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Effect of Probiotic Bacteria on Legumes Purees

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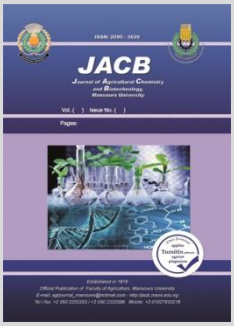

ABSTRACT

In this study three common legumes (Fababean, Chickpea and Soybean) were fermented using probiotic bacteria after isolated and identified by morphological, biochemical and molecular characteristics. The cultures containing *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium lactis* as probiotic bacteria were used to study their effect on the properties of some food products prepared from legumes. Results revealed that P Soy bean (puree) recorded the highest scores in appearance (8.5 ± 0.2), acceptable odor and mouth feeling scores were (8.4 ± 0.2) respectively .However P Faba bean (puree) and P Chickpea (puree) had the best color (8.5 ± 0.2). While,taste was the best for P Faba bean (8.2 ± 0.2). Results indicated that the highest values of chemical parameters were recorded for P Faba bean in moisture and ash which were (4.7±0.1, (4.6±0.2) respectively. At the same time, P Soy bean in lipids had (19.0±0.0), and in protein was (39.3±0.1).Results obtained of P Chickpea in carbohydrates was(62.6±0.1). Also, results obtained shows the effect of all three bacterial isolates *(Lactobacillus acidophilus , Bafidobacterhum lactis* and *Strentococcus thermophilus*) as probiotic -fermented Faba bean, Chick pea and Soy bean. shows that ten storage days value at (5.5 ± 0.3) for the Soy bean (5.5± 0.3)for the Faba bean and (5.8 ± 0.5) for the Soy bean. Finaly the results concluded that the counts of probiotic bacterial isolates were improved the sensory and chemicaly evaluation of fermentated legumes when increased.

Keywords: Probiotic bacteria;Legumes purees; *Lactobacillus* ;*Bifidobacterium*; *Streptococcus*; Food productes.

INTRODUCTION

Chandan (1999) stated that potential mechanisms by which probiotics may exert their beneficial effects are: (1) competition for nutrients with other microflora; (2) acids production which inhibits certain pathogens; (3) production of inhibitory metabolites or bacteriocin (like antimicrobial peptides, hydrogen peroxide and short-chain fatty acids); (4) immuno-modulation; (5) competition with other microflora for adhesion to intestinal mucosa.

Marteau *et al.*, (2001) reported that probiotics are viable microorganisms which, when ingested, exhibits a positive effects on health or physiology of the host. Probiotics are non-pathogenic microorganisms. Also Marteau and Rambaud, (2002) defined probiotics as 'components of microbial cells or microbial cell preparations that have helpful effects on the health and well-being.

Montero *et al.*, (2006) mentioned that health claims have been known for fermented milk products other than Yoghurt, prebiotic or probiotic properties are mostly mentioned. Consumers have been observed a lack of information regarding the adequate amounts to be consumed and the real benefits provided by the probiotic product. Nutrition claims have been found concerning the products with adequate content of specific nutrients; thi facts might create confusions as regards to really enriches foods. Fujimori and Penas (2009) Prebiotics are "nondigestible substances that produce beneficial physiological actions on the host by a selective stimulating of the favorable activity or growth of a limited number of indigenous bacteria".

Ng *et al.*, (2009) recorded that yoghurts containing *B. bifidum, B.infantis, L. acidophilus, S. thermophilus* and *L. bulgaricus* stimulated the response of fermentated foods.

The aim of this investigation to determine characteristics and the effect of probiotic bacteria (Lactobacilli, Streptococci and Bafidobacteria) on sensory, chemical and microbiological of legumes purees.

MATERIALS AND METHODS

Source of legumes:

Faba bean (*Vicia faba*), Chick pea (*Cicer arietinum*) and Soy bean (*Glycine max*) were purchased from Local market at Meet-Ghamr City, Dakahlia Governorate, Egypt.

