academicJournals

Vol. 9(5), pp. 307-313, May, 2015
DOI: 10.5897/AJFS2014.1217
Article Number: 4A3E9E653078
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Synergistic fermentative nutritional quality of *Lactobacillus delbrueckii* and *Bacillus pumilus* on date fruits (*Phoenix dactylifera*)

Bankefa, Emmanuel Olufemi[1]* and Oyedeji, Olumide Murtala[2]

[1]Federal University, Oye, Ekiti, Ekiti State, Nigeria.
[2]Federal University of Technology, Akure, Ondo State, Nigeria.

Received 14 October, 2014; Accepted 27 April, 2015

The work examined the effect of mixed cultural fermentation on the nutritional quality of date palm fruits (*Phoenix dactylifera*). This was done by isolating microorganism from samples subjected to natural fermentation. The isolated organisms were thereafter used as starter culture in a five day controlled fermentation. *Lactobacillus delbrueckii* and *Bacillus pumilus* were identified as the predominant microorganisms and were used as starter culture. pH, titratable acidity, proximate, amino acid and mineral composition of the fermented samples were determined. The sample fermented with mixed culture gave best nutritional value of 15.3% protein as compared to the 9.62% and 11.61% yield in *B. pumilus* and *L. delbrueckii*, respectively, on the fifth day of fermentation. Arginine was found to be on the high side among the amino acids discovered and the mineral composition of the products gave an appreciable amount of sodium, potassium and calcium.

Key words: Fermentation, Nutritional quality date palm fruits, *Lactobacillus delbrueckii*, *Bacillus pumilus.*

INTRODUCTION

Fermentation is a metabolic process that converts sugar to acids, gases and/or alcohol. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium. Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, carbon dioxide and hydrogen gas (H_2) (El-Sohaimy and Hafez, 2010). However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Lactic acid fermentation is commonly used in many parts of the world as a method for preservation of plant materials as well as importing desirable sensory and nutritional properties to the product (Cooke et al., 1987). Fermentation has been reported to improve the nutritional values, taste and aroma (Adegbehingbe et al., 2014).

Date fruit (*Phoenix dactylifera* L.) is a palm in the genus *Phoenix* belonging to the *Arecaceae* family, cultivated for its edible sweet fruit. Dates, is identified by its clusters of oval, dark, reddish-brown drupe with the skin of the dried fruit wrinkled and covered in a sticky waxy film. The date palm (*P. dactylifera* L.) is one of mankind's oldest

*Corresponding author. E-mail: femibankefa@gmail.com.

cultivated plants and has been used as food for about 6000 years (Sahari et al., 2007). It is an important food crop in Middle East and is considered to be one of the most important fruit tree particularly in North African, the Middle Eastern and Asian countries. The fruit contributes to the economy and social life within these regions (Bastway et al., 2008) and it is considered as a vital component of their diet (Vayalil, 2002). Date fruits are well known as a staple nutritious food and source of wealth for many years (Khan et al., 2008). Due to its high nutritional value, great yields and its long life, the date palm has been mentioned as the "tree of life" (Augstburger et al., 2002). Date fruits are considered as major source of carbohydrate which include simple sugars like glucose and fructose (Ahmed and Ahmed, 1995; Al-Hooti et al., 1997; Myhera et al., 1999) and sucrose (Guizani et al., 2010). They are good sources of dietary fiber and some important minerals which include iron, potassium, selenium, calcium and vitamins and it also contains vitamin C, B_1, B_2, A, riboflavin and niacin but it is low in fat and protein contents (Myhera et al., 1999; Guizani et al., 2010; Sawaya et al., 1983).

Al-Shahib and Marshall (2003) reported that the fruits (dates) of the date palm contain a high percentage of carbohydrate (total sugars, 44-88%), fat (0.2-0.5%), 15 salts and minerals, protein (2.3-5.6%), vitamins and a high percentage of dietary fibre (6.4-11.5%). There are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g date depending on the type of mineral. Additionally, the seeds contain aluminum, cadmium, chloride lead and sulphur in various proportions. The protein in dates contains 23 types of amino acids. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 thiamine, B_2 riboflavin, nicotinic acid (niacin) and vitamin. Date fruit is served mainly as desserts, these date fruits are generally chopped into tiny slices and scattered across cakes and puddings to ameliorate their flavor. Some people mix date paste with yogurt, milk, bread, or butter to enhance their flavor. Dates are also deseeded and stuffed with several sweet fillings like apricot. Besides the versatility of date preparation and consumption, these dry fruits also feature various nutritional values.

