried out using the quantity of water absorbed by beans in order to minimize the loss of nutrients and to make sure that the change in phytic acid content is due to enzymatic hydrolysis and doesn't belong to leaching out process.

** To stop the phytase activity 30 ml 0.67 M HCl was added to analytical samples only at the end of each step of the present study as recommended by Larsson & Sandberg (1992) .

extracted with 5 % trichloroacetic acid as outlined by Belavady & Banerjee (1953) . The phosphorus content of the different phosphorus compounds was determined using the spectrophotometric method that is based on the formation of phosphorus - molybdate complex (AOAC , 1980) . Samples belong to all treatments were dialyzed at 4°C for 7 days against distilled water , which was changed twice daily. The method of Serraino and Thompson (1984) was followed in this respect . Dialysis retentates were analyzed for phytic acid content as described previously .

The pepsin- pancreatin procedure of Saunders *et al* (1973) was used to determine the *in vitro* protein digestibility except that at the end of digestion , 30 ml of 1.6 M TCA were added to the digest and left for 2 hrs. prior to centrifugation . The supernatant was analyzed for TCA soluble nitrogen by the microKjeldahl method (AOAC, 1980) . The percentage of digestion was calculated with respect to the total nitrogen in the sample .

Fried Falafel samples were presented simultaneously to a panel of 10 panelists who were asked to rank each sample on a hedonic scale as 1 (very poor) ; 2 - 4 (poor) ; 5.6 (fair) , 7 - 8 (good) and 9 - 10 (excellent) for each of colour , flavour and consistency .

Data were subjected to statistical analysis using Analysis of Variance (ANOVA) and further subjected to Duncan's Multiple Range test as outlined by Steel and Torrie (1980) .

RESULTS AND DISCUSSION

1. Distribution of phosphorus compounds of Falafel :

Data given in Tables 1 and 2 indicate that the total phosphorus contents were quite comparable during soaking and fermentation steps and this was true for both phytase treatment and untreated samples . This may be due to the volume of water added in soaking process being equal to the hydration coefficient of cotyledons , thus leaching out was minimized.

Phytate phosphorus content represented 55.1% of the total phosphorus in bean cotyledons .In accordance phytate phosphorus was reported to account approximately for 50-85% of the total phosphorus stored in many raw cereals and legumes (Ravindran *et al* , 1994) .It was obvious that soaking and /or fermentation steps exhibited highly significant effect on phytate phosphorus. The percentages of the reduction of phytate phosphorus content were 6.0% & 22.9% for untreated samples and were 16.6% & 64.8% for phytase treated samples , after 12 hr soaking and after soaking followed by fermentation , respectively for 180 min at 37 °C.

On contrary to phytate phosphorus , the inorganic phosphorus was found to increase gradually as soaking and or fermentation were preceded. The percentage increases were very high for samples treated with phytase compared with their untreated counterparts. The increases of inorganic phosphorus were 96% & 320% (phytase treated samples) and were 25.8% & 158% (untreated samples) as affected by soaking for 12 hr and soaking followed by fermentation for 180 min , respectively (Table 1&2) .

Table 1 : Phosphorus compounds' content of Falafel samples as affected by endogenous phytase.

Treatment	Total phosphorus mg/100g	Phytic acid phosphorus (PAP)		Inorganic phosphorus		Organic phosphorus other than PAP	
		mg/100g	%	mg/100g	%	mg/100g	%
		Raw cotyledons	250.1 ^b	137.6 ^a	55.1	21.5 ^b	8.6
Soaked 12hr X	261.7 ^a	129.3 ^b	49.4	27.0 ^f	10.3	105.4 ^a	40.2
Y		125.9 ^b	48.1	28.8 ^f	11.0	107.0 ^a	40.8
Ferm. 30 min X	256.3 ^{ab}	126.3 ^b	49.2	33.0 ^e	12.9	97.0 ^b	37.9
Y		121.1 ^c	47.2	34.6 ^e	13.5	100.6 ^b	39.2
Ferm. 60 min X	257.7 ^{ab}	121.4 ^c	47.1	39.4 ^d	15.3	97.0 ^b	37.6
Y		118.0 ^{cd}	45.8	42.3 ^d	16.4	97.4 ^b	37.8
Ferm. 90 min X	266.2 ^a	117.9 ^{cd}	44.3	47.9 ^c	18.0	100.4 ^b	37.7
Y		115.4 ^d	43.4	51.1 ^b	19.2	99.7 ^b	37.5
Ferm. 120minX	260.3 ^a	115.2 ^d	44.3	49.5 ^b	19.0	95.6 ^{bc}	36.7
Y		112.9 ^{de}	43.3	53.9 ^{ab}	20.7	93.5 ^c	35.9
Ferm. 150minX	256.3 ^{ab}	109.8 ^e	42.8	52.0 ^a	20.3	94.5 ^c	36.9
Y		106.2 ^{ef}	41.4	54.9 ^a	21.4	95.2 ^c	37.1
Ferm. 180minX	256.5 ^{ab}	106.1 ^{ef}	41.3	55.4 ^a	21.6	95.0 ^c	37.0
Y		102.8 ^f	40.1	58.2 ^a	22.7	95.5 ^c	37.2

* Means in a column not sharing the same superscript are significantly different at P < 0.01 .

