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Improving the quality of free-fat bifidus milk using some plants' seeds

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ABSTRACT:

Synbiotics are food products that contain both prebiotics and probiotic microorganisms, wherein prebiotics encourage the growth of probiotics. In this study, The free-fat synbiotic Bifidus milk was improved by adding flaxseeds (FS), sunflower seeds (SS) or pumpkin seeds (PS) in powder form as prebiotics for Bifidobacteria (Bifidobacterium lactis and Bifidobacterium bifidum). Chemical analysis, microbiological examination including the viability of Bifidobacteria, synersis and sensory attributes were determined. The lowest fat, protein and fibers content were recorded by the control while, the highest fat content was for fermented milk supplemented with 4% SS. The highest protein content was reported for treatment supplemented with 4% PS followed by milk supplemented with 4% SS. The pH value decreased as the seeds concentrations increased and the lowest pH value was for Bifidus milk enriched with 4% FS powder at 21 days

The viability of Bifidobacteria increased during storage to reach the highest levels at day 14, and then it decreased. The biggest count was for treatments enriched with SS powder. All treatments which contained seeds have lower TVC numbers than control when fresh and either across the storage. The treatment supplemented with 4% FS recorded the lowest Syneresis value (2 ml/100ml). Sensory evaluation's results showed that the highest total scores were recorded by Bifidus milk enriched with 2 and 4% pumpkin seeds.

So, it can be recommended to enrich fermented dairy products with plants' seeds as prebiotics to enhance the nutritional value, overall acceptability and the viability of probiotics used in fermentation.

KEYWORDS: Bifidus milk, flaxseeds, sunflower seeds, pumpkin seeds, prebiotic

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1. INTRODUCTION:

Synbiotic foods are those that contain both probiotics and prebiotics, which may improve human health. The favorable benefits of synbiotics on the host have been linked to the encouragement of beneficial bacterial growth, the activation of various health-promoting bacteria's metabolism, and/or the survival of nutrientrich microbial communities in the digestive system (Shaghaghi et al., 2013 and Kearney & Gibbons, 2018; Pourjavid et al. 2022).

Probiotics are live microorganisms that, when taken in the right amounts, boost customers' health (Afzaal et al., 2019; Zendeboodi et al., 2020). Numerous studies have discussed the possible therapeutic benefits of probiotics, including improved innate immune response, lowered cholesterol, and therapy of gastrointestinal disorders (Miremadi et al., 2014; Bron et al., 2017; Han et al., 2020).

Probiotics use prebiotic compounds, which are indigestible and non-viable dietary components, as energy sources to increase their activity or development. Several studies have supported the synergy of probiotics and prebiotics in synbiotic foods (Fahimdanesh et al., 2013; Markowiak and Liewska, 2017; Mohanty et al., 2018; Xavier-Santos et al., 2022).

Probiotics refer variety to а of microorganisms, including bacteria. Bifidobacteria is a probiotic that has a number of beneficial benefits, including those that lower cholesterol, reduce lactose intolerance, prevent cancer, and modulate the immune system. (Yakoob and Pradeep, 2019; Lee et al., 2020). Bifidobacterium bifidum grew better in milk under anaerobic conditions than standard strains. This strain gave the best results when added at 5-10% to milk and when stored at pH 5.0-5.3, and storage at lower pH adversely affected growth and acid production. Also, B. lactis BB-12 is a widely used probiotic strain in

food manufacture like fermented milk, yogurt and ice cream because of its viability (Bozanic et al. 2011; Plessas et al. 2012; Jungersen et al. 2014; Yakoob and Pradeep 2019). In addition to other nutritious elements, drinking bifidus milk that contains a significant amount of this organism (10^8 cfu/g) will also provide L (+)-lactic acid, an antibacterial factor, and live Bifidobacteria (Misra and Kuila, 2001). Natural prebiotics from sources, such as grains, seeds and vegetables, are consumed in everyday meals and are considered as the prebiotic compounds. most important Flaxseed is reported to be full of nutrients such flavonoids, minerals, vitamins, and carbs, which may have a variety of positive effects on human health. In addition, it has alpha-linolenic acid (ALA), a precursor to omega-3 fatty acids, 20% protein, 7.7% moisture, 3.4% ash, and 30-40% oil. Additionally, flaxseed contains 35-45% fibers (Tvrzicka et al. 2011; Yaqoob et al. 2016; Mueed et al. 2022). Also, studies have found that sunflower seeds provide with dietary fiber, proteins, vitamin E, pentothenic acid and folic acid. Sunflowers seeds are also rich in minerals like calcium, copper, iron, magnesium, manganese, selenium, phosphorous potassium, sodium and zinc (Anjum et al. 2012; Rani et al. Moreover, Pumpkin seeds 2014). are useful packed with nutrients and nutraceuticals such as amino acids. phytosterols, unsaturated fatty acids. phenolic compounds, tocopherols, and valuable minerals (Dotto and Chacha 2020). Fermented dairy products such as yogurt and fermented milk are the major vehicle in delivering probiotics (Ranadheera et al. 2017), so the aim of this study is to make a functional free-fat fermented Bifidus milk using some nutritious seeds like flaxseeds, sunflower and pumpkin seeds.