Starter culture:

Commercial probiotic fermented milk (*Lactobacillus acidophilus, Bifidobacterium lactis* and *S. thermophilus*) were used as starter culture after isolated and identified .

Isolation and identification of starter cultures:

Probiotic bacteria were isolated from fermented milk sample using the pour plate techinique on M17 agar according to Terzaghi and Sandine (1975) or MRS broth De Man *et al* ., (1960) .The selected colonies were purified with streak plate techinique Dave and Shah, (1998). The purified isolates were examined to their morphological and biochemical characteristics. Then ,isolates were also identified by molecular characterization at Sigma Company, Cairo, Egypt.

Preparation and fermentation of legume puree:

Faba bean, Chickpea and Soy bean were soaked in tap water for 8 hours, boiled until cooked, at 55°C and

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cooled to room temperature Shah, (2003). Finally, legumes puree (P) was prepared by mixing of salt (2g/100g), lemon (15 drops/100g) and sesame paste (Tahina) (20g/100g) in the blinder.

Legumes fermentation by probiotic bacteria:

Puree of Faba bean, Chickpea and Soy bean were fermented using probiotic bacteria (*Lactobacillus acidophilus, Bifidobacterium lactis* and *S. thermophilus*). Samples were incubated at 37° C according to Kirii *et al.*, (2009).Samples were keeped in refrigerator at 5 ° C.

Sensory evaluation of fermented Legumes:

Sensory evaluation of the fermented legumes prepared from each mixture was estimated to determine their sensory characteristics. All members 15 panel of adult inviduals including the participants in our study according to Sanni *et al.*, (1998).Members were asked to evaluate samples the first day after manufacture for color, texture, taste, odor & thickness using a ten-point score system (10 excellent, 1 unacceptable).

Products chemical analysis:

Moisture, proteins, fats and ash were determined according to the methods of A.O.A.C. (2005).Carbohydrates were calculated by difference as following:

Carbohydrates = 100- (% moisture+ % proteins+ %fats +% ash)

Statistical analysis:

The collected data were statistical analyzed, using SPSS version 18.0 following descriptive statistics (mean and standard deviation) as well as analytical tests analysis of varianes (ANOVA).This test was used to explain the significance of difference between before and after the intervention.

RESULTS AND DISCUSSION

Identification of bacterial isolates

Morphological and biochemical characteristics of the bacterial isolates:

The results of the morphological and biochemical characteristics of the bacterial isolates are presented in Table (1) .The bacterial isolates YO1, LR61 andYO16 are belonging to *Lactobacillus acidophilus, Bifidobacterium lactis* and *Streptococcus thermophilus* respectively according to Axelsson ,(2004). *Streptococcus thermophilus* is known as a gram-positive bacterium, a fermentative facultative anaerobe, positive for catalase motile and does not form endospores. It has an optimal growth temperature range of 35 - 42 °C while *L. bulgaricus* has an optimal range of 43- 46 °C Tannock and Gerald, (2005).

Lactobacillus acidophilus is considered as a gram positive bacteria species in the genus of Lactobacillus. *L. acidophilus* is a microaerophilic, homo fermentative species, as it produces lactic acid by sugars fermentation, and can grow at low pH values (rather below pH 5.0). It's optimum growth temperature is at around 37 °C Fabre-Gea, (2000).

The form of Bifidobacterium cells varies between species, but generally they are slim, rod-shaped and with clubbed ends or slightly bulbous. Bifidobacteria tend to fork at one or both ends when nutrients are short Hoover, (2000).

Molecular identification of the selected bacteria isolate:

In order to confirm morphological and biochemical identification of the three bacterial isolates from Zabady(Y0) and Laben Rayb (LR), Molecular identification was done by Sigma Scientific Services Co.using 16SrRNA gene. The resulted nucleotide sequenceswas blasted in National Center for Biotechnology Information database (NCBI) (www.ncbi. nlm. nih.gov/blast) to identify the DNA sequence. To functionally characterize the isolated DNA fragment, similar sequence of ITS in many bacteria related to our targets species.