** X : before frying ; Y : after frying .

Table 2 : Phosphorus compounds content of Falafel samples as affected by fungal phytase.

Treatment	Total phosphorus mg/100g	Phytic acid phosphorus (PAP)		Inorganic phosphorus		Organic phosphorus other than PAP	
		mg/100g	%	mg/100g	%	mg/100g	%
Raw cotyledons	250.1 ^b	137.6 ^a	55.1	21.5 ^b	8.6	91 ^c	36.4
Soaked 12hr X	260.5 ^a	114.7 ^b	44.0	42.2 ^b	16.2	103.6 ^{cd}	39.8
Y		110.2 ^b	42.3	45.8 ^b	17.6	104.5 ^{cd}	40.1
Ferm. 30 min X	251.7 ^b	103.6 ^c	41.2	48.3 ^c	19.2	99.8 ^c	39.7
Y		97.8 ^c	38.8	51.1 ^c	20.3	102.8 ^{de}	40.8
Ferm. 60 min X	255.8 ^{ab}	89.9 ^d	35.1	60.9 ^e	23.8	105.0 ^{cd}	41.0
Y		84.9 ^d	33.2	64.0 ^e	25.0	106.9 ^c	41.8
Ferm. 90 min X	262.7 ^a	79.0 ^e	30.1	70.7 ^d	26.9	113.0 ^b	43.0
Y		74.6 ^e	28.4	72.2 ^d	27.5	115.9 ^b	44.1
Ferm. 120minX	257.3 ^{ab}	70.9 ^f	27.5	79.2 ^c	30.8	107.2 ^c	41.7
Y		67.7 ^f	26.3	81.1 ^{bc}	31.5	108.5 ^{bc}	42.2
Ferm. 150minX	255.0 ^{ab}	61.3 ^g	24.1	82.4 ^{bc}	32.3	111.3 ^a	43.6
Y		57.4 ^g	22.5	84.4 ^b	33.1	113.2 ^b	44.4
Ferm. 180minX	260.8 ^a	48.5 ^h	18.6	90.2 ^a	34.6	122.1 ^a	46.8
Y		44.5 ^h	17.1	92.1 ^a	35.3	124.2 ^a	47.6

* Means in a column not sharing the same superscript are significantly different at P < 0.01 . ** X : before frying ; Y : after frying .

The organic phosphorus other than phytate phosphorus content in phytase untreated samples seemed to be unchanged and generally fluctuated slightly on contrary to a considerable increase of the same component in phytase treated samples. The values of increase were 14% & 34% after soaking and after soaking followed by fermentation, respectively (Table 1 and 2).

Phytic acid content :

Tables 3 and 4 show that phytic acid content in faba bean cotyledons was 487.8 mg / 100g. Owing to the limited volume of soaking water, the reduction of phytic acid content during soaking and fermentation mainly was due to hydrolysis of phytate. The endogenous phytase resulted in a considerable reduction in phytic acid content by about 6% & 8% as a result of soaking for 12 hr and fermentation for 180 min, respectively. The low effect of endogenous phytase in bean cotyledons may be due to the low concentration of enzyme and / or its activity which is directly related to the rate of hydration. Endogenous phytase of faba bean was found to increase markedly during soaking at 20 °C up to 24 hr while soaking at 35 °C resulted in a decline of the endogenous faba bean phytase activity (Henderson & Ankrah, 1985). The optimum temperature for fungal phytase added in the present study was 37 °C. However, the addition of phytase resulted in a further significant decrease in phytic acid content by 5.5% and 23% after soaking for 12 hr and fermentation for 180 min, respectively. It was clear that the effect of fermentation at 37 °C for 3 hr was more pronounced than soaking process for 12 hr. This may be explained on the basis that soaking was applied for cotyledons (at 20 °C), while fermentation process was carried out for minced soaked cotyledons (at 37 °C). The mincing process leads to increase the surface area of bean particles and thereby, elevates the hydrolytic activity of added phytase.

