

## **Effect of lupine powder on rheological, chemical and microbiological properties of yoghurt**

**Abdel-Salam, A. F.\*; Jehan B. Ali\* and Abeer F. Zayan\*\***

\* Regional Center for Food and Feed (RCFF), Agric. Res. Center, Giza, Egypt.

\*\* Food Technology Research Institute (FTRI), Agric. Res. Center, Giza, Egypt.



### **ABSTRACT**

The effect of different concentrations (2, 4, 6 and 8%) of lupine powder on sensory, rheological, chemical and microbiological properties of yoghurt samples were investigated during refrigerated storage at 4°C for 15 days. The results indicated that the overall pH of lupine yoghurt lowered with increasing of % lupine powder as well as acidity increased gradually by adding lupine powder. The development of acidity in yoghurt (control) or which supplemented with lupine was increased gradually during storage with increase the lupine powder in yoghurt. The syneresis of fresh lupine yoghurt was the lowest value compared with the results after 7 and 15 days. The texture properties of lupine powder yogurt during storage were noted that control increased in firmness and gumminess, while cohesiveness, chewiness, springiness and resilience were decreased. On the other hand all concentrations recorded high values in firmness, cohesiveness, gumminess, chewiness, springiness and resilience during cold storage. There are significant ( $P \leq 0.05$ ) differences in the overall acceptability between control and those prepared by adding 2 & 4 %. Panel test showed that fresh yoghurt lupine prepared from control, 2 & 4% are more acceptable compared to that of 6 & 8%. Also the results revealed that the concentrate 8% of lupine powder represented the optimum concentration in decreasing total bacterial count and *Staphylococcus aureus* counts from  $9 \times 10^5$  and  $9 \times 10^3$  to  $9 \times 10^2$  and  $5 \times 10$  cfu/g respectively and increasing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from  $10 \times 10^4$  and  $8 \times 10^4$  to  $8 \times 10^6$  and  $5 \times 10^6$  cfu/g respectively. While the total coliform and faecal coliform bacteria were completely disappeared as well as yeast & Moulds were eliminated after 15 days during storage time at 4°C.

**Keywords:** Lupine yoghurt –antimicrobial activity- alkaloids - chemical and sensory properties.

### **INTRODUCTION**

Lupine is arguably the world's richest source of natural protein and fiber with virtually no starch. Lupines have been declared as Super Food by the scientists. The health benefits associated with lupines' are tremendous – Suppresses appetite, control blood sugar level, lower blood pressure, reduce blood cholesterol and improve bowel health. Consuming lupine beans for thirty days can result in reducing blood pressure, triglyceride, and cholesterol and can result in weight reduction.

Lupine is a good source of nutrients, not only proteins but also lipids, dietary fiber, minerals and vitamins (Martínez-Villaluenga *et al.*, 2006 & 2009; Torres *et al.*, 2005).

Lupine has been used for human consumption and as a medicinal plant in Egypt (Kattab, 1986 and ARC, 1994). Lupine is used in the treatment

of liver disorder, diabetes, hemorrhoid and eczema (Baser *et al.*, 1986; Baytop, 1999). Protein content in legume ranged from 17- 40%, contrasting with that of cereals 7-13% (Genovese and Lajolo, 2001). Lupines flour is added for nutritive value and also provides functional properties in bakery and pastry products, protein concentrates and other industrial products, as well as the elaboration of lactose free milk and yoghurt analogues (De Cortes Sánchez *et al.*, 2005).

These characteristics of wild Lupines varieties result in a revalorization of these crops as a protein and other healthy promoting compounds for human or animal consumption (Guemes-Vera *et al.*, 2012). *Lupinus termis* is one of the rich plants by alkaloid, amino acids, carbohydrates and proteins with moderate gelatin properties compared to soy proteins (Wäsche *et al.*, 2001). The quinolizidine alkaloids (QA), the main lupine alkaloids, play a chemical defensive role against herbivores and pathogenic microorganisms (Wink, 1988 and 1992).

It is widely known that the *Lupinus* genus contains endogenous concentrations of quinolizidine alkaloids, which are considered as chemotaxonomic markers, but at the same time are toxic compounds for humans, microorganisms, and even for some plant species (Wink, 1984). The latter property has led to the use of lupine extracts in biological control and in pharmacological trials (Zamora-Natera *et al.*, 2005 and 2008; Ruiz-Lopez *et al.*, 2010).

Many trials had been made to prolong the shelf life time of yoghurt. The short shelf life is mainly due to the mold growth. It causes economic losses by discoloration, poor appearance and off flavor during cold storage. Some molds are capable of producing toxic metabolites known as mycotoxins causing serious public health concern. Aflatoxins have been demonstrated as potent human carcinogenic, mutagenic and teratogenic. They are highly stable during processing and storage of yoghurt (Elena *et al.*, 2004).

So, the objective of this study was to investigate the effect of various concentrations (2, 4, 6 and 8%) of lupine powder on sensory, rheological, chemical and microbiological properties of yoghurt samples during refrigerated storage at 4°C for 15 days.

## **MATERIALS AND METHODS**

### **Materials:**

Lupine was purchased from the local Market. Cow milk (87% water, 13% total solids, 3.5% fat, 3.4% protein, 4.8% lactose, 0.8% minerals), was obtained from the Technology Center of Agriculture Production, Faculty of Agriculture, Cairo University.

Direct Vat Set (DVS) containing *Streptococcus thermophiles* and *Lactobacillus delbruckii sub sp. bulgaricus* (YCX31) was obtained from Chr. Hansen's laboratories, Denmark.

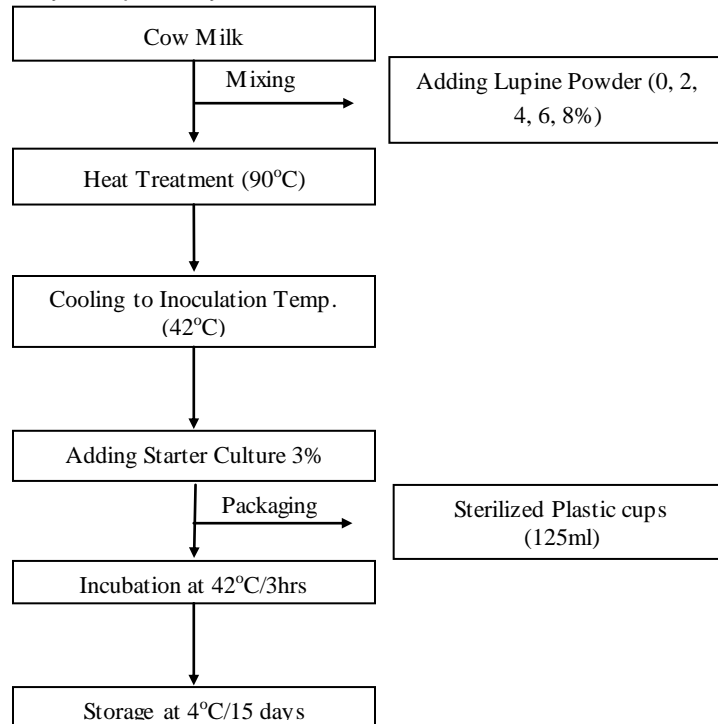
### **Experimental Procedure**

#### **Preparation of Lupine Powder:**

The lupine cereals were cleaned to remove extraneous materials, soaking in tap water for 72 hours at room temperature (~25 °C), and the water was changed every 2 hours, then dried and milled. The samples were packed and kept under refrigeration for analysis.

