

EFFECT OF FERMENTATION OF FABIA BEAN (*Vicia faba*) ON ITS NUTRITIVE AND SENSORY PROPERTIES

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ABSTRACT

Evaluation the effect of using different microbial strains in the fermentation of Faba Bean on its nutritive value and consumer acceptance was performed. Fermentation of processed Faba Bean (autoclaved and non autoclaved) with *Lactobacillus acidophilus* (LAB), *Bacillus Subtilis* (*B.subtilis*) and *Saccharomyces cerevisiea* (Yeast) were performed for 44 hrs after which analysis of chemical composition and anti-nutritional factors were performed to estimate the major differences before and after fermentation process.

The result showed marked decrease in the pH of the fermented product accompanied by slight changes in the amino acids pattern. Marked reduction of anti-nutritional factors was obtained after fermentation especially in phytic acid.

Estimation of True Digestibility (T.D), Biological Value (B.V) and Net Protein Utilization (N.P.U) were performed in a biology trial using Albino rats. Results revealed that, soaked and cooked Faba Bean fermented with *B. Subtilis* showed the greatest T.D, B.V and N.P.U.

By applying panel test to estimate the palatability of the fermented Faba Bean, results showed that, there was no significant difference between all fermented products.

INTRODUCTION

Faba Bean (*Vicia faba*) is an important food legume in China, Egypt, Italy, Brazil and Ethiopia. While China is considered as the major producing country in the world (Adsule and Akpapunam, 1996). *Vicia faba* is known by a variety of common names such as Faba Bean, field bean, broad bean, horse bean, tick bean, Windsor bean, haba and feve. It belongs to the family leguminosae (Fabaceae). *Vicia faba* is divided into three botanical varieties on the basis of seed size (1) *Vicia faba* L. var. minor Beck- tick bean with smaller seeds, (2) *Vicia faba* L. var. equina Pers- horse bean with medium sized seeds, (3) *Vicia faba* L. var major – broad beans with larger seeds and Faba Bean production is widespread in temperate and subtropical regions of the world and it ranks as the fourth most important pulse crop in the world after dry beans, dry peas and chickpea. It is the most important pulse crops in the world, being consumed in large quantities in the Middle East, Far East, and North Africa, particularly Egypt. Faba Bean, like other leguminous crops, plays a unique role due to its high protein content and ability to fix atmospheric nitrogen (El-sheikh *et al.*, 1999).

The yield of broad beans in Egypt according to the statistical study of FAO (2005) is 127,000, 31,496, 400,000, 26,000 Hary for dry area, dry yield, dry production and dry seed, respectively. (Hary = Hectare = 10,000 m²).

Faba Bean forms an important part of people diet in developing countries. It is consumed in the form of immature tender pods, green mature seed or as dry seeds after cooking. The methods of cooking and consumption differ widely according to the geographical location. Some of the popular Faba Bean dishes in Egypt include Fool Medammis, Taamia or Falafel and Fool Nabet (Askar, 1986).

Faba Bean seeds contain antinutritional factors such as protease inhibitors, tannins, favism inducing factors, haemagglutinins, flatus producing factors and phytates (Adsule and Akpapunam, 1996).

Many processing techniques were used to overcome the high antinutritional factor content of this high protein legume like, soaking, germination and dry and moist cooking which were found to be effective in this purpose (Ibrahim *et al.*, 2002).

Another approach is to use fermentation with certain types of bacteria and yeast to gain the same effect. These types of living organisms include *B. subtilis*, LAB and Yaset which showed significant reduction of the antinutritional factor contents in Faba Bean (Doblado *et al.*, 2003; Porres *et al.*, 2003 and Azokpota *et al.*, 2006).

The present work was designed to study the behaviour of micro-organism and pH values during the fermentation process, effect of fermentation process on amino acids contents, anti-nutritional factors, biological evaluation and consumer acceptance.

MATERIALS AND METHODS

- Dry Faba Bean (*Vicia faba*) Var. Giza-2- , the most common variety in Egypt, were kindly obtained from the Legume Research Section, Agriculture Research Center , Ministry of Agriculture, Giza Egypt.
- *B. subtilis*, LAB and yeast were kindly obtained from Food Safety and Biotechnology laboratory, Regional Center for Food and Feed, ARC, Giza.
- Adult male Albino rats for biological evaluation were obtained from the animal house in Research Institute of Ophthalmology, Academy of Scientific Research and Technology.
- Salt mixtures and vitamins mixture for biological evaluation were obtained from Sigma Chemical Co.

Preparation of micro-organism's cultures:

Cultures of micro-organisms were performed according to Kiers *et al.*, (2000). Before the experiment, the strains were inoculated into brain heart infusion broth and incubated at the optimum temperature (37°C for *B. Subtilis* and LAB and 25°C for yeast) for 18-24 hrs. The bacterial cultures were diluted with sterile solution contained 0.85% NaCl and 0.1% peptone and the yeast culture was diluted with sterile distilled water and 0.1% peptone to give approximately 10⁶ colony forming units /ml(CFU/ml).