The natural fermentation of legume pastes exhibited a significant decline in phytic acid content. The degree of degradation depends on the temperature and time of incubation (Chitra *et al*, 1996; Cuadrado *et al*, 1996 and Sharma and Khetarpaul, 1997). However, the maximum accepted period for fermentation of Falafel dough was 3 h/ 30°C (El-Sahn & Youssef, 1989).

The effect of frying on phytic acid content and other phosphorus compounds wasn't significant (Tables 1-4). The forming Falafel paste into patties along with the short time of frying lowered the heat penetration into Falafel patties (Youssef *et al*, 1988). However, phytates are generally considered to be heat-stable with only a small fraction being destroyed during heat processing (Deshpande and Cheryan, 1984 and Ziena *et al*, 1992, a).

Meanwhile, Falafel made from chick peas contained about 50% of the initial phytic acid content present in raw chickpeas. The reduction mainly attributed to leaching out during soaking in a plenty of water (Almana, 2000). It was clear that leaching out of water soluble phytates during soaking accompany with a significant decrease in phosphorus content and other related minerals. So, the degradation of phytic acid by another tool rather

Table 3 : Effect of dialysis on the forms of phytic acid of Falafel treatments without fungal phytase .

Treatment	Total mg/100g	Forms of Phytic Acid					
		Dialyzed		Undialyzed		Hydrolyzed	
		mg/100g	%	mg/100g	%	Mg/100g	%
Raw cotyledons	487.8 ^a	293.1 ^a	60.1	194.6	39.9	0.0	0.0
Soaked 12h X	458.5 ^b	262.9 ^b	53.9	192.7	39.5	32.2 ⁱ	6.6
Y	446.3 ^b	254.1 ^c	52.1	190.7	39.1	42.9 ^h	8.8
Ferm. 30min X	447.7 ^b	253.6 ^c	52.0	193.6	39.7	40.5 ^h	8.3
Y	429.3 ^c	247.8 ^c	50.8	181.5	37.2	58.5 ^g	12.0
Ferm. 60min X	430.5 ^c	245.8 ^c	50.4	188.8	38.7	57.1 ^g	11.7
Y	418.4 ^{cd}	237.5 ^d	48.7	180.9	37.1	69.3 ⁱ	14.2
Ferm. 90min X	418.1 ^{cd}	228.3 ^c	46.8	189.7	38.9	69.8 ⁱ	14.3
Y	408.2 ^d	220.9 ^{de}	45.3	187.3	38.4	79.5 ^e	16.3
Ferm. 120min X	408.7 ^{de}	219.0 ⁱ	44.9	189.7	38.9	79.0 ^e	16.2
Y	400.3 ^{de}	312.1 ⁱ	43.7	183.3	38.4	87.3 ^d	17.9
Ferm. 150min X	389.5 ^f	202.9 ^g	41.6	186.3	38.2	98.5 ^c	20.2
Y	376.6 ^{gh}	186.3 ^h	38.2	190.2	39.0	106.3 ^b	22.8
Ferm. 180min X	376.3 ^{gh}	193.2 ^h	39.6	182.9	37.5	111.7 ^b	22.9
Y	361.8 ^h	180.0 ⁱ	36.9	181.9	37.3	125.8 ^a	25.8

^a Percentage of Initial quantity .

** X : before frying ; Y : after frying .

+ Means in a column not sharing the same superscript are significantly different at P < 0.01 .

Table 4 : Effect of dialysis on the forms of phytic acid of Falafel treated with fungal phytase .
Forms of Phytic Acid

Treatment	Total mg/100g	Dialyzed		Undialyzed		Hydrolyzed	
		mg/100g	%	mg/100g	%	Mg/100g	%
Raw colyledons	487.8 ^a	293.1 ^a	60.1	194.6 ^a	39.9	0.0	0.0
Soaked 12h X	406.7 ^b	214.1 ^b	43.9	192.6 ^a	39.5	80.9 ⁱ	16.6
Y	350.8 ^c	197.1 ^c	40.4	193.6 ^a	39.7	97.1 ^k	19.9
Ferm. 30min X	367.5 ^d	174.6 ^d	35.8	193.1 ^a	39.7	119.9 ^j	24.6
Y	346.9 ^e	151.7 ^e	31.1	195.6 ^a	40.1	140.4 ⁱ	28.8
Ferm. 60min X	318.7 ^f	124.8 ^f	25.6	194.1 ^a	39.8	168.7 ^h	34.6
Y	301.3 ^g	112.2 ^f	23.0	189.3 ^a	38.8	186.3 ^g	38.2
Ferm. 90min X	280.2 ^h	86.3 ^g	17.7	192.7 ^a	39.5	208.8 ⁱ	42.8
Y	264.8 ⁱ	75.6 ^g	15.5	193.6 ^a	39.7	218.5 ⁱ	44.8
Ferm. 120min X	251.3 ^j	58.0 ^h	11.9	193.1 ^a	39.6	236.5 ^e	48.5
Y	240.1 ^j	42.4 ⁱ	8.7	198.0 ^a	40.6	247.3 ^e	50.7
Ferm. 150min X	217.5 ^k	46.3 ⁱ	9.5	171.2 ^b	35.1	270.2 ^d	55.4
Y	203.6 ^l	33.7 ^j	6.9	170.2 ^b	34.9	283.9 ^c	58.2
Ferm. 180min X	172.7 ^m	35.6 ^j	7.3	147.8 ^c	30.3	304.4 ^b	62.4
Y	166.2 ^m	34.1 ^j	7.0	135.6 ^c	27.8	318.0 ^a	65.2