Dates are opulent in vitamins, minerals, natural fiber and are also cholesterol-free. Dates may be considered as an almost ideal food, providing a wide range of essential nutrients and potential health benefits (Al-Shahib and Marshall, 2003). In Tunisia, the date palm sap from date palm is directly consumed as a fresh drink called "Legmi" or used as an alcoholic beverage after natural fermentation. The fresh sap is purgative, sweet, clear, translucent and rapidly fermented (Ben Thabet et al., 2007). Composition analysis of fresh sap from date palm revealed that sugars are the major components (92-95% dry matter basis) with the dominance of sucrose. It contains also 2.7-5% of proteins and 2.3-2.6% of minerals (Ben Thabet et al., 2007, 2009). Palm sap is rapidly fermented by autochthones microflora composed essentially of yeasts, lactic acid bacteria and acetic acid bacteria. This is why date fruit benefits are widely known across the globe. Over the years, several approaches have been adopted in order minimize production cost, make available varieties of fermented drinks in the market and also to explore the arrays of underutilized nutritional fruits. Development of new beverage from date fruits using indigenous microorganisms may be a promising idea to the beverage industries. The aim of this research was therefore to study the quality of the date fruit upon fermentation by indigenous organisms.

MATERIALS AND METHODS

Collection of date palm fruits

Date palm fruits were purchased from a main market of Akure, Nigeria and transported to the Microbiological Laboratory of Federal University of Technology, Akure, Nigeria. The fruits were sorted and the apparently healthy fruits were washed, subjected to natural fermentation at 25±2°C for five days and samples were examined at each day of fermentation. Colonies were isolated and subjected to biochemical identification (Erdoğrul and Erbilir, 2006; Gueimonde et al., 2004). New set of apparently healthy ones were subjected to controlled fermentation using the single culture and mixed culture of the predominant cultures obtained from the fruits in a modified bioreactor at 25°C for 5 days (Adegbehingbe et al., 2014). Samples were aseptically withdrawn from the solution throughout the fermentation period and subjected to chemical, nutritional and microbiological analyses. Samples were also filtered and presented for sensory evaluation.

Determination of pH

The pH of the sample was determined at each day of treatment for five consecutive days using Jenway pH meter (Jenway 3010; Jenway Ltd., Essex, UK) which had been standardized with buffer solution of 7.

Titratable acidity

This was determined using the method of Valverde et al. (2005) by titrating 20 ml of fermented sample with 0.1 M NaOH to an end point of permanent pink colour using phenolphthalein as indicator.

Proximate composition

This was analyzed using standard procedure to determine the moisture, protein, fat, fiber, ash and carbohydrate (AOAC, 2003 and 1984).

Processing of the fermented samples

Both fermented samples were processed on the fifth day of fermentation for sensory evaluation. The flow sheet showing the process used to obtain fermented samples is given in Figure 1.

Amino acid analysis

The amino acid composition of the fermented sample was

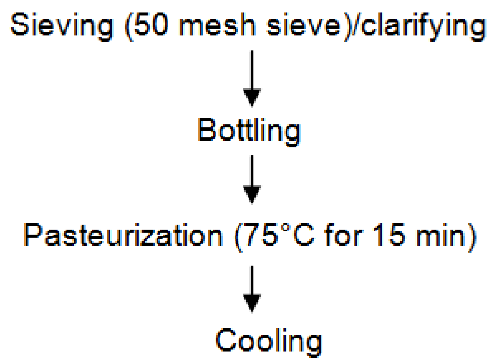

Figure 1. The flow sheet showing the process used to obtain fermented samples.

Table 1. Occurrence of organism during the fermentation periods.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B. pumilus	X	X	√	√	X
L. delbrueckii	X	√	√	√	√
B. pumilus + *L. delbrueckii*	X	√	√	√	√

determined by reverse phase HPLC by Kang-Lyung (2001).

