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Effect of Lupin (Lupinus albifrons) Flour on Microbial and sensory properties of local Yoghurt

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Abstract
This study conducted and implicated in the General State Company for Dairy Products, Abu-Ghraib/ Baghdad/Iraq in February to April /2014. Two different sweet lupin seed (Jordanian, Egyptian) origin were used for this study which been imported from the local markets.
Soaking and boiling process (de- bittering process) of lupin seeds was used to destroy thermo-labile anti-nutritional factors, such as trypsin inhibitors, phytic acid and to soften the seed.
It was been found that, the concentration (2, 4, 6)% of lupin seed supplementation for each origin (Jordan and Egypt) are the best in dairy product supplementation after conducting many different tests to see the best degree of agreement by the specialized panelists in the dairy company.
The physicochemical analysis of the supplemented yogurt with different concentration (2, 4, and 6) % of Jordanian and Egyptian lupin flour showed that, it does not affect the percent of fat content statistically. But, there were clear positive statistical significant differences in the total solid. Also, there were statistically positive correlation between lupin flour concentration addition and total solid of the yogurt product which does affect directly the texture of the yogurt. There were statistically positive correlation between lupin flour concentration supplement and the pH of the yogurt product which does affect directly the flavor and taste of the yogurt product by the consumers. Thus, increasing the concentration of the supplementation with lupin flour lead to decrease the acidity and increase the pH in yogurt products significantly.
Sensory evaluation of the supplemented yogurt product were showed that the 2 and 4% of lupin flour for both origin were the highest positive effect on physicochemical and sensory scores (flavor, taste, acidity, texture and consistency, appearance, and the total properties). Also, it was found that, the period of storage (1-14 days) did not affect the physicochemical and sensory evaluation clearly of the supplemented lupin dairy product.
Therefore, we recommended the possibility of supplementation of yogurt with sweet lupin flour for its healthier and structural effect on human health to these kind of products.
Keywords: Dairy product, Supplementation, Lupin flour, Physicochemical properties, Sensory evaluation, microbial content.

Introduction

Yoghurt is a fermented milk product and is accepted all over the world and Iraqi population with a great desire and with its high nutritive value and positive effects on human health, based on cutlers containing (Streptococcus thermophiles and Lactobacillus delbrueckii susbsp. Bulgaricus). Although it has acidic property, yoghurt shows contamination due to its high content of water (about 85%) during storage.

Elaboration fermented products may be difficult because of the problem of milk coagulation. Yoghurt texture is a very important property that affect its quality (appearance, mouth feel, firmness, consistency, and overall acceptability). In an attempt to increase the firmness and prevent syneresis, whey protein concentrate (Hashim et al. 2008), stabilizer and hydrocolloid have been added to yoghurt (Herro, 2006, Koksoy, 2004).

Lupines (lupines spp.) belong to the Geniteae family, Fabaceae or Leguminosae (Uzun, et al. 2007, Pastor, et al. 2009). From the genus Lupinus more than 400 species are known, four of them are of agronomic interested known as sweet lupine due to their low levels (0.003%) of bitter-tasting and potentially toxic alkaloids (Wasche, et al. 2001), so that lupin de-bittering important before using by the human.

Lupin is a good source of nutrients, not only proteins but also lipids, dietary fiber, minerals and vitamins (Zielinska, et al. 2008). Lupin seed contains higher amount of available soluble sugars and higher levels of soluble non-starch polysaccharides (30-40%) (Erbas, et al. 2005). Lupin contents of these dietary fibers , soluble sugars, and polysaccharides an important roles in yoghurt products for its structural and microbial properties). Lupin proteins possess important emulsifying properties and are expected to contribute to the stabilization of fat particle (Pozani, et al. 2002). Lupin seed proteins are highly soluble at PH> 5.5, and show good water-and fat –binding capacities, foaming capacity, and emulsifying ability (Hojilla, et al. 2004). In addition, their gel-forming ability allows them to strengthen the structure of a processed product (Drakos, et al. 2007). The emulsifying properties are thus promising functional characteristics for future development of lupin utilization (Pollard, et al. 2002).

