New Synbiotic Sweet Produced from Almond Skin and Some Probiotic Bacteria

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Abstract: Several studies in recent years have shown the benefits deriving from the ingestion of probiotics, and a large number of products containing lactobacilli and bifidobacteria have been released to the markets. The aim of this study to show a symbiotic relationship (combination of probiotic and prebiotic) in a hand-made almond sweet and for the first time that combines the properties of both almond, as prebiotic, and Lactobacillus, as probiotic, to be served to the market as a healthy promoting foods. In this study we used Lb. Rhamnosus GG and Lb. Plantarum, well-recognized probiotic, with almond skin which was reaming after almond milk production which has a probiotic characteristic, to build a symbiotic relationship. Also inulin was used, a well-recognized prebiotic, as another factor to maintain high viability and survival for both Lb. Rhamnosus GG and Lb. Plantarum during the procedure of processing and storage. Synbiotic sweet was sensory evaluation in food science department–College of Agriculture–University of Baghdad. The evaluation was conducted monthly, synchronous with microbiological assays. The result shown that no big different in the viability between Lb. Plantarum and Lb. Rhamnosus GG in the symbiotic sweet during the stored period in the refrigerator at 4°C ± 1. But there’s different between the control sweet and symbiotic sweet in total coliforms, yeasts and mold during the stored period and the Drop ratio for bacteria Lb. Plantarum and Lb. Rhamnosus GG at the end of the storage experiment was 8.5% and 7.6%, respectively. And the symbiotic sweet hold all its sensory properties during the storage period on the contrary the control sweet expired and reached the stage of sensory rejection.

Keywords: Almond Skin. Probiotic. Synbiotic Sweet. Prebiotic

1. Introduction

Probiotic defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host [1]. Lactobacillus is the most widely recognized as probiotic [2]. The use of probiotic in food production is not new, although in the early years it was used as food preservation then evolved towards benefits for health, such as reduction of cholesterol and enhance immune system [3]. The minimum recommended number of viable probiotic bacteria is $10^7$ cfu/g or mL of a product at the time of consumption [4]. Lb. Plantarum has significant antioxidant activities and also helps to maintain the intestinal permeability [5]. It is able to control the growth of gas producing microorganism in the intestines [6]. Lb. Plantarum increase hippocampal brain derived neurotropic factor which means it have a beneficial role in the treatment of depression [7]. Lactobacillus Rhamnosus GG has the ability to stop allergic reactions to peanuts in 80% of children [8], and has been shown many other benefits in the prevention of rotavirus diarrhea in children, also it has a role in the prevention and treatment of various types of diarrhea both in children and in adults [9]. Lactobacillus GG reduces the risk of obtaining respiratory tract infections in children that attend daycare [10]. Although the dairy industry is the major sector involved in probiotic products, other food areas have recently become involved in probiotic products, Almond shows various types of diarrhea both in children and in adults [9]. Almond skin is very useful in the gastrointestinal tract (GIT), Almond is very useful in the prevention and treatment of various types of diarrhea both in children and in adults [9]. Almond skin is by-product of almond milk and blanching process. The high amount of antioxidants in the skin may be a result of natural evolution for the purpose of protecting the oil-rich almonds from oxidation by penetrating atmospheric oxygen [17]. Other health promoting compounds present in almond skins are polyphenols which have been shown to be protective agents against cancer and cardiovascular disease, the polyphenols present in almond skins are also active as antimicrobials against a range of food-borne pathogens. There is an increased effort in trying to avoid foods with chemical preservatives and this is manifested by the food industries growing interest in finding natural compounds with antimicrobial activity [18]. Dairy is a very nutritious substrate, which satisfies the nutritional requirements of fastidious LAB. But, expanding trend of vegan lifestyles, the issues of lactose intolerance, and the demand for low-fat and low-cholesterol foods have created a growing demand for non-dairy probiotic products [19]. Synbiotic is the co-administration of probiotic and prebiotics with the expectation that the prebiotics will enhance the survival and growth of the probiotics [20].
2. Materials and Methods

2.1 Bacterial Strains and Culture Conditions

The strains used were Lactobacillus rhamnosus GG and Lactobacillus plantarum purchased from (NOVA, USA) well-recognized probiotics. One capsule of each Lb. Rhamnosus GG and of Lb. Plantarum individually was release in tuber contain 9 ml of Man, Rogosa and Sharpe (MRS) broth (Oxoid, UK), and incubated at 37c for 24 h, and was repeated 3 times. Then they were incubated individually in skim milk broth (12% w/v) at 37c till curd is settled down, and repeated 3 times before it used.