**Preparation of lupine yoghurt:**

Yoghurt was made from whole cow milk, lupine powder was added (0, 2%, 4%, 6% and 8%). Cow milk was poured to a stainless steel container, and then heated at 90 °C for 10 min followed by cooling to 42 °C. Starter was added at the rate of 3%. The samples were incubated at 42°C until coagulation occurred, then cooled and stored in a refrigerator at 4°C for 15 days as shown in Fig.(1). Samples were taken for sensory, rheological, chemical and microbiological analysis at zero time (just after manufacture), 7 and 15 days respectively.



**Fig. (1) Steps of yoghurt processing making**

**Methods of analysis**

**Chemical analysis:**

Milk and yoghurt samples were analyzed for total solid, protein content and total acidity according to AOAC, (2000). The pH was determined by using digital pH meter (Inolad model 720, Germany).

**Physicochemical analysis:**

Syneresis of yogurt was measured immediately after coagulation and during the storage period for 15 days at 4°C±1 (Shidlovskaya, 1979).

**Texture profile analysis:**

Samples for texture measurements were carried out with universal testing machine (Cometech, B type, Taiwan) provided with software. Back extrusion cell with 35 mm diameter compression disc was used. Two cycles were applied, at a constant crosshead velocity of 1mm/s, to 25% of sample depth, and then returned. From the resulting force-time curve, the values for texture attributes, i.e. firmness (N), gumminess (N), chewiness (N), adhesiveness (N.s), cohesiveness, springiness and resilience were calculated from the TPA graphic (Bourne , 2003).

**GC / MS / MS analysis:**

The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass- selective detector (MSD, Agilent 7000) equipped with an a polar Agilent HP-5ms (5%- phenyl methyl poly siloxane) capillary column (30 m × 0.25mm i.d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min.

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature, (Santana *et al.*, 2013).

**Microbiological analysis:**

Yoghurt samples were prepared according to the method recommended by ICMSF (1996) and analyzed at zero, 7 and 15 days for total bacterial count (Berrang *et al.*, 2001), total coliform and faecal coliform counts (Mercuri and Cox, 1979). Sabouraud agar medium was used for total molds and yeasts enumeration according to APHA (1992) , Pitt and Hocking, (1997). Total *Staphylococcus aureus* count was carried out according to Gouda (2002). *Sterptococcus thermophiles*, M17 agar (Difco) was used to enumerate *Streptococcus* in yoghurt samples and incubated aerobically at 37 C° for 72 hours according to Torriani *et al.*, (1996). *Lactobacillus bulgaricus*. MRS Rogosa agar (Difco) was used for enumeration according to Tharmaraji and Shah, (2003). Plates were incubated under anaerobic condition at 37 C° for 72 hours.

**Sensory evaluation**

The sensory characteristics of yoghurt samples were evaluated following the IDF standards (Anonymous.a a 1995). A trained panel of 4 members, composed of adult male was assigned to determine the quality of the fresh and mature lupine yoghurt- like (appearance, body and texture and flavor). The samples were randomized and presented using tag for each one. To determine the differences in judge's response, the mean scores were analyzed by Duncan's multiple range tests.

**Statistical analysis**

Statistical analysis was carried out using SPSS programme (2007). Five separate samples were analyzed and mean values were calculated. The data were assessed by analysis of variance (ANOVA) and by Duncan's Multiple Range Test with a probability  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Chemical compositions of lupine powder:

Data obtained in Table (1) showed that lupine powder had high dry matter, protein and fat contents as compared with those of lupine cereals. These results were in agreement with those reported by Abdelrahman, (2012) revealed that dry matter, protein and fat contents were higher in lupine powder compared with lupine cereals and similar with those described by Jiménez-Martínez *et al.*(2001) and Torres *et al.* (2005) who mentioned that the protein content of the lupine cereals was determined to be 43.7%. Mostafa *et al.* (2013) recorded that protein content was  $46.30 \pm 0.52$  g/100 g.

**Table 1: Chemical compositions of lupine cereals and lupine powder.**

Analysis	Samples	
	Lupine Cereals	lupine powder
Moisture	11.90 %	7.50%
Dry Matter (DM)	88.20%	92.60%
Curd Fiber (CF)	25.70%	8.50%
Protein	29.70%	43.70%
Fat	1.30%	8.70%

### Alkaloids content of lupine powder:

The alkaloid profile of lupine powder is shown in Table (2). Four quinolizidine alkaloids (mianserine, lupinine, epilupinine and lusitanine) were identified. Lupanine was the only alkaloid and its content was below the limit of 200 mg/kg; thus the applied process appears to be very efficient in removing the alkaloids considering the high alkaloid content of cv. Typ Top and boregine seeds. There are a number of reports on the alkaloid patterns of *Lupinus* species (Przybylak *et al.*, 2005; Sańchez *et al.*, 2005).The increasing consumption of lupine products by vegetarians and subjects interested to their nutraceutical properties appears justified that some Health Authorities have decided to fix a maximum limit of 200 mg/kg for quinolizidine alkaloids in lupine flours and foods.

**Table (2): Alkaloid composition and alkaloid content of lupine powder**

Name of alkaloid	Total alkaloid content %
Mianserine	26.77
Lupinine	40.83
Epilupinine	11.57
Lusitanine	20.83

### Physical and chemical properties of lupine yogurt samples:

#### pH changes during coagulation:

Data recorded in Figure (2) clearly showed that the pH decreased gradually with the increase of coagulation time. Our results are matched with Cristian *et al.* (2003) as the profile of pH decreased during the fermentation of cow and lupine milks was very similar in both cases. With a value around pH

4.0 after a period of 8 h. at 42°C. A pH value of 4.7 or less is important in this product, since it has been related to a good body (texture), flavour, aroma, and stability. The pH indicated a slight and gradually decreases during storage of all treatments. Also the changes in pH during fermentation were found to vary with the starter cultures and substrate concentration (carbon source), and this result is in agreement with the Donkor *et al.* (2007).

