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Influence of Fortification with Extracts of Three Varieties of Iraqi Dates on the Viability of *Lactobacillus plantarum* in Probiotic Fermented Milk Products

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Abstract: Therapeutic fermented milk is product owing to its particular physical, nutritional, probiotic and organoleptic properties containing prebiotics as enhancers of human natural micro-flora and probiotics with their positive effects on health when consumed regularly according to WHO recommendations, This study was conducted to fortified the Milk by the extracts of three variety of Iraqi dates (Zahdi, Khastawi and Khadrawy) on four different percentage levels of treatments T1 (5), T2 (10), T3 (15) and T4 (20) comparing with T0 (without any addition) in order to know its role in the impact on the vitality and numbers of Probiotic *Lactobacillus plantarum* expressed in terms of growth intensity when used as starter culture In the manufacturing of therapeutic fermented milk and their influence as Prebiotic enhancers, The lowest number appeared in logarithmic counts of viable bacteria cell were in the treatment of control T0 (10.80), comparing with the increase in the supported products with extracts for T4 treatment, which were similar to those of al-Zahdi and al-Kashtawi (12.86), while al-Khudrawi (12.96) and what it means that there is a direct increasing in the logarithmic number of probiotic bacteria with the higher percentage addition of date extracts, and was the most effective with the Khadrawi variety, there is a correlation between the growth of *Lb. plantarum* bacteria to get the best and smooth texture of the therapeutic fermented milk products, and to maintain the probiotic bacterial number at proper required accounts, That the results obtained give a positive indication of the role of date extracts of these varieties in increasing numbers and enhancing the viability of these probiotic bacteria in fermented milk products.

Keywords: Prebiotic, Date Extracts, *Lb. plantarum* , Probiotic Fermented Milk.

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Introduction:

Date palm (*Phoenix dactylifera* L.) is considered the oldest fruit tree in the world, there are more than 2000 different cultivars of date palm known worldwide, but only a few of them have been used for their agricultural productivity and fruit quality, Some reports cite that it has been used since 4000 BC in Mesopotamia and considered a highly valuable for human and animal nutrition in many countries, The Date Palm has been important nutritional constituent depending on the stage of maturity with proximate composition were carbohydrates 60%, moisture 24.2%, Dietary Fibre 8.6%, protein 2.3% and others (1). Iraq is one of the largest producers of dates in the world and it have an important locally food in which were consumed either directly as fruits at Tamar stage due to their availability all round year and their good storage stability or manufactured to several food products for nutritional and therapeutic value that made it as healthy food with rich source of

minerals, The total production of the three varieties of dates Zahdi, Khastawi and Khadrawy was, 54.2%, 10.5% and 5% respectively, In which accounted for 69.7% of the total quantity of dates produced in 2016 and estimated was 615.2 thousand tons played an vital role in the social life of the our people and their diet (2).

The advancement of human health awareness, the idea of rejecting chemical treatments according to their known health risks and developed instead of it the mechanism of dependence on natural resources, such as probiotics therapies, which are alternative treatments for many diseases with no side effects (3). probiotics fermented milk have been defined, according to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) as products food containing live microorganisms to actively enhance health by improving the balance of microflora in the human gut, it has been essential that products must met the criterion of a minimum concentration of probiotic bacteria of 10^6 colony forming units (cfu)/ mL at the expiry date, as the minimum daily therapeutic dose for human consumption was suggested to be (10^7-10^{10}) CFU/ mL, Lactobacilli which have a predominant flora in the gut are still being the most commonly starter culture using in therapeutic fermented milk processing Including *Lb. plantarum* species (4). This study was conducted to include the extracts of three varieties of the most Iraqi dates produced in the preparation of the milk mix used in the dairy fermented therapeutic industry and knowledge of its role as the source of prebiotic and increasing the growth and counts of probiotics *Lb. plantarum* and the compatibility to the definition of probiotics as well as the enhancement of nutritional value, chemical composition and good rheological properties in fermented milk products (5, 6).

Materials and Methods:

Preparation of Water Date Extraction:

Briefly, 500 grams of each of the three dates varieties were used with cleaning and seed removing, then soaked with 1500 ml of warm distilled water for half hour, then were good blended with the electrically laboratory blender and filtered through very fine sieve (0.5mm), then the date water extract stored in a refrigerated temperature at 4°C. The date extraction diluted with distilled water until its reached 14° Brix, then were used in each of 200 mL plastic cups for making Probiotics fermented milk as following step.

Date Extracts and Treatments:

The water extracts of three varieties of Iraqi dates from the production season of 2016, theirs chemical composition were shown in a (Table 1) were prepared according to procedure mentioned by Al-Mosawi *et al.* (7). For experimental purposes and addition treatments T0, T1, T2, T3 and T4 were 0, 5, 10, 15 and 20 respectively and separately for each of the skimmed milk mix preparation used in the manufacture of therapeutic milk ferments as shown in (Table 2).

Table (1): Chemical composition of three Iraqi Date Variety.