The objectives of this study were to investigate a new type of functional yoghurt by adding lupin flour

with different concentration as an alternative for texture and quality improvement, to characterize the flavor properties of the product; and to determine the effect of lupin addition on microbial content in yoghurt product.

Material and Methods

This study was carried out in the General State Company for Dairy Products , Abu-Ghraib/ Baghdad/Iraq in 2-5/2014.

Materials

Lupin seed

Two different lupin seed imported from north and south of each Jordan and Egypt farms were purchased from the local veg. market in Jordan and Egypt. .

Lupin seed flour and Skimmed Milk Mixed Ingredient

De- bittering of lupin seed

The de- bittering process of lupin seeds consisted of cleaning, soaking, boiling, and de bittering stages. Extraneous material, stone, immature and damaged seeds were removed. About 800 gram of chosen lupin samples were cleaned, presoaked in hot water for 12 hours, then repeated this process for another 12 hours, after each 4 hours we change the hot water, then we boiled the soaked seeds for 1-2 hours (1:3, seed: water) to destroy thermo-labile anti-nutritional factors, such as trypsin inhibitors, phytic acid and to soften the seed. The hull of the seed were taken off by pressing on the seed with hands finger, then washed with tap- water and soaked with water for at least 6 hours until the bitter taste off and accepted. Then dried, milled with coffee grinder, and packed into glass jar in refrigerator (Erbas, et al. 2010).

Yoghurt manufacture

The total solid content of milk was standardized to about 14% by adding 30 g/L skimmed milk powder, and then the mixture was blended with laboratory blender until all ingredients were dissolved in the milk. The prepared lupin flour were weight (2, 4, and 6% of Jordanian lupin flour JLF), and (2, 4, and 6% of Egyptians lupin flour ELF source) respectively, then placed in yoghurt plastic cups, then placed in electrical oven at 80°C/10 minutes, cooled to 44°C. The above Skimmed milk (30% low fat) also, was heated at 85°C for 15 min, cooled rapidly to 45°C to kill pathogens and then mixed with starter culture was a 1:1 mixture of Str. Thermophiles and L. bulgaricus. Inoculated milk was incubated at 42± 0.5°C then mixing with c different concentration of lupin flour for about 2-3 hours, until PH decreased to 4.7. Following the incubation, all samples were placed immediately in a cooler and stored at 4 ± 1°C for 1, 7, 14, and 21 days before testing. Control yoghurt without any addition also prepared. Preliminary studies indicated that yoghurt containing a high level of (8, 10, and 12%) lupin flour had a beany flavor, so we used the 2, 4, and 6% concentration only. Three replicates of set yoghurt were produced.

Analytical methods

PH and Titratable Acidity

The PH of the samples was determined using a Testo 230 PH meter. The measurements were done in duplicate.

Titratable acidity, expressed as percentage of lactic acid, was determined by mixing 10g of yoghurt with 20 ml of distilled water and titrating with 0.1 N NaOH using phenolphthalein as an indicator to an end-point of faint pink color. The measurements were done in duplicate.

Fat, T.S. determined as given in the AOAC .

Sensory Evaluation

Yoghurt manufactured were subjected to organoleptic evaluation by 20 panelists of Stuff member of Dairy Products general Company, Abu-ghraib/ Baghdad/Iraq was carried out according to scheme of (Salem, et al 2013), or according to Iraqi yoghurt Standard (2001). Samples were organoleptic ally scored for flavor (45 point), consistency with spoon (35 point), acidity (10 point), and appearance (10 points) when fresh and after storage for 7, 14,21 days at 5±1 °C.