Fermentation of Faba Bean:

Fermentation of Faba Bean was performed according to Kiers *et al.*, (2000). Cooked Faba Bean was transferred into 8 glass jars and 4 of them were autoclaved at 121°C for 30 min. After cooling, one jar from the

autoclaved and one jar from the non autoclaved groups were left as control and the other 6 jars were inoculated with 5 ml of the micro-organisms under study. After mixing, the beans were fermented at 37°C for bacterial fermentation and 25°C for yeast fermentation for 44 hrs. Sub samples were taken at 0, 6, 18, 24 and 44 hrs to perform total count of the used strains and to measure the pH during fermentation process. After 44 hrs, drying of the samples was performed according to AOAC 2006.

Estimation of the chemical composition:

The chemical composition including moisture, ash, crude fibres, total proteins and total lipids contents of the raw materials and the processed products were determined after fermentation according to the methods described in the AOAC (2006). Total carbohydrate was calculated by difference. Amino acids determination except for tyrosine and tryptophan was performed according to the method described by the Official Journal of the European Communities (1998). Amino acids determination in amino acids department in Regional Center for Food and Feed, ARC, Giza.

Determination of anti-nutritional factors:

Determination of tannins, phytic acid and trypsin inhibitor were determined according to (Price *et al.*, 1978; Wheeler and Ferrel, 1971 and Kakade *et al.*, 1969).

pH measurements and microbial analysis:

pH measurements and microbial analysis were carried out according to Kiers *et al.*, 2000.

Animal Feeding trial for biological evaluation:

Experiments were carried out according to the procedure of Eggum (1973) using adult male Albino rats. The animals are weighted at the beginning of the experiment as well as at the changing from pre-period to experimental period and again at the end of the experiment. Urine is collected in 50 ml 5% H₂SO₄ while the faeces in 100 ml H₂SO₄. At the end of the experiment, the rats were weighed and scarified with di-ethyl ether. An eventual feed rest is weighed.

Nitrogen of urine and faeces was determined according to the microkjeldahl method described AOAC (2006).

TD, BV and NPU were calculated according to the following equations:

$$TD = \frac{N \text{ intake} - (\text{Faecal N} - \text{metabolic N})}{N \text{ intake}} \times 100$$

$$BV = \frac{N \text{ intake} - (\text{Faecal N} - \text{metabolic N}) - (\text{Urinary N} - \text{endogenous})}{N \text{ intake} - (\text{Faecal N} - \text{metabolic N})} \times 100$$

$$NPU = \frac{T.D. \times B.V.}{100}$$

Where: N=nitrogen

Organoleptic evaluation:

Sensory evaluation for the fermentation products was carried out according to Lanza *et al.*, (1995). The panelists (n=15) were asked to evaluate color, taste, odor, texture and general acceptance as follows: very good (9-10), good (7-8), acceptable (5-6), weak (3-4), very weak (1-2).

Statistical analysis:

Statistical analysis of the obtained results was carried out according to the method of Snedecor and Cochran (1980) using SAS program (1987).

RESULTS AND DISCUSSION

1-Chemical composition of raw materials:

The effect of fermentation of autoclaved and non autoclaved Faba Bean using *B. subtilis*, LAB and yeast on its chemical composition was studied and the obtained results are shown in Tables (1) and (2).

Table 1: Effect of fermentation by *B.subtilis*, LAB and yeast on chemical composition of soaked, cooked and autoclaved Faba Bean.

Products	Protein%	Fat%	Ash%	Fiber%	Carbohydrate%
1	29.78	1.19	2.36	1.69	64.98
2	29.20	1.09	3.12	1.62	64.97
3	28.50	0.97	3.13	1.37	66.03
4	28.90	0.98	2.82	1.59	65.71

1) represented soaked, cooked and autoclaved Faba Bean. (2, 3 and4) represented soaked, cooked and autoclaved Faba Bean fermented with *B.subtilis*, LAB and yeast, respectively.

Table 2: Effect of fermentation by *B.subtilis*, LAB and yeast on chemical composition of soaked, cooked and non autoclaved Faba Bean.

Products	Protein%	Fat%	Ash%	Fiber%	Carbohydrate%
5	30.91	1.23	2.48	2.13	63.25
6	30.55	1.19	2.60	1.98	63.68
7	29.40	1.12	2.59	1.78	65.11
8	30.00	1.18	2.60	1.92	64.30

(5) represented soaked, cooked and non autoclaved Faba Bean. (6,7 and8) represented soaked, cooked and autoclaved Faba Bean fermented with *B.subtilis*, LAB and yeast, respectively.

From the results presented in Table (1) it could be noticed that, the fermentation process using *B. subtilis*, LAB and yeast was found to have low effect on chemical contents of autoclaved Faba Bean. However, ash contents of all treatments were increased compared to control sample. The percentage of increase were 32.2%, 32.63% and 19.49% for autoclaved Faba Bean which treated with *B.subtilis*, LAB and yeast, respectively.

However fat contents were slightly decreased. The percentage of decrease were 8.4%, 18.49% and 17.65% for autoclaved samples treated with *B.subtilis*, LAB and yeast, respectively.

