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Preparation of Functional Ice Milk Supplemented With Lupine Flour

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THIS study was proposed to investigate the possibility of utilization of sweet lupine (*lupines albus*) flour for producing functional ice milk for human consumption. Lupine considers a rich source of protein, minerals, antioxidant, and anti-microbial materials. Sweet lupine flour (SLF) was incorporated with skim milk powder at levels 5%, 15% and 25% respectively to produce healthy ice milk. The effect of replacement on chemical, physical, viscosity, meltdown, rheological, microbial quality and sensory evaluation were evaluated. Protein, fat, ash, TS contents as well as pH values and overrun were increased in all samples which supplemented with SLF compared to control. The acidity was decreased by increasing SLF compared to control. Also, the viscosity of mixes increased by increasing SLF compared to control. As well as melting down value decreased by increasing SLF. Texture profile was affected by replacement with SLF, the hardness increased by increasing the amount of SLF from2049.49 gf in control to 2347.98, 2937.19 and 3100.05 gf for 5, 15 and 25% SLF, respectively. The microbiological aspect was improved specially the sample contained 25% SLF which had the lowest total bacterial count. The sensory properties of ice milk especially sample contained 5% SLF had the highest overall acceptability compared with other samples.

Keywords: Sweet Lupine Flour, Ice milk, Physiochemical properties, Texture profile, Anti-microbial activity, and antioxidants

Introduction

As a source of bioactive compounds, species of the genus *Lupinus* are interesting legumes from a nutritional point of view, especially *Lupinus albus* (Ruiz-Lopez et al., 2019). Lupine has been used as a source of protein since ancient times. Currently interest in a wider utilization of this legume seed is rising. This is mainly due to its similarity with soybeans as a high source of protein and to the fact that it can be grown in wider climatic range. Moreover, its adaptation to poor (i.e. Leached) soil makes it economically feasible. Lupine is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in the other parts of the world (Kurzbaum et al., 2008). Sweet lupine is high in protein and dietary fiber. Incorporating sweet lupine flour with wheat produces more nutritious food (Kefale & Yetenayet, 2020).

On another view, Lupine flour is widely considered an excellent raw material for supplementing different food products owing to its high protein content with moderate gelatin properties, amino acid, and carbohydrate, non-starch, vitamins, minerals and none saturated fatty acid, such as bakery product –protein concentrates and other industrial as well as the elaboration of lactose free milk. Comparing to milk powder as a concentrated product of solid and looks like it in shape with different components, lupine flour can be considered as a cheap source of solids and even as a stabilizer martial in ice milk manufacture to improve its consistency and general acceptability (Shalaby et al., 2013)

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The production of non-dairy food products has been pointed out as a novel trend in the production of functional foods. Sweet lupine flour is used in the preparation of frozen desserts especially ice milk. Ice milk is a frozen mixture of a combination of some milk components (carbohydrates, proteins, fats, vitamins, and minerals), sweeteners, stabilizers emulsifiers and flavors. It may be defined as partially frozen foam with air contents of 40 to 50% by volume. It has higher nutritive, biological, and caloric value. According to the Egyptian Standard 1185-3/2005, the fat content of ice milk must not less than 3% (Atallah and Barakat et al., 2017).

For the application of Lupine in dairy field; Shalaby et al. (2013) prepared functional whipped cream by partial replacement (10%) of milk fat with different combinations of Sweet Lupine Flour (SLF) and Whey Protein Concentrate. While, Abdel-Salam et al. (2015) manufactured Zabadi with different concentrations (2, 4, 6 and 8%) of SLF and studied the effect of this addition on chemical, rheological, sensory, and microbiological properties of Zabadi samples.

Standard specifications of ice milk are a term used in the USA refer to standardized frozen desert class with a fat content between 2 and 8% ice milk is highly nutritive (Tharp and Young, 2013). Ice milk preparation typically involves a mix making, and freezing stage, with important processing steps being blending, pasteurization, homogenization, aging, flavoring, freezing, packaging, hardening and frozen storage. Consumption of ice milk has increased during the last few years due to its favorable nutritional properties as low-fat content in comparison with high fat ice cream. Up to now, no work has been published on producing ice milk supplemented with sweet lupine flour. Therefore, the aim of this study was to prepare innovative ice milk by partial replacing of skim milk powder with different combination of sweet lupine flour and studying the effect of this adding on chemical, physical, rheological, microbiological and sensory properties of ice milk.

Materials and Methods

Materials

Sweet lupine seeds (*lupinus albus*) were obtained from the Agriculture Research Center, Giza, Egypt. Fresh whole buffalo milk (Acidity = 0.16% and the pH value 6.65, Total protein = 3.52%, Fat =6.1% and Total solid =14.12%) was obtained from the farm of the faculty of Agriculture, Damietta University, Egypt. Skim Milk Powder (SMP) (contained 34% protein, 1.2% fat, 53% lactose) made in the USA was used in this study. Chemicals and microbial media were obtained from Sigma Chemical Co. Nasr city, Cairo, Egypt.