Microbial tests:

Prepared yoghurt supplement samples were examined for total viable count, total coliform count, total yeast and mold count. For total viable count of bacteria, colony count method was used according to Laboratory Methods in Dairy Products Company (IQS). The total number of viable bacteria per gram of yoghurt was obtained by multiplying the number of colony forming units (CFU) on the plate with respective dilution factor and then was counted by MPN method.) converted into logarithmic form. Total coliform ($MPNg^{-1}$)

Yeast and mold were determined according to the Standard Methods for Examination of Dairy Products By Iraqi Standard (IQS).

For microbial analysis, all yoghurt samples were homogenized and serial dilution were prepared. Yoghurt samples were analyzed at weekly intervals for two weeks. All relative data were transferred using a based-10 logarithm.

Statistical analysis

The results of researchers were estimated by using Completely Random Design and GIM Procedure of SAS Statistic Analysis Program (SAS, 2012). LSD test were used between the mean values of treatments comparison

and the control. Analysis are the averages of production which had five replications and made as parallel. Only results of dietary fibrous yoghurts were evaluated in this study.

Results and Discussion

Fat% in Yoghurt products: Table 1 shows the fat% values of vegetables yoghurt products. Effect of different addition of lupin flour does not affect the percent of the fat contents significantly and this comparable with the finding of (Hassan et al. 2009).

Total solid percentage (T.S %): Table 1 shows the T.S% values of lupin flour yoghurt products. Different concentrations addition of lupin flour affect the percent of the T.S% contents significantly, the highest content was 18% in yoghurt enriched with 4, and 6% of ELF, then 17% with in yoghurt enriched with 4, and 6% of JLF, then 14% in yoghurt enriched with 2% of each JLF and ELF flour. There were no significant interaction between storage time and T.S values, but there were significant effects of different concentration of lupin flour and the T.S%.

PH and the lupin flour addition: Table 1 shows the PH values of lupin yoghurt products. Effect of different concentration of lupin flour to the yoghurt and the PH was found significant ($p>0.05$) increased especially with 6% addition the PH increased to 5.0 compared with control which was 4.3. There were a significant differences between storage time and the PH values, except after storage of 14 days, that were no significant difference appeared, this value is similar to that found in yoghurt (Senol, and Elven 2011). The yoghurt culture showed good survival over the whole storage period.

Acidity: The acidity values for the entire yoghurt samples were similar compared with control. The acidity scores ranged from 8 to 9 points compared with control which was 10 points also, that means no significant differences in acidity, this indicate that lactose content was responsible for the coagulum formation. Singh and Muthukkumarappan (2008) indicated that there was no statistical difference in the acidity of control and calcium-enriched fruit yoghurt. Also, Hashim and Khalil (2008) reported that the addition of gelatin, ALG, and Ca did not affect the PH and the titratable acidity of the yoghurt.

Acidity and the different addition of the lupin flour: Table 1 shows the acidity values of lupin flour yoghurt products. Effect of different concentration of lupin flour to the yoghurt and the acidity was found significant ($p>0.05$). As the storage period advanced the acidity in all products of lupin yoghurt increased gradually with a very slow rate. The differences in acidity response to applied lupin addition were only significant in highest period of times 21 day, and could be due to the phase change of calcium phosphate from the soluble phase to the colloidal one. The phase change is thought to result from the liberation of hydrogen ion (Hattem et al., 2011). There was no significant interaction between storage time and the acidity values after 14 days of storage. Thus, a good index of the addition sweet lupin flour for an acceptance acidity, in addition of improving the nutritional properties of yoghurt and fermented dairy products (Zare, et al. 2011), by its higher contents of soluble fibers have repeatedly been shown to reduce blood cholesterol both in hypercholesterolemia and normal cholesterolemia individuals (Bengmark & Finocchiaro 2010).

Table 1: The average (2trial) treatments of %Fat, %T.S, PH, and %Acidity of the enriched yoghurt with different concentration of different lupin flour sources which stored at 4°C after1, 7, 14, 21 day from processing.