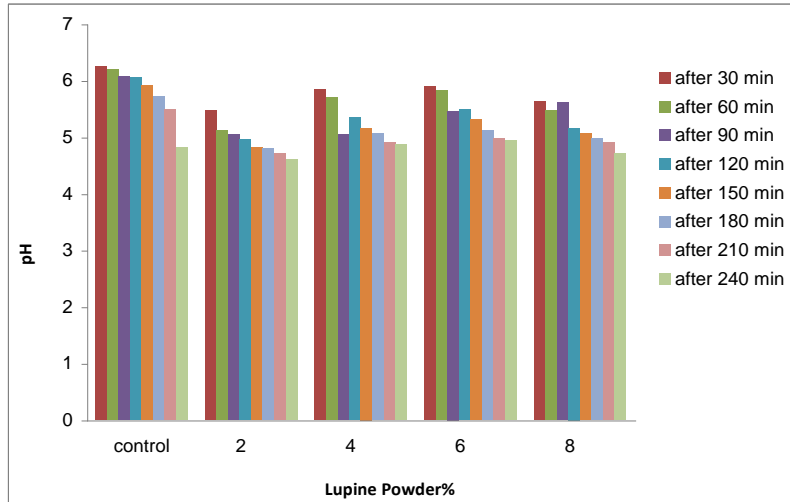


Fig. 2: pH changes of lupine yoghurt during coagulation.

**Chemical composition of fresh and stored lupine yoghurt samples:**

Data present in Table (3) showed that the total solid, ash and protein contents increased gradually with the increase of lupine powder in the yoghurt at 0, 2, 4, 6 and 8% concentrations. Total solid, ash, protein decreased in control and 2% concentration from fresh, after 7 and 15 days, on the other hands, the total solids, ash and protein decreased in control, 2 and 4% at storage time after 7 days, but in 6 & 8 % total solids, ash & protein increased. After 15 days the total solids, ash and protein decreased in control, 2 & 4% of Lupine powder, while total solids, ash & protein increased in 6 & 8%.

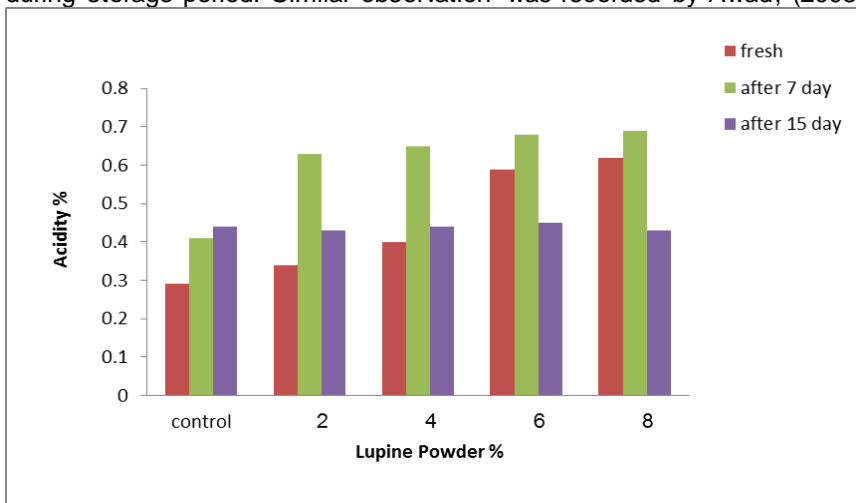
**Table (3): Chemical composition of fresh and stored lupine yoghurt stored at 4C°.**

Chemical composition %	Control	Lupine Powder %			
		2	4	6	8
Fresh					
Total Solids	14.0	15.0	16.7	16.8	19.2
Ash	0.59	0.74	0.77	0.80	0.92
Protein	3.29	5.38	5.52	5.87	6.40
After 7days					
Total Solids	13.2	14.7	16.6	17.3	20.2
Ash	0.55	0.56	0.65	0.83	0.94
Protein	3.00	3.60	4.50	5.00	6.59
After 15 days					
Total Solids	13.1	14.1	15.1	17.1	19.8
Ash	0.69	0.74	0.80	0.84	0.95
Protein	3.2	4.7	5.7	6.2	7.1

**Acidity of fresh and stored lupine yoghurt samples:**

Data in Figure (3) indicated that the acidity of fresh lupine yoghurt increased with the increase of the lupine powder concentrations. After 7 days the same trend was noticed.

Increasing the acidity of the combined product in comparison with the control product mainly depends on the malic acid production. This is due to the formation of by-products of homo fermentative lactic acid fermentation, in particular lactic acid. Organic acids are produced in varying degrees in the process of fermentation and storage of yoghurt (Fernandez-Garcia & McGregor, 1994). Changes of acidity had an opposite trend to that occurred in pH being higher in treatments of lupine paste with slight proportional increase during storage period. Similar observation was recorded by Awad, (2003).



**Fig. (3): Acidity of fresh and stored lupine yoghurt.**

**Syneresis of fresh and stored lupine yoghurt samples:**

The obtained data in Figure (4) exhibited that the syneresis of fresh lupine yoghurt presented the lowest value compared with the results after 7 and 15 days. Also control yoghurt was the lowest values of syneresis compared to 2, 4, 6 and 8 % concentration of lupine yoghurt- like. In treatments with 2% lupine concentration syneresis increased gradually during the storage. These results were in agreement with that reported by Yazici *et al.* (1997).

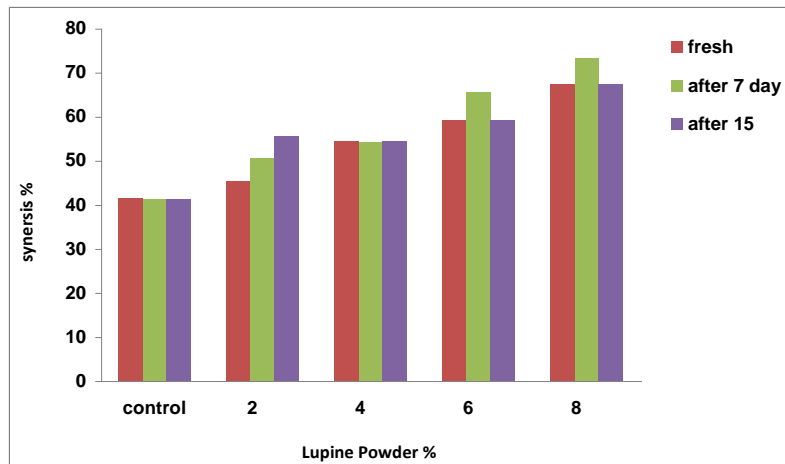


Fig. (4): Syneresis of fresh and stored lupine yoghur.