Concerning non autoclaved Faba Bean, the results presented in Table (2) showed no clear effect on chemical contents of samples treated with *B.subtilis*, LAB and yeast with exception of fiber contents which showed slightly decrease. The percentage of decrease were 7.04%, 16.43% and 9.86 % for non autoclaved samples treated with *B.subtilis*, LAB and yeast, respectively.

These results also confirmed those reported by Soares *et al.*, (2005), who reported that fermentation had no marked effect on the chemical composition of legumes.

2- Behavior of micro-organism and pH values during the fermentation process:

The results in Table (3) indicated that, *B. subtilis* bacteria increased in number after 6 hrs of fermentation period then remained stable till the end of fermentation period. The pH values markedly decreased during fermentation period. It decreased from 7.49 to 5.2 after 44 hrs. LAB showed an increase in the colony count accompanied with a gradual decrease in the pH values (5.09 at the end of fermentation period). During fermentation with yeast the colony count showed an increase during the first 18 hrs of fermentation process followed by a gradual decrease. The pH values decreased markedly during the fermentation process and reached to 5.23 at the end of the experiment.

Table 3: Effect of incubation period on the count of the examined bacterial species and the pH of non autoclaved sample.

	0 hr	6 hr	18 hr	24 hr	44 hr
<i>B.subtilis</i> *CFU	1×10 ⁵	50×10 ⁵	10 ⁵	10 ⁵	10 ⁵
<i>B.subtilis</i> pH	7.49	5.33	5.07	5.08	5.20
LAB *CFU	1×10 ⁵	103×10 ⁵	230×10 ⁵	10 ⁵	226×10 ⁵
LAB pH	7.50	6.11	5.72	5.43	5.09
Yeast *CFU	7×10 ⁵	3×10 ⁵	5×10 ⁷	11×10 ⁵	92×10 ⁵
Yeast pH	7.50	6.30	5.46	5.42	5.23

The count of the used microorganism in the fermentation process and the pH. Changes during fermentation period of soaked, boiled and autoclaved Faba Bean are illustrated (Table 4). The obtained results indicated that, *B. subtilis* bacteria increased in number during the first 18 hrs of fermentation period and then decreased in number gradually till the end of fermentation period. The pH values markedly decreased during fermentation period and reached to 5.54 after 44 hrs. LAB showed a marked increase after 6 hrs followed by stability in count till the end of fermentation period. A gradual decrease in the pH values was noticed during fermentation with LAB and reached to 5.05 at the end of the fermentation period. During fermentation with yeast the count showed a gradual increase accompanied with a decrease in the pH values which reached 6.46 at the end of the experiment.

The decrease in the pH values during fermentation period in all treatments could be due to the ability of the used micro-organisms to use sugars as substrate for growth resulting in formation of organic acids which causes reduction of the pH. This finding is similar to that obtained by Onilude

et al., (1999) who reported a decrease in the pH value of fermented legumes using yeast and lactic acid bacteria. These results also agreed with the findings of Kiers *et al.*, (2000) who found a decrease in the pH values during fermentation of soya beans with *B. subtilis*. Porres *et al.*, (2003) also found that, the pH value of some fermented legumes using lactic acid bacteria was decreased during the whole fermentation time.

Table 4: Effect of incubation period on the count of the examined bacterial species and the pH of the autoclaved sample.

	0 hrs.	6 hrs.	18 hrs.	24 hrs.	44 hrs.
Control pH	7.27	7.35	7.18	6.95	6.38
<i>B.subtilis</i> *CFU	50×10 ⁵	3×10 ⁶	7×10 ⁷	14×10 ⁷	21×10 ⁸
<i>B.subtilis</i> pH	7.21	6.94	6.18	5.86	5.92
LAB *CFU	3×10 ⁵	5×10 ⁸	20×10 ⁸	300×10 ⁸	161×10 ⁸
LAB Ph	7.28	7.28	6.46	5.70	5.49
Yeast *CFU	2×10 ⁵	1×10 ⁶	1×10 ⁶	14×10 ⁷	10×10 ⁷
Yeast pH	7.29	7.31	7.09	6.72	6.50

Bacillus Subtilis (*B. subtilis*) - *lactobacillus acidophilus* (LAB)- *Saccharomyces cerevisiea* (Yeast)- Colony Forming Unit *CFU

The value of the obtained pH in the bacterial fermentation process ranged from 5.05 to 5.54 which was also found in agreement with (Sherfi and Hamad 2001) who reported that, the pH value of fermented legumes with *Bacillus* species and LAB was about 5.

The increase which was followed by a reduction in the colony count of the used micro-organisms might be due to the normal life span of living micro-organisms which begins with a lag phase followed by growth phase then stationary phase, in which the number remains stable, and at last the death phase. These findings are agreed with those of (Feng *et al.*, 2005) who noticed a marked increase in the count of lactic acid bacteria used in fermentation of some legumes and cereals.

From the previous results it is clear that fermentation with lactic acid bacteria yielded the lowest pH value due to consumption of the present carbohydrates forming lactic acid.

However, the low pH value was found to enhance the quality of fermented high- protein plant biomass as reported by Shurkhno *et al.*, (2005).

