The Scientific Journal of King Faisal University

Basic and Applied Sciences

The Effect of Sweet Lupine Seed Hulls on the Probiotic Viability of Strained Yogurt

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LINK	RECEIVED	ACCEPTED	PUBLISHED ONLINE	ASSIGNED TO AN ISSUE
https://doi.org/10.37575/b/agr/210061	08/09/2021	30/11/2021	30/11/2021	01/12/2021

NO. OF WORDS	NO. OF PAGES	YEAR	VOLUME	ISSUE
4639	5	2021	22	2

ABSTRACT

Recent advances in gut microbial flora research have shown the health benefits of probiotic bacterial strains on the small and large intestines. These strains, particularly Bifidobacterium, offer health advantages, including obesity, atopic diseases, inflammatory bowel diseases, and intestinal cancers in the human body. However, food components, additives, and processing-related factors can have an impact on probiotic survival. As a result, adding appropriate supplements to increase the viability of probiotics may be necessary for some food products. This study investigated the influence of sweet lupine (*Lupineus albus* L.) seed hulls (SLSH) on the probiotic viability of strained yogurt. Traditional strained yogurt prepared using Bifidobacterium bifidum and the two starter culture strains Streptococcus thermophiles, and Lactobacillus bulgaricus. The strained yogurt was supplemented with different concentrations of SLSH (1, 2, and 3%). Over 7 to 14 days of cold storage, the total bacterial cells were enumerated. The result showed B. bifidum strains increasing more than double with a slight decrease in the starter culture strains. The overall acceptance of strained yogurt supplemented with 1% SLSH was convenient, compared to 2% and 3% of SLSH.

KEYWORDS

Probiotics, viability, lupine, B. bifidum, strained yogurt

CITATION

Al-Asmari, F. (2021). The effect of sweet lupine seed hulls on the probiotic viability of strained yogurt. *The Scientific Journal of King Faisal University: Basic and Applied Sciences*, **22**(2), 125–9. DOI: 10.37575/b/agr/210061

1. Introduction

Over the last few decades, the consumption of probiotics has increased due to their therapeutic and beneficial health impacts on the host when consumed adequately. The effect of probiotics, including intestinal microflora improvement, minimizing cholesterol level in the blood, increasing immune response, and strengthening the body's natural defense mechanisms, has been reported (Chen *et al.*, 2019; Fernandez and Marette, 2017; Jackson and Jewell, 2019; Lau and Chye, 2018; Mani-López *et al.*, 2014). The bacterial strains belonging to Bifidobacteria and Lactobacilli, the most important probiotic microorganisms allied with the human digestive tract, have been introduced in current food products such as yogurt, ice cream, and cheese (Bayar *et al.*, 2018; Moineau-Jean *et al.*, 2019; Mollakhalili *et al.*, 2017).

The most commonly used probiotics are *Lactobacillus acidophilus* NCFB 1748, *Lactobacillus casei* Shirota, *Lactobacillus johnsonii* LA1, and *Bifidobacterium bifidum*. These organisms have shown significant health effects when consumed (Founden *et al.*, 2000). There is a minimum amount of live bacterial cells consumed to observe the beneficial effect of probiotics. The suggested number of daily intake is between 108 and 109 CFU/g (Knorr, 1998). Likewise, regular yogurt contains about 108 to 1011 CFU/g of bacterial cells (Vanderhoof and Young, 1998).

The quantity of bacterial cells in commercial products is often not as much concern as the probiotic instability during the storage period (Sodini *et al*, 2002). Several studies have examined improving the viability of probiotics, but rarely are studies carried out to explore the effect of sweet lupine seed hulls on probiotic viability. A study conducted by Martinez-Villaluenga *et al.* (2006) found that a significant growth level of *Bifidobacterium* spp. was observed when a lupine kernel fiber diet was ingested and compared with the control. Another study carried out by Smith *et al.* (2006) showed that the Raffinose family of oligosaccharides extracted from lupine seeds acted as prebiotics in fermented milk probiotics.

Sweet lupine (*Lupineus albus* L.), known as white lupine, is an annual legume cultivated across the continents, particularly in the Mediterranean basin and South America. It is rich source of protein (34% - 44%), non-starch polysaccharides (30–40%), crude fiber (15% - 18%) and oil (5–15%). Also, it has been used in folk medicine to treat skin and diabetes diseases (Santiago Quiles *et al.*, 2010). Additionally, when compared to many other legumes, lupine has a lower level of antinutritional factors such as lectins and protease inhibitors that reduce protein digestibility (Carvajal-Larenas *et al.*, 2016). The current food applications are utilizing lupine flour and proteins in bakery, and lupine hulls in meat products as a fat replacer and a dietary fiber source (Gulewicz *et al.*, 2008; Visvanathan *et al.*, 2020).