Sensory evaluation

Sensory characteristic of the fermented processed drinks were assessed by 5 untrained panelists for their colour, texture, odour, taste and overall acceptability. The panelists recorded quality characteristics of each sample on an 8- point hedonic scale (8 = like extremely, 1=dislike extremely).

Statistical analysis

All analyses were carried out in triplicates. Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test was used for comparison of means SPSS software (version 16.0 for Windows, SPSS Inc., Chicago). Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

This study revealed that *B. pumilus* and *L. delbrueckii* (Table 1) are the predominant fermentative microorganisms of date fruits with the occurrence even after 24 h of fermentation. This observation is in line with the findings of Okafor (2007) and On-Ong et al. (2012) who reported similar organisms as part of organisms that are associated with the fermentation of the fruit. The absence of *B. pumilus* on the fifth day of fermentation may be an indication that the environmental condition of the fermenting medium is not conducive for its metabolic activities. This observation may be supported by the result of the total acidity (Table 3) of the medium. *B. pumilus* is an alkaline active organism so the acidity produced by the fermentation of the date fruits makes the substrate unfavorable for its reproducibility. The use of starter cultures is in line with the research of Kimaryo et al. (2000) as an appropriate approach for control and optimization of the fermentation process in order to alleviate problems of variations in organoleptic quality and microbial stability observed in African fermented foods.

The observed increase in protein content could be due to the activity of *B. pumilus* which is mostly used for alkaline protease production. The reduction in the carbohydrate content can be attributed to the utilization of the sugars present in the fruits by the utilizing microorganisms (Akande et al., 2010). The decline in the carbohydrate content during the fermentation period may be connected to the ability of fermenting microorganisms to metabolize carbohydrate as carbon source in order to synthesize cell biomass (Vadivel and Janardhanan, 2005), thereby producing alcohol or acids. Ogunbanwo et al. (2004) reported the effect of fiber in food as an aid to digestion. The insignificant increase in the crude fiber may also be an indication that the product will have easy digestion when consumed. Fermentation using the mixed culture of *L. delbrueckii* and *B.pumilus* gave the best nutritional value in all the days of fermentation (Figures 2 to 4). This finding agrees with the work of Achi and Akubor (2000) who stated that the use of mixed cultures improve the nutrition of fermented foods while working on the effect of bacteriocinogenic *Lactobacillus* spp. on the shelf life of fufu, a traditional fermented cassava product.

The observed decrease in the pH of all the samples (Table 2) and subsequent increase in the titratable acidity justifies the claim that the fermentation process by the organisms is not only alcohol based but also acidic based. In food safety, fast reduction in pH is desirable in order to inhibit the growth of microorganisms especially Gram negative and acid sensitive food borne pathogens (Orji et al., 2003). The observed increase in total titratable acidity of the fermented sample during the fermentation period could also be linked to the ability of the organisms to secrete acid while utilizing the available nutrient for their metabolic activities (Holzapfel, 2002). The results of the sensory evaluation (Table 4) from this study has shown that product from combined starter cultures of *B. pumilus* and *L. delbrueckii* had higher acceptability as compared to the spontaneously fermented product. The observed amino acids content of the product is an indication that the product can be recommended or even used as fortifier in nutrient food deficient in amino acids (Table 6). The observed high value of one of the essential amino acid arginine may also make the product recommendable for patient having low erection as arginine has been reported as amino acid used by the