Exprimentals

Preparation of sweet lupine flour (SLF)

SLF was obtained after grinding the lupine grains in a laboratory hammer mill (Retsh-Germany) until they could pass through a 250 µm screen (Abdelrahman, 2014).

Preparation of ice milk

According to Atallah and Barakat (2017), soft ice milk was prepared, skim milk powder had been substituted by sweet lupine flour at 3 substitution levels as (0:100) Control (5:95, 15:85 and 25:75%) replacement as shown in Table 1. The amount of calculating fresh Buffalo's milk was added to the dry blend and mixed wells in a high-speed mixer for 1 min. All soft ice milk mixtures were heated at 85 °C for 15 min, then cooled down to 5 °C and aged for about 24 h. Natural vanilla powder portion was added to the aged mixes before freezing. Soft ice milk mixtures were frozen in the batch freezer, and then the resultant frozen soft ice milks were packed in plastic cups and stored at -18±1 °C. All experiments were carried out in triplicates and the mean values were tabulated.

TABLE 1. Different formula of soft ice milk supplemented with sweet lupine flour (SLF).

Ingredients	Soft ice milk with different SLF levels			
	0% (control)	5 %	15 %	25 %
Fresh whole Buffalo's milk, ml	500	500	500	500
SLF, g	---	6	18	30
SMP, g	120	114	102	90
Sugar, g	160	160	160	160
CMC, g	2	2	2	2
Vanilla, mg	15	15	15	15

SLF: Sweet lupine flour **SMP**: Skim Milk Powder **CMC**: Carboxyl methyl cellulose

Chemical analysis

Moisture content was determined by using an air-drying oven at 105 °C until arrived at constant weight. Total Solid (TS) was calculated according to AOAC (2010) using the following equation:

$$\text{Total solid (g/100 g)} = \frac{\text{Weight of sample after drying (g)}}{\text{Weight of sample before drying (g)}} \times 100$$

Total nitrogen was determined by micro–kjeldahl procedure, Crude protein content of the samples was calculated by multiplying the percent of nitrogen by 6.25. The ether extract content was determined using petroleum ether (40- 60 °C) in a Soxhelt apparatus for 16 hr. The ash content using a muffle furnace at (525-550 °C) and crude fibers of the samples were performed according to the methods described by AOAC (2010). Fat content of ice milk product was determined using Gerber tube according to Ling (1963). Total carbohydrates content was calculated by difference, according to the following equation: (%) =100- the sum of (% protein+ % fat+ % ash+ % Crude Fiber) as reported by (Tadrus, 1989). Total Energy was calculated according to AOAC (2010) using the following equation: Total Energy (Kcal) = 4 (%Carbohydrate + %Protein) + (9× %Fat). Minerals as potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P) as well as Heavy metals, zinc (Zn), copper (Cu) iron (Fe) were determined by Atomic Absorption Units (GBC 932 AA) according to the method described in AOAC (2010). Vitamins as thiamin, riboflavin and niacin were carried out by HPLC technique according to (Batifoulier et al., 2005). Amino acids profile was determined by acid hydrolysis as described by (Block et al., 1958) using a Beckman amino acid analyzer (Modle 119CL). Fatty Acids analysis was carried out by gas chromatography (Perkin Elmer Auto System XL) according to (Christie, 1993). Antioxidant activity was carried out by using 2, 2 diphenyl-1-picrylhdrazyl (DPPH), 2,2-azino-bis/3-ethil-benothiazoline-6-sulfonic acid (ABTS) and Ferric reducing activity power (FRAP) assay according to Hwang and Do Thi (2014).

Physicochemical characteristics of ice milk

The pH Value of ice milk samples was measured by using a laboratory pH meter (Acumen portable AP61, Fisher Scientific) as described by AOAC (2010). Total acidity was determined by titrating (10 mL) of ice milk samples with 0.1N NaOH using (ph th) as indicator. The results were expressed as acid according to AOAC (2010).

Calculation of overrun percentage

Overrun was measured by comparing the weight of mix and ice milk in a fixed volume container by using a 250 mL beaker. The overrun percentage was determined according to the following equation:

On % = 100 (Wm–Wic)/Wic

Where: On (%) is the overrun percentage; Wm (g) is the weight of a given volume of the mix; Wic (g) is the weight of the same volume of ice milk (Pon et al., 2015).

Viscosity determination

Apparent viscosities of ice milk mixes were determined using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden) attached to a workstation loaded with software V88 viscometery program. The system C30 was filled with the ice milk mixture at the measured temperature of 20 °C. The viscosity was carried out in the up mode at shear rate ranging from 34 to 270 1/s. Apparent viscosity was expressed as mPa (Atallah and Barakat, 2017).