Several studies have identified various phytochemicals in the lupine hulls; antioxidant isoflavone genistein, apigenin derivatives with antioxidant properties (Ranilla *et al.*, 2009), vitamin E isomers α-, β-, γ- and δ-tocopherol (Torres, Frias, and Vidal-Valverde, 2005), anti-tumor phytosterols (Bhardwaj *et al.*, 2004), anti-inflammatory phytosterol β-sitosterol (Loizou *et al.*, 2010) in the lupine hulls. Therefore, the study aimed to evaluate the effect of sweet lupine seed hulls (SLSH) on probiotic viability in strained yogurt products.

2. Materials and Methods

2.1. Preparation of Sweet Lupine Seed Hulls (SLSH):

The collection of the sweet lupine seeds was from local shops in Cairo, Egypt. The seeds were washed and soaked at 5°C for about 5 hours, then blanched at 90°C for one hour in boiling water and, cooled using tap water. The seeds then peeled, and the hulls dried at 43°C for 30 hours. The hulls were ground into a fine powder using a kitchen grinder.

2.2. Bacterial Strains:

Commercial freeze-dried yogurt starter cultures strains of *Streptococcus thermophilus* TH-4, *Lactobacillus bulgaricus* YCX31 (freeze-dried DVS-type), and a single probiotic strain, *Bifidobacterium bifidum* UABb-10, were obtained from Chr. Hansen, Denmark.

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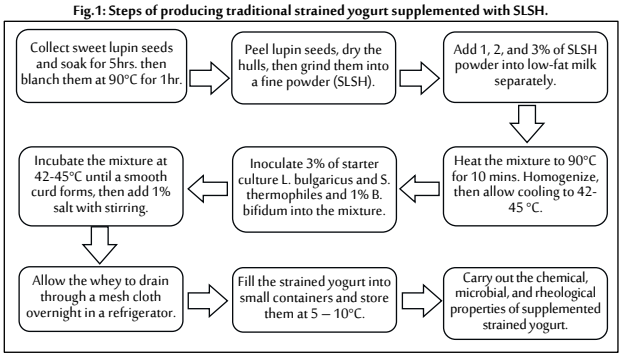
2.3. Preparation of Lupine Hulls Strained Yogurt:

Strained yogurt was prepared using a traditional method of eliminating excess whey from low-free milk yogurt described by Yamani and Abu-Jaber (1994). Three samples of fresh milk—each containing 2 L—were supplemented with different concentrations of SLSH as (1%, 2%, and 3%) represented as (T_1, T_2, and T_3), respectively. All mixtures were heated to 90°C for 10 min and homogenized to mix all ingredients thoroughly. The heated mixtures were cooled to 42°C, while 3% of starter culture and 1% of *B. bifidum* were inoculated. Upon attaining pH 4.6, the mixture was cooled to 7°C to stop the fermentation process. The formed yogurt was placed in cloth bags and left to drain by gravity at 5 – 10°C overnight and then stored in the refrigerator. Physicochemical, microbiological, and organoleptic properties were analyzed and assessed after storage days one, seven, and 14 (Fig. 1).

Fig.1: Steps of producing traditional strained yogurt supplemented with SLSH.



2.4. Physicochemical Analysis:

Physicochemical analyses of SLSH including pH, and acidity, were carried out according to official analysis methods (AOAC, 2010). Acetic and lactic acid content was measured by HPLC according to Akalin *et al.* (2002). For extraction of acids, 4 g of strained yogurt sample was diluted to 25 mL with 0.1N H_2SO_4, homogenized, and centrifuged at 5000x for 10 min. The aliquots 2 mL of supernatant were filtered through Whatman 0.20 µm and analyzed through HPLC as described by Bevilacqua and Califano (1989).

2.5. Organoleptic Assessment:

Appearances, consistency, flavor, and overall acceptance parameters of strained yogurt, supplemented with different concentrations of SLSH, were evaluated by ten panelists.

2.6. Enumeration of Microbial Cells:

For the enumeration, appropriate dilution of each sample was plated, in triplicates, using Petri dishes. Strains *L. bulgaricus* and *S. thermophilus* were enumerated on MRS and the *S. thermophilus* agars, respectively. The *B. bifidum* was enumerated, using a double-layered technique, on Lithium chloride-sodium propionate agar as described by Lapierre, Undeland, and Cox, (1992). Incubation of the plates was at 37°C for 48 hours.