**Texture profile analysis of fresh lupine yogurt:**

The texture properties of fresh lupine yogurt are shown in Table (4). As shown, firmness, gumminess and chewiness were improved in treatments 2 & 4% lupine powders concentration.

**Texture profile analysis of lupine yogurt after 7 days:**

Concentrations 2, 4 % lupine powder showed higher values in firmness, cohesiveness, gumminess, chewiness and springiness. Results obtained indicated that 2% is the best texture properties.

**Texture profile analysis of lupine yogurt after 15 days:**

Concentrations 2, 4 % lupine powder showed higher values in firmness, cohesiveness, gumminess, chewiness. Results obtained indicated that 2% is the best texture properties.

Anyway our data are disagree with the results reported by Yang *and li* (2010) as cohesiveness of sogurts (Soy-Yogurt) maintained the same level during soybean seedling growth, indicating that germination did not diminish the strength of internal bonds of all samples. Also our data is disagree with the results of Yazici *et al.* (1997). Soy milk yogurt had greater hardness (gel strength) with slightly more springiness than regular commercial yogurt. Values for cohesiveness were comparable or slightly higher than the reference sample. The profiles showed that calcium fortification reduced the gel strength but not springiness.



Table (4): Texture profile analysis of fresh and stored lupine yoghurt at 4°C.

Parameters	Control	Concentrations %			
		2	4	6	8
<b>Fresh</b>					
Firmness	0.69 <sup>l</sup>	0.98 <sup>e</sup>	0.83 <sup>g</sup>	0.74 <sup>n</sup>	0.49 <sup>nn</sup>
Cohesiveness	0.70 <sup>te</sup>	0.64 <sup>ll</sup>	0.57 <sup>l</sup>	0.55 <sup>l</sup>	0.38 <sup>l</sup>
Gumminess	0.48 <sup>ll</sup>	0.55 <sup>g</sup>	0.53 <sup>g</sup>	0.28 <sup>k</sup>	0.27 <sup>k</sup>
Chewiness	0.42 <sup>gnn</sup>	0.47 <sup>e</sup>	0.43 <sup>gnn</sup>	0.17 <sup>k</sup>	0.16 <sup>k</sup>
Springiness	0.86 <sup>e</sup>	0.85 <sup>e</sup>	0.81 <sup>l</sup>	0.61 <sup>l</sup>	0.58 <sup>l</sup>
Resilience	0.17 <sup>e</sup>	0.13 <sup>tg</sup>	0.12 <sup>g</sup>	0.09 <sup>n</sup>	0.05 <sup>l</sup>
<b>After 7days</b>					
Firmness	0.93 <sup>t</sup>	1.57 <sup>b</sup>	0.98 <sup>e</sup>	0.74 <sup>n</sup>	0.54 <sup>l</sup>
Cohesiveness	0.67 <sup>g</sup>	0.76 <sup>d</sup>	0.72 <sup>e</sup>	0.69 <sup>g</sup>	0.64 <sup>n</sup>
Gumminess	0.62 <sup>t</sup>	1.19 <sup>b</sup>	0.71 <sup>e</sup>	0.47 <sup>n</sup>	0.37 <sup>l</sup>
Chewiness	0.41 <sup>ln</sup>	1.0 <sup>b</sup>	0.55 <sup>d</sup>	0.44 <sup>g</sup>	0.30 <sup>l</sup>
Springiness	0.66 <sup>n</sup>	0.78 <sup>g</sup>	0.89 <sup>d</sup>	0.93 <sup>c</sup>	0.80 <sup>gr</sup>
Resilience	0.15 <sup>te</sup>	0.13 <sup>tg</sup>	0.21 <sup>d</sup>	0.27 <sup>c</sup>	0.07 <sup>n</sup>
<b>After 15 days</b>					
Firmness	1.17 <sup>d</sup>	2.16 <sup>a</sup>	1.22 <sup>c</sup>	0.65 <sup>l</sup>	0.59 <sup>k</sup>
Cohesiveness	0.64 <sup>n</sup>	0.95 <sup>b</sup>	1.06 <sup>a</sup>	0.83 <sup>c</sup>	0.64 <sup>n</sup>
Gumminess	0.76 <sup>d</sup>	1.83 <sup>a</sup>	1.14 <sup>c</sup>	0.47 <sup>n</sup>	0.41 <sup>l</sup>
Chewiness	0.40 <sup>l</sup>	1.65 <sup>a</sup>	0.94 <sup>c</sup>	0.45 <sup>te</sup>	0.43 <sup>gn</sup>
Springiness	0.46 <sup>k</sup>	0.93 <sup>c</sup>	0.98 <sup>b</sup>	1.05 <sup>a</sup>	0.99 <sup>b</sup>
Resilience	0.13 <sup>tg</sup>	0.14 <sup>tg</sup>	0.37 <sup>b</sup>	0.42 <sup>a</sup>	0.05 <sup>l</sup>

**Microbiological analysis:**

Data presented in Table (5) indicated that total bacterial count, total coliform, faecal coliform, total moulds and yeasts, *Staph aureus*, *L. bulgaricus* and *S. thermophilus* counts exhibited approximately the same in plain and lupine yoghurt samples at zero time with exist markedly increasing in *L. bulgaricus* and *S. thermophilus* counts of lupine yoghurt samples and disappearance of molds in all examined yoghurt samples. These data are agreed with Jimenez- Martinez *et al.* (2003) where Lupines *campestris* milk was obtained with 6.3% protein by using an alkaline thermal treatment. The product was pasteurized and inoculated with a culture of *Streptococcus thermophiles* and *Lactobacillus delbrueckii ssp bulgaricus*. A lupine yogurt showed pH 4.02, 0.87% lactic acid, and a lactic acid bacteria count ( $3.2 \times 10^8$  cfu ml<sup>-1</sup>) and viscosity similar to commercial cow's milk yogurt.