2. MATERIALS AND METHODS: 2.1. Preparation of probiotic bacterial culture

A pure culture of probiotic bacteria, Bifidobacterium lactis namely. and Bifidobacterium bifidum were obtained from Dairy Microbiology Laboratory, National Research Center (NRC), Dokki, Egypt. Under microaerophilic Giza. conditions, both strains (1:1) were activated for 24 hours at 37 °C, first in MRS broth medium and subsequently in 10% (w/v) sterilized skim milk. Newly activated cultures were used in this investigation.

2.2. Seed preparation

The Flaxseeds (FS), pumpkin seeds (PS) and sunflower seeds (SS) that were bought from the Abu-Auf Company were dehulled and ground into a fine powder using an electric grinder to use in powder form. After being sifted, the powder was put in plastic bags and stored in the refrigerator until needed.

2.3. Preparation of synbiotic Bifidus milk

Fresh skimmed buffalo's milk (obtained from faculty of agriculture's farm, Fayoum, Egypt) was used to make the synbiotic drink according to Nasr et al. (2018). Milk was divided to seven portions as shown in Fig. (1). The prepared drinks were poured into 100 ml sterilized plastic cups, kept in a cooling incubator at 5-10 ° C, and then examined for chemical composition when they were fresh even after 21 days of storage. The pH values, microbiological examination, and sensory evaluation were also determined when the drinks were fresh and after 7, 14, and 21 days of storage. The fresh fermented milk beverages' syneresis rate was also assessed.

2.4. Chemical analysis

The resultant synbiotic samples were analyzed for their moisture (oven drying method), total nitrogen (macro Kjeldahal method), fat% (Gerber method) and total dietary fibers as described in A.O.A.C (2012). The pH values were measured by using pH meter Thermo Scientific Orion Star (A 214). All chemicals that used in this study were analytical grade and purchased from El- Nassr, Sigma and Technogen Companies.

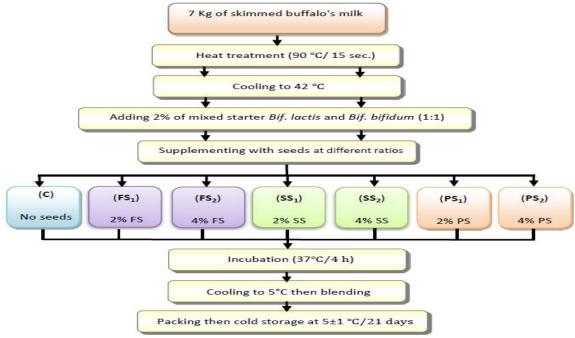


Fig. 1. Preparation of synbiotic Bifidus milk

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2.5. Microbiological examination

The count of probiotic bacteria was determined by plating on MRS-bile agar (Merck Co., Germany) and incubation for three days at 37 °C under anaerobic conditions (Afjeh et al., 2019). The total viable counts (TVCs) of cheese samples were determined using plate count agar medium. Fungi counts of cheese samples were determined on potato dextrose agar medium. Coliform bacterial counts of cheese samples determined on MacConkey agar medium.

2.6. Determination of syneresis

Syneresis was determined as described by Akin (2014). Syneresis (%) was expressed as volume of drained whey per 100 ml drink.

2.7. Sensory evaluation

Samples of synbiotic fermented milk were scored using the score card sheet of Bodyfelt et al. (1988) intervals the storage period: fresh, 7, 15, and 21 days. The total score (100 points) was broken down into 45 points for flavor, 40 points for body & texture and 15 points for appearance and color.