5.7. Statistical Analysis:

All data are presented as mean ±SD for three replicates for each sample. The comparisons were performed using the one-way analysis of variance of the SPSS software. The differences among the means at $P< 0.05$ were compared, using Duncan's multiple analysis methods.

3. Results and Discussion

3.1. Physicochemical Analysis:

The physicochemical characteristic of strained yogurt supplemented with different concentrations of SLSH, were illustrated in Table 1. There were no significant differences in pH values between the control and the supplemented samples (T_1, T_2, and T_3) over the same storage conditions. During the first seven days of storage the strained yogurt stored at 5°C to 10°C, showed the pH value dropped from 4.63 to 3.33 in (T_1), from 4.68 to 3.41 in (T_2), and from 4.84 to 3.48 in (T_3), compared to the control that dropped from 4.58 to 3.41. In contrast, as pH values were dropped during the first 7 days, a slight increase was noted on the 14th day of storage. This result agrees with the previous study conducted by Jakubowska and Karamucki (2020) on natural yogurt. He observed a slight increase in the pH value after 21 days of storage from 4.13 to 4.16. The result obtained by Eissa *et al.* (2010) differed from the above result, showing a steady decrease in the pH value with prolonged storage time of natural yogurt. This figure may attribute to the high water-binding capacity of lupine insoluble fiber (Turnbull *et al.*, 2005), which can dilute the produced acidity.

An assessment was made of the acidity levels of the strained yogurt supplemented with different concentrations of SLSH. In the (T_1) sample, the acidity increased from 1.35 to 2.13, from 1.31 to 2.06 in T_2, and from 1.30 to 1.89 in (T_3.) The acidity of the control sample increased from 1.28 to 1.62 under the same conditions. The result showed no significant differences between the control sample and the supplemented samples. The increase of acidity observed after 14 days of storage could be due to the phase change of calcium phosphate from the soluble phase to the colloidal one, which resulted from the liberation of hydrogen ions (Hattem *et al.*, 2011). This result is consistent with the report published by Alqahtani *et al.* (2021), who produced strained yogurt with sweet lupine husk with no significant differences in pH values and acidity between the control and the supplemented samples. In general, the acidity of yogurt is affected by the activity of its microflora, which is diverse in its activities of acidifying, proteolytic and lipolytic processes (Jakubowska and Karamucki, 2020)

Also, the lactic acid amount produced in strained yogurt was increased from 4.49 mg/kg in control to 5.96 mg/kg in T_2 during 14 days of storage. Although the capacity of the hull's fiber in diluting the form acidity, the amount of lactic acid formed in T_2 was significant. Although lactic acid production in a fermented dairy product is an essential indication for starter culture viability, in this case, most of the lactose that provides a carbon source for the microorganisms was removed by eliminating excess whey during the strained yogurt processing. In addition, the acetic acid value was increased in all supplemented samples compared to the control sample over the storage period.

Table 1: The physicochemical characteristics of strained yogurt supplemented with different concentrations of sweet lupine seed hulls (SLSH)
Each value represents mean (n =3) ±SD

Chemical properties	Day	Control	T_1 SLSH (1%)	T_2 SLSH (2%)	T_3 SLSH (3%)
pH	1	4.58 ±0.02	4.63 ±0.02	4.68 ±0.02	4.84 ±0.02
	7	3.41±0.02	3.33±0.02	3.41±0.02	3.48±0.02
	14	3.78±0.02	3.75±0.02	3.68±0.02	3.81±0.02
Acidity %	1	1.28 ±0.16	1.35 ±0.01	1.31 ±0.02	1.30 ±0.01
	7	1.62 ±0.02	2.13 ±0.05	2.06 ±0.05	1.89 ±0.01
	14	2.23±0.02	2.05±0.08	2.18±0.01	1.934±0.01
Acetic Acid (mg/kg)	1	0.39 ±0.005	0.55±0.01	0.61±0.01	0.41±0.02
	7	0.60±0.01	1.30±0.01	1.25±0.01	0.90±0.01
	14	0.80±0.01	1.21±0.01	1.22±0.01	1.121±0.02
Lactic Acid (g/kg)	1	3.44±0.01	4.55±0.01	4.96±0.01	4.98±0.02
	7	4.03±0.06	5.40±0.005	5.40±0.01	5.20±0.01
	14	4.49±0.01	5.95±0.01	5.96±0.15	5.93±0.05