Table 1. Some morphological and biochemical characteristics of the probiotic bacterial isolates

Tests	Bacterial isolates No.		
Morphological characters	**YO1**	**YO16**	**LR61**
Gram stain	+	+	+
Cell shape	Long rods	Short rods	cocci
Spore forming	-	-	-
Motility	+	+	-
Capsule formation	-	-	-
Measurement (μm)	(4 x 1.2)	(1.5-(3:4)	(1.5)
Biochemical characters			
Indole production	-	-	-
Voges Proskauer test	+	+	+
Methyl Red test	+	+	+
Citrate utilization	+	+	+
Catalase production	+	+	+
Starch hydrolysis	+	+	+
Casein hydrolysis	+	+	+
Gelatin liquefaction	+	+	+
Cellulase production	-	-	-
Glucose assimilation	+	+	+
Manitol assimilation	-	-	-
Sucrose assimilation	+	+	+
Fructose assimilation	+	-	-
Lactose assimilation	+	-	-
Dextrin assimilation	-	-	-
Xylose assimilation	-	-	-
Glycerol assimilation	-	-	-

Genomic DNA for the three isolates were subjected to PCR to amplify the 16SrRNA GENE using universal primers. The amplified PCR amplicones (350 dp) were then subjected to DNA sequencing. The resultant 16SrRNA Sequences (Table 2) were analyzed using the basic local alignment search tool (BLASTN) at NCBI database. The results showed that two isolates were identified as *Lactobacillus acidophilus* with sequence identity 98%, *Streptococcus thermophilus* with sequence identity 99%, and third isolate as *Bafidobacterium lactis*. The obtained 16SrRNA sequences were submitted to Gen Bank under accession numbers as shown in Table (2).

Sensory evaluation:

Sensory attributes of boiled Faba bean, Chick pea and Soy bean purees fermented by probiotic bacteria are represented in Table (3).Data indicated that Probiotic (P) Soy bean puree recorded the highest scores in appearance (8.5 ± 0.2), however P Faba bean and P Chickpea puree had the best color (8.5 ± 0.2), (8.5 ± 0.2). Taste was the best for P Faba bean (7.9 ± 0.2), and the most acceptable odor, mouth feeling and overall acceptability scores were observed for P Faba bean puree (8.2 ± 0.2), (7.9 ± 0.2) and (8.1 ± 0.1). On the other hand; the lowest sensory attributes

was noticed for P chickpea in mouth feeling (7.6 ±0.2)and P soy bean in odor (7.8±0.2), taste (7.5± 0.3) , overall acceptability (7.9 ± 0.1) and color (8.3± 0.3). Also P Faba bean had the lowest scores in appearance (7.6 ± 0.3). Studies indicated that lactic acid bacteria can contribute to the taste, overall appearance, aroma and generally produce moreover pleasing sourness. These results come in agreement with Devlin, (2006) in appearance, color, mouth feeling and over all acceptability for different in taste and odor. Also, Modu, (2010) in appearance, mouth feeling and over all acceptability, but different in color, taste and odor.