Dates Variety	Chemical Composition %					
	Moisture	Carbohydrate	Protein	Fat	Fiber	Ash
ZAHDI	8.50	86.50	2.8	0.40	3.14	1.8
KHASTAWI	7.50	87.18	3.19	0.38	2.05	1.75
KHADRAWY	9.50	84.65	2.9	0.45	3.44	2.5

Table (2): Chemical composition % of the reconstituted skimmed milk.

Reconstituted Skimmed Milk	Chemical composition %				
	Total Sold	Protein	Fat	Titratable Acidity	pH
	12.00	4.30	0.18	0.16	6.60

Starter Culture:

Probiotic *Lactobacillus plantarum* in (Figure 1) was purchased as capsules from QUEST Probiotic UK. Activation was done by emptying the contents of the capsule at MRS Broth with incubation at 37°C for 24 hours. Repeating this step twice for activation and use then as a 5% inoculums in reconstituted milk (12% w/v) and repeat three times under the same incubation conditions for early preparation Active starter cultures used in the production of therapeutic milk preparations.

Figure (1): Package of Probiotic Lb. plantarum.

Products Processing:

The therapeutic fermented milks were prepared according to what mentioned by Tamime *et al.* (8). In the manufacture of therapeutic ferments by using Probiotic *Lb. plantarum* by 5% inoculum size in 12% reconstituted skimmed milk used in manufacturing.

Chemical Analysis:

The A. O. A. C. (9). Methods was adopted for estimation of Protein, moisture, ash and fat, The carbohydrates estimated according to Jimoh *et al.* (10). Three pH replicates were performed using a pH-meter and the acidity was developed according to the method described by Almutairi (11).

Microbiological Count:

The total number of viable probiotic bacterial cell per gram was calculated after incubation under anaerobic conditions on 37°C using the MRS Agar by multiplying the number of (CFU) on the plate with respective dilution factor and then was converted into logarithmic form (12).

Rheological Properties:

Viscosity Determination:

The method which mentioned by Donkor *et al.* (13). was followed as described by using Brookfield Digital device model DV-E, with 4-spindle spindle, 10 cycles/min and 150 mL for the sample. Leaving the spindle to rotate inside the sample for 60 seconds, after the gel has been broken by moving it 10 times in the direction of the hourglass and 10 times in reversed direction, viscosity were calculated in centipoises according to manufacturers manual.

Wheying off Determination:

Wheying off was estimated according to Amatayakul *et al.* (14). by placing 50 ml of the product in a bowl at a 45 ° angle for two hours at 5°C. After that, remove the water from the surface using the syringe, then re-weight the plastic cup again and perform the operation within 10 seconds to avoid excessive effusion.

Statistical Analysis:

The results of this study were the averages of three replications analyzed by using Completely Random Design and GIM Procedure of SAS Statistic Analysis Program (15). LSD test were compared between the mean values of treatments and the control.

Result and Discussion:

(Table 3) shows the effect of fortification by extracts of three variety of dates (Zahdi, Khastawi and Khadrawy) on four levels of treatments T1, T2, T3 and T4 in the viability of *Lb. plantarum* and its logarithmic live numbers, as well as the chemical composition of the therapeutic fermented dairy products. the highest logarithmic number of live bacteria in the products supported by the three dates variety extracts was obtained at T3 pinpointed between (12.86-12.96), which was similar to their minimum for DE1 and DE2, while the highest limit was the DE3, with this level of fortification in which exceeded the values of others comparing with T0 the log. number of the control (10.80), with significant differences in ($P < 0.05$) while there were no significant between all treatments related to the addition of the three date extracts, which the lowest was in the treatment of T4 for Khastawi extract (11.79) and remained higher than the control treatment although not significantly different with it. In line with what Marzie and Alireza (16). found to contain the date juice extract on monosaccharide's as rich material and approximately equal proportions of glucose and fructose which has an active role in promoting bacterial growth.

The viable counts of *Lb. plantarum* was found to be highly cultured in fermented dairy products and was associated with growth in lower the pH to less than 4 and extreme acidic conditions (17). There has been a direct increase in the logarithmic count of probiotic bacteria, whenever the higher milk fortification with the date extract for the purpose of preparing the therapeutic fermented milks, with the superiority of the Khadrawy extract compared to the extracts of the other two varieties in the qualities of the products studied. In addition, the proportion of carbohydrates in all

products supported by the extracts of three dates variety was significantly increased by T4 (7.55% - 7.56) in accordance with Aljasass *et al*. (18). The nutritional value of fermented milk was increased with the addition of date extract and increasing of carbohydrates and total solids, The highest value of protein and fat was in the control treatment T0 (4.26, 0.18) respectively, compared with the other treatments with the extracts of the three dates, with no significant difference (P <0.05) between them. This is consistent with other studies (19, 20). Low content of Yoghurt protein and humidity with the increase in the proportion of total solids when the addition of dates. The increase in the total solids in all therapeutic fermented dairy products with *Lb. plantarum* when increasing the rate of support with the extracts of the three dates varieties was observed, and the highest was the treatment T4 (14%) compared to control treatment T0 (12%) with significant differences in the level of probability (P <0.05). As for the Ash ratio, the treatment with T4 Khadrawy Dates extract was higher 0.85 and the lowest in control was T0 (0.57) with significant differences (P <0.05) between them and other T4 treatments of date extracts, while no significant differences were observed between them in all the T1, T2 ,T3 and T4 treatments for the three varieties, and these results are consistent with the study (21).