* Percentage of Initial quantity .

+ Means in a column not sharing the same superscript are significantly different at P < 0.01 .

** X : before frying ; Y : after frying .

than leaching out process (e.g. treatment with phytase) will help the phosphorus and the other related minerals to release while avoid proteins (and enzymes) to form phytate – protein complex.

Dialyzed and undialyzed phytic acid :

Data for dialysis experiment are shown in Tables 3 and 4. It was clear that dialyzed phytate (i.e. free phytic acid & phytate-mineral complex) represented ~ 60 % of the total phytates , while the undialyzed portion was ~ 40 % (i.e. phytate –protein complex & phytate – minerals – protein complex) . A gradual decrease in dialyzed phytates after soaking for 12 hr and during fermentation was noticed . The reduction of dialyzed phytates were up to 20 % and 53 % from the total phytate for untreated and phytase treated samples , respectively as a result of soaking followed by fermentation (Tables 3&4) . On contrary to dialyzed phytates , the undialyzed phytates portion seemed to be constant after soaking and during fermentation process for both phytase-treated and untreated samples with an exception of phytase-treated pastes which were fermented for 150 & 180 min, since the undialyzed phytate portion was found to decrease significantly . It seemed that both endogenous bean phytase and the added microbial phytase acted on the dialyzable phytates specially free phytic acid rather than the soluble phytate – mineral complex . However , the hydrolyzed phytates increased gradually up to 23 % and 62 % from the initial phytates for untreated and phytase – treated samples , respectively as a result of soaking followed by fermentation . It was obvious that the fermentation process was efficient in lowering phytate rather than soaking and frying . The effect of added phytase was 3 folds of that for the endogenous phytase itself (Table 3 and 4) .

Although the heat treatment during frying process had a slight but generally not significant effect on total phytic acid content , only the hydrolyzed phytate portion was found to increase markedly as a result of frying .

II . The *in vitro* protein digestibility :

In general, the method applied for Falafel preparation results in elevation of the *in vitro* protein digestibility . It was clear that just soaking process increased the digestibility of protein by ~ 8 % . However , the frying added another 5 – 6 % (Table 5) . Addition of phytase resulted in an additional increase in the *in vitro* protein digestibility by 8.5 % and 12.5 % after soaking for 12 hr and after fermentation for 3 hr , respectively . However , the figure of 88.8 % was obtained for the *in vitro* digestibility of Falafel prepared from phytase – treated cotyledons . It was reported that the *in vitro* protein digestibility of Falafel on Alexandria markets was generally low and ranged between 67 and 70 % (Ziena *et al* , 1992 , b) .

III . Sensory evaluation of Falafel :

Data for sensory evaluation of Falafel samples are shown in Table 6 . The colour scores of all samples under study were more or less the same and ranged between 6.9 and 7.6 . On the other hand , the consistency of Falafel samples was improved markedly by fermentation process .

Table 5 : The *in vitro* protein digestibility (%) of Falafel subjected to different treatments .