Melting resistance determination

Meltdown of frozen ice milk was determined according to Arndt & Wehling (1989), by carefully cutting the foamed plastic cups from the ice milk samples (~50g), placing the samples onto wire mesh over a glass funnel fitted on conical flask, and weighing the amount of ice milk drained into the conical flask at 25±2 °C every 10 min until the entire sample had melted.

Texture profile analysis "TPA"

A texture analyzer (TA-XT2i, multi test 1-d mecmesin, west succex, UK) was used to determine the hardness, adhesiveness, cohesiveness, gumminess, and springiness of the ice milk samples. The analysis was carried out at 25 °C. Samples were carried out using a 2 mm stainless steel cylindrical probe (P2) attached to a 5 g load cell. The penetration depth at the geometrical center of the samples was 20 mm and the penetration speed was set at 3.3 mm/s (Pon et al., 2015).

Sensory evaluation

Members belonging to the dairy science Department, Faculty of Agriculture, Damietta University were asked to evaluate the prepared ice milk samples towards flavor (45 point), body/texture (30 point), melting properties (15 point), color (10 point) and overall acceptability (100 point) according to Arbuckle (1986).

Microbial analyses

Total bacterial count (TBC) was determined using nutrient agar medium as described by (Oxoid, 1992). Mould and Yeast count was determined by using potato dextrose agar medium according to (Difco, 1984). *Salmonella* and *Shigella* count were enumerated using S.S. agar medium used as described by Byran (1991). Total and fecal coliforms were determined by using MacConkey Broth with inverted Durham tubes (Tassew & Seifu, 2011). Psychotropic Bactria count was detected following Difco (1984) method using nutrient agar medium. Differential tests were used in the identification of *Escherichia coli* and *Enterobacter* (Speck, 1976). Standard methods were used for determining the coagulase positive *Staphyococcus* was carried out using the method of Cher and Clerk (1968).

Statical analysis

The results were statistically analyzed by analysis of variances by SPSS (1997). Significant differences between individual means were analyzed by Duncan's multiple range tests (Duncan, 1955).

Results and Discussions

Chemical composition of Sweet Lupine flour (SLF)

As shown in Table 2, moisture, crude protein, crude fat (ether extracts), crude fiber, ash, carbohydrate and total energy contents for SLF were 5.12, 34.76, 13.23, 6.11, 2.86, 37.92% and 409.79 Kcal as dry weight basis (DWB), respectively. This finding agreed with those declared by (El-sayed, 2013, Jahreis et al., 2015, Elsamani, 2016, Al-Hamdan, 2017 and Abrha & Kefal, 2018) whose reported that SLF contained 5–14% moisture, 30–40 % protein, 4–20% crud fat, 3-36% crude fiber, 2–7% ash and 11–51% carbohydrate. In this concern, it has been reported that although lupine belongs to the legumes and is not described as an oil seed crop. The lupine seeds contain a considerable amount of oil ranged between 4% and 20% crude oil of whole seed weight (Ciesiolska et al., 2005 and Uzun et al., 2007).

Minerals and vitamins contents of SLF

The data presented in Table 3 show the minerals and vitamins content of SLF. The contents of P, K, Ca, Mg, Na, Fe, Zn and Cu of SLF were 6.8, 4300, 235, 850, 150, 1.31, 1.6 and 0.32 mg/Kg, respectively. These results are in agreement with those reported by (Yousif & Faid, 2014; Jahreis et.al. 2015; Kouris-Blazos & Belski, 2016; Prusinski, 2017 and Abrha & Kefal, 2018) as they found that the Ca content ranged from 1.2 to 2.5 g/kg, P content from 2.8 to 4.9g/kg, Mg content from 0.96 to 1.6g/kg, K content from 4.1 to 12.0g/kg, Na content from 0.1 to 1.1g/kg for SLF. Meanwhile SLF contained 4.6 mg/100g Fe, 4.8mg/100g Mn and 0.5 mg/100g Cu. Also, the same Table shows the vitamins as thiamine, riboflavin and niacin for SLF were 5, 4 and 40mg/100g, respectively. These results coincided with results of (Erbas, *et al*. 2005, Kouris-Blazos & Belski, 2016) who's reported that thiamine content ranged from 3 to 4 mg/100 g, riboflavin from 1.90 to 2.5 mg/100 g and niacin from 39 to 40 mg/100g for SLF. In general, data revealed that SLF are good source of minerals and vitamins and can be used to supplement food substances, which are deficient in proper growth of children and good health of adult.