Concerning soaked boiled non autoclaved Faba Bean, it was noticed that lactic acid bacteria grew slower than the other used microorganisms and this finding agreed with the investigation of Isu and Njoku (1997) who reported that, a marked increase in the number of *Bacillus* cells was observed. On the contrary of LAB which increased slowly during fermentation of soya beans.

With respect to soaked boiled autoclaved Faba Bean, it was noticed that LAB grew very fast during the first 6 hrs due to the absence of competitor bacteria which destroyed by autoclaving.

3-Amino acids contents of Faba Bean products:

Effect of fermentation process on amino acid contents of Faba Bean products:

The results presented in Table (5) represent the effect of fermentation using *B. subtilis*, LAB and yeast organisms on the amino acid pattern of soaked cooked autoclaved and soaked cooked non autoclaved Faba Bean. The results indicated that, soaked cooked autoclaved Faba Bean showed no differences in all amino acids pattern before and after fermentation except for cysteine with fermentation by *B. subtilis* and yeast and leucine with fermentation by yeast which showed a slight increase in their values. In soaked cooked non autoclaved Faba Bean, the amino acids pattern was almost the same before and after fermentation except with the fermentation done by *B. subtilis* which caused a slight increase for threonine, leucine, lysine, serine, glutamic and glycine contents. However, fermentation with LAB species was found to have an increasing effect only on threonine, isoleucine, leucine, aspartic and serine contents.

Table 5: Effect of fermentation process amino acids contents by *B. subtilis*, LAB and yeast on soaked, cooked, autoclaved and non autoclaved Faba bean on soaked, cooked and autoclaved and non autoclaved Faba Bean.

Essential amino acids	Autoclaved				Non Autoclaved			
	1	2	3	4	5	6	7	8
Methionine	0.79	0.74	0.79	0.71	0.79	0.72	0.7	0.73
Threonine	3.69	3.43	3.67	3.23	3.28	3.54	3.52	3.16
Cysteine	1.25	1.55	1.32	1.93	1.45	1.31	1.03	1.38
Valine	4.86	4.83	4.73	4.7	4.83	4.77	4.71	4.83
Isoleucine	4.08	4.02	3.82	3.9	3.86	3.75	4.07	3.81
Leucine	7.80	7.71	7.71	8.01	7.38	7.60	7.88	7.91
Phenylalanine	4.39	4.35	4.38	4.17	4.66	4.06	4.57	4.21
Lysine	6.59	6.27	6.27	6.3	6.18	6.23	5.56	6.05
Total determined essential amino acids	33.45	32.90	32.69	32.95	32.43	31.98	32.04	32.08
Aspartic	10.88	10.26	10.24	10.79	11.28	11.04	11.86	11.23
Serine	4.80	4.13	4.5	3.65	3.80	4.27	4.71	4.1
Glutamic	18.11	17.93	17.12	17.48	15.59	17.72	15.4	15.47
Proline	4.36	4.32	4.5	4.29	4.35	4.06	4.32	4.65
Glycine	4.48	4.17	4.13	4.15	4.21	4.23	4.01	4.68
Alanine	5.01	4.83	4.69	4.78	4.45	4.37	4.3	5.05
Histidine	3.79	3.21	2.5	2.91	4.76	3.91	2.73	3.84
Arginine	9.09	8.93	9.11	8.62	11.83	10.02	10.69	10.88
Total Non essential amino acids	60.52	57.78	56.79	56.67	60.27	59.62	58.02	59.9
Total determined amino acids	93.97	90.68	89.48	89.62	92.70	91.60	90.06	91.98

(1) Represented soaked-cooked-autoclaved Faba Bean. (2, 3 and 4) Represented soaked-cooked-autoclaved Faba Bean fermented with *B. subtilis*, LAB and yeast, respectively. (5) Represented soaked-cooked-non autoclaved Faba Bean. (6, 7 and 8) Represented soaked-cooked-non autoclaved Faba Bean fermented with *B. subtilis*, LAB and yeast, respectively.

Moreover, fermentation with yeast showed no effect on amino acids pattern except for a slight increase was observed in leucine, serine, proline, glycine and alanine contents. The reduction of amino acids using LAB species and yeast may be attributed to the used species which are known to be proteolytic in nature. Also, the differences in the behavior of amino acids pattern between autoclaved and non autoclaved Faba Bean may be due to the effect of different microbial species included in the non autoclaved treatment.

The obtained results are similar to those obtained by (Wang and Fields 1978) who observed a decrease in methionine during fermentation of corn meal by some microorganisms including yeast. Teniola and odunfa, (2001) found also some decrease in amino acid pattern during fermentation of maize grains.

4-Antinutritional factors of Faba Bean products:

The effect of fermentation of soaked-cooked-autoclaved/non autoclaved Faba Bean by *B.subtilis*, LAB and yeast on its antinutritional factor contents (on dry weight basis %) were also studied and the obtained results are shown in Table (6).

Table 6: Effect of fermentation by *B.subtilis*, LAB and yeast on antinutritional factors of soaked-cooked-autoclaved/non autoclaved Faba Bean:

Treatment	Total tannins (%)	Phytic acid %	Trypsin inhibitor mg/100g
1	0.33	0.23	33
2	0.28	0.18	22
3	0.31	0.20	27
4	0.20	0.19	25
5	0.33	0.30	45
6	0.20	0.23	29
7	0.20	0.25	32
8	0.22	0.28	28

(1)Represented soaked-cooked-autoclaved Faba Bean. (2, 3 and 4) Represented soaked-cooked-autoclaved Faba Bean fermented with *B.subtilis*, LAB and yeast, respectively.