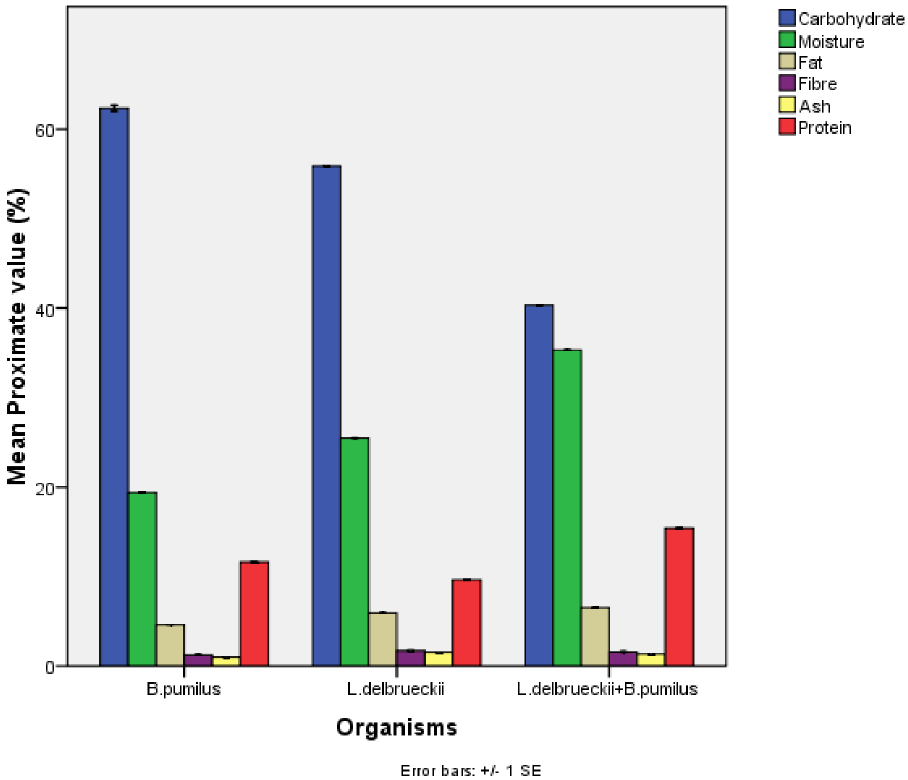

Figure 2. Proximate composition of fermented samples at the end of fermentation period (5th day).

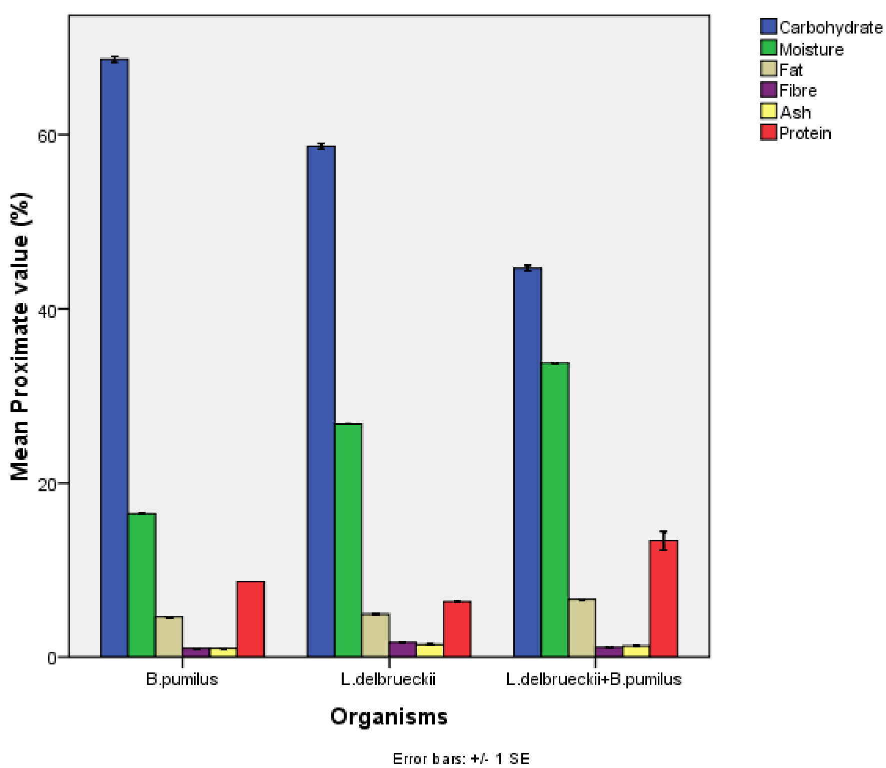

Figure 3. Proximate composition of fermented samples at the 3rd day of fermentation.

