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Effect of Storage of Therapeutic Product on LAB Bacterial Counts and Acidity

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Abstract: This investigation carried out to utilize whey and rice in preparation of a therapeutic product. The raw materials were mixed at a ratio of 1:10 rice to whey (w/w). Five treatments were prepared by fermentation with either *Lb. acidophilus* and *Lb. casei*, or mixes of both. The treatments were: T1 (*Lb. acidophilus*), T2 (*Lb. casei*), T3, T4, T5 which were mixes of (*Lb. acidophilus*+*Lb. casei*) starters with ratios of 1:1, 1:2, 2:1, respectively. The best fermentation period was two days as highest therapeutic bacterial counts resulted. The treatments were divided into two groups; first group stored at -18°C for four months, the second group dried at 45°C, then stored at 25°C for three months. The frozen treatments revealed significant differences ($p \leq 0.05$) in titrable acidity which were 0.43-0.85% for T2, T3, respectively. The pH values were (3.63-4.72) for T4, T2, respectively. LAB counts dropped for all treatments during frozen storage. The drop was between 19.14-60.05% for T1 and T4, respectively. The titrable acidity for dried treatments was 0.33-0.66% for T2, T1, respectively. Then increased to 0.46-0.75% after three months of storage for T2, T5, respectively. Highest pH value of 4.5 obtained for T2. There was a slight drop in pH value for all treatments after 3 months of storage. LAB counts dropped for all treatments after drying. The highest number of 5.48 x 10^{10} cfu/gm was for T4. LAB counts were acceptable to impart therapeutic character to the product.

Key words: Therapeutic, lactic acid bacteria, *Lb. acidophilus*, *Lb. casei*, whey

INTRODUCTION

The therapeutic foods are very common around the world with different shapes, forms and included in traditional foods, supplementary additives and medical purposes (Lankaputhra and Shah, 1995; Sanders, 2000).

In spite of the dairy product had a great importance being at the top of the these products for carrying microorganisms particularly to lactic acid bacteria (LAB), this would not effect the existence of other products such as sauerkraut and sausages that can be a good carriers to live populations of LAB, that kept during processing procedures and during products storage, (Heller, 2001; Huebner *et al*., 2008).

The supermarkets in Arab Gulf region, particularly United Arab Emarates and Saudi Arabia exhibit these products in huge quantities and different shapes (pharmaceutical product, food and dairy products), due to the developments and advancement of medical and culture knowledge of populations, while being previously favoured in Europe and Japan particularly (Senok, 2009).

The aim of this study was to process a therapeutic product from rice and whey containing probiotic (LAB) and store it by both freezing and drying, then study the effect of storage methods on the chemical properties and LAB counts of the product to prove and asses it as a therapeutic food.

MATERIALS AND METHODS

Raw materials: Thailand rice was purchased from a local market in Baghdad city-Iraq. Whey was obtained from the dairy factory-College of Agriculture-Baghdad University.

Preparation of the therapeutic product: The product prepared from rice and whey at ratio of 1:10 w/w respectively, then autoclaved at 121°C for 15 min. Five treatments of product has been prepared by fermentation with two types of LAB in different rates Table 1. The treatments were subjected to fermentation for eight days. The best fermentation period was two days as highest therapeutic bacterial counts were resulted. The product treatments were stored by two methods; freezing and drying. Five treatment of product were stored at -18°C for four months, titrable acidity, pH values, LAB bacterial count were determined by the end of storage period. A sample of 100 ml of each treatment were dried at 45°C for 48-54 h in sterilized environment. The dried product were milled in sterilized grinder to obtain smooth powder, then stored at 25°C for 3 months. Titrable acidity and pH were performed, in addition to viable lactic acid bacteria counts were conducted towered the end of storage period.