Treatment	Without phytase				With phytase			
	Before frying		After frying		Before frying		After frying	
	Value	% improv.	Value	% improv.	Value	% improve	Value	% improv.
Raw cotyledons	63.8±0.8 ^{cd}	-			63.8 ± 0.8 ^f	-		
Soaked 12 h	71.7±1.1 ^c	12.38	75.5±1.3 ^d	18.34	77.1±1.3 ^e	20.85	80.5±0.7 ^f	26.18
Ferm. 30 min	71.9±1.0 ^{bc}	12.69	75.9±1.3 ^{cd}	18.97	78.2±1.1 ^{de}	22.57	81.6±0.8 ^{ef}	27.90
Ferm. 60 min	73.0±0.7 ^{bc}	14.42	76.2±1.1 ^{cd}	19.44	79.5±1.1 ^{cd}	24.61	82.8±1.2 ^{de}	28.86
Ferm. 90 min	73.5±0.9 ^{bc}	15.20	77.0±1.2 ^{bc}	20.69	80.7±0.8 ^{bc}	26.48	84.1±1.1 ^{cd}	31.81
Ferm. 120 min	73.9±0.8 ^{ab}	15.83	78.3±1.1 ^{ab}	22.73	82.5±0.7 ^{ab}	29.31	85.9±1.2 ^{bc}	34.64
Ferm. 150 min	74.7±1.0 ^a	17.08	78.8±1.0 ^{ab}	23.51	82.9±1.0 ^a	29.94	87.0±0.9 ^{ab}	36.36
Ferm. 180 min	76.1±1.2 ^a	19.28	79.1±0.9 ^a	24.45	84.2±1.1 ^a	31.97	88.8±1.0 ^a	39.18

The *in vitro* digestibility of casein = 98.8 %

* Mean ± Standard error .

** Means in a column not sharing the same superscript are significantly different at P < 0.01 .

Table 6 : Sensory evaluation⁺ of Falafel subjected to different treatments .

Treatment	Without phytase				With phytase			
	Colour	Flavour	Consistency	Overall accep.	Colour	Flavour	Consistency	Overall accep.
Soaked 12 h	7.2±0.2 ^{ab}	7.8±0.3 ^{bc}	7.4±0.3 ^c	7.5±0.3 ^{ab}	7.5±0.1 ^a	8.0±0.3 ^{ab}	7.5±0.2 ^b	7.8±0.3 ^a
Ferm. 30 min	7.5±0.2 ^a	8.1±0.2 ^{ab}	8.2±0.3 ^{ab}	7.8±0.2 ^a	7.3±0.2 ^{ab}	8.3±0.2 ^a	8.1±0.3 ^a	7.9±0.2 ^a
Ferm. 60 min	6.9±0.1 ^b	8.3±0.3 ^a	8.5±0.4 ^a	7.8±0.2 ^a	7.6±0.1 ^a	8.1±0.1 ^a	8.1±0.1 ^a	7.7±0.1 ^a
Ferm. 90 min	7.3±0.2 ^a	7.5±0.2 ^c	8.0±0.1 ^b	7.4±0.3 ^b	6.9±0.2 ^b	7.7±0.2 ^b	8.3±0.3 ^a	7.4±0.2 ^b
Ferm. 120 min	6.9±0.1 ^b	7.6±0.2 ^c	8.1±0.1 ^{ab}	7.2±0.2 ^b	7.3±0.2 ^{ab}	7.0±0.1 ^a	8.0±0.3 ^a	7.0±0.1 ^c
Ferm. 150 min	7.4±0.3 ^a	6.4±0.2 ^d	8.3±0.3 ^{ab}	6.5±0.2 ^c	7.6±0.1 ^a	6.1±0.1 ^d	8.4±0.2 ^a	6.3±0.2 ^d
Ferm. 180 min	7.6±0.2 ^a	5.3±0.3 ^e	8.0±0.2 ^b	5.9±0.2 ^d	7.5±0.2 ^a	5.0±0.1 ^e	8.2±0.1 ^a	5.4±0.2 ^e

+ Each characteristic is evaluated by hedonic scale (1 to 10) .

* Mean ± Standard error .

** Means in a column not sharing the same superscript are significantly different at P < 0.01 .

Moreover, the flavour of Falafel samples was improved significantly as the fermentation period was elongated. It was clear that the maximum time for fermentation period was 120 min. and this was true for both the two experiments (i.e. without and with phytase). This may be due to the developing of sourness as reported by El- Sahn and Youssef (1989) who found that the continuous increase of total aerobic mesophilic lactic acid bacteria during the fermentation of Falafel paste prior to frying is the cause of developing of sourness in the final fried Falafel. The overall acceptability of Falafel was influenced mainly by the flavour property rather than the other two properties specially during the fermentation process. The point of interest is that the addition of fungal phytase had no deteriorative effect on the sensorial properties of *Falafal*.

CONCLUSION

In the light of data presented here, the addition of fungal phytase during the processing of Falafel can be recommended. It significantly decreased the phytate content and thereby increased the bioavailability of phosphorus. As well, the *in vitro* protein digestibility of Falafel increased significantly.