(5) Represented soaked-cooked-non autoclaved Faba Bean. (6, 7 and 8) Represented soaked-cooked-non autoclaved Faba Bean fermented with *B.subtilis*, LAB and yeast, respectively.

From data obtained from Table (6) it is clear that, fermentation of soaked-cooked-autoclaved Faba Bean with *Saccharomyces cerevisiae* had the greatest effect in reduction of tannins content by 39.4% followed by *B. subtilis* (15.15%) then LAB which caused 6.06% reduction if compared with non fermented soaked-cooked-autoclaved Faba Bean (control). While fermentation of soaked-cooked-autoclaved Faba Bean by *B. subtilis* had the greatest effect on both phytic acid (21.7%) and trypsin inhibitor (33.3%) followed by yeast (17.4% and 24.2%) then LAB which made a reduction percent of 13% and 18.2%, respectively.

Also from the same Table it is clear that, fermentation of soaked-cooked-non autoclaved Faba Bean with *B. subtilis* had the greatest reducing effect on total tannins (39.4%) and phytic acid content (23.3%) compared to LAB (39.4% and 16.7%) and yeast (33.3% and 6.7%), respectively, while

yeast had the greatest effect on trypsin inhibitor reduction (37.8%) followed by *B. subtilis* (35.6%) then LAB (28.9%).

These data agreed with those obtained by Isu and Ofuya, (2000) who found marked decrease in the anti-nutritional factors contents due to fermentation of some legumes with useful bacteria. Also McKay (1992) suggested a potential use of microorganisms or microbial enzymes for detoxification of Faba Bean. Porres *et al.*, (2003) evaluated the role of lactic acid bacteria in the reduction of phytate content in beans.

Ene-obong and Obizoba (1996) mentioned that, fermentation had a marked reducing effect on phytate levels in legumes. The role of *Bacillus* species in reduction of antinutritional factors is supported by data obtained by (Ramachandran *et al.*, 2005) who used *Bacillus* fermentation in the reduction of tannins and phytates in legumes.

Data clarified the role of microbial fermentation in the reduction of trypsin inhibitor in legumes is supported with those obtained by (Vidal-Valverde *et al.*, 1993; Ibrahim *et al.*, 2002 and Doblado *et al.*, 2003), who found marked reduction in trypsin inhibitor using fermentation of legumes by useful bacteria such as LAB and *Bacillus* species. Kiers *et al.*, (2000) and Amro *et al.*, (2006) reported the positive effect of microbial enzymes during fermentation on antinutritional factors reduction in legumes and cereals.

5- Biological evaluation of different Faba Bean products.

Growing Albino rats were fed on various Faba Bean products in addition to casein for comparison at 10% protein level for 9 days. The nutritional quality of the Faba Bean protein was evaluated by the following biological parameters: true digestibility (T.D), biological value (B.V) and net protein utilization (N.P.U). The results obtained for biological evaluation are shown in Table (7).

Effect of processing on the true digestibility (T.D) of diets containing Faba Bean products at 10% protein level:

Concerning the fermented products, the results indicated that, soaked cooked Faba Bean fermented with *B. subtilis* and soaked cooked and autoclaved Faba Bean fermented with yeast showed TD values higher than control treatment (soaked cooked Faba Bean). The values were 93.04, 90.80 and 89.65%, respectively.

However, the results of statistical analysis showed that TD values of all Faba Bean products were significantly lower than that of casein diet.

The same results showed also that, there were no significant differences in the effect of fermentation by *B. subtilis*, LAB and yeast in case of the soaked cooked Faba Bean on the TD values.

The same results in Table (7) showed that, fermentation of soaked cooked and autoclaved Faba Bean with *B. subtilis*, LAB and yeast did not affect significantly the TD of the diets compared to that contained soaked cooked and autoclaved Faba Bean without fermentation. However, TD of the diet contained soaked cooked autoclaved Faba Bean fermented with yeast was significantly different from that of the diet contained soaked cooked autoclaved Faba Bean fermented with LAB. Kiers *et al.*, (2000) found that, fermentation process improved the digestibility of legumes in healthy rats.

Also Amro et al., (2006) found that, protein digestibility of grains after fermentation was greatly improved.

Table 7: Biological effect of different Faba Bean products.