2.8. Statistical analysis

Data were statistically analyzed using General Linear Models procedure of Statistical Package for Social Sciences (SPSS, 2008) Version 17.0.0 software. Duncan's (1955) multiple range tests were used to compare between the means.

3. RESULTS AND DISCUSSION:

3.1. Chemical composition

Results presented in Table (1) showed the Chemical composition (moisture, fat, protein and fibers content) of synbiotic free fat Bifidus milk as affected by adding different seeds. The control, Free-fat fermented milk (C) contained higher moisture content (91.75%), while fermented drink which supplemented with 4% PS, SS and FS (PS2, SS2 and FS2) contained the lowest moisture content (88.52, 88.48 and 88.40%, respectively).

These results are in accordance with Sayed (2012) and Nassar et al. (2016), who studied some chemical properties of yogurt drink and reported the same average values of moisture.

It is clear that the fat, protein and fibers content are significantly (p < 0.001) affected by adding different seeds. The lowest fat, protein and fibers content were recorded by the control (C, free-fat fermented milk without additives) of 0.10, 6.95 and 0.00%, respectively. The highest fat content was for fermented milk supplemented with 4% sunflower seeds (SS2; 1.78%), it may due to the high lipid content of these seeds. On the other hand, the highest protein content was reported for PS2; fermented Bifidus milk supplemented with 4% pumpkin seeds (12.44%) followed by Bifidus milk supplemented with 4% SS then FS (12.05 and 11.93%, respectively). While fibers content of milk with flaxseeds was significantly (p < 0.001) the highest of 0.25%.

Analysis of flax averaged 41% fat, 10-20% protein, 28% total dietary fiber and 3.4% ash, which is the mineral-rich residue left after samples are burned. The protein content of the seed decreases as the oil content increases (Kaushik 2015; Naik R. et al. 2020). Sunflower seed contains 15-22% protein, 44-58% total lipid (fat), and 24% carbohydrate with 675 kcal total energy per 100 g (Rani et al. 2014; Prado et al., 2020; Filho and Egea, 2021). Moreover, Pumpkin seed is high in crude protein, roughly 35%, and this translates to a significant and different amount of amino acids, Studies show that protein isolates from pumpkin seed resemble those of soybean with high values of bioavailability of amino acids. It contains about 35% fat and 13-15% fibers (Rezig et al. 2019; Dotto and Chacha 2020).

3.2. The pH values

The pH values of synbiotic Bifidus milk were affected significantly ($P \le 0.001$) by

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adding different seeds and storage periods at 5 ± 1 °C. results in **Fig. (2)** show that the pH ranged between 4.8 - 4.50 in all fresh samples. The lowest pH value was 3.14 for free-fat Bifidus milk enriched with 4% flaxseeds (FS2) at 21 days. It's clear from results that pH values decreases across all storage times. On the other hand, the pH value decreased as the seeds concentrations increased. Similar results are in found by some researchers who observed that a pH

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reduction in fermented milk composed of flaxseed and milk correlated with the cell count. The pH dropped below 4.0, which is evidence of over fermentation. This result was consistent with the findings of another study, which showed that the pH value decreased in all samples of yoghurt enriched with flaxseeds. It was also reported that an increase in acidity correlated with the activity of probiotic bacteria (Smolová et al. 2017; Basiri et al. 2018; Alhssan et al. 2023).

 Table 1. Chemical composition of synbiotic free fat bifidus milk as affected by adding different natural seeds.

Component	Treatments*								
(%)	С	FS1	FS2	SS1	SS2	PS1	PS2	Sig.	SE±
Moisture Fat	91.75^{a} 0.10^{f}	90.61 ^b 0.54 ^e	88.48° 1.07°	90.25 ^b 0.88 ^d	88.40° 1.78ª	90.33 ^b 0.82 ^d	88.52° 1.63 ^b	* ***	0.03 0.01
Protein	6.95 ^e	10.65 ^d	11.93°	11.54°	12.05 ^b	12.05 ^b	12.44 ^a	***	0.11
Fibers	0.00^{f}	0.12°	0.25ª	0.08 ^d	0.17 ^b	0.04 ^e	0.09 ^d	***	0.01

a, b,... and f: Means having different superscripts within each column are significantly different (p < 0.001).

