Study The Physicochemical, Microbiological and Sensory Characteristics of Soft Cheese Incorporated with Lupine (*Lupinus albus* L.) Powder in Different Proportion

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Abstract. In this paper the effect of adding different proportions of lupine powder on the physiochemical, microbial and sensory characteristics of soft cheese was studied. Lupine powder at 10, 20 and 40% was added into crude soft cheese. The T1, T2, T3 and T4 treatments in proportions of lupine powder was added (0, 10, 20, 40)% and then stored for 0, 7, 14 and 21 days at refrigeration at (8±2) oC. The proximate analysis was conducted such as moisture, ash, fat, protein, acidity and pH for each treatment. The moisture content in T2, T3 and T4 treatment was (62.72-59.31) %, (60.70-58.20) % and (70.69-65.30) %, respectively for (0 – 21) days of storage and the fat was (15.76), (16.40) and (16.30) % in T2, T3 and T4 treatment, respectively. While, the percentage of the protein was significantly high in all treatments such as T2 (15.91-15.65) %, T3 (16.06-16.10) % and T4 (16.02-16.00) % for 0 and 21 days. The total viable account, total coliform, yeast and mold count were conducted, all the treatments had no detected of coliform bacteria and molds growth during 0, 7, 14 days of storage while, they were rejected after storage for 21 days. The results of the sensory evaluation such as color, taste, flavor, texture and appearance showed the soft cheese was incorporated with lupine powder at 10% and 20% had the highest levels in comparison with 40% and control treatment. Therefore, this study was presented that utilizing of lupine powder could improve the nutritional, storage and organoleptic properties of soft cheese as well as the results indicated the inhibitory role of lupine powder in reducing the microbial load and extended the storage period.

1. Introduction

Lupine belongs to the genus *Lupinus* and family of Genisteeae, and also known as Fabaceae or Leguminosae [1]. It is one of the annual herbs belonging to the legume family, lupine has been known and cultivated in the Mediterranean and the Andes mountain since ancient times to fertilize the soil. Boiled and roasted seeds have been used as a human food [2]. There are several types of lupine: *L. albus* L., *L. angustifolius* L., *L. luteus* L. and *L. mutabilis* Sweet [3]; [1]; [4] The common names for these types are white lupine, narrow-leaved (blue) lupine, yellow lupine and pearl lupine, respectively. The most important types are white lupine (sweet lupine) and yellow lupine (bitter lupine) [5]. Bitter and sweet lupine is similar to fiber and essential nutrients in chemical composition such as carbohydrates, proteins and fats, but bitter lupine loses some nutrients such as minerals and antioxidants during soaking [6] Lupine can be considering a high source of protein and in the Middle East is consumed as a snack and in other parts of the world can be used as a high-protein soy replacer [4]. Lupine proteins have a better digestibility and bioavailability than other legumes [7]. As mentioned by [8] that lupine flour had 38% protein content as compared to wheat flour was 11%, and when wheat flour was supplemented with lupine flour, it increased protein efficiency ratio (PER) in rats and the lupine protein digestibility was 85% as compared to casein protein digestibility was 87%. In addition, lupine protein is one of
the richest nutritional sources of amino acids such as Arginine. It also exceeds soybeans with high levels of amino acids such as Arginine, Lysine, Leucine, and Phenylalanine [9].

Lupine seeds are characterized as the main component of carbohydrates is non-starchy sugars, that an important source of dietary fiber and has the ability to bind of water as compared to other legumes [10]. Reduced total carbohydrate content in lupine and reached 100g/kg lupine flour as compared to wheat flour, green peas and coarse wheat flour as (685, 600, 667) g/kg, respectively [11]. Meanwhile, the highest percentage of lupine fiber as compared to other crops was 350 g/kg lupine flour, it contains the most nutritionally beneficial fiber that the ratio of soluble fiber to insoluble fiber was 21.5: 2.2 in lupine seeds, where foods with high levels of insoluble fiber reduce the level of low density lipoprotein [12]. [13] conducted that the lupine albus variety is the best for obtaining raffinose sugar (12.3%) that used in functional foods. On the other hand, [14] found that the lupine seed was a healthy nutritional combination due to it contained 8% crud fat which included 86%unsaturated fatty acids, 44% oleic acid and 26% linoleic acid. In addition, lupine is a rich source of mineral elements such as calcium, phosphorus, potassium, sodium, magnesium, iron, manganese and zinc as 2.1-4.7, 4.3-7.2, 8.6-11.1, 0.1-0.2, 1.2-2.2, 0.078-0.093,1.675-1.8, and 0.0536-0.0403, respectively [15]. Finally, Lupin seed proteins have high soluble at PH> 5.5, and a good binding capacities with water and fat, foaming capacity, and emulsifying ability [16]. Furthermore, their gel-forming have the ability of strengthen the structure of final product [17]. Thus, Lupine seeds have an important functional property of emulsifying characteristics that can be fortified with food products in the future such as cheese [18]. However, the process of fortifying cheese should not affect the specific characteristics of the cheese, especially its taste, appearance and hardness [19, 20] showed that the possibility of using potassium chloride salt and emulsifying salts as an alternative to sodium chloride in the manufacture of processed cheese for maintaining consumer health, especially people with high blood pressure, through studying its chemical, microbial, sensory and texture properties. In addition, it was found the process of fortifying soft cheese in low quantities zinc salts did not lead to any significant effects of the organoleptic properties of soft cheese, while the treatment of high additives of zinc salts led to high significant effects in both texture and sensory properties [21].