REFERENCES

- Agte, V.V. and S.R. Joshi (1997). Effect of traditional food processing on phytate degradation in wheat and millets. *J. Agric. Food Chem.*, 45:1659-1661.
- Almana, H.A. (2000). Extent of phytate degradation in breads and various foods consumed in Saudi Arabia. *Food Chemistry*, 70: 451- 456.
- AOAC (1980). Official Methods of Analysis. 13thed., Association of Official Analytical Chemists. Washington, DC.
- Ayet, G.; C. Burbano; C. Cuadrado; M.M. Pedrosa; L.M. Robredo; M. Muzquiz; C. La Cuadra; A. Castano and A. Osagie (1997). Effect of germination, under different environmental conditions, on saponins, phytic acid and tannins in lentils (*Lens culinaris*). *J. Sci. Food Agric.*, 74: 273-279.
- Belavady, B. and S. Banerjee (1953). Studies on the effects of germination on the phosphorus values of some common Indian pulses. *Food Research*, 18: 223-226.
- Brooks, J.R. and C.V. Morr (1984). Phosphorus and phytate content of soybean protein components. *J. Agric. Food Chem.*, 32: 672-674.
- Chen, L.H. and S.H. Pan (1977). Decrease of phytates during germination of pea seeds (*Pisum sativa*). *Nutr. Rep. Int.*, 16: 125-131.
- Cheryan, M. (1980). Phytic acid interactions in food system. Review. *CRC Critical Reviews in Food Science and Nutrition*, 13: 297-335.
- Chitra, U.; U. Singh and V.P. Rao (1996). Phytic acid, *in vitro* protein digestibility, dietary fiber and minerals of pulses as influenced by processing methods. *Plant Foods for Human Nutrition*, 49: 307-316.

- Cuadrado, C.; G. Ayet; L.M. Robredo; J. Tabera; R. Villa; M.M. Pedrosa; C. Burbano and M. Muzquiz (1996). Effect of natural fermentation on the content of inositol phosphates in lentils. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 203:268-271.
- Deshpande, S.S. and M. Cheryan (1984). Effects of phytic acid, divalent cations, and their interactions on \downarrow amylase activity. *J. Food Sci.*, 49: 516-519.
- El-Sahn, M.A. and M.M. Youssef (1989). Microbial changes during the natural fermentation of Falafel paste. *Microbiologie-Aliments-Nutrition*, 7: 267-272.
- El-Shimi, N.M. (1980). Changes in Nutritional Value and Microstructure of Faba Bean Seeds during Germination. Ph.D. Thesis, Faculty of Agric., Univ. of Alex. Alexandria, Egypt.
- Eskin, N.A.M. and S. Wiebe (1983). Changes in phytase activity and phytate during germination of two faba bean cultivars. *J. Food Sci.*, 48: 270-271.
- Fredrikson, M.; P. Biot; M.L. Alminger; N.G. Carlsson and S. Sanberg (2001). Production process for high quality pea protein isolate with low content of oligosaccharides and phytate. *J. Agric. Food Chem.*, 49: 1208 - 1212.
- Griffiths, D.W. and T.A. Thomas (1981). Phytate and total phosphorus content of field beans (*Vicia faba* L.). *J. Sci. Food Agric.*, 32: 187-192.
- Hamza, M.A. and M.M. Youssef (1988). Effects of decortication, soaking and germination on amino acid composition, *in vitro* digestibility and some antinutritional factors of faba bean (*Vicia faba* L.). *Alex. J. Agric. Res.*, 33: 103-114.
- Harland, B.F. and G. Narula (1999). Food phytate and its hydrolysis products. (Review). *Nutrition Research*, 19: 947-961.
- Henderson, H.M. and S.A. Ankrah;(1985). The relationship of endogenous phytase, phytic acid and moisture uptake with cooking time in *Vicia faba minor* cv. Aladin. *Food Chemistry*, 17: 1-11.
- Hincks, M.J. and D.W. Stanley; (1987). Multiple mechanisms of bean-hardening. Proceedings of the Second Workshop, Antigua, Guatemala, 1-7 June, (1986). In: IDRC Manuscript Report, MR 157e, June (1987). PP. 16-65.
- Juliano, B.O., A. Hussain; A.P. Resurreccion and W. Bushuk (1991). Interference of phytate with extraction of protein from brown rice using SM acetic acid. *Cereal Chemistry*, 68: 317-318.
- Khan, N.; R. Zaman and M. Elahi (1988). Effect of processing on the phytic acid content of Bengal grams (*Cicer aritinum*) products. *J. Agric. and Fd Chemistry*, 36: 1274-1276.
- Larsson, M. and Sandberg, A.S. (1992). Phytate reduction in oats during malting. *J. Food Science*, 57: 994-997.
- Lopez, H.W.; A. Ouvry; E. Bervas; C. Guy; A. Messenger; C. Demigne and C. Remesy (2000). Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. *J. Agric. Food Chem.*, 48: 2281 - 2285.