Treatment	Fat	% T.S. after				PH after				Acidity % after			
	%	1d	7d	14d	21d	1d	7d	14d	21d	1d	7d	14d	21d
2 % JLF	4.0	14	13	13	13	4.3	4.2	4.0	3.6	106	103	108	112
4 % JLF	4.0	17	16	16	16	4.5	4.4	4.3	4.1	98	102	105	113
6 % JLF	4.0	17	16	16	16	4.9	4.6	4.1	3.9	90	88	99	101
2 % ELF	4.0	14	13	13	13	4.2	4.1	4.4	3.7	103	103	103	102
4 % ELF	4.0	18	17	16	16	4.5	4.6	4.5	4.2	76	79	78	82
6 % ELF	4.0	18	18	17	17	5.0	4.4	4.3	4.2	88	91	92	93
Control	3.9	13	13	12	12	4.3	4.7	4.5	4.3	103	103	106	108
LSD value	0.77 NS	2.58 *	3.06 *	2.79 *	2.57 *	0.52 *	0.38 *	0.64 NS	0.42 *	8.5 *	7.4 *	8.5 *	8.2 *

Sensory Evaluation after 1 day of storage

Sensory evaluation is widely used for food quality control and product development. Table 2 present sensory evaluation scores with different concentration of different lupin flour sources supplemented yoghurt products.

Flavor plays an important role in food choice of consumers. Different addition of the lupin flour affect the flavor significantly, table **2** shows the higher score of the flavor was 44 for the 4% ELF addition, then 43 points for each 4% JLF and 2% ELF addition which were comparable to the control. Whereas, the lowest score of the flavor was 38 for the 6% ELF addition which is far away compared with control, the flavor was beany and

rejected by all the panelists, I could present that 2, and 4% concentration for addition of lupin flour to fermented product.

Texture: texture is one of the most essential components of yoghurt quality. Elaboration of yoghurt is depending on various factors, which have been extensively studied with the goal to improve the quality of the yoghurt. Most applications of dietary fiber to yoghurt are related to using water soluble dietary fiber because of water binding properties. Dietary fibers in yoghurt have been used for increasing the viscosity of the product as a stabilizer (Koksoy & Kilic 2004), preventing syneresis and improving textural properties as creaminess. Pectin in lupin improves the textural properties by its anionic hydrocolloids which interact with the positive charges on the surface of casein micelles to strengthen the casein network and reduce the syneresis which were impaired by (Oya, et al. 2013). Also, the addition of dietary fiber to yoghurt is an effective tool for reducing calories and fat (Nulufer, et al. 2004) for consumers.

Table **2** shows no significant differences between the different concentration of different lupin flour sources supplemented yoghurt products and the texture scores. The highest score was 34 points which was even higher than control, the lowest score 30 points for each 6% addition of JLF and ELF. That was a good index of adding lupin flour as a stabilizer in addition off nutritional health of lupin supplements which is comparable with the observation that was by (Nulufer, et al. 2004).

Appearance: Appearance scores of 6% JLF and ELF were significantly lower than those of 2, 4% of each JLF and ELF ($P<0.05$).

The overall total: The overall scores of 6% JLF and ELF were significantly lower than those of 2, 4% of each JLF and ELF ($P<0.05$). The result of this study conducted that 2 and 4% of lupin flour addition improve the yoghurt product sensationally and healthily.

Table 2: The average (2trial) treatments of sensory evaluation for enriched yoghurt with different concentration of different lupin flour sources which stored at 5°C after 1 day from processing.

Treatments	Flavor 45	Texture & consistency35	Acidity 10	Appearance10	Total 100
2 % JLF	42	33	9	10	94
4 % JLF	43	34	9	9	94
6 % JLF	39	30	8	8	85
2 % ELF	43	33	9	9	95
4 % ELF	44	33	9	9	94
6 % ELF	38	30	8	7	84
Control	44	33	10	10	96
LSD value	4.22 *	4.09 NS	3.15 NS	2.58 *	5.52 *
* (P<0.05).					