C: control, free-fat fermented bifidus milk without additives

FS₁: free-fat fermented bifidus milk + 2% Flaxseeds powder

FS₂: free-fat fermented bifidus milk + 4% Flaxseeds powder

SS1: free-fat fermented bifidus milk + 2% Sunflower seeds powder

SS2: free-fat fermented bifidus milk + 4% Sunflower seeds powder

PS₁: free-fat fermented bifidus milk + 2% Pumpkin seeds powder

PS₂: free-fat fermented bifidus milk + 4% Pumpkin seeds powder

SE: Standard error Sig.: Significance NS: Not significant

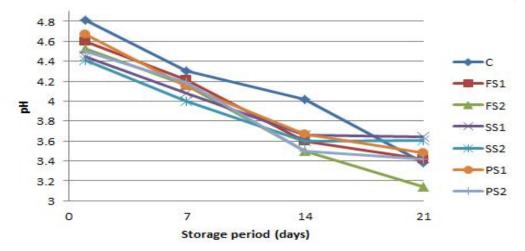


Fig. 2. Changes in pH values of synbiotic Bifidus milk as affected by adding different seeds and storage periods at 5±1°C.

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3.3. The Bifidobacterial count

The viability of bifidobacteria (B. lactis and B. bifidum) was affected significantly (P \leq 0.001) by treatments and storage period as shown in **Fig** (3). At day one after fermentation, the highest viability was for treatment enriched with 4% sunflower seeds (SS2; 6.03 × 10⁸ cfu/ml) while, the lowest viability was recorded by the control (C; 1.39× 10⁸ cfu/ml).

Generally, the viability of Bifidobacteria increased during storage to reach the highest levels at day 14, and then it decreased. This result may therefore be by post-acidification caused due to persistent metabolic activity of the product's micro flora, which decreased Bifidobacteria count. The biggest count was for SS2 then SS1 at day 14 (102.22 and 91.85×10^8 cfu/ml, respectively).

It can be concluded from results that the best viability of Bifidobacteria was for the fermented milk which supplemented with sunflower seeds (SS1 and SS2) followed by pumpkin seeds (PS1 and PS2) and then flaxseeds (FS1 and FS2).

The growth behavior of B. Lactis, Propionibacterium

freudenreichii NCC 1177 and Lactobacillus gasseri was monitored in a 10% (w/v) sunflower seeds flour suspension and a 10% (w/v) sunflower protein suspension by <u>Fritsch</u> et al. (2015) and Tangyu et al. (2022). The selected strains showed a good growth behavior on the different sunflower substrates.

According to Alhssan et al. (2013), the addition of flaxseeds increased B. lactis growth when compared to the control, and the maximum Bifidobacterium lactis count was achieved on day 14. FS is a prebiotic substance that combines the neutral polysaccharides rhamnogalacturonan and arabinoxylan. These substances enhance the survival of B. lactis. (Naran et al. 2008). Another study suggested that flaxseed oil with high level of α -linolenic acid (ALA) had a beneficial effect on the proliferation (Smolová Bifidobacterium of 2017). Furthermore, PS, SS and FS which are rich in dietary fibers can be used to form a new prebiotic product for use in the food industry (Roberfroid et al.2000; Fodje et al.2009).

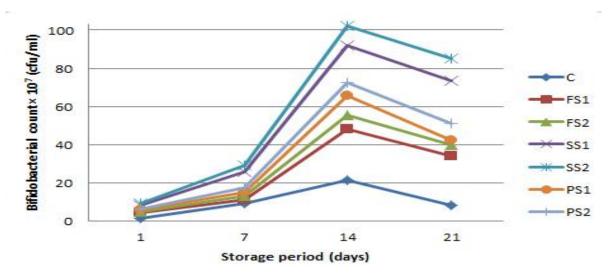


Fig. 3. Changes in Bifidobacteria counts (cfu/ml) of synbiotic Bifidus milk as affected by adding different seeds and storage periods at 5±1°C.

3.4. Total viable counts, fungi and coliform

Total viable count was significantly (P<0.001) affected by different treatments and storage periods (Fig.4). The control (C) recorded the highest TVC number $(85.25 \times 10^3 \text{ cfu/ml})$ at the end of storage (21) days) but the lowest TVC numbers at the same time were for treatments contains sunflower seeds (SS1 and SS2); 25.56 and 20.55×10^8 cfu/ml. It is clear from the results that TVC increased as storage periods increased. Generally, all treatments which contained seeds have lower TVC numbers than control when fresh and either across the storage.