2.2 Ingredients for sweet Production

Sweet was produced by using the following commercial ingredients: roasted almond peel 20% (obtained from almond milk processing), condensed sweeten milk 10% (Dawn company, origin Singapore), inulin 10% (Now company, origin USA), cooking chocolate 50% (AL-wijdan company, origin Iraq).

2.3 Synbiotic Sweet Production

1) Solid ingredients Almond peel and inulin mix well until homogenization for 3 m.
2) Liquid ingredients 10% starter of Lb.GG and Lb.pla add individually and condensed milk after good mixing and continuous blending until the mixture is homogeneous for 5 m.
3) Cooking chocolate added after melting it in a water bath at temperature 60 c until it became runny and easy to stirring and then taken out and left to cool down to 40 c then it added to the mixture and kept mixing it until it became well-mixed.
4) Rolling pin use to spread the mixture with the desired thickness then put it in frozen for 10 minutes to become hard and easy to cut with knife or steel cutter mold.
5) The sweet wrapped with aluminum foil and stored in the refrigerator.

![Synbiotic Sweet Production Diagram](image)

Figure 1: Synbiotic sweet procedure

3. Microbial Analysis

Microbiological analyses were carried out on sweet samples synbiotic and control at 0, 30, 60, 90, 120 days of refrigerator storage, according to the following procedure. Ten grams of each sweet was aseptically transferred into a sterile ceramic mortar, diluted with 90 ml of sterile peptone water (0.1%W/V), and homogenized for 6 min [21]. One milliliter of the third dilutions was used to obtain $10^{-3}$-fold serial dilutions, which were used for microbial counts. Both probiotic bacteria were counted under anaerobic conditions on MRS agar (Oxide, UK) for 48 h of incubation at 37 c, yeasts and mold were quantified on potato dextrose agar medium (Oxide, UK) for 5-7 days of incubation at 28 c. Total coliforms were counted on MacConkey agar (Oxide, UK) for 24 to 48 h of incubation at 37c. Total count were estimated (just for the control sweet) on nutrient agar (Oxide, UK) for 24 h of incubation at 37c.

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4. Storage Experience

To acknowledge the efficiency of symbiotic sweet in maintaining an appropriate viability number of probiotics was stored in the refrigerator at temperature of 4°C.

5. Sensory Evaluation

Symbiotic sweet was evaluated by 10 individuals’ professors and postgraduate students in food science department–College of Agriculture –University of Baghdad. The evaluation was conducted monthly, synchronously with microbiological assays. According to the form mentioned by Popov-Raljic [22]. Five terms were used: Color, Appearance, Hardness, Taste and Odor, with a scale from 1 to 10 for all the five terms. The samples stored at 4°C and were removed from the refrigerator and drop out for 5 min at 25°C before sensory analysis.

6. Results and Discussion

6.1 Starter Total Count

The result shown in figure 2 both probiotic bacteria Lb.GG and Lb.pla in symbiotic sweet count was extremely high 12.3 log/g and 12.4 log/g respectively, due to the high amount of starter culture that were added and because of inulin (Probiotic) which stimulates the growth of both probiotic bacteria Lb.GG and Lb.pla. The result shown that even after 30 days of cold storage theirs no drop in the numbers of Lb.pla and the drop ratio for Lb.GG was 1.6% this is because of inulin and the sweet matrix which provided protection to the probiotic bacteria during storage period in the refrigerator but at the end numbers of probiotic bacteria was decreased so slowly after 120 days of storage, where the rate of decline for Lb.GG was after 30, 60, 90, and 120 days of storage 1.6, 6.6, 6.6 and 7.6% respectively, and for Lb.pla was 0, 5.7, 5.7 and 8.5% respectively. In that matter inulin can provide a high numbers and maintain good viability for probiotic bacteria and also the solid food matrix has an effective role for protection the probiotic during long storage period and within the human digestive system against extreme conditions compared to liquid matrix such as milk. Studies on probiotic chocolate by Possemiers have used a simulator of human GI tract (SHIME) to model the viability inside the GI tract. The observation is viability of chocolate is 5-fold higher than the viability of milk inoculated with the same probiotics and inoculum size [23]. Other studies have suggested that the metabolism and utilisation of prebiotics by probiotics as a means of enhancing probiotic survival and proliferation [24], [25]. So non-dairy products particularly solid has its advantage in providing better viability and protection to the probiotic compared to dairy products especially which have liquid matrix like fermented milk, also lactobacillus spp. show more survival comparing with Bifidobacterium spp. [26].