Amino acids composition of SLF

Results in Table 4 show essential amino acids composition (g/100g) in SLF compared to FAO/WHO requirement (1991). Glutamic acid was the most predominate amino acid in SLF which recorded 7.79 g/100 g followed by aspartic acid, which recorded 3.65 g/100 g. From the same Table, it could be also concluded that, SLF was rich with leucine, isoleucine and aromatic amino acids (Phenylalanine and tyrosine). As well it was considered to be a good source of lysine,

TABLE 2. Chemical composition of sweet lupine flour (SLF).

Components	SLF
Moisture, %	5.12±0.14
Protein, %	34.76±0.48
Ether extract, %	13.23±0.24
Ash, %	2.86±0.11
Carbohydrate, %	37.92±2.48
Crud fiber, %	6.11±1.08
Total energy, Kcal	409.79

but generally was poor in the content of sulfur-amino acids; methionine and cysteine (Martinez-Villaluenga et al., 2006). These findings are in agreement with those results was previously reported by Yousif & Faid (2014) whose found that, the essential amino acids content of SLF (Lysine – Lucien – phenylalanine – isoleucine –threonine –valine- methionine – cysteine –Histidine – Tyrosine) was (4.57g/100g – 7.00g/100g – 2.40g/100 g– 3.36g/100 g– 3.36g/100 g– 3.34g/100 g– 1.44g/100g – 1.02g/100g – 3.00g/100g – 3.53g/100g, respectively. It is clearly shown that total essential amino acids content of SLF (11.99%) was lower than the recommended pattern FAO/WHO (1991) for school children (21.2%), preschool children (30%) and adults (19.8%).

Fatty acids composition of SLF

The fatty acids content of SLF was determined and the obtained results were tabulated in Table 5. From the obtained data, it could be observed that the highest fatty acids content in SLF was oleic acid (C18:1), which score 44.3% flowed by Palmitic acid (C16:0) which score 19.47%, Linoleic acid (C18:2) which score 19.29%, Stearic acid (C18:0) which score 4.66%, Abietic acid(C20:0) which score 3.53%, Capric acid (C10:0) which score 3.36 and Docosanoic acid (C22:0) which score 2.63%. These results are agreement with Prusinski (2017) who found that the fatty acids content of SLF were 54.3, 14.9, 8.57, 7.22, 4.14, 1.57, 0.81, 16.1, 1.59, 58.8 and 15.0% for oleic acid, linoleic acid, Palmitic acid, linolenic acid, Gadoleic acid, Stearic acid, Arachidic acid, Saturated fatty acid, Erucic acid, mono-saturated fatty acid and Poly un-saturated fatty acid, respectively.

TABLE 3. Minerals and vitamins content of sweet lupine flour (SLF).

Minerals (mg/kg)	SLF
P	6.8
K	4300
Ca	235
Mg	850
Na	150
Fe	1.31
Zn	1.6
Cu	0.32
Vitamins (mg/100 g)	
Thiamine	5
Riboflavin	4
Niacin	40

TABLE 4. Amino acids composition (g/100 g protein) of SLF compared to FAO/WHO reference (1991)

Essential Amino Acids (EAA)	SLF	FAO/WHO (1991)		
		Preschool Children	School Children	Adult
Valine	0.87	3.5	2.5	1.3
Methionine	0.59	2.5	2.2	1.7
Histidine	2.04	1.9	1.9	1.6
Threonine	1.20	3.4	2.8	0.9
Tyrosine	0.46	3.5*	2.2*	1.9*
Isoleucine	1.94	2.8	2.8	1.3
Leucine	1.64	6.6	2.4	1.6
Phenylalanine	1.23			
Lysine	1.52	5.8	4.4	1.6
Cystine	0.50			
Total (EAA)	**11.99**	**30**	**21.2**	**19.8**

* Tyrosine + Phenylalanine. **SLF**: Sweet Lupine Flour

Antioxidant activity of SLF

Table 6 showed the antioxidant activity of SLF with DPPH, ABTS and FRAP. Results clearly revealed that, SLF exhibited antioxidant activity with DPPH, ABTS and FRAP which recorded (19.82%, 38.27% and 13.68 µmol Fe^{+2}/g, respectively). These results are closed with (Ahmed, 2014 and Aniess et al., 2015) whose found that, antioxidant capacity of SLF with DPPH ranged from 20.6% to 20.7%, and anti-oxidant capacity with ABTS ranged from 41% to 43%. The antioxidant activity of SLF may have been due to that, lupine seeds have higher levels of phenolic and flavonoids than other legumes (Abdurrahman, 2014 and Angelika Król et.a.l, 2018).