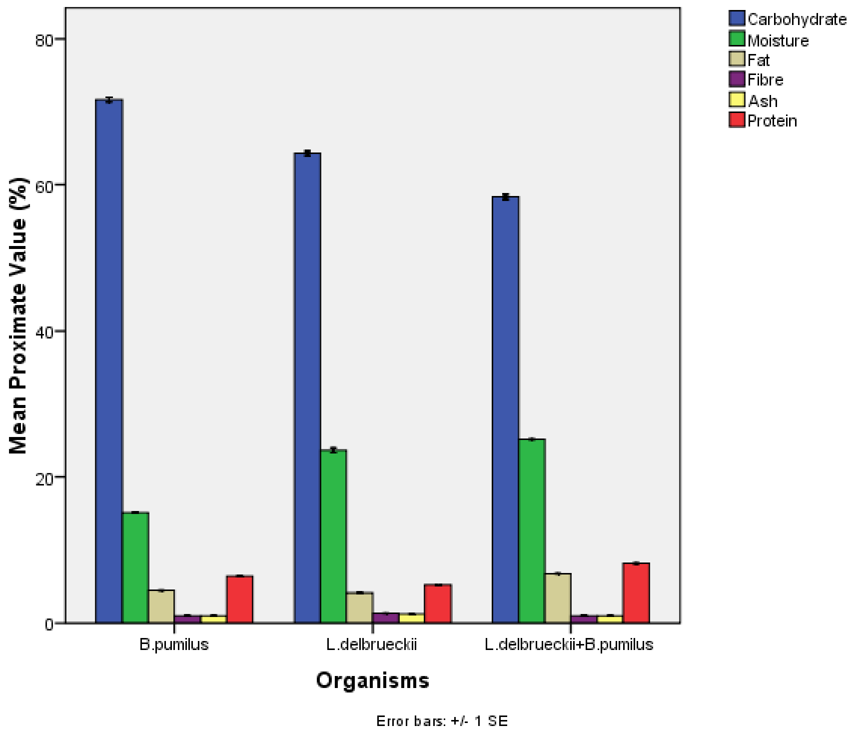

Figure 4. Proximate composition of fermented samples at the 1st day of fermentation.

Table 2. pH during fermentation period.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B.pumilus	7.2±0.0^b	6.7±0.06^b	6.8±0.05^b	6.4±0.1^c	6.5±0.0^c
L.delbrueckii	7.0±0.0^b	6.7±0.06^a	6.4±0.05^a	6.0±0^b	5.7±0.1^b
B.pumilus+L. delbrueckii	6.8±0.17^a	6.5±0.14^a	6.3±0.05^a	5.4±0.0^a	4.7±0.1^a

Samples with the same superscripts down the column are not significantly different.

Table 3. Total titratable acidity during fermentation period.

Organism	Fermentation periods (days)				
	1	2	3	4	5
B.pumilus	1.1±0.0^a	1.3±0.0^a	1.33±0.1^a	1.7±0.0^a	1.7±0.0^a
L.delbrueckii	1.2±0.1^b	1.5±0.0^c	1.7±0.0^b	1.9±0.0^b	2.1±0.1^a
B.pumilus+ L.delbrueckii	1.2±0.0^{ab}	1.37±0.1^b	1.7±0.0^b	2.03±0.1^c	3.27±1.3^a

Samples with the same superscripts down the column are not significantly different.

Table 4. Sensory evaluation of the processed samples.

Fermented drinks	Appearance	Sourness	Odour	Taste	Acceptability
B. pumilus fermented drink	4.0±0.0^a	5±0^a	6.8±0.89^a	4±0.0^a	4±0.0^a
L. delbrueckii fermented drink	5.2±0.45^b	6.2±0.45^b	4.5±0^a	5.4±0.54^b	6.4±0.55^b
B. pumilus+ L. delbrueckii fermented drink	5.6±0.89^b	6.2±0.45^b	5.2±0.44^b	6.2±0.44^c	7±0.0^c

Samples with the same superscripts down the column are not significantly different.

Table 5. Mineral composition of the processed samples (µg/ml).