**Table (5): Effect of different concentrations of lupine powder on the microbial load (cfu/g) of lupine yoghurt at zero time.**

Samples	Type of Microorganisms							
	T.B.C	T.C	F.C	T.M	T.Y	<i>Staph aureus</i>	<i>L. bulgaricus</i>	<i>S. thermophilus</i>
Control	9×10 <sup>5</sup>	9×10 <sup>3</sup>	9×10 <sup>3</sup>	0	7×10 <sup>4</sup>	9×10 <sup>3</sup>	10×10 <sup>4</sup>	8×10 <sup>4</sup>
2%	7×10 <sup>5</sup>	7×10 <sup>3</sup>	6×10 <sup>3</sup>	0	5×10 <sup>4</sup>	5×10 <sup>3</sup>	4×10 <sup>5</sup>	10×10 <sup>4</sup>
4%	4×10 <sup>5</sup>	5×10 <sup>3</sup>	4×10 <sup>3</sup>	0	3×10 <sup>4</sup>	5×10 <sup>3</sup>	6×10 <sup>5</sup>	3×10 <sup>5</sup>
6%	4×10 <sup>5</sup>	2×10 <sup>3</sup>	2×10 <sup>3</sup>	0	2×10 <sup>4</sup>	3×10 <sup>3</sup>	7×10 <sup>5</sup>	3×10 <sup>5</sup>
8%	2×10 <sup>5</sup>	10×10 <sup>4</sup>	10×10 <sup>4</sup>	0	2×10 <sup>4</sup>	2×10 <sup>3</sup>	9×10 <sup>5</sup>	6×10 <sup>5</sup>

T.B.C: Total bacterial count T.C: Total coliform F.C: Faecal coliform T.M: Total molds  
T.Y: Total yeasts

Data illustrated in Table (6) showed that total bacterial count, total coliform, faecal coliform and *Staph aureus* counts decreased from 5×10<sup>6</sup>, 4×10<sup>4</sup>, 2×10<sup>4</sup> and 4×10<sup>3</sup> to 8×10<sup>3</sup>, 4×10<sup>2</sup>, 2×10<sup>2</sup> and 2×10 cfu/g respectively in yoghurt with 8% of lupine powder after 7 days of yoghurt storage. Total yeasts decreased from 2×10<sup>5</sup> to 6×10<sup>2</sup> cfu/g at concentration 4% and completely disappeared in yoghurt with 6 and 8% lupine powder respectively. *L. bulgaricus* and *S. thermophilus* increased from 6 ×10<sup>4</sup> and 4×10<sup>4</sup> to 6×10<sup>6</sup> and 3×10<sup>6</sup> cfu/g respectively in yoghurt at concentration 8% of lupine powder. All examined yoghurt samples were negative for molds.

**Table(6):Effect of different concentrations of lupine powder on the microbial load (cfu/g) of lupine yoghurt after 7 days of stored at 4°c.**

Samples	Type of Microorganisms							
	T.B.C	T.C	F.C	T.M	T.Y	<i>Staph aureus</i>	<i>L. bulgaricu</i>	<i>S. thermophilus</i>
Control	5×10 <sup>6</sup>	4×10 <sup>4</sup>	2×10 <sup>4</sup>	0	2×10 <sup>5</sup>	4×10 <sup>3</sup>	6×10 <sup>4</sup>	4×10 <sup>4</sup>
2%	2×10 <sup>5</sup>	3×10 <sup>3</sup>	2×10 <sup>3</sup>	0	4×10 <sup>3</sup>	6×10 <sup>2</sup>	8×10 <sup>5</sup>	3×10 <sup>5</sup>
4%	8×10 <sup>4</sup>	10×10 <sup>4</sup>	8×10 <sup>2</sup>	0	6×10 <sup>2</sup>	3×10 <sup>2</sup>	10×10 <sup>5</sup>	5×10 <sup>5</sup>
6%	2×10 <sup>4</sup>	7×10 <sup>2</sup>	4×10 <sup>2</sup>	0	0	5×10	4×10 <sup>6</sup>	7×10 <sup>5</sup>
8%	8×10 <sup>3</sup>	4×10 <sup>2</sup>	2×10 <sup>2</sup>	0	0	2×10	6×10 <sup>6</sup>	3×10 <sup>6</sup>

T.B.C: Total bacterial count T.C: Total coliform F.C: Faecal coliform T.M: Total molds  
T.Y: Total yeasts

Data recorded in Table (7) clearly showed that total bacterial count and *Staph aureus* counts decreased from 9×10<sup>5</sup> and 8×10<sup>3</sup> to 9×10<sup>2</sup> and 5×10 cfu/g respectively in yoghurt with 8% of lupine powder, total coliform decreased from 9×10<sup>4</sup> to 7×10 cfu/g at 6% concentration and completely disappeared with 8% of lupine powder, faecal coliform decreased from 6×10<sup>4</sup> to 2× 10<sup>2</sup> cfu/g with 2 and 4% of lupine powder but completely disappeared with 6 and 8%. Total yeasts completely disappeared in yoghurt beginning from 4% of lupine powder.

The viability of *L. bulgaricus* and *S. thermophilus* remained high during 15 days of storage for the examined lupine yoghurt samples such increased from 4×10<sup>4</sup> and 2×10<sup>4</sup> to 8× 10<sup>6</sup> and 5×10<sup>6</sup> cfu/g respectively at concentration 8%. Total molds began appear after 15 days of storage in yoghurt samples at concentrations 2 and 4% lupine powder but didn't appear in yoghurt with 6 and 8% of lupine powder.

**Table (7): Effect of different concentrations of lupine powder on the microbial load (cfu/g) of lupine yoghurt after 15 days of stored at 4°C.**

Samples	Type of Microorganisms							
	T.B.C	T.C	F.C	T.M	T.Y	<i>Staph aureus</i>	<i>L. bulgaricus</i>	<i>S. thermophilus</i>
Control	9x10 <sup>5</sup>	9x10 <sup>4</sup>	6x10 <sup>4</sup>	15x10 <sup>5</sup>	9x10 <sup>4</sup>	8x10 <sup>3</sup>	4x10 <sup>4</sup>	2x10 <sup>4</sup>
2%	2x10 <sup>4</sup>	8x10 <sup>2</sup>	2x10 <sup>2</sup>	5x10 <sup>5</sup>	3x10 <sup>2</sup>	8x10 <sup>2</sup>	9x10 <sup>0</sup>	6x10 <sup>0</sup>
4%	7x10 <sup>3</sup>	4x10 <sup>2</sup>	2x10 <sup>2</sup>	8x10 <sup>4</sup>	0	6x10 <sup>2</sup>	2x10 <sup>0</sup>	7x10 <sup>0</sup>
6%	2x10 <sup>3</sup>	7x10	0	0	0	8x10	6x10 <sup>0</sup>	9x10 <sup>0</sup>
8%	9x10 <sup>2</sup>	0	0	0	0	5x10	8x10 <sup>0</sup>	5x10 <sup>0</sup>

T.B.C: Total bacterial count T.C: Total coliform F.C: Faecal coliform T.M: Total molds  
T.Y: Total yeasts

The above results were in agreement with Venizelou *et al.* (2000) who reported that the presence of flavoring materials added to yoghurt have little effect on the survival of *L. bulgaricus* and *S. thermophilus*. The inhibition of the growth of mold and yeast in lupine yoghurt may be attributed to the action of iso coumarine which naturally present in traces in lupine (Höhn and Künsch, 2003).