The aim of this study was to investigate a new type of functional soft cheese by incorporating lupine powder in different proportion to improve the texture and quality of final product under storage at (10 ± 2 °C) for 0, 7, 14 and 21 days, and also to determine the effect of addition lupine powder on microbial content of soft cheese.

2. Material and Methods
2.1 Materials
Lupine seed was provided from the farms medical and aromatic plant research unit -college of agricultural engineering since -Baghdad -Iraq.

2.2 Removing the bitterness from lupine seed process
Removing the bitterness from lupine seed applied by cleaning, soaking, boiling, and remove the bitterness levels. The exotic material, stone, unripe and broken seeds were removed. Around 800 g of lupine samples were cleaned and kept in hot water for 12 hours, then repeated this process for 12 hours, each 4 hours the hot water was changed. Then, the soaked seeds were boiled for 1-2 hours (1:3, seed: water) to destroy thermodlabile anti-nutritional factors, such as trypsin inhibitors, phytic acid and to make the seed softer. The hull of the seeds was taken off by pressing the seeds with hands finger, then they were washed with tap-water and soaked with water nearly 6 hours until the bitter became taste off. Then, dried and milled with coffee grinder and they packed into glass jar and kept them in cold storage using refrigerator [22].

2.3 Manufacture of soft cheese
The process of filtration of full fat milk carried out by pasteurization at a temperature 63 °C for 30 minutes. Then, the samples of milk were cooled until 38°C with continuous stirring. The dry rennet was used and added according to the company's recommendations (1g/25kg milk) after dilution with water and stirring for 3 minutes. After that, the heat rennet for 45 minutes with milks samples until the state of coagulation was formed. The coagulate was caught by knife in size 1 cm longitudinal and wide then left it for 5 minutes. Whereas, the flipping process continues quietly to help the whey out and coagulation. The coagulation of each sample was collected and they were used in the manufacture of soft cheese treated as T1,control. The samples of crud soft cheese treatments were prepared by mixing with lupine powder in 10%, 20% and 40% as T2, T3 and T4 treatment, respectively. Then, the salt was added to all samples around 1.5 % from crud weight, and samples (T1, T2, T3, T4) were pressed in container made from stainless-steel to gain the desired shape and texture.
Finally, all samples were stored at a temperature of (8± 2) °C for 0, 7, 14 and 21 days for the purpose of conducting the required tests.

2.4 Physicochemical analysis of soft cheese treatments

2.4.1 Proximate analysis

The soft cheese samples were carried out to determine the proportion of protein, moisture, fat and ash. Moisture and ash content using AOAC method 926.08 [23], the percentage of ash was estimated by burning the samples at the Muffle furnace at 550 °C for 6 hours or until the weight was stable. whereas the percentage of fat and protein was conducted by [24].

2.4.2 Total Acidity and pH Value

pH value was analyzed according to [24] by weighed 2.00 g of sample into 200 ml glass bottle, and 100 ml of deionizer water was added. The glass bottle was boiled for 10 min. Then, 50 ml of filtered extract was used for pH value determination with a pH meter. Measurements were taken in triplicate. The proportion of total acidity was calculated as lactic acid by weighed 3.00 g of sample and mixed with 10 ml of distill water using mortar, and titrate with 0.1 N of NaOH solution [25].

2.4.3 Microbiological analysis of soft cheese treatment

Soft cheese samples (T 1, T 2, T 3 and T 4) were examined for total viable count, total coliform count and total yeast and mold count. The colony count method for all above of microbial count of bacteria was used according to the Standard Laboratory Methods for examination of Dairy Products Company by Iraqi standard (IQS). The total number of viable and coliform bacteria for per gram of soft cheese was obtained by colony forming units (CFU) with respective dilution factor and the MPN method was counted by converted into logarithmic form (MPNg-1). All the samples were homogenized and prepared of serial dilution, then, soft cheese samples were analyzed for 0, 7, 14 and 21 days. All relative data were transferred using a based-10 logarithm.