3.2. Bacterial Enumeration:

The bacterial enumerations of *S. thermophilus*, *L. bulgaricus*, and *B. bifidum* was illustrated in Table 2. The microbial cell counts of *S. thermophilus* fluctuated during the storage period regardless of the concentration of SLSH. The initial counts of (T_1), (T_2), and (T_3) were 7.5,

7.4, and 7.2 Log_{10} CFU/g respectively, then increased to 8.0, 8.0, and 8.2 Log_{10} CFU/g respectively on day 7, then reduced again to 7.0, 6.9 and 6.5 Log_{10} CFU/g respectively on day 14. Meanwhile, the control sample showed a steady decrease in microbial cell counts during the storage period. Similarly, *L. bulgaricus's* initial count declined from 8.7, 8.4, and 8.1 Log_{10} CFU/g in (T_1), (T_2), and (T_3) samples to 7.9, 7.5, and 7.3 Log_{10} CFU/g, respectively after 14 days. Meanwhile, the count of the control sample also declined from 8.5 to 7.8 Log_{10} CFU/g along the storage timeline.

Table 2 shows the variation in the microbial cell count of strained yogurt supplanted with SLSH. The maximum decrease in microbial cell counts was observed at 14 days of storage after 3% of SLSH was added. The microbial cell counts of *L. bulgaricus* decreased more slowly than *S. thermophilus* counts under the same conditions. Therefore, *Lactobacillus* bacteria showed more tolerance towards the storage temperatures (*i.e.*, 4 – 5°C) than *Streptococcus* bacteria. An explanation is the one-way antagonistic effects of *L. bulgaricus* against other starter cultures, particularly at higher temperatures (> 5 °C). At temperatures ranging from 5 to 10°C, the *L. bulgaricus* strains grow faster and produce lactic acid and hydrogen peroxide. These substances are the most important viability-reducing factors during cold storage (Mortazavian and Sohrabvandi, 2006; Shah *et al.*, 1995). In general, the increase in storage temperature increased the metabolic activities of bacterial cells, increasing cell death (Mortazavian *et al.*, 2006).

Sady *et al.* (2007) reported that the concentration of *S. thermophilus* and *L. bulgaricus* in natural yogurt was increased slightly during the first three days, then decreased to the lowest level after 14 days storage. Also, the reduction in *S. thermophilus* was higher than *L. bulgaricus*, as *S. thermophilus* is less tolerant to high acidity, while *L. bulgaricus* strains can survive in an environment with a pH below 4.0 (Jakubowska and Karamucki, 2020). Generally, the yogurt whey containing large numbers of *S. thermophilus*, and *L. bulgaricus*, was lost during the production of the strained yogurt. Thus, the bacterial count that remained in strained yogurt is usually similar to those in regular yogurt.

The viability of *B. bifidum* was assessed in the strained yogurt, supplemented with different concentrations of SLSH. The microbial cell counts of *B. bifidum* obtained from the (T1) sample increased significantly compared to the control. The microbial cell counts were increased more than double from 0.2 Log_{10} CFU/g in the control sample to 0.6 Log_{10} CFU/g in (T_1) after 14 days of storage. Whereas microbial cell counts of (T_2) and (T_3) almost remained the same during the storage period. The improvement of microbial cell counts *B. bifidum* may attribute to the carbohydrate levels in SLSH or its alkaloids (Al-hamdani *et al.*, 2015).

The result agrees with previous studies on oat mash, showing an increase in viability and survival of probiotics (Akalın *et al.*, 2012; Champagne *et al.*, 2011). A study carried out by Phuapaiboon *et al.* (2013) revealed that the probiotic viability of natural yogurt supplemented with pineapple improved during storage. Elsanhoty and Ramadan (2018) carried out a study that found the probiotic viability of yogurt supplemented with barley β-glucan improved over the storage period. Also, a recent study conducted by El-Sattar *et al.* (2020), who used guava seeds powder in natural yogurt, found an increase in *B. bifidum* and *L. acidophilus* viability during storage compared to control samples. Therefore, adding a small amount of SLSH (*i.e.*, 1%) to fermented dairy products may improve the probiotic viability during the storage period and, further study on the action mechanism of SLSH may be needed.

Table 2: Bacterial count of strained yogurt samples supplemented by different concentrations of sweet lupine seed hulls (SLSH) over the storage period.