Table 2. The resultant 16SrRNA Sequences

Isolate No.	Bacterial name	Partial sequence
YO I	*Lactobacillus acidophilus* KF724899	CGGGTTTAGATATAGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGCA ACTGACGCTGAGGCTCGAAAGCCCATGGGTAGCGAACAGGATTTAGATACCCTGGTAGT CCATGGGCCGTAAACGATGAGTGCTAAGTGTTGGGAAACGGTTTCCGCCTCTCACGTGCT GCAGCACTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAA AGGAATATGACGGCGGGCCCGGCACACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA ACGCGAAGAACCTTACCAGGTCTTGACAAAT.
LR 61	*Streptococcus thermophilus* KF724885	GGTCCCCCCGGACCTCACCTCATATCCACCAATCATCTGTCCCACCTTAGGCGGCTGGCT CCAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGT GTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCC GACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGATTGGCTTTAAGAGATTA GCTCGCCGTCACCGACTCGCAACTCGTTGTACCAACCATTGTAGCACGTGTGTAGCCCAG GTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCAATTAAAAA.
YO 16	*Bifidobacterium lactis.* KF725389	TCCCCCCGGACCTCACCTCATATCCAGGTGAGGTAACGGCTCACCAAAGGCGATGAGACA CGGCCCAAACTCCTACCCGGGAGGCAGCTGAGGCTCGAAAGCCCATGGGTAGCGAACAG GATTTAGATACCCTGGTAGTCCATGGGCCGTAAACACTCCGCCTGGGGAGTACGACCGCA AGGTTGAAACTCAAAGGAATATGACGGCGGGCCCGGCACACGACTCGCAACTCGGTCAT AAGGGGCATGATGATTTGACGGGCCCAAACTCCTACCCGGAGCCCGCGGCGGCGTATTA GCTAGTTGGTGAGGTAACGGCTCACCAAAGGCGATGAAATACGTAGCCCGAACTGAGA.

Table 3. Sensory attributes of legumes puree fermented probiotic bacteria

Samples	Appearance	Color	Taste	Odor	Mouth feeling	Overall acceptability
P. Faba bean	7.6± 0.3 [a]	8.5± 0.2 [a]	7.9± 0. 2 [a]	8.2± 0.2 [a]	7. 9± 0.2 [a]	8.1± 0.1 [a]
P. Chick pea	8.3± 0.2 [b]	8.5± 0.1 [a]	7.8± 0.2 [a]	7.9± 0.2 [a]	7.6± 0.2 [a]	8.0± 0.1 [a]
P. Soy bean	8.5± 0.2 [b]	8.3± 0.2 [a]	7.5± 0. 3 [a]	7.8± 0.2 [a]	7.7± 0.2 [a]	7.9± 0.1 [a]

Means in column with different letters are significantly different (P<0.05).

Chemical evaluation:

Chemical composition of Faba bean, Chickpea and Soy bean purees fermented by probiotic bacteria are shown in Table (4). Results indicated that the highest values of chemical parameters were recorded for P Soy bean in moisture which were (6.6±0.1), P Faba bean in ash was (4.6±0.2), P Soy bean in lipids had (19.0±0.0),, protein was (39.3±0.1), P Chickpea in carbohydrates was (62.6±0.1). On the other hand the lowest contents were in P Chickpea in moisture, ash, P Faba bean in lipids, proteins also P Soy bean in carbohydrates. The results were in Hassan ,(2013) found that moisture content of legumes was 3.9%, while 5.9%moisture .Hosono and Hisamatsu, (1995) reported 1.50% fat content in legumes found a range from 0.91% to 1.70%, and 23.36% fat for Soybean, 33.4% protein content for legumes, and 30.89 to37.14% . Kadooka *et al*., (2010) found that carbohydrate content of 36.8% for legumes, while 54.2% was found50.8% carbohydrate in Faba bean, and range from 60.80 to 62.12%.

Table 4.Chemical composition of legumes puree fermented probiotic bacteria

Samples	Moisture	Ash	Lipids	Proteins	Carbohydrates
P. Faba bean	4.7^{b}±0.1	4.6^{a}±0.2	1.3^{b}±0.1	27.6^{b}±0.3	61.8^{a}±0.2
P. Chickpea	4.2^{c}±0.2	4.3^{ab}±0.1	1.5^{b}±0.1	28.0^{b}±0.3	62.6^{a}±0.4
P. Soy bean	6.6^{a}±0.1	4.4^{ab}±0.2	19.0^{a}±0.2	39.3^{a}±0.1	31.3^{b}±0.1

Means in column with different letters are significantly different (P<0.05).