Sensory Evaluation after 7 days of storage

Table **3** present sensory evaluation scores with different concentration of different lupin flour sources after 7 days storage time of the supplemented yoghurt products.

Different concentrations addition of the lupin flour affect also the flavor significantly, table **3** shows the higher score of the flavor was 41 for each of the 2%, 4% ELF lupin flour addition, then decreased to 39 points for each 2, 6% JLF and 6% ELF addition, which were comparable to the control. The high concentration 6% of lupin flour affects the flavor which was beany and rejected by all the panelists, I could present that 2, and 4% concentration for addition of lupin flour are the best addition to the fermented product.

Table **3** shows also the positive significant differences between the different concentration of different lupin flour sources supplemented yoghurt products and the texture scores. The highest score was 32 points for the 4% addition of ELF which was even higher than control; the lowest score 27 points for 6% addition of JLF. That was a good index of adding lupin flour as a stabilizer instead of nutritional health of lupin supplements (Nulufer, et al. 2004).). Also, particle (Pozani, et al. 2002) concluded that Lupin contents of these dietary fibers, soluble sugars, and polysaccharides an important role in yoghurt products for its structural and microbial properties. Lupin proteins possess important emulsifying properties and are expected to contribute to the stabilization of fat.

Acidity: The acidity values for the entire yoghurt samples were similar compared with control. The acidity scores ranged from 8 to 9 points compared with control which was 9 points also, that is means no significant differences in acidity, this indicate that lactose content was responsible for the coagulum formation, and The yoghurt culture showed good survival over the whole storage period.

Appearance: Appearance scores show no significant differences among all the different concentration

of lupin flour, which were equal or comparable to the control. Thus means lupin flour supplemented to the yoghurt product an excellent effect on the appearance of the yoghurt, which attract the consumer.

The overall total: The overall total scores of 6% JLF and ELF were significantly lower than those of 2, 4% of each JLF and ELF (P<0.05). The result of this study conducted that 2, and 4% of lupin flour addition for each sources improved the yoghurt product sensationally and healthily.

Table 3: The average (2trial) treatments of sensory evaluation for enriched yoghurt with different concentration of different lupin flour sources which stored at 5°C after 7 day from processing.

Treatments	Flavor 45	Texture & consistency35	Acidity 10	Appearance10	Total 100
2 % JLF	39	30	8	8	86
4 % JLF	41	31	8	9	89
6 % JLF	39	27	9	8	83
2 % ELF	41	30	8	9	89
4 % ELF	41	32	8	8	90
6 % ELF	39	29	8	8	84
Control	43	30	9	8	90
LSD value	3.97 *	3.25 *	3.11NS	2.71 NS	5.62 *
* (P<0.05).					

Sensory Evaluation after 14 days of storage

Table **4** present sensory evaluation scores with different concentration of different lupin flour sources supplemented yoghurt products.

Different concentrations addition of the lupin flour affect the flavor significantly, table **4** shows the higher score of the flavor was 42 point for the 2% ELF, then 41 points, and 40 points for 4, and 2% of JLF lupin flour addition respectively. The lowest score was 32 points for 6% JLF addition. The 4% ELF addition, had 39 point score which was comparable to the control. The high concentration of lupin flour affects the flavor which was beany and rejected by all the panelists, I could present that 2, and 4% concentration for addition of lupin flour are the best addition to the fermented product.

Table **4** shows positive significant differences between the different concentration of different lupin flour sources supplemented yoghurt products and the texture scores. The highest score was 32 points for the 2, and 6% addition of JLF which was even higher than control; the lowest score 27 points for 6% addition of ELF. That was a good index of adding lupin flour as a stabilizer instead of nutritional health of lupin supplements (Nulufer, et al. 2004).