- O'Dell, B.L. and A. De-Boland (1976). Complexation of phytate with proteins and cations in corn germ and oilseeds. *J. Agric. Food Chem.*, 24: 804-808.
- Ologhobo, A.D. and B.L. Fetuga (1984). Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. *J. Food Science*, 49: 199-201.
- Porres, J. M.; P. Etcheverry; D.D. Miller and X.G. Lei. (2001). Phytase and citric acid supplementation in whole-wheat bread improves phytate – phosphorus release and iron dialyzability. *J. Food Science*, 66: 614 – 619.
- Ravindran, V.; G. Ravindran and S. Sivalogan (1994). Total and phytate phosphorus contents of various foods and foodstuffs of plant origin. *Food Chemistry*, 50: 133-136.
- Rimbach, G. and J. Pallauf (1993). Enhancement of zinc utilization from phytate – rich soy protein isolate by microbial phytase. *Zeitschrift fuer Ernahrungswissenschaft*, 32: 308 – 315.
- Rizk, S.S.; G.A. El-Sherbiny and M.A. El-Shialy (1986). Improving the nutritional value of patti beans. *Egypt. J. Food Sci.*, 14: 111-118.
- Sandberg, A. S.; L.R. Hulthen and M. Turk (1996). Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J. Nutrition*, 126: 476 – 480.
- Sandberg, A.S. and U. Svanberg (1991). Phytate hydrolysis by phytase in cereals: Effect on *in vitro* estimation of iron availability. *J. Food Science*, 56: 1330 – 1333.
- Saunders, R.M.; M.A. Canner; A.N. Booth; E.M. Brekoff and G.O. Kohler (1973). Measurement of digestibility of alfalfa protein concentrate by *in vitro* and *in vivo* method. *J. Nutr.* 103: 530-535.
- Saxen, R. (1990). A new enzyme reduces mineral deficiencies. *Kemish Tidskrift*, 102: 20 – 21.
- Serraino, M.R. and L.U. Thompson (1984). Removal of phytic acid and protein-phytic acid interactions in rapeseed. *J. Agric. Food Chem.*, 32: 38-40.
- Sharma, A. and N. Khetarpaul (1997). Effect of fermentation on phytic acid content and *in vitro* digestibility of starch and protein of rice- blackgram dhal whey blends. *Journal of Food Science and Technology, India*, 34: 20-23.
- Silva, L.G. and L.C. Trugo (1996). Characterization of phytase activity in lupin seed. *Journal of Food Biochemistry*, 20: 329-340.
- Srivastava, S. and S. Khokhar (1996). Effects of processing on the reduction of α -ODAP (α -N-oxalyl-L-2,3-diaminopropionic acid) and anti-nutrients of khesari dhal, *Lathyrus sativus*. *J. Sci. Food Agric.*, 71: 50-58.
- Steel, R.G.D. and T.H. Torrie (1980). Principles and Procedures of Statistics. McGraw Hill Co., USA.
- Tabekhia, M.M. and B.S. Luh (1980). Effect of germination, cooking and canning on phosphorus and phytate retention in dry beans. *J. Food Science*, 45: 406-408.

