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Effect of lactic acid bacteria on fermented legumes

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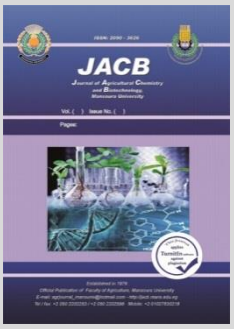

ABSTRACT

The bacterial cultures from yogurt (Y) containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as lactic acid bacteria were used to study their effect on the properties (such as sensory ,chemicaly and microbiologicaly) of some food products prepared from legumes . This lactic acid bacteria were isolated and identified by morphological, biochemical and molecular characteristics. Results revealed that Y Soy bean (puree) recorded the highest scores in appearance (8.5 ± 0.2), color (8.3±0.2) and mouth feeling (8.4±0.2) , however Y Faba bean (puree) had the best taste and over all acceptability scores (8.2 ± 0.2), (8.2 ± 0.1) respectivily. Odor was the best for Y Chick pea (8.2 ± 0.2). Results indicated that the highest values of chemical parameters were recorded for Y Soy bean in moisture , ash, lipids and proteins which were (5.9±0.1) ,(5.0±0.1), (20.0±0.1) and (37.8±0.1) respectively . While , Y Chickpea and Y Faba bean were the best in carbohydrates (62.2±0.2), (62.1±0.1) respectively. Results obtained shows the effect of *Lactobacillus bulgaricus* of yogurt -fermented Faba bean, Chick pea and Soy bean. Generally, results represent shows that ten storage days value at (5.6 ± 0.3) for the Chik pea and Soy bean. While , results represent show that ten storage days value at (5.8 ± 0.5) for the Soy bean with *Streptococcus thermophilus* . Finaly , the results concluded that the counts of yogurt bacterial isolates were improved the sensory and chemicaly evaluation of fermentated legumes when increased with storage days.

Keywords: Lactic acid bacteria; Legumes purees; Lactobacilli; Streptococci; Food productes.

INTRODUCTION

LAB are known for their fermentative ability and thus promoting food safety, improving sensory attributes, increasing health benefits and enriching nutrients. Many LAB species play an important role in cheese manufacture through the ripening process, especially in improving the consistency, flavor and aroma Hosono and Hisamatsu ,(1995). Certain LAB strains characterized by their ability to transform lactose and therefore improves the digestibility of fermented dairy products Wang and Hesseltine,(1981) as well as their preservative effects. LAB also is utilized for improvement of the texture, viscosity and taste in the dairy products manufacture. Fermented foods and beverages vegetables, milk and milk products can be used for recovering of lactic acid bacteria. Lactic acid bacteria (LAB) are a group of non- sporulating , gram positive, anaerobic or facultative aerobic rods or cocci, which produce lactic acid as one of the major fermentation products of the carbohydrates metabolism Axelsson ,(2004). Lactic acid bacteria (LAB) have been used for food fermentation since ancient days and today their main applications are still in the feed and food industry used as starter cultures Berente *et al.*, (1993). The aim of this investigation to determine characteristics and the effect of lactic acid bacteria (Lactobacilli and Streptococci) on sensory, chemical and microbiological of legumes purees .

MATERIALS AND METHODS

Source of legumes

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

Starter cultures

Yogurt milk (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were used as starter cultures after isolated and identified .

Isolation and identification of yogurt cultures

Lactic acid bacteria were isolated from yogurt samples using the pour plate techinique on M17 / MRS agar according to International Dairy Federation (IDF Standaed 306 , 1995) . M17 agar with aerobic incubation was used for *St. thermophilus* while , MRS agar was applied for *L. bulgaricus* (Fathallah , 2019). 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 of legume purees

Legumes puree (P) was prepared by mixing of salt (2g/100g), lemon (15 drops/100g) and sesame paste (Tahina) (20g/100g) in the blinder after soaked legumes according to Shah , (2003) .

Fermentation of legumes

Faba bean, Chickpea and Soy bean were fermented using yogurt bacterial isolates (*Lactobacillus bulgaricus* and *St. thermophilus*) as starter by adding 10% yogurt , samples were incubated at 37° C until pH 5.2 was reached according to Kabeir *et al.*, (2005) and stored in refrigerator at 5 ° C.

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Sensory evaluation of fermented Legumes

Sensory characteristics of fermented legumes were evaluated using 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 and thickness using a ten-point score system (10 excellent, 1 unacceptable).

Chemical analysis of fermented legumes

Moisture, proteins, fats and ash were determined according to the methods of A.O.A.C. (2005).

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 varians (ANOVA).

RESULTS AND DISCUSSION

Identification of yogurt 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 No. 6 &9 are belonging to *Streptococcus thermophilus* and *Lactobacillus bulgaricus* respectively according to Axelsson ,(2004) reported that lactic acid bacteria (LAB) are a group of non-sporulating , gram positive , anaerobic or facultative aerobic rods or cocci , which produce lactic acid as one of the major fermentation products of the carbohydrates metabolism .