The major compounds with inhibitory potential in sunflower seeds are represented by esterified hydroxycinnamic acids. mainly chlorogenic acid, as well as free phenolic acids (caffeic acid, quinic acid). A vast variety of microorganisms, including pathogens like Escherichia coli. Staphylococcus aureus. Salmonella typhimurium, and Bacillus subtilis, are susceptible to the effects of chlorogenic acid. Chlorogenic acid's mechanism of action has been well understood, and it suggests that the loss of barrier functions is caused by increased plasma membrane permeability. Due to the inability to sustain membrane potential. the various cytoplasmic macromolecules, such as nucleotides, are lost (Lou et al., 2011; Fritsch, 2016).

Also, phenolic acid, flavonoids, and lignans are primarily responsible for flaxseed's antimicrobial action and flaxseed contains a considerable amount of lignans and secoisolariciresinol diglucoside (Alphonse & Aluko, 2015; Akl et al. 2020; Yanjun et al. 2022).

Pumpkin seeds show an antimicrobial activity against Staphylococcus aureus, Bacillus subtilis and Klebsiella pneumoniae. Also, reported higher antimicrobial zone of inhibition against Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa by pumpkin methanolic and aqueous extracts (Dubey et al. 2010; Chonoko and Rufai, 2011; Abdullahi and Santhose, 2018).

On the other hand, Bifidobacterium strains can provide several health benefits, such as antimicrobial and immunomodulatory effects. Some strains inhibit growth or cell adhesion of pathogenic bacteria, including multidrug-resistant bacteria, and their antibacterial activity can be intensified when combined with certain antibiotics (Lim and <u>Shin</u>, 2020; Miksusanti and Hidayat, 2022).

Throughout the storage period, all treatments were free of fungus and coliform bacteria. It is because fermented drinks are made with appropriate hygiene practises, and it may also be because of the antimicrobial effect of Bifidobacteria and used seeds, suggesting good hygiene and sanitary conditions at the start of the fermentation process and during the storage time.

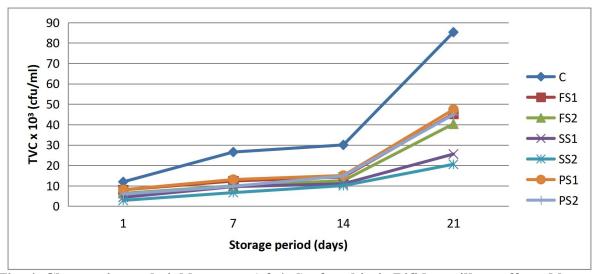
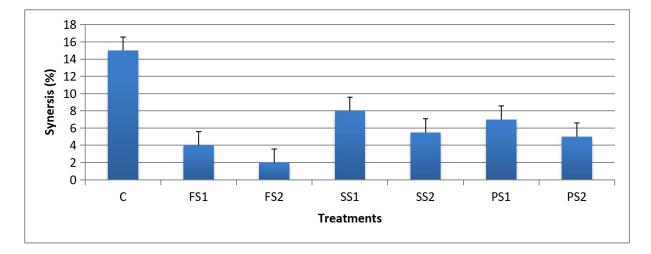


Fig. 4. Changes in total viable counts (cfu/ml) of synbiotic Bifidus milk as affected by adding different seeds and storage periods at 5±1°C.

3.5. Syneresis values

The syneresis values (the quantity of whey which has separated from 100 ml of sample) of fresh samples are recorded (Fig. 5). The treatment FS2; Bifidus milk which supplemented with 4% flaxseeds recorded the lowest Syneresis value (2 ml/100ml) followed by FS1 which contained 2% flaxseeds, while the highest syneresis value was for control (C); 15 ml/100ml. this results due to the water holding capacity of

flaxseeds as mentioned by Naik et al. (2020). They reported that Flaxseeds have functional properties like solubility, thermal emulsifying stability. properties and electrostatic charge density, water holding and fat absorption capacities polyunsaturated fatty acids (PUFA) omega-3 family, soluble dietary fibers, lignin's, proteins and carbohydrates. The same with PS and SS because of its content of fibers.





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2.7. Sensory evaluation

Enriching the free-fat Bifidus milk with FS, SS or PS had a significant effect on the sensory attributes. Body & texture were highly influenced by the addition of all seeds, the highest scores of body & texture was for treatments supplemented with FS (FS2 and FS1; average 39 points for each) compared to average 32 points for control.

The best flavor among control and other treatments were Bifidus milk enriched with pumpkin seeds; PS1 and PS2 (43 and 42 points, respectively) while, treatments contained flaxseed recorded the lowest flavor scores.