Table 1: Starters used to prepare treatments

Treatment symbol	Ratio	Starter type
T1	pure	*Lactobacillus acidophilus*
T2	pure	*Lactobacillus casei*
T3	1:1	*Lactobacillus acidophilus+Lactobacillus casei*
T4	2:1	*Lactobacillus acidophilus+Lactobacillus casei*
T5	2:1	*Lactobacillus acidophilus+Lactobacillus casei*

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Table 2: Number of viable lactic acid bacteria for treatments stored by freezing for 4 months

LAB counts treatment	At zero time x 10^{10} cfu/ml	After 4 months x 10^{10} cfu/ml	Counts decrease (%)
T1	2.35*	1.90	19.14
T2	2.20	1.50	31.81
T3	5.85**	3.21	41.50
	2.80***	1.85	
Total	8.65	5.06	
T4	2.50	0.52	60.05
	1.18	0.95	
Total	3.68	1.47	
T5	3.50	1.50	47.78
	0.79	0.74	
Total	4.29	2.24	

*Each figure represents the average of three replicates
**Upper figures represents the number of *Lb. acidophilus*
***Lower figures represents the number of *Lb. casei*

Table 3: Effect of drying and storage of the fermented product at 25°C for a period of three months on the lactic acid bacteria counts

LAB counts treatment	Before drying x10^{10} cfu/g	At zero time x10^{10} cfu/g	Decrease after drying (%)	After one month x10^{10} cfu/g	After two months x10^{10} cfu/g	After three months x10^{10} cfu/g	Decrease after three months (%)
T1	2.35*	1.02	56.59	0.99	0.80	0.55	46.07
T2	2.20	1.10	50.0	0.98	0.75	0.45	59.1
T3	8.65	5.48	36.64	3.60	2.98	1.55	71.71
T4	4.47	3.76	15.88	2.33	2.10	1.20	68.08
T5	3.68	2.15	41.57	2.05	1.92	1.40	34.88

*Each figure represents the average of three replicate

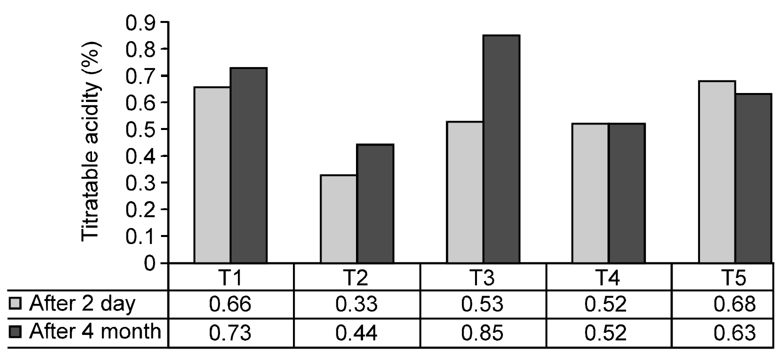


Fig. 1: Effect of frozen storage time at (-18°C) on the titrable acidity percentage of the fermented product

pH determination: The pH values was conducted according to the method mentioned in A.O.A.C (2005) using a pH meter.

Total titrable acidity: The total titrable acidity were determined according to method stated in A.O.A.C (2005).

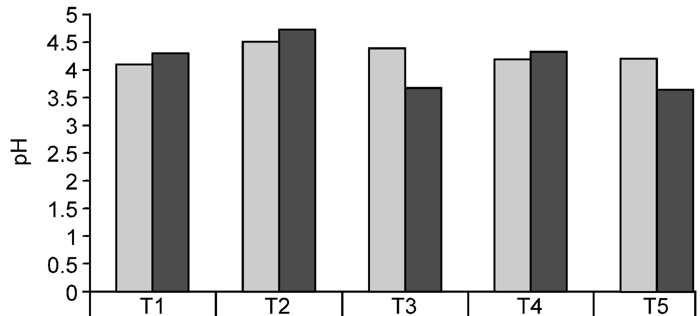
$$\text{Acidity}(\%) = \frac{\text{Volume of 0.1 N NaOH} \times (0.009)}{\text{Sample weight}} \times 100$$

where, the number of viable LAB was counted by pour plate method as mentioned by Benson (2002) using MRS-C Agar.

RESULTS AND DISCUSSION

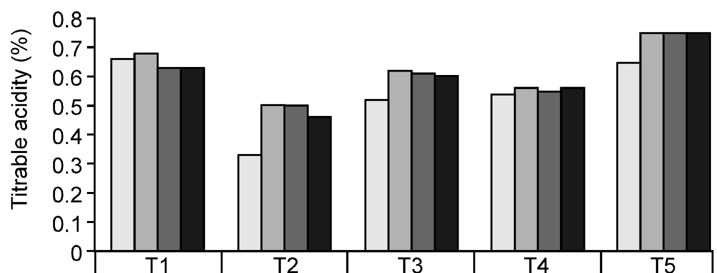
Effect of frozen storage on total titrable acidity and pH values: Figure 1 show the total titrable acidity values at zero time (after two days of fermentation) for the fermented product treatments that ranged from 0.33-0.68% for T2, T4, respectively. The values were increased significantly ($p \leq 0.05$) to reach 0.73% for T1 and 0.85% for T3 after 4 months of frozen storage.