Molecular identification of the selected bacterial isolates

Molecular identification was done by Sigma Scientific Services Co.using 16S rRNA gene. The resulted nucleotide sequences was 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 yogurt bacterial isolates

Characters	Bacterial isolates No.	
	9	6
Morphological		
Gram stain	+	+
Cell shape	rods	cocci
Spore forming	-	-
Motility	+	-
Capsule formation	-	-
Measurement (µm)	(1 x 4)	(1.5)
Biochemical		
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 two 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 *Streptococcus thermophilus* with sequence identity 99% and *Lactobacillus bulgaricus* with sequence identity 98%. The obtained 16SrRNA sequences were submitted to Gen Bank under accession numbers as shown in Table (2).

Table 2. The resultant 16S rRNA Sequences

Isolate No.	Scientific name	Partial sequence
9	*Lactobacillus bulgaricus*	CGGGTTTAGATATAGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGCA ACTGACGCTGAGGCTCGAAAGCCCATGGGTAGCGAACAGGATTTAGATACCCTGGTAGT CCATGGGCCGTAAACGATGAGTGCTAAGTGTTGGGAAACGGTTTCCGCCTCTCACGTGC TGCAGCACTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCA AAGGAATATGACGGCGGGCCCGGCACACAAGCGGTGGAGCATGTGGTTTAATTCGAAG CAACGCGAAGAACCTTACCAGGTCTTGACAAAT.
6	*Streptococcus thermophilus*	GGTCCCCCCGGACCTCACCTCATATCCACCAATCATCTGTCCCACCTTAGGCGGCTGGCT CCAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTG TGTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGATCCGCGATTACTAGCGATTC CGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGATTGGCTTTAAGAGATT AGCTCGCCGTCACCGACTCGCAACTCGTTGTACCAACCATTGTAGCACGTGTGTAGCCC AGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCAATTAAAAA.

Sensory evaluation

Sensory attributes of boiled Faba bean, Chick pea and Soy bean purees fermented by yogurt bacteria are represented in Table (3). Data indicated that yogurt (Y) Soy bean puree recorded the highest scores in appearance (8.5 ± 0.2), however Y Faba bean had the best taste (8.2±0.2)and Y Chickpea puree had the best odor(8.2 ± 0.2). On the other hand; the lowest sensory attributes was noticed for Y Faba bean in mouth feeling (7.5 ±0.3)and Y Soy bean in overall acceptability (7.7±0.1). Also, Y Faba bean had the lowest scores in appearance (8.2 ± 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) and Modu, (2010) but different in color, taste and odor.

Table 3. Sensory attributes of legumes puree fermented by yogurt bacteria

Samples	Appearance	Color	Taste	Odor	Mouth feeling	Overall acceptability
Y. Faba bean	8.2± 0.3 [a]	7.9± 0.2 [a]	8.2± 0. 2 [a]	6.9± 0.4 [a]	7. 5± 0.3 [a]	8.2± 0.1 [a]
Y. Chick pea	8.3± 0.2 [a]	7.9± 0.2 [a]	8.0± 0.2 [a]	8.2± 0.2[b]	7.9± 0.2[b]	8.0± 0.1[b]
Y. Soy bean	8.5± 0.2 [a]	8.3± 0.2 [a]	8.0± 0. 2 [a]	7.8± 0.2 [b]	8.4± 0.2 [b]	7.7± 0.1 [b]

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 yogurt bacteria are shown in Table (4). Results indicated that the highest values of chemical parameters were recorded for Y Soy bean in moisture which were (5.9±0.1), in lipids had (20.0±0.1), , protein was (37.8±0.1), Y Chickpea in carbohydrates was (62.2±0.2). The results were in Hassan ,(2013) found that moisture content of legumes was 5.9% .Hosono and Hisamatsu, (1995) reported that fat content in legumes were a range from 0.91% to 1.70%, and protein content for legumes were 30.89 to 37.14% . Kadooka *et al*., (2010) found that carbohydrate content of 36.8% for legumes, while found that carbohydrate in Faba bean were a range from 60.80 to 62.12%.

Table 4. Chemical composition of legumes puree fermented by yogurt bacteria

Samples	Moisture	Ash	Lipids	Proteins	Carbohydrates
Y. Faba bean	3.7±0.1[a]	4.5±0.0[a]	1.3±0.0[a]	28.1±0.1[a]	62.1±0.1[b]
Y. Chickpea	5.0±0.2[b]	4.2±0.1[b]	1.6±0.0[a]	27.9±0.1[a]	62.2±0.2[b]
Y. Soy bean	5.9±0.1[c]	5.0±0.1[c]	20.0±0.1[b]	37.8±0.1[b]	31.1±0.1[a]

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

Microbiological evaluation

Results represent in Table (5) shows that zero storage days value at Faba bean ,Chick pea and Soy bean was (4.8± 0.3, 5.3± 0.2 and 5.1 ± 0.3) respectively. On the other hand, the zero storage days value of the Chick pea was (5.3± 0.2)it could be noticed from this data a highest value while the lowest value with the Faba bean (4.8± 0.3) .