2.4.4 Sensory Evaluation

Soft cheese samples were subjected to evaluate by 20 panelists according to scheme of [26]. The panelists were selected from experienced residents of teaching and Department of Food Science staff - Collage of Agricultural engineering science - Baghdad University. All treatments were evaluated using scored for taste and flavor (45 point), texture with spoon (35 point), color (10 point), and appearance (10 points) when fresh and after storage for 0, 7, 14 and 21 days at 8±2°C.

2.4.5 Statistical analysis

Completely Random Design and GIM Procedure of SAS Statistic Analysis Program were used to study the influence of various parameters [27]. LSD test were used between the mean values treatment.

3. Results and Discussion

3.1. Physicochemical analysis of soft cheese treatments

The table 1 showed, the proportion of chemical composition such as moisture, fat, protein and ash of soft cheese incorporated with lupine powder. The proportions of lupine powder were added to soft cheese was 0, 10%, 20%, and 40% as T 1, T 2, T 3 and T 4, respectively.

The results showed that the low moisture was observed in T 1, T 2, T 3 and T 4 treatment was (61.21-53.95), (62.72-59.31), (60.70-58.20) and (70.69-65.30), respectively for 0 and 21 days of storage at a temperature 8±2°C. The results showed, significant decrease in the moisture content (P ≤ 0.05) of T 1 treatment and no significant decrease (P ≤ 0.05) for each T 2, T 3 and T 4 treatment due to the moisture-holding characteristic of lupine proteins and is considered a positive condition for lupine powder. Through the results, the moisture content after 21 days of cold storage at 8±2°C was more increased in T 2 (59.31), T 3 (58.20) and T 4 (65.30) treatment than T 1 (53.95) sample because of the added lupine powder to soft cheese samples contains proteins that have the property of holding moisture and thus preserved the texture and softness of the cheese during storage period.

Accordingly, both fat and protein proportion were increased and no significant (P≤0.05) increase in ash content. In soft cheese control (T 1) treatment, high significant differences in fat proportion (15.38-21.00) for 21 days of storage due to the loss of moisture during storage period and this in turn will be affecting in the high proportions of the other components. While, there are no significant differences (P≥0.05) in the T 2, T 3 and T 4 treatment, this comparable with the finding of (Hassan et al., 2009) due to added a percentage of fat using lupine powder to the ingredients of the cheese curd. In addition, lupine proteins also have the ability to retain
fat, which explains the decline fat proportion. In zero time, there were no significant differences (P ≤ 0.05) in fat proportions and it was increased for all treatments T2 (15.76), T3 (16.40) and T4 (16.30) treatment as compared to control treatment T1 (15.38) sample because of lupine contain a certain percentage of fat. As a result, the percentage of fat for T2, T3 and T4 treatment was 15.80, 15.29 and 15.00, respectively, has decreased after storage for 21 days at a temperature 8±2 °C due to the high percentage of both protein and moisture, which was reflected on the fat percentage.

The Table 1 showed no significant differences (P≤0.05) was observed in the protein during storage period (0 and 21 days) in T1, T2, T3 and T4 treatment, but there was a significant difference (P≤0.05) among the T1 (15.71-15.69) and other treatments. In addition, the percentage of protein was significantly high in all treatments such as T2 (15.91-15.65), T3 (16.06-16.10) and T4 (16.02-16.00) which was incorporated with lupine powder due to the lupine powder contained high proportion of protein and lower in moisture content. These results were corresponded with [28], they found that only 10% lupine flour can be fortified with refined wheat flour before quality is reduced.

Table 1. The chemical Composition of Soft cheese treatments incorporated with lupine powder in different proportion during storage period at 8 ± 2 °C. *

| Treatment | Age of Days | Moisture (%) | Fat (%) | Protein (%) | Ash | Acidity | pH |
|-----------|-------------|--------------|---------|-------------|-----|---------|----|
| T1        | 0           | 61.21        | 15.38   | 15.71       | 3.22 | 0.63    | 6.61 |
|           | 21          | 53.95        | 21.00   | 15.69       | 3.21 | 0.52    | 5.43 |
| T2        | 0           | 62.72        | 15.76   | 15.91       | 3.29 | 0.61    | 6.45 |
|           | 21          | 59.31        | 15.80   | 15.65       | 3.19 | 0.52    | 5.47 |
| T3        | 0           | 60.70        | 16.40   | 16.06       | 4.39 | 0.60    | 6.53 |
|           | 21          | 58.20        | 15.29   | 16.10       | 4.40 | 0.52    | 5.49 |
| T4        | 0           | 70.69        | 16.30   | 16.02       | 5.28 | 0.63    | 6.58 |
|           | 21          | 65.30        | 15.00   | 16.00       | 5.27 | 0.52    | 5.44 |
| LSD       |             | 7.063 *      | 3.226 * | 1.073 NS    | 0.993 * | 0.188 NS | 0.892 * |