The alkaloid extract showed significant activity on *B. subtilis*, *S. aureus* and *P. aeruginosa* while it was weakly active on *E. coli* (Erdemoglu *et al.*, 2007). The quinolizidine alkaloids (QA) as the main lupine alkaloids have been shown to have antimicrobial activity by several researchers (Wink, 1984; Wippich & Wink, 1985 and Tyski *et al.*, 1988). In Wink's study (1984), sparteine was reported to possess antimicrobial activity against bacteria and phytopathogenic fungi. Moreover, Wippich and Wink (1985) and Tyski *et al.*, (1988) reported that pure QA isolated from *Lupinus angustifolius* var. *Mirela*, *lupanine*, 13a-hydroxylupanine and angustifoline and the ethanolic extract of the seed of the plant and compound sparteine showed to have bacteriostatic effects against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *B. thuringiensis*. Besides, these researchers declared that bacteriostatic effects of QA were supported the allelopathic function of alkaloids (Tyski *et al.*, 1988). All data obtained in this study supported that QA may be involved in the antimicrobial defense system of lupins (Wink, 1984; Wippich and Wink, 1985). The G-ve bacteria were more resistant to the plant extract than gram-positive bacteria such as *Ps. aeruginosa* exhibited more resistant than *B. subtilis* when they were tested with *L. termis* extract (Mahmoud *et al.*, 2014).

The alkaloidal patterns of various plant organs (leaves, flowers, stems, roots, pods and seeds) are documented, Screening for antimicrobial activity of these plant extracts of lupine demonstrated substantial activity against *Candida albicans*, *Aspergillus flavus* and *Bacillus subtilis* (El- Shazly *et al.*, 2001). Tyski *et al.*, (1988) assumed that QA play a role in antimicrobial defence (besides flavonoids and is flavones) of lupines. Lupine alkaloids to heighten the antimicrobial activity (Wink, 1984). Other approaches using alkaloid fractions from *L. angustifolius* reported a weak effect in some *E. coli* strains (Erdemoglu *et al.*, 2007).

**Sensory characteristics of lupine yoghurt:**

Table (8) shows the results of sensory characteristics of lupine yoghurt that was made using different levels of lupine powder (2, 4, 6 and 8%). Treatment with 8% lupine powder concentration gained the highest score for appearance whether in fresh or after 7 days of cold storage. Regarding the body & texture there is no significant difference between the different treatments as shown in the same table. Regarding to the flavor of the different treatments, flavor of treatment with 2% lupine powder was the better after control treatment whether fresh or after 7 or 15 days of cold storage.

The overall acceptability of all types of lupine yoghurt was enhanced during maturation (fresh, 7 and 15 days). The results obtained agree with the findings of Cristian *et al.* (2003) who reported that the overall acceptability of lupine yoghurt was increased during storage.

**Table (8): Sensory characteristics of lupine yoghurt stored at 4°c. Fresh**

Treatments	Organoleptic properties		
	Appearance (10%)	Body& Texture (35%)	Flavor (40%)
Control	7.0000 <sup>abc</sup>	29.500 <sup>ab</sup>	38.250 <sup>a</sup>
2%	6.2500 <sup>ced</sup>	28.000 <sup>b</sup>	34.000 <sup>abcd</sup>
4%	6.7500 <sup>cd</sup>	29.750 <sup>ab</sup>	29.750 <sup>edf</sup>
6%	7.2500 <sup>ab</sup>	29.750 <sup>ab</sup>	26.750 <sup>f</sup>
8%	7.2500 <sup>ab</sup>	31.250 <sup>ab</sup>	21.750 <sup>g</sup>

**After 7days**

Treatments	Appearance (10%)	Body& Texture (35%)	Flavor (40%)
Control	5.5000 <sup>ef</sup>	23.750 <sup>c</sup>	30.250 <sup>cedf</sup>
2%	7.2500 <sup>ab</sup>	30.000 <sup>ab</sup>	28.500 <sup>ef</sup>
4%	7.0000 <sup>abc</sup>	29.750 <sup>ab</sup>	26.250 <sup>fg</sup>
6%	7.0000 <sup>abc</sup>	30.500 <sup>ab</sup>	26.250 <sup>fg</sup>
8%	7.7500 <sup>a</sup>	30.250 <sup>ab</sup>	26.750 <sup>f</sup>

**After 15days**

Treatments	Appearance (10%)	Body& Texture (35%)	Flavor (40%)
Control	7.5000 <sup>ab</sup>	29.0000 <sup>ab</sup>	36.250 <sup>ab</sup>
2%	7.0000 <sup>abc</sup>	30.0000 <sup>ab</sup>	34.750 <sup>abc</sup>
4%	6.0000 <sup>ed</sup>	30.750 <sup>ab</sup>	33.250 <sup>bcd</sup>
6%	6.0000 <sup>ed</sup>	31.750 <sup>ab</sup>	31.500 <sup>ced</sup>
8%	5.0000 <sup>ef</sup>	32.500 <sup>a</sup>	30.250 <sup>cedf</sup>

**CONCLUSION**

The uses of lupine powder in yoghurt production were advantageous due to its sensory, chemical and microbiological properties. The results indicated that processing yoghurt with 2% lupine powder was proved to be of good quality, long shelf life and could be kept at 4° C for 15 days. The dipping

process of lupine powder in water didn't induce elimination completely of all the present alkaloids in the seeds.