Acidity: The acidity values for the entire yoghurt samples were similar compared with control. The acidity scores ranged from 7 to 9 points compared with control which was 8 points also, that is means no significant differences in acidity, this indicate that lactose content was responsible for the coagulum formation, and The yoghurt culture showed good survival over the whole storage period.

Appearance: Appearance scores shows a significant differences among all the different concentration of lupin flour, which were equal or comparable to the control. Thus means lupin flour supplemented to the yoghurt product, even after 14 days of storage time does not effect the appearance of the yoghurt, and acceptance by consumers.

The overall total: The overall total scores of 6% JLF and ELF were significantly lower than those of 2, 4% of each JLF and ELF (P<0.05). The result of this study conducted that 2, and 4% of lupin flour addition improve the yoghurt product sensationally and healthily.

Table 4: The average (2trial) treatments of sensory evaluation for enriched yoghurt with different concentration of different lupin flour sources which stored at 5°C after 14 day from processing.

Treatments	Flavor 45	Texture & consistency35	Acidity 10	Appearance10	Total 100
2 % JLF	40	32	8	10	90
4 % JLF	41	23	9	9	82
6 % JLF	32	32	9	9	82
2 % ELF	42	31	9	9	92
4 % ELF	39	30	8	8	94
6 % ELF	33	27	7	7	73
Control	39	29	8	7	82
LSD value	3.30 *	4.28 *	2.75 NS	2.41 *	4.75 *
* (P<0.05).					

Comparison of microbial characteristics of different lupin yoghurt supplement concentration:

Microbiological characteristics are indicators of safety, quality and shelf life of prepared yoghurt. Total Viable Count (TVC), total coliform and yeast and mold count of the supplemented yoghurt were determined at weekly intervals for two weeks. All relative survival data were transformed using a based-10 logarithm.

Table 5 show the average viable counts of coliform bacteria, during 2 weeks after storage periods. The effect of lupin flour addition on the counts of viable bacteria were found significant, may be due to inhibit the growth of yoghurt bacteria positively with the increasing of the lupin concentration, because of the high carbohydrate levels in lupin flour or by its alkaloids. There was a little contamination of both Yeast and Mold in the samples of low concentrations of lupin flour, but the contamination lies within the limit of the Iraqi standard of the dairy product (IQS).

Table (1): The microbial contamination of the different concentration different lupin flour sources in yoghurt products.

Treatments	Coliform (CFU/g)	Mold No. (CFU/g)	Yeast No. (CFU/g)
2 %JLF	3×10^2 a	2×10^3 a	5×10^4 a
4 %JLF	1×10^2 a	2×10^2 b	Nil e
6 %JLF	Nil c	Nil c	Nil e
2 %ELF	4×10^2 a	Nil c	1×10^3 b
4 %ELF	2×10^2 a	Nil c	2×10^1 d
6 %ELF	8×10^1 b	1×10^2 b	Nil e
Control	1×10^1 b	Nil c	1×10^2 c
LSD value	45.62 *	61.93 *	97.04 *

* ($P \leq 0.05$).

Conclusion and recommendation

From the results it can be concluded that it is feasible to produce yoghurt with good nutritional value and sensory characteristics from lupin flour LF supplementation on yoghurt. The addition of 2 and 4% resulted in yoghurt with high yoghurt quality and good overall acceptability. The sensory evaluation also indicated that 2 and 4% LF yoghurt was the most acceptable yoghurt.

The research was conducted to develop supplemented lupin flour yoghurt with acceptable chemical, sensation, and microbial quality. Two different concentrations of lupin flour (2, and 4% of both JLF and ELF) were improved the sensory evaluation and chemical characteristics of the yoghurt products stored at the refrigeration temperature within two weeks period time only, this study were been done without any addition of texture improver, so we must work more on this side of addition with different stabilizers, and it was safe and healthily products for consumers who ware about healthier products. In addition of that, it was economically to product better than depending on the importing products only.

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