Source of protein	True digestibility % (T.D)	Biological value % (B.V)	Net protein utilization % (N.P.U)
1	89.27 ± 1.28 ^{bcdef}	64.65 ± 5.16 ^{abcde}	57.73 ± 4.85 ^{bcd}
2	87.17 ± 3.51 ^{def}	60.5 ± 10.53 ^{bcdef}	52.68 ± 0.59 ^{bcd}
3	85.07 ± 3.15 ^{ef}	51.4 ± 11.64 ^{ef}	43.87 ± 10.81 ^{ef}
4	90.80 ± 2.70 ^{bcd}	63.93 ± .00 ^{abcdef}	58.11 ± 5.21 ^{bcd}
5	89.65 ± 3.25 ^{bcde}	68.72 ± 3.50 ^{abc}	61.59 ± 3.58 ^{bc}
6	93.04 ± 5.48 ^{bc}	67.39 ± 5.49 ^{abcd}	62.51 ± 2.10 ^{bc}
7	89.37 ± 2.60 ^{bcde}	58.11 ± 2.48 ^{bcddef}	51.96 ± 3.10 ^{cda}
8	88.77 ± 1.41 ^{cdef}	61.4 ± 8.39 ^{bcddef}	54.50 ± 6.91 ^{bcdde}
Casein	97.48 ± 0.87 ^a	76.68 ± 4.79 ^a	74.74 ± 4.59 ^a
L.S.D. (0.05)	* 4.220	*** 11.663	** 9.851

- Faecal nitrogen and urine nitrogen of nitrogen Free diet were (13.02%) and(1.2%), respectively.
- Each value is the mean of 5 replicates; Means ± Standard error; Means in the same column with the same letter are not significantly different at P< 0.05; **: Highly significant values; *: Low significant values.
- *B.subtilis*¹, LAB² and yeast³.

Effect of processing on biological value (B.V) of diets containing Faba Bean products at 10% protein level:

Results in Table (7) showed that the B.V of the diet containing 10% casein was 76.68%. This value is in agreement with the values reported by (Youssef , 1999) who found that the B.V of casein was 76.50 %.

Results in the same Table indicated that, B.V of the experimental diets could be classified into two groups:

B.V. of the first group of diets was not significantly different from that of the casein diet. Those diets contained soaked- cooked fermented Faba Bean with *B.subtilis* (67.39%), soaked- cooked autoclaved Faba Bean fermented with yeast (63.93%).

The B.V values of the rest of the experimental diets were significantly lower than that of the casein diet. B.V of this group of diets were for soaked cooked Faba Bean fermented with yeast (61.4%), soaked cooked autoclaved Faba Bean fermented with *B.subtilis* (60.5%), soaked cooked Faba Bean fermented with (LAB) (58.1%) soaked cooked autoclaved Faba Bean fermented with LAB(51.4%). Hussein (1982) found that the B.V value of steam cooked Faba Bean was 40%.

Also one can notice that fermentation of either soaked cooked Faba Bean or soaked cooked autoclaved Faba Bean had no significant effect on the B.V of the diets contained these products.

Effect of fermentation on net protein utilization (N.P.U) of diets containing Faba Bean at 10% protein level:

Results in Table (7) indicated that N.P.U of the diet contained casein was 74.74%. This value is in agreement with the values reported by (Youssef, 1999) who found the N.P.U of the casein was 74.30%.

However, the observed lower T.D, B.V and N.P.U values of some Faba Bean products under study might be due to their higher content of tannins (Table 6) which may have depressed the utilization of dietary protein by interacting with protein to form indigestible complexes, by inactivating proteolytic enzymes, by interference with the epithelial protective mucus of the intestine or by altering the absorption of digested nutrients (Oh and Hoff, 1989).

6- Estimation of consumer acceptance by applying the panel test.

Results presented in Table (8) represent the mean scores for the panel test of the parameters: color, taste, odor, texture, and overall acceptability. Higher score had been given for product supplemented with yeast (autoclaved or yeast non autoclaved) respectively followed by product supplemented with *B. subtilis* (autoclaved) or *B. subtilis* (nonautoclaved) respectively. The lowest score was given to product supplemented with LAB (non-autoclaved), whereas, supplementation with LAB (autoclaved) given acceptable score as compared to control products.

There were no significant differences between products supplemented with all tested organisms. Also, the autoclaved samples were more acceptable than the others according to Lanza *et al.*, (1995).

Table 8:-Sensory evaluation of Faba Bean supplemented with LAB, *B. subtilis* and yeast N=15

Parameters	Taste	Odor	texture	color	Acceptance
Control non autoclaved	8.7 ± 0.75 ^a	8.5±0.95 ^{ab}	8.75±0.47 ^a	8.25±0.47 ^a	8.75±0.47 ^{ab}
Control autoclaved	9.5 ± 0.28 ^a	9.87±0.12 ^a	8.75±0.47 ^a	8.75±0.25 ^a	9.25±0.47 ^a
LAB non autoclaved	2.5 ± 0.64 ^b	1.0±0.0 ^c	7.75±0.25 ^a	8.28±0.25 ^a	2.25±0.25 ^c
LAB autoclaved	8.0 ± 0.40 ^a	8.0±0.40 ^{ab}	7.75±0.25 ^a	8.50±0.28 ^a	7.5±0.28 ^b
<i>B. subtilis</i> non autoclaved	8.0 ± 0.57 ^a	7.87±0.51 ^b	7.75±0.25 ^a	7.75±0.25 ^a	7.5±0.28 ^b
<i>B. subtilis</i> autoclaved	7.5 ± 0.64 ^a	8.0±0.81 ^{ab}	7.75±0.25 ^a	7.75±0.25 ^a	7.5±0.28 ^b
Yeast non autoclaved	8.5 ± 0.86 ^a	8.5±0.64 ^{ab}	7.75±0.25 ^a	8.5±0.28 ^a	7.75±0.74 ^b
Yeast autoclaved	8.6 ± 0.89 ^a	8.25±0.47 ^{ab}	7.75±0.25 ^a	8.5±0.28 ^a	8.75±0.64 ^{ab}

- Values are expressed as means ± SE.
- Means with the different letter superscripts in the same column denote significance at P < 0.05.