In Table (5) the three storage days value at Faba bean , Chick pea and Soy bean was (4.9 ± 0.3, 5.4± 0.3, and 5.3 ± 0.3) this data increase value in Chick pea was (5.4± 0.3) and decrease value in Faba bean was (4.9 ± 0.3).

Table (5) also shows that seven storage days value at Faba bean , Chick pea and Soy bean was (5.3 ± 0.5, 5.5 ± 0.3and 5.5± 0.3) . It was the highest value for Soy bean and Chick pea was (5.5 ± 0.3) and decreased value was in Table (5) shows that in Faba bean(5.3 ± 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 (5.4± 0.5) as the highest value was for the Chick pea and Soy bean (5.6± 0.3).

Table 5. *Lactobacillus bulgaricus* counts (cfu x10^3/g) as yogurt-fermented Faba bean, Chick pea and Soy bean sauces during storage periods

Samples	Storage days			
	0	3	7	10
Faba bean	4.8±0.3 [a]	4.9±0.3 [a]	5.3±0.5 [a]	5.4±0.5 [a]
Chick pea	5.3±0.2 [a]	5.4±0.3 [a]	5.5±0.3 [a]	5.6±0.3 [a]
Soy bean	5.1±0.3 [a]	5.3±0.3 [a]	5.5±0.3 [a]	5.6±0.3 [a]

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

Results obtained in Table (6) show the effect *Streptococcus thermophilus* of yogurt -fermented Faba bean, Chick pea and Soy bean and reavaled that zero storage days value at Faba bean , Chick pea and Soy bean was (4.7± 0.3, 5.3± 0.5 and 5.2 ± 0.3) respectively.

While, the zero storage days value of the Chick pea was (5.3± 0.5)it could be noticed from this data a highest value and the Faba bean was (4.7± 0.3) 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(4.8 ± 0.2, 4.8± 0.2, and 5.4 ± 0.3) this data increase value in Soy bean was (5.4± 0.3) and decrease value in Faba bean and Chick pea (4.8 ± 0.2) .

Table (6) also shows that seven storage days value at Faba bean , Chick pea and Soy bean was (5.3 ± 0.3, 5.4 ± 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 (5.3 ± 0.3). 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 with Faba bean was (5.3± 0.3) and the highest value with 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).

Table 6. *Streptococcus thermophilus* counts (cfu x10^5/g) as yogurt -fermented Faba bean, Chick pea and Soy bean sauces during storage periods

Samples	Storage days			
	0	3	7	10
Faba bean	4.7±0.3 [a]	4.8±0.2 [a]	5.3±0.3 [a]	5.3±0.3 [a]
Chick pea	5.3±0.5 [a]	4.8±0.2 [a]	5.4±0.2 [a]	5.6±0.2 [a]
Soy bean	5.2±0.3 [a]	5.4±0.3 [a]	5.5±0.3 [a]	5.8±0.5 [a]

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

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تأثير بكتيريا حمض اللاكتيك على البقوليات المتخمره

عايدة حافظ عفيفى[1] ، أحمد فتح الله فتح الله الرفاعى[2] ، محمد عبدالله العوضى سليم[1] و فاطمه محمد الزمزمى[2]

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إستخدمت بكتيريا حمض اللاكتيك (بكتيريا الزبادى) فى إعداد بعض البقوليات المتخمره مثل الفول و الحمص و الصويا بعد نقعهم و طهيهم حيث أجريت الإختبارات المورفولوجيه والبيوكيميائيه وكذلك الجزيئيه لتعريف هذه البكتيريا على أنها *Lactobacillus bulgaricus* and *St. thermophilus* وقد قيمت المنتجات من البقوليات المتخمره حسيا و كيميائيا و ميكروبيولوجيا. أظهرت النتائج أن أفضل درجات القابليه و الطعم فى بيوريه فول الصويا والتذوق فى بيوريه الحمص والنكهه و المظهر فى بيوريه الفول البلدى .وأظهرت النتائج الكيميائيه ان أعلى القيم التى سجلت فى الرطوبه و الرماد و الدهون والبروتين فى بيوريه فول الصويا واما الكربوهيدرات ففى بيوريه الحمص وكما أظهرت النتائج الميكروبيولوجيه ان افضل القيم كانت لبكتيريا *Lactobacillus bulgaricus* فى بيوريه الحمص و فول الصويا خلال عشرة أيام وبكتيريا *thermophilus St.* سجلت أعلى القيم فى بيوريه فول الصويا و قد سجلت النتائج النهائية أن زيادة أعداد بكتريا حمض اللاكتيك تحسن من الخصائص الحسية والكيميائية لهذه البقوليات .