The pH values for the five treatments at zero time ranged from 4.1 to 4.5 for T1, T2, respectively. While by the end of storage period, the values ranged from 3.66-4.72 for T3 and T2, respectively. Figure 2 also show that frozen storage didn't affect the pH values except T3 as the value decreased as the total acidity values increase.



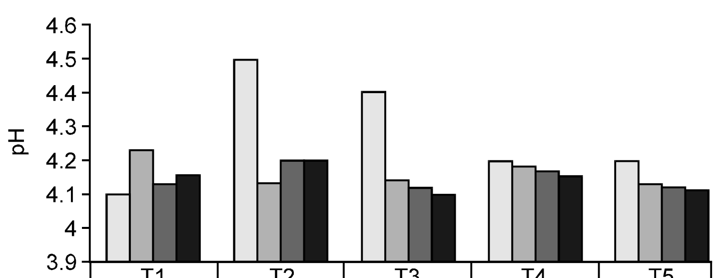
	T1	T2	T3	T4	T5
After 2 day	4.1	4.5	4.4	4.2	4.2
After 4 month	4.3	4.72	3.66	4.33	3.63

Fig. 2: Effect of frozen storage time at (-18°C) on the pH values of the fermented product



	T1	T2	T3	T4	T5
After 2 day	0.66	0.33	0.52	0.54	0.65
After 1 month	0.68	0.5	0.62	0.56	0.75
After 2 month	0.63	0.5	0.61	0.55	0.75
After 3 month	0.63	0.46	0.6	0.56	0.75

Fig. 3: Effect of storage time at 25°C on titrable acidity values of the dried femented product



	T1	T2	T3	T4	T5
After 2 day	4.1	4.5	4.4	4.2	4.2
After 1 month	4.23	4.13	4.14	4.18	4.13
After 2 month	4.13	4.2	4.12	4.17	4.12
After 3 month	4.16	4.2	4.1	4.15	4.11

Fig. 4: Effect of storage time at 25°C on pH values of dried fermented product

Effect of frozen storage on LAB counts: Table 2 show the number of lactic acid bacteria for all product treatments at zero time which ranged from 2.2-8.65 x 10^{10} cfu/ml for T2 and T3, respectively. By the end of the frozen storage, the number of LAB decreased in all treatments, the drop percentage ranged from 19.4-60.05% for T1 and T5, respectively. In spite of that, the bacterial count remained acceptable to impart therapeutic character of products.

Effect of dry storage on total titrable acidity and pH values: Figure 3 show that the titrable acidity values were ranged from 0.33-0.66% for T2 and T1, respectively for dried product treatments. There were significant differences ($p<0.05$) in titrable acidity among the product treatments after 3 months of storage at 25°C, ranged from 0.75-0.46% for T5 and T2, respectively.
Figure 4 show that pH values for the dried product were ranged from 4.1 to 4.5 for T1, T2, respectively. There was

a slight drop in pH values in all dried product treatments after 3 months of storage. The values ranged from 4.2-4.1 for T2, T3, respectively.

Effect of dry storage on LAB counts: Table 3 show the effect of drying and storage of the fermented product at 25°C for a period of three months on lactic acid bacteria counts.

The number of LAB before drying ranged from 2.2-8.6 x 10^{10} cfu/g for T2, T3.

The number of LAB after drying were dropped in all treatments, they were ranged from 1.02-5.48 x 10^{10} cfu/g for T1, T3, respectively.

The percentage of decreasing ranged from 15.88-56.59% for T4, T1, respectively.

The number of LAB decreased gradually during 3 months of storage in all treatments. The decreasing percentage ranged from 34.88-71.71% for T5, T3, respectively.

In spite of that, the LAB counts remained acceptable to impact therapeutic character to the product and this agreed with Heller, 2001; Shah, 2000.

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