REFERENCES

Adsule , R. N. and M. Akpapunam (1996). Faba Bean (*Vicia faba* L.) in: Food and feed from legumes and oilseeds. Edited by E. Nwokolo J. Smartt. Pubished by Champman and Hall. London- Glasgow-New York- Tokyo.

Amro, B.; Hassan ; A. Isam; A. Mohamed; N.Osman; M. Eltayeb; G.Osman; And E.Elfadil; (2006). Effect of Processing Treatments Followed by Fermentation on Protein content and Digestibility of Pearl Millet (*pennisetum typhoideum*) Cultivars.J. of Nutrition 5 (1): 86-89.

AOAC Association of Official Analytical Chemists (2006). Official Methods of Analysis of AOAC International, 18th Edition, Published by AOAC International Maryland, USA.

Askar, A.(1986). Faba Bean (*Vicia faba* (L.)) and their role in the human diet. Food Nutr. Bull., 8, 15-24.

- Azokpota, P.; D.J. Hounhouigan and M.C. Nago (2006). Microbiological and chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce afitin, iru and sonru, three traditional condiments produced in Benin. *J Food Microbiol.* 1,107(3):304-9.
- Doblado, R.; J. Frias; R. Munoz and C.Vidal-Valverde (2003). Fermentation of *Vigna sinensis* var. *carilla* flours by natural microflora and *Lactobacillus* species. *J Food Prot.* 66(12):2313-20.
- Eggum, B.O. (1973). A study of certain factors influencing protein utilization in rats and Pigs. *Kommission hos landhusholdningssselskabets forlag Trykt frederikberg Bogtrykkeri.*
- El-Sheikh, E.A.E.; A.H. El Tinay and I.A. Fadul (1999). Effect of nutritional status of Faba Bean on proximate composition, anti-nutritional factors and in vitro protein digestibility (IVPD). *Food Chemistry* 67,379-383.
- Ene-obong, H.N. and I.C. Obizoba (1996). Effect of domestic processing on the cooking time, nutrients, antinutrients and in vitro protein digestibility of the African yambean (*Sphenostylis stenocarpa*). *Plant Foods Hum Nutr* 6;49(1): 43-52.
- Feng, X.M. ; A.R. Eriksson; and J. Schnurer (2005). Growth of lactic acid bacteria and *Rhizopus oligosporus* during barley tempeh fermentation. *J Food Microbiol.* 23
- Food Agricultural Organization (FAO) (2005). FAOSTAT. Prod STAT, Crops (<http://faostat.fao.org/>).
- Hussein, L.A. (1982). Antinutritional factors in Faba Bean in : Faba Bean improvement. Edited by G. Hawtin and C. Webb. Published by Martinus Nijhoff, Netherlands. For the ICARDA / IFAD Nile Valley Project.
- Ibrahim, S. S. ; R. A. Habiba; A. A. Shatta and H. E. Embaby (2002). Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Nahrung.* 46(2):92-5.
- Isu, N.R. and H.O. Njoku (1997). An evaluation of the microflora associated with fermented African oil bean (*Pentaclethra macrophylla* Bentham) seeds during ugba production. *Plant Foods Hum Nutr* 51(2): 145-57.
- Isu, N.R. and C.O. Ofuya (2000). Improvement of the traditional processing and fermentation of African oil bean (*Pentaclethra macrophylla* Bentham) into a food snack—'ugba'. *J. Food Microbiol* 10,59(3):235-9
- Kakada, M.L.; N. Simons and I.E. liner (1969). An evaluation of natural synthetic substrates for measuring the antitryptic activity of soy bean samples. *Cereal Chem.*, 46: 518-526
- Kiers, J.L. ; M. J. Robert Nout and F.M. Rombouts (2000). In vitro digestibility of processed and fermented Soy bean, Cowpea and maize. *J. Sci Food Agric* 80:1325-1331.
- Kiers, J.L.; A.E. Van Laeken; F.M. Rombouts and M.J. Nout (2000). In vitro digestibility of bacillus fermented soya bean. *J. of Food Microbiol* 25, 60(2-3):163-9.
- Lanza, C.; E. Pagiaini and F.Tamseli, (1995): "Sensory and chemical evaluation of frozen blood orange juice., *Agric. Mediterranean.*, 125:421-426