Fermented drinks	Mineral concentration (µg/ml)		
	Na	K	Ca
B. pumilus fermented drink	113.4±0.61[b]	354.1 ± 0.61[a]	118.7±0.58[a]
L.delbrueckii fermented drink	110.3±0.21[a]	355.0 ± 0.51[a]	118.5±0.58[a]
B. pumilus+ L. delbrueckii	114.8±0.5[c]	364.0 ± 0.72[b]	124.7±0.58[b]

Samples with the same superscripts down the column are not significantly different.

Table 6. Amino acid composition of the processed drink.

Amino acid	Concentration (µml/ml)
Alanin	0.78
Arginine	346.45
Aspartic	126.24
Cystine	3.47
Glutamin	87.31
Methionine	7.42
Proline	286.43
Threoine	43.21
Valine	76.32
Glycine	101.32
Histidine	178.90
Isoleucine	43.29
Leucine	30.63
Lysine	30.18
Phenylalanine	26.74
Serine	13.93
Tyrosine	15.42

body to make nitric oxide (NO), which plays a role in the development of erection by enhancing the circulation of blood (Onibon et al., 2007). Fermentation of date fruit gave an appreciable mineral content (Table 5). The high potassium content observed in all the fermented products conforms with the work of Beaumont (2002) while working on nutritional and anti-nutritional composition of some Nigerian fruits. The high odour reported by the panel in the *B. pumilus* fermented product may be due to their ability to secrete ammonia a bye product during the utilization of the amino acids present in the product.

Conclusion

The result of this investigation showed that a new beverage with acceptable organoleptic properties can be produced from date palm fruits using co-cultural fermentation of *B. pumilus* and *L. delbrueckii*. This fruits can be adopted by beverage industries to introduce new product with high nutritional quality into the market.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

Federal University, Oye, Ekiti, Ekiti State, Nigeria and Federal University of Technology, Akure, Ondo State, Nigeria are acknowledged for their support.

REFERENCES

Achi OK, Akubor PI (2000). Microbiological characterization of yam fermentation for elubo (yam flour) production. World J. Microbiol. Biotechnol. 16:3-7.

Adegbehingbe K, Adetuyi FC, Akinyosoye FA (2014). Effect of fermentation on nutrient and anti-nutrient contents of ground-cooked Lima bean (*Phaseolus lunatus*) seeds using *Bacillus subtilis* and *Bacillus pumilus*. Br. Microbiol. Res. J. 4(11):1285-1298.

Ahmed IA, Ahmed WK (1995). Chemical composition of date's varieties as influenced by the stage of ripening. Food Chem. 54: 305-309.

Akande KE, Abubakar MM, Adegbola TA, Bogorose SE, Doma UD (2010). Chemical evaluation of the nutritive quality of pigeon (Cajanus cajan (L.) Millsp.). Int. J. Poult. Sci. 9 (1):63-65.

Al-Hooti S, Sidhu JS, Al-Otaibi J, Al-Ameeri H, Quabazard H (1997). Date bars fortified with almonds, sesame seeds, oat flakes and skim milk powder. Plant Foods Hum. Nutr. 51: 125-135.

Al-Shahib W, Marshall RJ (2003). The fruit of the date palm: its possible use as the best food for the future?. Int. J. Food Sci. Nutr. 54 (4): 247-259.

AOAC (1984). Official methods of Analysis. Washington; DC: Association of Official Analytical Chemists.

AOAC (2003). Official methods of analysis of the association of official's analytical chemists, 17th edn. Association of official analytical chemists, Arlington, Virginia.

Augstburger F, Berger J, Censkowsky U, Heid P, Milz J, Streit C (2002). Date Palm. Naturland. Germany.

Bastway M, Hasona NA, Selemain H (2008). Protective effects of extract from dates (*Phoenix dactylifera* L.) and ascorbic acid on thioacetamide induced hepatotoxicity in rats. J. Pharm. Res. 7: 193-201.

Beaumont M (2002). Flavouring composition prepared by fermentation with Bacillus spp. Int. J. Food Microbiol. 75: 189 -196.

Ben Thabet I, Attia H, Besbes S, Deroanne C, Francis F, Drira NE, Blecker C (2007). Physicochemical and Functional Properties of Typical Tunisian Drink: Date Palm Sap (*Phoenix dactylifera* L.). Food Biophys. 2: 76-82.