Microbial analyses of SLF

The results showed that, the initial total microbial load of SLF which recorded 96X10^3. These results may be due to the treatments which using during preparation of SLF which decreased the amount of bacteria (Elsamani et al., 2014). Detection tests of pathogenic bacteria (*E. coli*, coliform, Enterobacter, Salmonella, Shigella and staphylococci), mold and yeast were carried out of SLF. All tested sample showed negative result for contamination by those. This result reflects the good sanitary condition followed during preparation, additionally, the anti-microbial activity of sweet lupine seeds Romeo et al. (2018).

Evaluation of ice milk supplemented with SLF

Physicochemical characteristics of ice milk samples

The physicochemical properties of ice milk supplemented with different levels of SLF were recorded in Table 7. There were significant differences between control ice milk and other samples. The data pointed out that, the TS content of control ice milk was 46.8% and increased significantly after adding SLF to 47.8%. The treatments contained 25% SLF had the highest content of TS. The data also revealed that, the protein content was ranged between 7.8% and 8.1% of soft ice milk samples. Protein in soft ice milk supplemented with different levels of SLF increased significantly (P<0.05) compared to the control sample. This may be due to that protein content of SLF higher than protein content of SMP. These results were consistent with observations of Abdullah et al. (2003);

TABLE 5. Fatty acids composition of sweet lupine flour.

Fatty acids	Carbon No.	Fatty acid of SLF, %
Capric acid	**C10:0**	3.36
Lauric acid	**C12:0**	0.09
Lauroleic acid	**C12:1**	0.25
Myristic acid	**C14:0**	--
Myristoleic acid	**C14:1**	0.15
Pentadecanoic acid	**C15:0**	--
Palmitic acid	**C16:0**	19.47
Margaric acid	**C17:0**	0.04
Stearic acid	**C18:0**	4.66
Oleic acid	**C18:1**	44.33
Linoleic acid	**C18:2**	19.29
γ- Linolenic acid	**C18:3**	--
Abietic acid	**C20:0**	3.53
Arachidonic acid	**C20:1**	0.90
Docosanoic acid	**C22:0**	2.63

SLF: Sweet Lupine Flour

TABLE 6. Antioxidant capacity of sweet lupine flour.

Antioxidant	SLF
DPPH, %	19.82
ABTS, %	38.27
FRAP, µmol Fe^{+2}/g	13.68

Pourahmad & Ahanian (2015); Aboulfazli et al. (2014); Atallah & Barakat (2017) and Aguilar-Raymundo and Velez-Ruiz (2018) who found that TS and protein content of soy ice milk increased with increasing the amount of soybean flour. Moreover, the fat content of control ice milk (5.1%) was increased by increasing the SLF to be 5.3% and 5.5% in sample with 15 and 25% SLF, respectively. These results agree with Abdullah et al. (2003) who showed that fat of soy ice milk content increased with increasing the amount of soybean flour. At the same trend ash content increased by increasing the added of SLF. The lowest ash content was recorded in control ice milk sample (1.5%) followed by ice milk with 5% SLF (1.6%) but no significant between other ice milk sample which recorded ash content (1.7%) which highest value noted in samples of ice milk. This may be due to the high content of ash in SLF (Table 2). These results agree with those achieved by Abdullah et al. (2003); Pourahmad & Ahanian (2015); Aboulfazli et al. (2014); Atallah & Barakat (2017) and Aguilar-Raymundo & Velez-Ruiz (2018) who reported that the ash content increased in treatments containing high amounts of soy flour.

While the carbohydrate content in Ice Milk at levels 15 and 25% SLF decreased by increasing the amount of added SLF significantly ($P>0.05$). It was 33% in control and decreased to be 32.9, 32.7 and 32.4% for ice milk with 5, 15, 25% SLF, respectively. These results agree with Abdullah et al. (2003) who reported that carbohydrate content of soy ice milk decreased by increasing the amount of soybean flour.

It could be seen from the same table that, replacement with different levels of SLF increased pH significantly ($P<0.05$) compared with control (6.6), and increased to 6.8 by increasing replacement ratios. Similar results were obtained by Abdullah et al. (2003) who reported that the pH value of soy ice milk was increased by adding soy flour. The same Table showed that different levels of added SLF has significant effect ($P<0.05$) on acidity of soft ice milk samples. The lowest value of acidity was 0.16% in samples with 5% SLF compared with control ice milk sample, which recorded 0.18%. Such conclusion may be related to pH value which was high in SLF at different levels. Those achieved by Abdullah et al. (2003) who reported that the acidity of soy ice milk decreased by adding soy flour.

From the obtained data in Table 7, it could be observed that, replacement with different levels of SLF in ice milk mixes increased over run significantly ($P<0.05$) compared with control. The overrun value in ice milk samples was increased by increasing levels of SLF, which ranged between 18.2% in sample with 5% SLF and 21.3% in samples with 25% SLF compared with control (12.5%). These results agreed with that found by Atallah and Barakat (2017) who cited that with increasing total solid, overrun increased and the highest amount of total solid can be observed in treatments containing the highest amount of soy flour. Also, the overrun increased when soy protein content increased. These results agree with Abdullah et al. (2003); Pourahmad and Ahanian (2015), Aboulfazli et al. (2014), Atallah & Barakat (2017), Aguilar-Raymundo & Velez-Ruiz (2018) and Zaki et al (2019).