That date juice contains many vitamins and minerals as well as sugars that help in the growth of bacteria and metabolic activities whenever the concentration of dates juice increases in the medium. By the time pH value of the control product T0 (4.70) was significantly higher than that of the three date extracts addition, which were the lowest of the T3 (4.48), and T4 in DE3 (4.58) with significant differences (P <0.05) by adding the date extract to the product causing to reduce the pH value, On the other hand, the acidity of the control product T0 (0.69) compared with all the products supported by the three dates fruits, the highest of which was treated with T3 (0.95), with significant differences (P <0.05) in which consistent with Al-Mosawi *et al.*(7). The acidity is increased by increasing the concentration of the added date extracts and These are consistent with what are mentioned in (22, 23).

The highest Wheying off were obtained in the control treatment T0 (9.5) compared with all products supported individually by the extracts of the three dates varieties, the lowest of which was T4 in DE2 (4.4) with significant differences in (P <0.05) Which means that the added dates extracts contribute to the growth of the bacteria and its positive effect on the improvement of the consistency of the milk fermentation and its cohesion to preventing Wheying off and may be due to the increase of its secondary metabolites Exopoloysaccharide (EPS). On the other hand, the viscosity was increased by the increase in the ratio of date extracts to the three cultivars in all the fermented products, which were highest at T4 (1640) centipose compared to the control product T0 (1410) centipose with significant differences in (P <0.05). In order to demonstrate the role of date extract ingredients in increasing viscosity with the increasing level of support required to improve the quality of products in terms of this physicochemical characteristic. Sensory evaluation

results (unpublished data) for therapeutic lactic fermentation were shown acceptable to the consumer, especially when treated with T3 extracts of the three date varieties and their contribution to giving good tissue and strength in the products manufactured by using Probiotic *Lb. plantarum*.

Table (3): Viability of Probiotic Lb. plantarum, Chemical and Rheological assays for Therapeutic fermented Milk.

Bacteria	Date Extracts	Treatments	Attributes Studied					
			Log. Bacterial Count	Chemical composition %				
				C	P	F	A	T
Probiotic *Lb. plantarum*	DE0	T0	10.80	5.65	4.26	0.18	0.57	12.00
	DE1	T1	11.85	6.15	4.24	0.17	0.60	12.50
		T2	11.86	6.65	4.21	0.16	0.64	13.00
		T3	12.86	7.10	4.18	0.15	0.73	13.50
		T4	11.82	7.56	4.14	0.14	0.82	14.00
	DE2	T1	11.83	6.13	4.25	0.17	0.61	12.50
		T2	11.85	6.64	4.23	0.16	0.63	13.00
		T3	12.86	7.14	4.17	0.15	0.70	13.50
		T4	11.79	7.55	4.16	0.14	0.81	14.00
	DE3	T1	11.93	6.12	4.24	0.17	0.63	12.50
		T2	11.94	6.64	4.20	0.16	0.66	13.00
		T3	12.96	7.13	4.14	0.15	0.74	13.50
		T4	11.92	7.55	4.12	0.14	0.85	14.00
LSD			1.32	1.41	0.26	0.02	0.17	1.25

Table (3): Continued.

Bacteria	Date Extracts	Treatments	Attributes Studied			
			pH	% Acidity as lactic acid	Rheological properties	
					Wheying off %	Viscocity centipoise
Probiotic *Lb. plantarum*	DE0	T0	4.70	0.69	9.5	1410
	DE1	T1	4.65	0.70	6.9	1600
		T2	4.63	0.77	5.3	1610
		T3	4.52	0.90	5.0	1620
		T4	4.61	0.84	4.5	1640
	DE2	T1	4.66	0.68	7.0	1610
		T2	4.65	0.75	6.0	1620
		T3	4.54	0.85	5.0	1630
		T4	4.64	0.80	4.4	1640
	DE3	T1	4.63	0.72	6.5	1610
		T2	4.60	0.78	5.6	1620
		T3	4.48	0.95	5.0	1630
		T4	4.58	0.86	4.5	1640
LSD			0.10	0.02	0.31	24.20

DE0: Control, DE1: Zahdi Extract, DE2: Khastawi Extract, DE3: Khadrawy Extract, C: Carbohydrate, P: Protein, F: Fat, A: Ash and T: Total Sold.

Conclusion:

The research was conducted to used for fortification of date extracts which were rich in dietary fiber, minerals as well as many nutrients and bioactive compounds in developing Probiotic fermented milk to higher viable

bacterial counts, Improves the sensory evaluation and chemical characteristics. It is obvious that the extracts of three varieties of Iraqi dates have been a positive role as bio-enhancers in increasing the bacterial growth, by adding them individually to milk by 3% and especially for Khadrawy extract without any addition of texture improver, so must work more on this side of fortification by using other variety extracts or different Probiotics lactic acid bacteria whether single or mixed strains for more than one genus or species to produce multi healthier dairy fermented products.

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