## REFERENCES

- Abdelrahman, R., A. (2012). Technological and Nutritional Studies on Sweet Lupine Seeds and its Applicability in Selected Bakery Products, TU, Berlin, Germany.
- Anonymous.a a 1995. Sensory Evaluation of Dairy Products. *FIL-IDF 99B. Brussels: FIL-IDF.*
- APHA, American Public Health Association, ( 1992). Compendium of methods for the microbiological examination of foods. 3rd Ed. Washington DC, USA, p: 300-344.
- AOAC, (2000). Official Methods of Analysis of AOAC International. 17<sup>th</sup> ed. AOAC International, Arling, VA.
- ARC, (1994). Agricultural Research Center Min. Agriculture of Egypt. Bulletin 226: 1-8.
- Awad, R. A. (2003). Impact of potato puree as a cheese base replacement in the manufacture of processed cheese. *Egypt. J. Dairy Sci.* 2, 375-387.
- Baser, K. H. C.; G. Honda and W. Miki (1986). Herb drugs and herbalists in Turkey. *Studia Culturae Islamicae*, No. 27, ILCAA, Tokyo.
- Baytop, T. (1999) .Therapy with medicinal plants in Turkey (past and present), 2<sup>nd</sup> edn. Nobel Tip Kitabevleri, Istanbul.
- Berrang, M.E; S. R. Ladely and R. J. Buhr (2001). Presence and level of *Campylobacter*, coliform, *Escherichia coli* and total aerobic bacteria recorded from broiler parts without skin. *Food Prot.*, 64 (2): 184-188.
- Bourne, M. (2003). Food texture and viscosity: Concept and measurement. Elsevier press, New York/London.
- Cristian, L.; J. D. Lear and W. F. DeGrado (2003). Use of thiol-disulfide equilibria to measure the energetics of assembly of transmembrane helices in phospholipid bilayers. *Proc. Natl. Acad. Sci.* 100: 14772–14777.
- De Cortes Sánchez, M. A.; P. Altares, M. ; M. Pedrosa; C. Burbano; C. Cuadrado; C. Goyoaga; M. Muzquiz; C. Jiménez-Martinez and G. Dávila-Ortiz (2005). Alkaloid variation during germination in different lupin species. *Food Chemistry* 90, 347-355.
- Donkor, Ossana, N.; A. Henriksson, T. Vasiljevic and N. P. Shah (2007). Rheological Properties and Sensory Characteristics of Set-Type Soy Yogurt, *J. Agric. Food Chem*, 55, 9868–9876.
- Elena, M.; G. Ferdenandz; L. Weneferida and M. Joya (2004). Development of  $\beta$ -carotene rich juice. *Milchwissenschaft*, 19: 200-206.
- El- Shazly, A.; A. M. Ateya and M. Wink (2001). Quinolizidine Alkaloid profiles of *Lupinus varius orientalis*, *L. albus albus*, *L. hartwegii*, and *L. densiflours*. *Z. Naturforsch.* 56c, 21-30.

- Erdemoglu, N.; S. Ozkan and F. Tosun (2007). Alkaloid profile and antimicrobial activity of *Lupinus angustifolius* L. alkaloid extract, *Phytochem. Rev.*, 6: 81-85.
- Erickson, J. P. (1985). Lupins show potential as protein source for livestock. *Feedstuffs* 57, 22-24.
- Fernandez-Garcia, E. and J.U. McGregor (1994). Determination of organic acids during the fermentation and cold storage of yogurt. *J.Dairy Sci.* 77, 2934–2939.
- Genovese, M.I. and F.M. Lajolo (2001). Atividade inibitória de tripsina do feijão (*Phaseolus vulgaris* L.): avaliação crítica dos métodos de determinação. *Archivos Latinoamericanos de Nutrición*, 51: 386-394.
- Gouda, H. (2002). Microbiological studies on some fish aquacultures in Egypt. M.Sc. Thesis, Faculty of Agriculture. Cairo University PP. 52-69.
- Guemes-Vera, N.; J. Martinez-Herrera; J. F. Hernandez-Chavez; J. Yanez-Fernandez and A. Totosaus (2012). Comparison of chemical composition and protein digestibility, carotenoids, tanins and alkaloids content of wild *Lupinus* varieties flour. *Pakistan Journal of Nutrition* ,11 (8): 676-682.
- Höhn, E. and U. Künsch (2003). Carrot flavor-acceptance, sweetness and bitterness. *Agrarforschung*, 10: 144-149.
- ICMSF, International Committee on Microbiological Specification for Foods, (1996). *Microorganisms in Foods: Their Significance and Methods of Enumeration*, 2<sup>nd</sup> ED., University of Toronto and Buffalo, Canada.
- Jiménez-Martínez, C.; H. Hernández-Sánchez; G. Álvarez-Manilla ; N. Robledo-Quintos ; J. Martínez-Herrera and G. Dávila-Ortiz (2001). Effect of aqueous and alkaline thermal treatments on chemical composition and oligosaccharide, alkaloid and tannin contents of *Lupinus campestris* seeds. *Journal of the Science of Food and Agriculture*, 81: 421-428.
- Jimenez- Martinez,C.; H. Hernández-Sánchez and G. Dávila-Ortiz (2003). Production of a yogurt- from *Lupinus campestris* seeds. *Journal of the Science of Food and Agriculture*, 83, (6): 515-522.
- Kattab, H.A. (1986). Plant wealth in ancient Egypt. *Min. Agriculture of Egypt*.
- Martínez-Villaluenga C.; E. Sironi; C. Vidal-Valverde and M. Duranti (2006). Effect of oligosaccharide removing procedure on the protein profiles of lupin seeds. *Eur. Food Res. Techn.* 223, 691-696.
- Mercuri, A. J. and N. A. Cox (1979). Coliform and Enterobacteriaceae isolated from selected Foods. *J. of food Protection*, 42, (9); 712-714.
- Martínez-Villaluenga, C.; H. Zieliński ; J. Frias, ; M. K. Piskula; H. Kozłowska, and C. Vidal-Valverde (2009). Antioxidant capacity and polyphenolic content of high-protein lupine products. *Food Chemistry*, 112: 84-88.
- Mahmoud,I.; A. Shahhat; G.M. Ghazal and S. Ghada Mohamed (2014). Effect of Ascorbic acid and Niacin on Protein, Oil Fatty Acids and Antibacterial Activity of *Lupinus termis* Seeds. *International Journal of Pharm acognosy and Phytochemical Research* ; 6(4); 866-873.