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On the other hand, PS1 and PS2 gained the highest scores of color & appearance and FS treatments were the lowest because of the rough fibers of flaxseeds powder. Increasing the concentration of FS decreased the appearance score. Moreover, the control recorded the lowest scores of color and appearance at the end of storage (10 points) because of high synerisis percentage.

To sum up, the highest total scores was recorded by PS1 followed by PS2 (Bifidus milk enriched with 2 and 4% pumpkin seeds; 98 and 96 points) when fresh and till 7th day of storage.

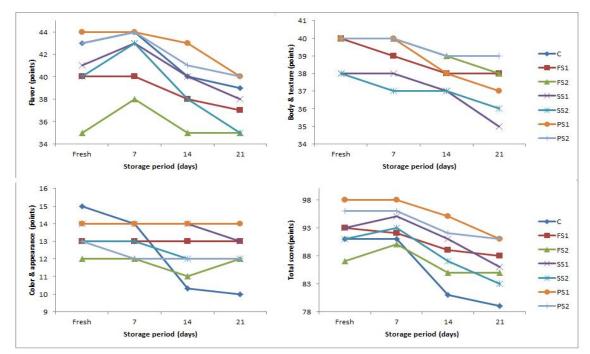


Fig. 6. Changes in sensory attributes of synbiotic Bifidus milk as affected by adding different seeds and storage periods at 5±1°C.

CONCLUSION:

In this study, the addition of plants' seeds (Sunflower, pumpkin or flax seeds powder) to fermented Bifidus milk significantly improved the survival of B. bifidum and B. lactis, as well as affecting the other chemical, microbiological and sensory properties. Therefore, it could be concluded that those seeds has good potential to improve Bifidus milk's quality in respect of its health benefits. The synbiotic combination of selected probiotic bacteria and each kind of seeds may provide health promotion via synergism of probiotic growth in the gut.

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الملخص العربي

تحسين جودة وحيوية لبن البيفيدوس خالي الدسم باستخدام بعض البدور النباتية نسرين محمد نصر، وداد عزب متري، عزة محمود أحمد إبراهيم

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يطلق مصطلح (Synbiotic) على المنتجات الغذائية التي تحتوي على كل من البكتريا الحيوية و منشطاتها والتي تشجع نموها وحيويتها. في هذه الدراسة ، تم تحسين لبن البيفيدوس خالي الدسم عن طريق إضافة بذور الكتان ، بذور عباد الشمس أو بذور القرع في صورة مسحوق كلا على حده وتم عمل التحليل الكيميائي والفحص الميكروبيولوجي بما في ذلك حيوية البيفيدوبكتريا وانفصال الشرش والتقييم الحسي. تم تسجيل أقل محتوى من الدهون والبروتين والألياف بواسطة العينة القياسية (الكنترول) ، بينما كان أعلى محتوى للدهون للمعاملة المدعمة بمسحوق بذور عباد الشمس ثم بنور عباد الشينة البيفيدوس المحتوي على 4% من مسحوق بذور القرع أعلى محتوى من الدوتين يليه بذور عباد الشمس ثم بذور الكتان. كانت أدنى قيمة للأس الهيدروجيني للبن البيفيدوس المدعم بنسبة 4٪ من بذور الكتان عند نهاية فترة التخزين (21 يوما).

زادت حيوية البيفيدو بكتريا أثناء التخزين لتصل إلى أعلى المستويات في اليوم 14، ثم انخفضت بعد ذلك. وكانت أعلى حيوية للعينات المدعمة بمسحوق بذور عباد الشمس. بالنسبة للعدد الكلي للبكتريا، وجد أن جميع المعاملات المدعمة بالبذور سجلت أعداد أقل من الكنترول سواء طازجة أو خلال فترة التخزين. وسجلت المعاملة المحتوية على 4% من بذور الكتان أقل معدل انفصال للشرش. كما أظهرت نتائج التقييم الحسي أن أعلى الدرجات الإجمالية تم تسجيلها بواسطة لبن البيفيدوس المدعم ببذور القرع بمعدل 2% ثم 4%. لذلك، يمكن التوصية بتدعيم منتجات الألبان المتخرة ببذور النباتات كمنشطات للمدعمات الحيوية وذلك لتعزيز القيمة الغذائية، والخواص الحسية، وحيوية البكتريا الحيوية المعتمرة ببذور النباتات التخمير.