Viscosity of lupine ice milk samples

The viscosity of ice milk mixes replacing SLF compared to control mix are shown in Fig. 1. The viscosity of the soft ice milk mixtures samples was determined at share rate (34-270)1/s. Results indicated that replacing with SLF had a significant effect on viscosity of prepared soft ice milk. Interestingly, 25% SLF sample had the highest viscosity at share rate 34 1/s, which recorded 1656 mpa.

TABLE 7. Physiochemical characteristics of lupine ice milk replaced with different levels of SLF

Ice milk samples	TS%	Protein, %	Fat, %	Ash, %	Carbohydrate, %	pH	Acidity, %	Over run%
Control	46.8±0.18^{c}	7.8±0.01^{b}	5.1±0.06b^{c}	1.5±0.10^{c}	33.0±0.15^{a}	6.6±0.07^{b}	0.18±0.01^{c}	12.5±0.10^{e}
				Ice mill with SLF, %				
5	47.6±0.18^{b}	8.1±0.01^{a}	5.1±0.2b^{c}	1.6±0.04^{b}	32.9±0.11^{a}	6.8±0.04^{a}	0.16±0.01^{e}	18.2±0.01^{cd}
15	47.7±0.18^{a}	8.1±0.03^{a}	5.3±0.06^{b}	1.7±0.03^{a}	32.7±0.15^{b}	6.8±0.02^{a}	0.17±0.00^{d}	18.2±0.01^{cd}
25	47.8±0.16^{a}	8.05±0.01^{a}	5.5±0.10^{a}	1.7±0.04^{a}	32.4±0.14^{c}	6.8±0.03^{a}	0.17±0.00^{d}	21.3±0.01^{b}

Means followed by different subscripts within column are significantly different at the 5% level, (n= 3),

SE: standard error **SLF**=sweet lupine flour **TS%** = Total solid

Many researchers demonstrated that, viscosity of ice milk mixture containing soy flour increased due to higher content of soy flour protein and capacity of soy flour protein for interaction and binding with water (Atallah and Barakat, 2017). Moreover, lupine proteins have higher water holding capacity; this ability is one of the most important functional properties of lupine based ingredients (El-sayed, 2013) which, the protein content of SLF was (34.76%). These results agree with (Abdullah et al., 2003, Battawy et al., 2019 and Zaki et al., 2019).

Meltdown of ice milk samples

The slow meltdown, slow serum drainage, good shape retention, and slower foam collapse are some of the desired important quality parameters of ice milk (Goff et al., 1993). Table 8 showed the meltdown of frozen ice milk containing different levels of SLF compared with control ice milk. After 10 min, samples with different levels of SLF had significant effect on the amount of melted ice milk compared with control ice milk. Meltdown reduced from 5.2g/100 g in Control to 2.1 g/100 g in samples with 25% SLF. After 20 min on ward, the lowest amount of melted ice milk was significantly ($P<0.05$) in sample with 25% SLF (10.0g/100 g) compared to control ice milk (22.9g/100 g). After 40 min the highest meltdown value was recorded in control (43.1g/100 g). These results are in agreement with those found by other researchers such as Moeenfard & Tehrani (2008) and Alakali et al. (2009) whose found that melting resistance increased as mix viscosity and overrun increased. Additionally, Badawi et. al. (2002) mentioned that, the lowest rate of melting could be attributed to the increase in mix viscosity. Sofjan & Hartel (2004) found that ice milk with low overruns melted quickly, whereas ice milk with high overruns began to melt slowly and had a good melting resistance. This slower melting rate in the ice milk with high overruns was attributed to a reduced rate of heat transfer due to a larger volume of air. Additionally, emulsifiers that promote destabilization and partial coalescence of fat globules greatly decrease the melting rate of ice milk and promote shape retention (Muse and Hartel, 2004).

Texture analysis profile of ice milk with different levels of SLF

The data presented in Table 9 shows the texture profile analysis of ice milk samples. Values of Hardness, Springiness, Cohesiveness, Gumminess and Chewiness for control ice milk sample was 2049.49 gf, 0.99 mm, 0.88, 1947.02 gf and 1927.50 gf mm, respectively, while in samples with 25% SLF was 3100.05 gf, 0.70 mm, 1.31, 3847.73 gf and 3001.22 gf mm, respectively.