- Mostafa A.; A. Awad-Allah and H. Elkatry (2013). Effect of Debittering Process on Characterization of Egyptian Lupine Seeds Oil (*Lupinus albus*). *Australian J. Basic and Applied Sci.*, 7(2): 728-734.
- Pitt, J.I. and A.D. Hocking (1997). *Fungi and Food Spoilage*. Blackie Academic and Professional Press, Chapman and Hall, London, p: 413- 455.
- Przybylak J. K.; D. Ciesiołka; W. Wysocka; P.M. García-Lo'pez; M. A. Ruiz-Lo'pez; W. Wysocki and K. Gulewicz (2005). Alkaloid profiles of Mexican wild lupine and an effect of alkaloid preparation from *Lupinus exaltatus* seeds on growth and yield of paprika (*Capsicum annum* L.). *Indust Crops Prod* 21(1):1-7.
- Ruiz-Lopez, M.A.; P.M. Garcia-Lopez; R. Rodriguez-Macaas; J.F. Zamora-Natera; M. L. Isaac-Virgen and M. Mozquiz (2010). Mexican wild lupines as a source of quinolizidine alkaloids of economic potential, *Polibotanica*, 29:159-164.
- Santana, P.M., M. Miranda, J. A. Payrol, M. Silva, V. Hernandez and E. Peralta, (2013). Gas Chromatography-Mass Spectrometry study from leaves fractions obtained of *Vernanthur Patens* (Kunth) H. Rob, *International Journal of organic Chemistry*, 3: 105-109.
- Sa'ñchez M.C.; P. Altares; M. M. Pedrosa; C. Burbano; C. Cuadrado; C. Goyoaga; M. Muzquiz; C. Jimenez-Martinez and G. Da'vila-Ortiz (2005). Alkaloid variation during germination in different lupin species. *Food Chem* 90(3):347-355.
- Shidlovskaya, V.P. (1979). Evaluation of syneresis of cultured milk products. *Molochnaya Promyshlennost*, No. 4: 23-25.
- SPSS Inc. (2007). *SPSS 16.0 Command Syntax Reference*. Chicago, IL: SPSS Inc.
- Tharmaraji, N. and N.P. Shah (2003). Selective enumeration of *Lactobacillus bulgaricu*, *Streptococcus thermophilus*, *Bifidobacteria*, *Lactobacillus casei* and Propionibacteria. *J. Dairy Sci.*, 86: 2288-2298.
- Torriani, S.F.; M. Gardini; E. Guerzoni and F. Dellaglio (1996). Use of response surface methodology to evaluate some variable affecting the growth and acidification characteristics of yoghurt cultures. *Int. Dairy J.* 6: 625-636.
- Torres, A.; J. Frias and C. Vidal-Valverde (2005). Changes in chemical composition of lupin seeds (*Lupinus angustifolius*) after selective  $\alpha$ -galactoside extraction. *Journal of the Science of Food and Agriculture*, 85: 2468-2474.
- Tyski, S. ; M. Markiewicz, K. Gulewicz and T. Twardowski (1988). The effect of lupine alkaloids and ethanol extracts from seeds of *Lupinus angustifolius* on selected bacterial strains. *J. Plant Physiol.* 133:240-242.
- Venizelou, M.; C. Kehagias; A. Samona and S. Koulouris, (2000). Survival of yoghurt characteristic microorganisms in fruit yoghurt prepared under various conditions. *Food Biotec.*, 44: 169-190.
- Wäsche A.; K. Müller and U. Knauf (2001). New processing of lupin protein isolates and functional properties. *Nahrung* 45: 393-395.

- Wink, M. (1984). Chemical defense of leguminosae. Are quinolizidine alkaloids part of the microbial defense system of lupins? Z. Naturforsch 39c, 548-552.
- Wink, M. (1988). Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor Appl Gen, 75:225–233.
- Wink, M. (1992). The role of quinolizidine alkaloids in plant insect interactions. In: Bernays EA (ed) Insect–plant interactions, vol IV. CRC-Press, Boca Raton, pp133–169.
- Wippich, C. and M. Wink (1985). Biological properties of alkaloids. Influence of quinolizidine alkaloids and gramine on the germination and development Powderly mildew, *Erysiphe graminis f. sp. hordei*. Experientia 41:1477–1479
- Phytochem Rev 123.
- Yang, M. and Li, L. (2010): Characteristics of Probiotic Soy Yogurt, Food Technol. Biotechnol. 48 (4) 490–496.
- Yazici, F., V.B. Alvarez, and P.M.T. Hansen (1997). Fermentation and Properties of Calcium-fortified Soy Milk Yogurt.
- Zamora-Natera, J.F.; A. Bernal Alcocer and M. Ruiz-Lopez (2005). Perfil de alcaloides de semillas de *Lupinus exaltatus* Zucc. (Fabaceae) y la evaluación antifúngica del extracto alcaloideoy la lupanina contra fitopatogenos, *Rev. Mex. Fitopatol.* 23, 124-129.
- Zamora-Natera, F.; P. Garca Lopez; M. Ruiz-Lopez and E. Salcedo-Pérez (2008). Composition of alkaloids in seeds of *Lupinus mexicanus* (Fabaceae) and antifungal and allelopathic evaluation of the alkaloid extract, *Agrociencia* 42, 185-192.

## تأثير بودرة الترمس على الخواص الريولوجية و الكيمائية و الميكروبيولوجية للزبادى

أحمد فريد عبد السلام\* ، جيهان بسطامى على\* و عبير فؤاد زيان\*\*  
\* المركز الإقليمي للأغذية والأعلاف- مركز البحوث الزراعية  
\*\* معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية

تم دراسة تأثير اضافة التركيزات المختلفة من بودرة الترمس بنسب ٢, ٤, ٦, ٨ ٪ الى اللبن البقرى وذلك لدراسة على الخصائص الحسية والريولوجية والكيمائية والميكروبيولوجية للزبادى الناتج من الخلطات المختلفة اثناء التخزين على ٤ م لمدة ١٥ يوم. أظهرت النتائج انخفاض تدريجى فى درجة الـ pH بزيادة تركيز بودرة الترمس. مع حدوث زيادة تدريجية فى الحموضة اثناء التخزين بزيادة التركيز المضاف فى الزبادى. حدث زيادة فى التشيريش بزيادة تركيز بودرة الترمس المضاف وايضاً اثناء فترة تخزين الزبادى مدة اقل فى التشيريش بعد ٧ الى ١٥ يوم. تشير خواص القوام الزبادى الطازج والمخزن الى ان اضافة بودرة الترمس يزيد من التماسك وقوة المضغ ويقلل كلاً من الثبات ودرجة الطراوة والمرونة. اشار التقييم الحسى ان الزبادى المدعم بودرة الترمس لا يوجد به اختلافات معنوية فى القبول العام بين الكونتروول و ٢ و ٤ ٪ . و زبادى الترمس سواء الكنتروول و ٢ و ٤ ٪ كان اكثر قبولا من ٦ و ٨ ٪ . اوضحت النتائج ان تركيز ٨ ٪ يمثل التركيز الامثل فى خفض العد الكلى للبكتريا واعداد *Staphylococcus aureus* من  $10 \times 9$  و  $10 \times 9$  الى  $10 \times 9$  و  $10 \times 5$  وحدة خلية/جرام على التوالي. وزيادة أعداد *Lactobacillus bulgaricus* and *Streptococcus thermophiles* من  $10 \times 10$  الى  $10 \times 8$  و  $10 \times 5$  وحدة خلية/جرام على التوالي. على الجانب الآخر لم يتواجد كل من المجموعة القولونية والقولونية البرازية والفطريات والخمائر بعد ١٥ يوم من التخزين على ٤ م.