- McKay, A.M.(1992). Hydrolysis of vicine and convicine from fababeans by microbial beta-glucosidase enzymes. *J Appl Bacteriol.* 72(6):475-8.
- Official Journal of the European Communities (1998).Manual of high performance Amino Acid Analyzer, 7300 (Analyzer used).EZ Chrom (soft ware used for data collection and processing.
- OH, H. J. and j.E. Hoff (1989). Effect of condensed grape tannins on in vitro digestion of proteins. *Nutr. Report Inter* 34:51-58. C.F. Bakr, A.A. and Bayomy, M.F.*Plant Foods Hum Nutr* 50 (1) 81-91. (1997).
- Onilude, A.A.; A.I. Sanni and M.I. Ighalo (1999).Effect of process improvement on the physico-chemical properties of infant weaning food from fermented composite blends of cereal and soybeans.*Plant Foods Hum Nutr.* 54(3):239-50.
- Porres, J.M. ; P., Aranda; M. Lopez-Jurado and G.Urbano (2003). Effect of natural and controlled fermentation on chemical Composition and nutrient dialyzability from beans (*Phaseolus vulgaris* L.). *J Agric Food Chem.* 13,51(17):5144-9.
- Price, M. L.; S.V. Scoyoc; and L.G. Butler (1978). A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. *J. Agric .Food Chem.* 26 (5):1214-1217.
- Ramachandran, S.; A. Bairagi and A.K. Ray (2005).Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings after fermentation with a fish gut bacterium. *Bioresour Technol.* 96(13):1465-72.
- SAS, (1987). SAS / STAT, guide for personal computers, version 6. SAS Institute, INC., Cary, NC.
- Sherfi, S.A. and S.H. Hamad (2001). Microbiological and biochemical studies on Gergoush fermentation. *J Food Microbiol.* 5,67(3):247-52.
- Shurkhno, R.A. ; R.G. Gareev ; A.G. Abul'khanov; S.h. Validov; A.M. Boronin and R.P. Naumova (2005). Fermentation of a high-protein plant biomass by introduction of Lactic acid bacteria. *Prikl Biokhim Mikrobiol.*41(1):79-8
- Snedecor, G.W. and W.G. Cochran (1980). *Statistical methods.* 7th Ed. Published by the Iowa state university press, Ames ,Iowa, U.S.A.
- Snedecor, G.W. and W.G. Cochran (1980). *Statistical methods.* 7th Ed. Published by the Iowa state university press, Ames ,Iowa, U.S.A.
- Soares, V. F.; L.R. Castilho; E.P. Bon and D.M. Freire (2005). High-yield *Bacillus subtilis* protease production by solid-state fermentation. *Appl Biochem Biotechnol.* 121-124:311-9
- Teniola and S.A. Odunfa (2001).The effects of processing methods on the levels of lysine, methionine and the general acceptability of ogi processed using starter cultures.*J. of Food Microbiol* 22,63(1-2):1-9.
- Vidal-Valverde, C.; J. Frias; M. Prodanov; J.Tabera; R. Ruiz and J.Bacon (1993).Effect of natural fermentation on carbohydrates, riboflavin and trypsin inhibitor activity of lentils.*Z Lebensm Unters Forsch* 197(5):449-52.
- Wang, Y.D. and M.L. Fields (1978). Feasibility of home fermentation to improve the amino acid contents of corn meal.*J. food Sci* 43,1104.

Wheeler E.L. and R.E. Ferrel (1971).A method for phytic Acid determination in wheat and wheat Fractions. American Association of cereal chemists 55, 312-20.

Youssef G. M.(1999).Effect of processing on antinutritional factors and biological value of major legumes. Ph.D. thesis, Fac. of Agric. Cairo Uni, Egypt.

تأثير عملية تخمير الفول على القيمة الغذائية و الخواص الحسية له
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** قسم التغذية وعلوم الأطعمة ، كلية الاقتصاد المنزلي ، جامعة حلوان

تم إجراء تقييم أثر استخدام سلالات الأحياء الدقيقة المختلفة في تخمير الفول على قيمته الغذائية ومدى قبول المستهلك له. تم تخمير الفول بعد تجهيزه (بالاتوكلاف وبدون الاتوكلاف) مع *Lactobacillus acidophilus, Bacillus Subtilis and Saccharomyces cerevisia* لمدة 44 ساعة وبعد ذلك تم تحليل التركيب الكيميائي والعوامل المضادة للتغذية وذلك لتقدير مدى وجود اختلافات قبل وبعد عملية التخمير .

وأظهرت النتائج وجود انخفاض ملحوظ في درجة pH للمنتج مع وجود تغييرات طفيفة في نوع الأحماض الأمينية . كذلك وجد انخفاض ملحوظ في العوامل المضادة للتغذية بعد عملية التخمير وخاصة حامض الفيتك .

تم تقدير كفاءة الهضم و القيمة البيولوجية ودرجة الاستفادة من البروتين في الفئران التي تم تغذيتها على هذه المنتجات، وأظهرت النتائج أن الفول الذي تم نقعه وطهيه والذي تم تخميره باستخدام سلالة *B. Subtilis* قد أعطي أكبر النتائج لمعدل كفاءة الهضم و القيمة البيولوجية و أيضا معدل كفاءة الاستفادة من البروتين.

من خلال تطبيق التقييم الحسي لتقدير مدى قبول المستهلك لوجبات الفول المخمرة ، أظهرت النتائج أنه لم يكن هناك فروق كبيرة بين جميع المنتجات المخمرة.

قام بتحكيم البحث

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