Bacteria strain	Day	Control	T1 (1% SLSH)	T2 (2% SLSH)	T3 (3% SLSH)
S. thermophilus (Log_{10} CFU/g)	1	8.3	7.5	7.4	7.2
	7	8.0	8.0	8.0	8.2
	14	7.1	7.0	6.9	6.5
L.bulgaricus (Log_{10} CFU/g)	1	8.5	8.7	8.4	8.1
	7	8.3	8.3	8.1	7.7
	14	7.8	7.9	7.5	7.3
B. bifidum (Log_{10} CFU/g)	1	1.3	2.7	2.3	2.1
	7	1.6	2.0	0.6	0.4
	14	0.2	0.6	0.3	0.2

3.3. Organoleptic Assessment of Strained Yogurt Supplemented with SLSH:

An organoleptic assessment of strained yogurt supplemented with different concentrations of SLSH is illustrated in Table 3. In general, the results did not show a statistically significant effect of using 1% and 2% SLSH on stained yogurt.

Appearance scores slightly decreased with the increase of SLSH concentration during the storage period. The score of the control sample ranged from 8.33 to 7.33 during storage. The score of the (T_3) sample decreased during storage from 6.17 on day 1 to 5.50 on day 14. Thus, a lower concentration of SLSH (*i.e.*, T_1 and T_2) showed a better score. Also, an increase of SLSH concentration was led to a slight increase in the consistency in (T_1) and (T_2) samples. By contrast, the consistency of the strained yogurt showed a decrease in the (T_3) sample compared to the control sample. So, a suitable amount of SLSH in strained yogurt would increase its consistency because of its capacity to act as a stabilizer. The SLSH as polysaccharides can hold water and bond between the particles (Hussein *et al.*, 2011). In turn, adding more SLSH may cause grittiness in texture.

Furthermore, the flavor score of strained yogurt slightly decreased from 7.83 in the control sample to 7.17 in the (T_1) sample over 14 days of storage. However, the flavor scores declined after higher concentrates of SLSH were added under the same conditions. This reduction is probably because of the adverse effects of curd fiber presented in SLSH which reflect the strained yogurt's flavor (Fernández-Garía *et al.*, 1998; Alqahtani *et al.*, 2021).

Overall, the sensory evaluation of strained yogurt supplemented with SLSH varied depending on the concentration of SLSH. In general, the effect of 1% SLSH was convenient in terms of appearance, consistency, and flavor over the storage period. However, a higher concentration of SLSH led to lower acceptance in the same previous parameters.

Table 3: Organoleptic assessment of strained yogurt supplemented with different concentrations of sweet lupine seed hulls (SLSH)

Parameters	Day	Control	T_1 (1% SLSH)	T_2 (2% SLSH)	T_3 (3% SLSH)
Appearance	1	8.33 ±0.52	7.50±0.55	7.33±0.52	6.17±0.75
	7	7.50±0.55	7.17±0.75	6.50±0.55	6.33±0.52
	14	7.33±0.52	6.33±0.52	6.33±0.52	5.50±0.55
Consistency	1	8.67±0.52	8.67±0.52	8.50±0.55	7.50±0.55
	7	8.00±0.63	8.67±0.52	8.17±0.75	7.17±0.75
	14	8.00±0.63	8.17±0.41	8.17±0.75	7.00±0.63
Flavor	1	7.50±0.84	7.43±0.75	7.50±0.55	6.33±0.82
	7	7.33±0.82	7.33±0.52	6.50±0.55	6.50±0.55
	14	7.83±0.41	7.17±0.75	6.50±0.55	6.00±0.63
Overall acceptance	1	8.17±0.41	7.17±0.41	6.67±0.52	6.67±0.82
	7	8.17±0.41	7.17±0.75	6.17±0.75	6.33±0.82
	14	7.33±0.52	6.83±0.75	6.33±0.52	5.83±0.75

Each value represents mean (n=6) ±SD

4. Conclusion

This study presented the effect of sweet lupine seed hulls (SLSH) on the probiotic viability of strained yogurt. Total acidity, acetic acid, and lactic acid production in strained yogurt increased across all concentrations of SLSH. Over the 14-day storage period, the *S. thermophilus* count decreased with an increasing amount of SLSH, as opposed to a slight increase in the *L. bulgaricus* count from 7.8 Log_{10} CFU/g in the control sample to 7.9 Log_{10} CFU/g in the (T_1) sample. This decreased again over the remaining concentrations under the

same conditions. The *B. bifidum* strain's count more than doubled after 14 days of storage, from 0.2 Log10 CFU/g in the control sample to 0.6 Log10 CFU/g in the (T_1) sample. While the rest of the SLSH concentration showed no improvement in B. bifidum viability. The sensory evaluation of strained yogurt supplemented with SLSH including, appearance, consistency, and flavor, were consistent with 1% SLSH. A higher concentration of SLSH may cause poor appearance and flavor.

Biography

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