Ben Thabet I, Besbes S, Attia H, Deroanne C, Francis F, Drira NE, Blecker C (2009). Physicochemical characteristics of date sap lagmi from Deglet Nour palm (*Phoenix dactylifera* L.). Int. J. Food Prop. 12: 659-670.

Cooke RD, Twiddy DR, Reilly PJA, (1987). Lactic acid fermentation as a low cost means of food preservation in tropical countries. FEMS

Microbiol. Rev 46:369-379.
El-Sohaimy SA, Hafez EE (2010). Biochemical and nutritional characterization of date palm fruits. Int. J. Appl. Sci. Res. 6(8):1060-1067.
Erdoğrul Ö, Erbilir F (2006). Isolation and characterization of *L. bulgaricus* and *L. casei* from various foods. Turk. J. Biol. 30:39-44.
Gueimonde M, Delgado S, Mayo B, Ruas-Madiedo P, Margolles A, de los Reyes-Gavilan G (2004), Viability & diversity of probiotic Lactobacillus and Bifidobacterium populations included in commercial fermented Milks. Food Res. Int. 37:839-850
Guizani N, Al-Saidi GS, Rahman MS, Bornaz S, Al-Alawi AA (2010). State diagram of dates: glass transition, freezing curves and maximal-freeze- concentration condition. J. Food Eng. 99: 92-97.
Holzapfel WH (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. Int. J. Food Microbiol. 75:197-212.
Kang-Lyung W (2001). Amino acid analysis protocol. Methods Mol. Biol. 159:141.
Khan MN, Sarwar A, Wahab F, Haleem R (2008). Physico-chemical characterization of date varieties using multivariate analysis of plums. Food Chem. 81:321-326
Kimaryo VM, Massawe GA, Olasupo NA, Holzapfel WH (2000). The use of a starter culture in the fermentation of cassava for the production of “kivunde”, a traditional Tanzanian food product. Int. J. Food Microbiol. 56:106-108.
Myhera RM, Karkdas J, Taylor MS (1999). The composition of maturing Omani dates. J. Agric. Food Chem. 76: 1345-1350.
Ogunbanwo ST, Sanni AI, Onilude AA (2004). Effect of bacteriocinogenic *Lactobacillus* spp. on the shelf life of fufu, a traditional fermented cassava product. World J. Microbiol. Biotechnol. 20: 57-63.
Okafor N (2007). Modern Industrial Microbiology and Biotechnology. Science Publishers, Enfield. pp. 263-308.
Onibon VO, Abulude FO, Lawal LO (2007). Nutritional and anti-nutritional composition of some Nigerian fruits. J. Food Technol. 5:120-122.
On-Ong C, Kornwika P, Donalphora T (2012). Pineapple wine fermentation with yeast isolated from fruit as a single and mixed starter culture. Asian J. Food Agro-Ind. 5 (02): 104-111.
Orji MU, Mbaka TI, Aniche GN, Ahonkhai I (2003). The use of starter cultures to produce ‘pito’, a Nigerian fermented alcoholic beverage. J. Microbiol. Biotechnol. 19:733-736.
Sahari MA, Barzegar M, Radfar R (2007). Effect of varieties on the composition of dates (*Phoenix dactylifera* L.). Food Sci. Technol. Int. 13:269-275.
Sawaya W, Miski A, Khalil J, Khatchadonian H, Mashadi A (1983). Physical and chemical characterization of the major date varieties grown in Saudi Arabia; I Morphological measurements, proximate and mineral analyses. Date Palm J. 2: 1-25.
Vadivel V, Janardhanan K (2005). Nutritional and antinutritional characteristics of seven south Indian wild legumes. Plant Food Hum. Nutr. 60 (2): 69-75.
Valverde JM, Valero D, Martinez-Romero D, Guillen F, Castillo S, Serrano M (2005). Novel edible coating based on *Aloe vera* gel to maintain table grape quality and safety. J. Agric. Food Chem. 53:7807-7813.
Vayalil PK (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. *Arecaceae*). J. Agric. Food Chem. 50: 610-617.