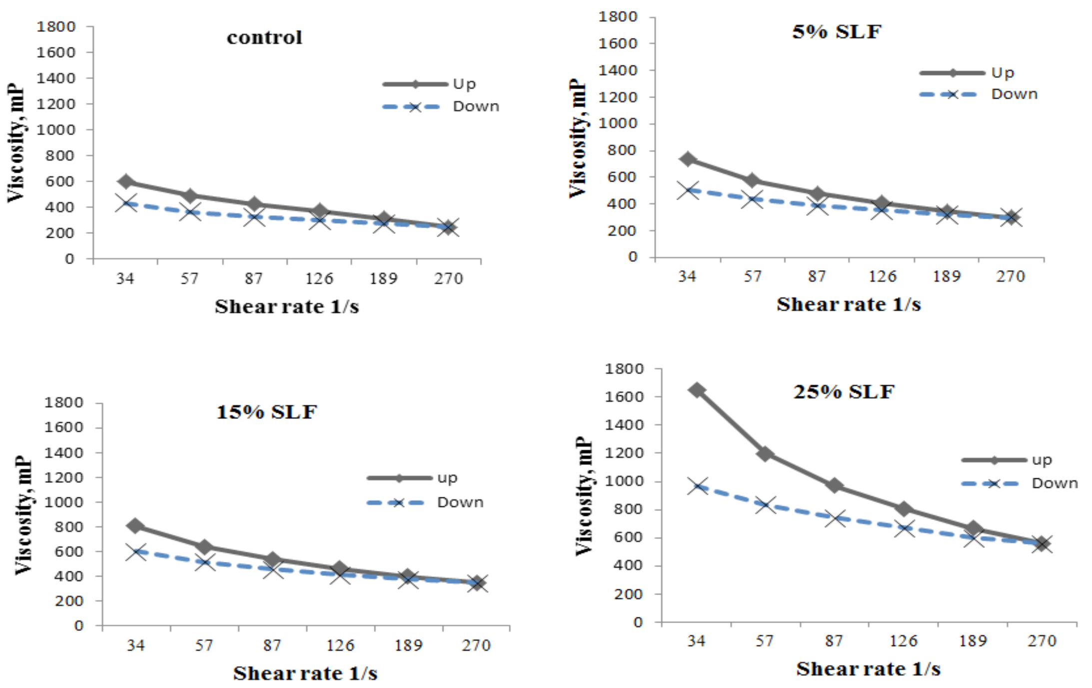

Fig. 1.Viscosity of ice milk supplemented with different levels of SLF

TABLE 8. Meltdown of ice milk replaced with different levels of SLF.

Samples	Meltdown of frozen ice milk (g/100g)			
	10 min	20 min	30 min	40 min
Control	5.2±0.22[a]	22.9±0.20[a]	33.8±0.34[a]	43.1±0.36[a]
		with SLF%		
5	3.0±0.12[bc]	14.4±0.37[b]	25.1±0.15[b]	39.2±0.26[b]
15	2.8±0.0.06[c]	12.9±0.26[c]	24.3±0.51[bc]	38.6±0.37[bc]
25	2.1±0.11[d]	10.0±0.10[d]	23.1±0.13[d]	37.0±0.15[c]

[a, b...] Means followed by different subscripts within column are significantly at the 5% level, (n= 3)

SLF=sweet lupine flour

TABLE 9. Texture profile analysis of ice milk with different levels of SLF.

Samples	Hardness, gf	Springiness, mm	Cohesiveness	Gumminess, gf	Chewiness, gf*mm
Control	2049.49	0.99	0.88	1947.02	1927.50
					with SLF, %
5	2347.98	0.82	0.95	2066.22	1693.59
15	2937.19	0.78	1.01	3100.51	2817.94
25	3100.05	0.70	1.31	3847.73	3001.22

SLF: Sweet Lupine Flour

From tabulated data, it could be observed that samples with SLF at different concentration affected on the hardness of the ice milk. This may be due to the micro-structure change, namely phase volume, ice crystal size, and fat stability in the ice milk (Muse & Hartel, 2004). This finding was in line with the previous discussion that the replacement and the concentration of SLF affect the total solid, determining amount of ice formation. With high water content, ice crystal is packed closer to each other (Muse, 2003). A larger force is required to be applied to the surface of the ice milk that is being categorized as hard. Besides hardness, samples with deferent levels of SLF also significantly increased the cohesiveness, Gumminess and Chewiness of result ice milk samples. Higher cohesiveness represents an improvement in sustainability of the ice milk as it is compressed in the mouth before it breaks (Radoĉaja et al., 2011). The sustainability of ice milk with SLF in the mouth was also supported by the increment of gumminess; gumminess is defined as the product of hardness and cohesiveness. It refers to the energy required in disintegrating the ice milk before swallowing. However, there was no significant difference in springiness for all the ice milk samples; similar amounts of force were required for the ice milk from the end of the first bite to the start of the second bite (Elsamani, 2016).