Microbiologcal evaluation:

Results obtained in Table (5) shows the effect *Lactobacillus acidophilu*s of probiotic -fermented Faba bean, Chick pea and Soy bean. Results represent in Table (5) shows that zero storage days value at Faba bean and Chick pea was (4.9± 0.3, 5.2± 0.2 and 5.0 ± 0.3) for the Soy bean. On the other hand, the zero storage days value of the Chick pea was (5.2± 0.2)it could be noticed from this data a highest value and the Faba bean was (4.9± 0.3) it could be noticed from this data a lowest value.

In Table (5) the three storage days value at Faba bean , Chick pea and Soy bean was(5.1 ± 0.3, 5.3± 0.3, and 5.2 ± 0.3) this data increase value in Chick pea was(5.3± 0.3) and decrease value in Faba bean was (5.1 ± 0.3).

Table (5) also shows that seven storage days value at Faba bean, Chick pea and Soy bean was (5.1 ± 0.5, 5.4 ± 0.3and 5.4± 0.3). It was the highest value for Soy bean and Chick pea was (5.4 ± 0.3) and decreased value was in Table (5) shows that in Faba bean (5.1 ± 0.5). On the other hand Table (5) shows the ten storage days value of Faba bean, Chick pea and Soy bean, also shows that decreased value at Faba bean was (5.2± 0.5) as the highest value was for the Chick pea and Soy bean (5.5± 0.3).

Table 5. *Lactobacillus acidophilus* as probiotic -fermented Faba bean, Chick pea and Soy bean sauces during storage period

Samples	Storage days			
	0	3	7	10
Faba bean sauce	4.9±0.3 [a]	5.1±0.3 [a]	5.1±0.5 [a]	5.2±0.5 [a]
Chick pea sauce	5.2±0.2 [a]	5.3±0.3 [a]	5.4±0.3 [a]	5.5±0.3 [a]
Soy bean sauce	5.0±0.3 [a]	5.2±0.3 [a]	5.4±0.3 [a]	5.3±0.3 [a]
LSD	0.6	0.9	0.7	0.8

Means in column with different letters are significantly different (P<0.05).

Results obtained in Table (6) shows the effect *Bafidobacterium lactis* of probiotic -fermented Faba bean, Chick pea and Soy bean. Results represent in Table (5) shows that zero storage days value at Faba bean and Chick pea was (5.0± 0.2, 4.3± 0.1 and 4.6 ± 0.0) for the Soy bean.

On the other hand, the zero storage days value of the Chick pea was (5.0± 0.2)it could be noticed from this data a highest value and the chick pea was (4.3± 0.0) it could be noticed from this data a lowest value.

In Table (6) the three storage days value at Faba bean , Chick pea and Soy bean was(5.0 ± 0.2, 4.6± 0.4, and 4.3 ± 0.3) this data increase value in Faba bean was (5.0± 0.2) and decrease value in Soy bean was (4.3 ± 0.3).

Table (6) also shows that seven storage days value at Faba bean , Chick pea and Soy bean was (5.3 ± 0.2, 5.1 ± 0.3and 5.1± 0.5)it was the highest value for Faba bean was (5.3 ± 0.2) and decreased value was shows that in Chick pea,Soy bean (5.1 ± 0.3).

Table 6. *Bafidobacterium lactis* as probiotic -fermented Faba bean, Chick pea and Soy bean sauces during storage period

Samples	Storage days			
	0	3	7	10
Faba bean	5.0±0.2 [b]	5.0±0.2 [a]	5.3±0.2 [a]	5.5±0.3 [a]
Chick pea	4.3±0.1 [a]	4.6±0.4 [a]	5.1±0.3 [a]	5.4±0.3 [a]
Soy bean	4.6±0.0 [b]	4.3±0.3 [a]	5.1±0.5 [a]	5.3±0.3 [a]
LSD	0.0	0.1	0.9	0.9

Means in column with different letters are significantly different (P<0.05).

On the other hand Table (6) shows the ten storage days value of Faba bean, Chick pea and Soy bean, also shows that decreased value at Soy bean was (5.3± 0.3) as the highest value was for the Faba bean and Soy bean (5.5± 0.3).