- Tuerk, M. and A.S. Sandberg (1992). Phytate degradation during breadmaking: Effect of phytase addition. *J. cereal science*, 15: 281-294.
- Vijayakumari, K.; Siddhuraju, P. and Janardhanan, K. (1995). Effect of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *valgaris* L. *Plant Food for Human Nutrition*, 48: 17-29.
- Vijayakumari, K.; Siddhuraju, P. and Janardhanan, K. (1996). Effect of soaking, cooking and autoclaving on phytic acid and oligosaccharide contents of the tribal pulse, *Mucuna monosperma* DC. *Ex. Wight. Food Chemistry*, 55: 173-177.
- Vijayakumari, K.; Siddhuraju, P.; Pugalenthi, M. and Janardhanan, K. (1988). Effect of soaking and heat processing on the levels of antinutrients and digestible proteins in seeds of *Vigna aconitifolia* and *Vigna sinensis*. *Food Chemistry*, 63: 259-264.
- Wheeler, E. L. and Ferrel, R.E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*. 48: 312-320.
- Youssef, M.M.; Hamza, M.A.; Abd-El-Aal, M.H.; Shekib, L.A. and A.A. El-Banna (1986). Amino acid composition and *in vitro* digestibility of some Egyptian foods made from faba bean (*Vicia faba* L.). *Food Chemistry*, 22: 225-230.
- Youssef, M.M.; Ziena, H.M. and Abdel-Nabey, A.A. (1988) Acceptability and trypsin inhibitor of Falafel as affected by particle size and roasting of faba bean cotyledons. *Alex. J. Agric. Res.* 33: 121-133.
- Zhang, X.; Roland, D.A.; McDaniel, S.R. and Rao, S.K. (1999). Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. *Poultry Sci.* 78: 1567-1572.
- Ziena, H.M.S. (1989). Hard-to-Cook Phenomenon in Relation to Physical, Chemical and Biological Properties of Faba Bean (*Vicia faba* L.). Ph.D. Thesis, Faculty of Agric., Alex. Univ., Alexandria, Egypt.
- Ziena, H.M.; M.H. Abdel-Aal and M.M. Youssef (1988). Formulation and characteristics of new recipes of Egyptian patti beans (*Falafel*). *Plant Foods for Human Nutrition*. 38: 225-234.
- Ziena, H.M.; M.M. Youssef and A.R. El-Mahdy (1992.a). Amino acid composition and some antinutritional factors of cooked faba bean (*Medammis*): Effects of cooking temperature and time. *J. Food Science*, 56: 1347-49 & 52.
- Ziena, H.M.; Youssef, M.M and Hamza, M.A. (1992,b). Effect of method of processing on quality of instant Falafel. 2nd Alex. Conf.Fd. Sci. Tech. March 2-4, 1992, Alexandria, Egypt. 150-163.
- Zyta, K. (1992). Mould phytases and their application in the food industry *World J. Microbiology & Biotechnology*, 8: 467 - 472.

تأثير إنزيم الفيتاز الفطرى على تحطم حامض الفيتيك خلال إعداد الفلافل

حامد مرسى سعد زينة

قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - فرع دمهور - جامعة الاسكندرية - دمهور -
٢٢٥١٦ - ج.م.ع

كان الهدف من هذا البحث هو استخدام الفيتاز الفطرى لتحطيم حامض الفيتيك أثناء إعداد الفلافل - والتي تعد الغذاء الشعبى للغالبية العظمى بمصر - وقد أضيف الفيتاز إلى ماء النقع بمعدل 10 جم/ك فلفلات ، حيث استخدم للنقع قدر من الماء يعادل مكافئ التشرب وذلك تجنباً لتأثير عملية الإرتشاح . هذا وقد تم دراسة تأثير عمليات النقع لمدة ١٢ ساعة على درجة حرارة الغرفة والتخمر لمدة تصل إلى ١٨٠ دقيقة على ٣٧ °م والقلى على محتوى كل من حامض الفيتيك والمركبات الفوسفورية الأخرى وأجزاء حامض الفيتيك القابلة للدليسة وغير القابلة للدليسة وتلك المحللة مائياً وكذا تأثيرها على الهضمية المعملية للبروتين والخواص العضوية الحسية للفلافل .

وأوضحت النتائج إنخفاضاً معنوياً لمحتويات حامض الفيتيك (وفوسفور الفيتيك) بنسب ٦% ، ٢٣% (للعينات غير المعاملة بالإنزيم) ، ١٧% ، ٦٥% (للعينات المعاملة بالإنزيم) وذلك عقب النقع وبعد النقع المتبوع بالتخمر على الترتيب . وقد ازداد الفوسفور غير العضوى معنوياً بينما لم يتأثر محتوى الفوسفور العضوى باستثناء فوسفور الفيتيك . كما أوضحت الدراسة أن الجزء من حامض الفيتيك القابل للدليسة يعادل ٦٠% من فيتات الفلفلات الخام وهو الجزء الذى تسلثر بانزيم الفيتاز .

وتبين أن تأثير القلى على المكونات السابقة كان طفيفاً ، أما الهضمية المعملية للبروتين فقد ازدادت معنوياً وخاصة لتلك العينات المعاملة بانزيم الفيتاز بينما لم تتأثر الخواص العضوية الحسية للفلافل ، غير أن عملية التخمر الطبيعى قد أثرت معنوياً فى النكهة والتقبل الكلى للفلافل وعموماً فإن تخمر عجينة الفلافل لمدة تصل ٢٠ دقيقة لم تكن مصحوبة بأى تأثير سلبى على الخواص العضوية الحسية للفلافل ولذا فإنه ينصح بإضافة مستحضرات الفيتاز أثناء إعداد الفلافل وذلك للتغلب على مثالب تواجد حامض الفيتيك - باعتباره أحد مانعات التغذية - ومن ثم إمكانية زيادة إتاحة الفوسفور كعنصر معدنى مفيد للإنسان .