The numbers in the table represent a rate for repeaters. * (P≤0.05). T1=0% lupine powder, T2=10% lupine powder was incorporated with soft cheese, T3=20% lupine powder was incorporated with soft cheese and T4=40% lupine powder was incorporated with soft cheese.

With regard to ash content was noted high significant differences (P≤0.05) for each T3 (4.39-4.40) and T4 (5.28-5.27) treatment in comparison with T2 (3.29-3.19) treatment due to high proportion of lupine powder was added to T3 and T4 treatment which in turn will improve the quality properties of final product (Table 1). The acidity values for the soft cheese treatments were similar as compared with control as shown in Table 1. That means no significant differences (P≤0.05) in the T1, T2, T3 and T4 treatment, respectively. These results were agreed with [29], they found that there is no significant differences between the samples and control when they incorporated the lupine flour with yoghurt in different proportion.

Table 1 was noted significantly low (P≤0.05) in PH value of all the treatments at a temperature (8 ± 2 °C) for 21 days, these values is similar to that found by[30]. The decrease in PH values of those treatments had a significant role for maintaining of soft cheese products (T1, T2, T3 and T4 treatment) and inhibited of microbial growth during storage period.

3.2. Microbiological analysis of soft cheese treatment
The total viable count, total coliform and yeast and mold count were determined at (8 ± 2°C) for 0, 7, 14 and 21 days. From Table 2 conducted that all treatments had no detected of coliform bacteria and molds growth, and there were high significant (P≤0.05) differences in viable count of microbial cells after storage period 7 and 14 days. While, all the treatment were rejected after storage for 21 days because of its deterioration.

In zero time, the total viable count of bacteria was increased in T2 (1.40×10^3), T3 (1.21×10^3) and T4 (2.95×10^3) as compared to T1 (1.51×10^3) due to lupine powder has not been sterilized when combined with soft cheese in different proportion to avoid deterioration of the functional properties of lupine protein. But these values were significantly decreased (P≤0.05) as compared with control treatment (T1) in zero time due to the inhibition affect of lupine powder as well as that lead to increase the storage period for 21 days (Table 2).
For T1 treatment, it was observed the appearance of coliform bacteria growth after 7 days ($4 \times 10^1$) and significantly high after 14 days ($1.00 \times 10^2$). Whereas, it wasn't observed any coliform bacteria growth after 14 days of storage in the T2, T3 and T4 treatment. As shown in Table 2, the coliform bacteria appeared in an acceptable number after 21 days of storage in T2 and T3 treatment, while in T4 treatment did not appear because of that the role of lupine powder inhibition with an increase in its proportion in T4 treatment (lupine powder by 40%).

Table 2 the microbiological analysis CFU/g of soft cheese treatments incorporated with lupine powder in different proportion during storage period at 8 ± 2 °c. *

| Treatment | Age of Days | Total Count | E. coli | Molds |
|-----------|-------------|-------------|---------|-------|
| T1        | 0           | $10^3 \times 1.51$ | Nill    | Nill  |
|           | 7           | $10^4 \times 2.32$ | $10^1 \times 4.00$ | Nill  |
|           | 14          | $10^4 \times 8.00$ | $10^2 \times 1.00$ | $10^2 \times 4.00$ |
| T2        | 21          |              |         |       |
|           | 0           | $1.40 \times 10^3$ | Nill    | Nill  |
|           | 7           | $2.83 \times 10^3$ | Nill    | Nill  |
|           | 14          | $5.41 \times 10^3$ | Nill    | Nill  |
| T3        | 21          | $6.12 \times 10^3$ | $2.00 \times 10^4$ | $3.00 \times 10^2$ |
|           | 0           | $1.21 \times 10^3$ | Nill    | Nill  |
|           | 7           | $1.40 \times 10^3$ | Nill    | Nill  |
|           | 14          | $2.43 \times 10^3$ | Nill    | Nill  |
| T4        | 21          | $2.72 \times 10^4$ | $3.00 \times 10^4$ | $3.00 \times 10^2$ |
|           | 0           | $2.95 \times 10^3$ | Nill    | Nill  |
|           | 7           | $6.05 \times 10^3$ | Nill    | Nill  |
|           | 14          | $6.65 \times 10^3$ | Nill    | Nill  |
| LSD       |              | 52.487 *      | 16.330 * | 32.508 * |

The numbers in the table represent a rate for repeaters. * (P≤0.05). T1=0% lupine powder, T2=10% lupine powder was incorporated with soft cheese, T3=20% lupine powder was incorporated with soft cheese and T4=40% lupine powder was incorporated with soft cheese.