Microbiological analysis of ice milk samples:

Total bacterial count of microorganisms (TBC) was assessed in ice milk control and ice milk replaced with different levels of SLF and the resultant data are shown in Table 10. The recorded results showed that, the initial total microbial load of ice milk control sample was 124x10^3 cfu/g. From the same obtained data, it could be observed that TBC in ice milk was decreased by increasing the levels of SLF. These results were agreement with Romeo et al. (2018) who reported that sweet lupine seed had highest anti-microbial activity. From the same table *Psychrotrophic bacteria* was assessed in ice milk control and replaced with different levels of SLF. The results showed that *Psychotropic bacteria* count of control ice milk was 35 x10^{3}cfu/g. *Psychotropic bacteria* were not detected in all ice milk samples with SLF.

Detection tests of pathogenic bacteria *E. coli*, coliform, *Enterobacter*, *Salmonella*, *Shigella* and staphylococci were carried out in ice milk control and ice milk with different levels of SLF. All tested samples showed negative result for contamination by those bacteria at different levels of SLF and ice milk control. This result reflects the good sanitary condition followed during preparation, additionally, the anti-microbial activity of sweet lupine seeds (Romeo et al., 2018). Mold and yeast count detection tests were carried out in ice milk control and ice milk replaced with different levels of SLF. All tested samples showed negative result for contamination by those microorganisms at different levels of replaced with SLF and ice milk control. This result reflects the good sanitary condition followed during preparation, additionally, the anti-microbial activity of sweet lupine seeds. These results agree with Atallah and Barakat (2017) who is reported that *E. coli, Salmonella* and *Shigella* not detected in all samples of ice milk with soy flour. This can be attributed to the hygienic condition granted during process soy flour had highest anti-microbial activity.

Sensory evaluation of ice milk samples

The sensory evaluation of ice milk with different levels of SLF was presented in Table 11. It was noted from the obtained data that the flavor of ice milk samples was influenced by replacing SLF. The flavor mean score of SLF samples were significantly decreased for most treatments compared with control, where the flavor of sample with 15% SLF was the lowest of score (16.9) followed by sample with 25% SLF (17.6) for all samples including control (19.3). These results may be due to high ratio of fiber in SLF, which there are mainly types of raffinose, stachyose and insoluble sugars. This result is found in agreement with Atallah and Barakat (2017). From the same Table (11) mean score for color pointed out that the ice milk with 15 and 25% SLF have the lowest scores. With regard to texture of studied ice milk treatments, results revealed that, there were slightly significant different between the control sample and some treatments. Where, ice milk with 15 or 25% SLF has the lowest scores with no significant differences. The melting properties mean score of ice milk with different levels of SLF showed significant differences for all samples compared to control sample. Ice milk with 15% SLF had the lowest significant mean score. The mean score of overall acceptability of ice milk samples exhibited comparable score between ice milk with SLF at different levels and control sample, where declared that the replacement with SLF in ice milk of significant differences from the control, this result was noted

TABLE 10. Microbial count of ice milk samples

Treatments	*Psychrotrophic* count, X10^3 cfu/g	TBC, X10^3 cfu/g
Control	35	124
	with SLF, %	
5	ND	30
15	ND	14
25	ND	7

ND = Not detected **TBC**= total bacterial count **SLF**=sweet lupine flour.

TABLE 11. Sensory evaluation of ice milk samples.

Samples	Mean±SE				
	Flavor (20)	Color (10)	Texture (40)	Melting properties (30)	Overall acceptability (100)
Control	19.3±0.95^a	10.0±0.0^a	38.9±3.14^a	28.5±3.17^b	96.7±6.12b^b
			with SLF, %		
5	19.3±1.57^a	9.9±0.32^a	38.8±2.10^a	29.3±1.56^a	97.3±4.71^a
15	16.9±2.02^c	9.5±0.85^b	37.9±2.81^b	26.2±3.76^d	90.2±7.32^d
25	17.6±1.84^{bc}	9.6±0.84^b	37.4±2.22^b	27.2±3.42^c	92.8±5.35^c

$^{a,b,...}$ Means followed by different subscripts within column are significantly at the 5% level, (n= 3)
SLF: sweet lupine flour.

for most treatments under investigation. It could be concluded that replaced with 5% SLF gave good sensory characters comparable to that of 15%, 25% SLF and control samples. These results are agreement with Atallah & Barakat (2017), who's reported that soy flour replacement up to 50%, has no effect negative on sensory properties of soy ice milk.

Conclusion

Ice milk samples containing different levels of sweet lupine flour (SLF) would serve as function food because of the high superior nutritionally compared with ice milk without SLF. The best ratio to replacement was sweet lupine flour up to 15%.

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