![Figure 2: Lb.GG and Lb.pla count in symbiotic sweet during 120 days of storage](image)

6.2 Coliforms test

The result show in figure 4 that total coliforms in symbiotic sweet that contain Lb.GG was 1.8 CFU/g at zero time and decline to 1.1 CFU/g and droop to zero after 60 days of storage. The same result shown in the symbiotic sweet that contain Lb.pla were total coliforms number decline form 2 CFU/G at zero time to zero after 60 days of storage, while in the control sweet total coliforms number raised up from 1.5 CFU/g to 9 CFU/g after 120 days of cold storage. The decline in the total coliforms number in the symbiotic sweet maybe to the antagonism activity for both probiotic bacteria Lb.GG and Lb.pla such as produce of hydrogen peroxide [27], bacteriocins [28], diacetyl & acetaldehyde, which is antimicrobial agent against spoilage and pathogenic bacteria in foods and it has a more efficient effect against Gram-negative bacteria than Gram-positive bacteria [29].

![Figure 4: Coliforms count during storage period](image)

6.3 Mold and yeasts test
The result show in figure 5 the numbers of mold and yeasts in synbiotic sweet that contain Lb.GG and Lb.pla individually at zero time the count of mold and yeasts was 1 CFU/g and 2 CFU/g respectively, and after 30 days of storage the numbers run-down to zero. While in the control sweet the numbers raised up from 1 CFU/g to 7 CFU/g after 120 days of cold storage. The decline in the mold and yeasts numbers in the synbiotic sweet maybe to the antagonism activity between Lb.GG and Lb.pla which control the growth of spoilage microorganism enzymes duo to the hydrogen peroxide, Bacteriocins, Diacetyl & acetaldehyde, which is antimicrobial agent against mold and yeasts [30].

6.4 Sensory Evaluation

The result shown in table 1 that the Lb.GG and Lb.pla synbiotic sweet kept all five terms quality (Color, Appearance, Hardness, Taste and Odor) after 120 days of refrigerator storage, and the addition of 10% starter culture to the sweet didn’t affect the five terms of sensory evaluation, comparing with the control sweet all sensory terms color, appearance, hardness, taste and odor was decreed during the storage period, droop from 10 to 7.3, 9.4 to 7, 9.8 to 6, 9.2 to 2, 9.3 to 5.6 respectively after 120 days of storage. That mean the control sweet was unacceptable after 60 days of storage, comparing with the synbiotic sweet the quality of all five terms kept excellent properties during the 120 days of cold storage period may be due to the antagonism activity by the probiotic bacteria which control the growth of spoilage microorganism like Coliforms, mold and yeasts [31], or the suppression of spoilage microorganism enzymes due to the production of hydrogen peroxide, bacteriocins, diacetyl & acetaldehyde [32]. Also the activity of probiotic dead cells show antagonism activity toward the spoilage microorganism [33], and the probiotic bacteria Lb.GG and Lb.pla show antioxidant activity that may keep the lipids from deterioration [34].

| Table 1: Sensory evaluation of synbiotic sweet* |
|-----------------------------------------------|
| Days | Sweet Treatment | Color | Appearance | Hardness | Taste | Odor |
|------|-----------------|-------|------------|----------|-------|------|
| Zero time | Lb.GG | 9.7 | 10 | 9.3 | 10 | 9.1 |
| | Lb.pla | 9.5 | 10 | 9.6 | 9.8 | 9.7 |
| | Control | 10 | 9.4 | 9.8 | 9.2 | 9.3 |
| 30 days | Lb.GG | 9.9 | 10 | 9.4 | 10 | 9.4 |
| | Lb.pla | 9.4 | 10 | 9.6 | 9.7 | 9.9 |
| | Control | 9.4 | 9.2 | 9.5 | 9 | 9.3 |
| 60 days | Lb.GG | 9.7 | 9.6 | 10 | 9.6 | 10 |
| | Lb.pla | 9.4 | 10 | 9.7 | 10 | 9.7 |

* An average of 10 people were taken each time a sensory evaluation was performed.

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