As observed in Table 2, The appearance of molds in the control treatment (T1) after 14 days of storage at a temperature of (8 ± 2°C), while the growth of molds appeared after 21 days of storage in the treatments T2, T3 and T4 they can be considered acceptable when compared to the standard specifications. In general, the results indicated the apparent inhibitory role of lupine powder in reducing the microbial load; this in turn extended the shelf life of the soft cheese product during the cold storage period at (8 ± 2°C) for 21 day. These results are corresponded with[29], they found the effect of lupine flour addition on the number of viable bacteria by inhibiting the yoghurt bacteria growth positively with the increasing of the lupine concentration when combined the lupine flour with yoghurt in different proportion.

3.3. Sensory evaluation

Sensory evaluation is necessary measures to determine the quality of food product by means of acceptability. [31] defined sensory evaluation as a scientific discipline used to interpret and determine the perception of the panelists (consumer) to evaluate the characteristics of food products. Soft cheese treatments were subjected to sensory evaluation by the experienced group of food science staff. In table 3 it was showed a significant decrease (P≤0.05) in color, taste, flavor and overall appearance of soft cheese after 14 days in the control treatment (T1). Whereas, the texture was decreased significantly (P≤0.05) after one week of cold storage. As for the treatments T2, T3 and T4, there were no significant differences (P≤0.05) in the overall appearance and texture during 21 days of storage period at (8 ± 2°C). Thus, during the storage period all the treatments were significantly superior in texture as compared with the standard treatment.

During storage period at (8 ± 2°C), there were no significant (P≤0.05) differences in color and taste between T2 and T3 treatment due to the high proportion (10%) and (20%) of lupine powder added in the T2 and T3 treatment, respectively, that led to the appearance of bean taste and change the color of the final product.
Table 3. Sensory evaluation of soft cheese treatments incorporated with lupine powder in different proportion during storage period at 8 ± 2 °C. *

| Treatment | Age of Days | Color (10) | Taste and flavor (45 Texture (35)) | Appearance (10) |
|-----------|-------------|------------|-----------------------------------|-----------------|
| T1        | 0           | 10         | 45                                | 35              |
|           | 7           | 10         | 39                                | 30              |
|           | 14          | 7          | 38                                | 27              |
|           | 21          |            |                                   |                 |
| T2        | 0           | 10         | 45                                | 35              |
|           | 7           | 10         | 45                                | 35              |
|           | 14          | 10         | 45                                | 33              |
|           | 21          | 10         | 42                                | 33              |
| T3        | 0           | 10         | 45                                | 35              |
|           | 7           | 10         | 45                                | 35              |
|           | 14          | 9          | 42                                | 33              |
|           | 21          | 8          | 40                                | 32              |
| T4        | 0           | 10         | 45                                | 35              |
|           | 7           | 9          | 40                                | 33              |
|           | 14          | 7          | 35                                | 33              |
|           | 21          | 6          | 32                                | 29              |
| LSD       |             | *2.307     | 6.115*                            | * 4.961         | * 2.059         |

The numbers in the table represent a rate for repeaters. * (P ≤ 0.05). T1=0% lupine powder, T2=10% lupine powder was incorporated with soft cheese, T3=20% lupine powder was incorporated with soft cheese and T4=40% lupine powder was incorporated with soft cheese.

4. Conclusion
We can produced a new type of functional soft cheese by incorporating lupine powder in different proportion to improve the texture and quality of final product under storage at (10 ± 2 °C) for 0, 7, 14 and 21 days, and also determining the effect of addition lupine powder on microbial content of soft cheese.

Acknowledgement
We extend our appreciation to the Food Sciences Dept. College of Agricultural engineering sciences, University of Baghdad, Medical and Aromatic Plants Research Unit, College of Agricultural engineering sciences, University of Baghdad and Dairy Factory of Agricultural College, University of Baghdad, Iraq for funding the work and their keen interest in accomplishing of this work.

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