Results obtained in Table (7) show the effect *Streptococcus thermophilus* of probiotic -fermented Faba bean, Chick pea and Soy bean and reavaled that zero storage days value at Faba bean and Chick pea was (4.5± 0.3, 5.2± 0.5 and 5.1 ± 0.3) for the Soy bean.

On the other hand, the zero storage days value of the Chick pea was (5.2± 0.5)it could be noticed from this data a highest value and the Faba bean was (4.5± 0.3) it could be noticed from this data a lowest value.

In Table (7) the three storage days value at Faba bean , Chick pea and Soy bean was(4.8 ± 0.2, 4.8± 0.2, and 5.3 ± 0.3) this data increase value in Soy bean was (5.3± 0.3) and decrease value in Faba bean and Chick pea (4.8 ± 0.2) .

Table (7) also shows that seven storage days value at Faba bean , Chick pea and Soy bean was (5.0 ± 0.3, 5.3 ± 0.2and 5.5± 0.3)it was the highest value for Soy bean was (5.5 ± 0.3) and decreased value shows that in Faba bean (5.0 ± 0.3).

Table 7. *Streptococcus thermophilus* as probiotic - fermented Faba bean, Chick pea and Soy bean sauces during storage period

Samples	Storage days			
	0	3	7	10
Faba bean	4.5±0.3 [a]	4.8±0.2 [a]	5.0±0.3 [a]	5.3±0.3 [a]
Chick pea	5.2±0.5 [a]	4.8±0.2 [a]	5.3±0.2 [a]	5.6±0.2 [a]
Soy bean	5.1±0.3 [a]	5.3±0.3 [a]	5.5±0.3 [a]	5.8±0.5 [a]
LSD	0.3	0.3	0.5	0.7

Means in column with different letters are significantly different (P<0.05).

On the other hand Table (7) shows the ten storage days value of Faba bean, Chick pea and Soy bean, also shows that decreased value at Faba bean was (5.3± 0.3) as the highest value was for the Soy bean (5.8± 0.5).

Lactic acid bacteria (LAB), in particular, contribute to the fermentation process and ensure the safety of legumes puree Ashenafi, (1994).

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تأثير بكتريا البروبيوتيك على بعض المنتجات المعده من البقوليات

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فى دراسة لتأثيرالبكتريا الحيويه على بعض المنتجات المعده من البقوليات أجريت الإختبارات المورفولوجيه والبيوكيميائيه وكذلك الجزيئيه لتعريف هذه البكتيريا ثم إعدادها لتخمير الفول و الحمص و الصويا بعد نقعهم و طهيهم وذلك بمزرعة مختلطة من هذه البكتيريا الحيوية *Lactobacillus acidophilus,Bifidobacterium lactis* and *S. thermophiles*وقد قيمت المنتجات حسيا و كيميائيا و ميكروبيولوجيا. أظهرت النتائج أن أفضل درجات الرائحة فى بيوريه فول الصويا واللون فى بيوريه الحمص والطعم فى بيوريه الفول البلدى وكان التأثير عند التذوق فى بيوريه فول الصويا واما القابلية العامة سجلت في الفول البلدى .وأظهرت النتائج الكيميائيه ان أعلى القيم التى سجلت فى الرطوبه كانت فى بيوريه الفول البلدى وكذلك فى الرماد فى بيوريه الفول البلدى أما الدهون والبروتين فى بيوريه فول الصويا واما الكربوهيدرات ففى بيوريه الحمص وكما أظهرت النتائج الميكروبيولوجيه ان افضل القيم كانت لبكتيريا *Lactobacillus acidophilus* فى بيوريه الحمص وبكتيريا *Bifidobacterium lactis*.وكانت فى بيوريه الفول البلدى وبكتيريا *S. thermophilus* فى بيوريه فول الصويا و قد سجلت النتائج النهائية أن زيادة أعداد البكتريا الحيوية تحسن من الخصائص الحسية والكيميائية لهذه البقوليات .