

Enrichment of Probiotic Yogurt with Broccoli Sprout Extract and its Effect on *Helicobacter pylori*

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Abstract

Background and Objective: Antibiotic consumption is the main way to cure infection induced by *Helicobacter pylori*. On the other hand, antibiotics have side effects on human body. So, finding an efficient way to replace antibiotic consumption seems necessary. The aim of this study was to investigate the effect of broccoli sprout extract on the viability of probiotic bacteria and yogurt's physicochemical properties, and examine the synergistic effect of this extract with probiotics on *Helicobacter pylori* growth inhibition.

Material and Methods: Four levels of broccoli sprout extract (22.5, 45, 90 and 180 mg ml⁻¹) were prepared and their effect on probiotic yogurt samples was examined. Moreover, their anti- *Helicobacter pylori* effect was determined.

Results and Conclusion: The research results revealed that Broccoli sprout extract did not have any inhibitory effect on *Bifidobacterium lactis* and *Lactobacillus acidophilus*. The variations in acidity of the samples were not significant during storage. A positive correlation was observed between broccoli sprout extract concentration and syneresis. The findings showed the synergistic effect of broccoli sprout extract and probiotics on *Helicobacter pylori* growth inhibition. Therefore, using broccoli sprout extract and probiotic bacteria, we can produce a yogurt that is effective on the growth inhibition of *Helicobacter pylori*.

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1. Introduction

Helicobacter (H.) pylori has been known as the cause of ulcer and gastric cancer. It is one of the most prevalent infectious factors in human and more than half of the world people are its hosts [1]. The main treatment of this disease is the consumption of antibiotics; this necessitates exploring other treatments for this infection because of the treatment duration and the damages caused by antibiotic consumption. Combined use of probiotics and herbal medicine seems to result in a decrease in the side effects of drugs and in their better efficiency. It can also be a proper replacement for the treatment of this disease [2].

Probiotics are microorganisms which can have potential health-promoting effects on their hosts if they are consumed sufficiently [3]. Yogurt is one the most popular dairy products for the transfer of probiotics [4]. The daily intake of yogurts containing probiotics, especially *Bifidobacterium* and *Lactobacillus*, could have a suppressing effect on *H. pylori* growth [5]. Some researchers reported that intake of probiotic yogurts containing *Bifidobacterium*

and *Lactobacillus* solely or in combination with antibiotic consumption can influence the eradication of *H. pylori* [6,7].

Owing to containing pharmaceutical compounds, most plants can be successfully used in curing various diseases [8]. Up to now, the antibacterial activity of several herbal extracts against *H. pylori* has been assessed [9,10] and the results have indicated that some herbs are able to inhibit the growth of *H. pylori*.

Broccoli (*Brassica Oleraceae Italica*) is one member of the cabbage family. The sulforaphane content of broccoli sprout is much higher than that of the mature broccoli, and its *in vitro* activity against *H. pylori* has been revealed [11]. Since it contains a variety of polyphenols, it is an excellent source of the compounds necessary for human health [12]. Some researchers, studying the effect of sulforaphane (an abundant component in broccoli) on *H. pylori* infection, observed that this compound reduced this infection and prevented tumor formation [13].

Enrichment of the probiotic yogurt with broccoli gives a product rich in phenolic and antioxidant compounds, minerals and vitamins; it also improved the sensory properties of the yogurt in terms of odor and flavor. Furthermore, regarding the effects of probiotics on *Helicobacter*, it can have a synergistic effect on the growth inhibition of *H. pylori* [14].

To our knowledge, no research so far has been carried out on the utilization of broccoli extract in production of probiotic yogurt and its synergistic effect on the growth inhibition of *H. pylori*. The aim of this study, therefore, was to evaluate the effect of broccoli sprout extract on the viability of probiotic bacteria *Bifidobacterium* (*B.*) *lactis* and *Lactobacillus* (*L.*) *acidophilus*, and on yogurt's physicochemical properties during storage, and finally, examine the synergistic effect of this extract on *H. pylori* growth inhibition.

2. Materials and Methods

2.1. Preparation of microbial strains

DVS yogurt starter (YC-X11), and DVS probiotic starter (*B. lactis* BB12 and *L. acidophilus* La5) were all supplied from Chr-Hansen Co. (Denmark).

2.2. Preparation of hydro-alcoholic broccoli extract

Broccoli var *Brassica oleracea italic* was purchased from a local market (Tehran, Iran). The dried material was weighed and used to prepare the stock solution (180 mg ml⁻¹). Serial dilution aliquots were made from this stock solution to examine the minimum and maximum effects of the extract on *H. pylori*. At first after rinsing out, it was dried and chopped into small pieces. They were then shadow-dried for 30 days. Next, they were homogenized to be powdered and passed through a sieve with a mesh of 60 (250 µm). 0.1 g of the obtained powder was weighed (SARTORIUS AX 623, Germany) and mixed with 20 ml of methanol 80% (Merck, Germany). The resulting mixture was shaken vigorously and set aside for 24 h. Then, it was filtered using a filter paper (Whatman No.1) and made to the volume with methanol 80% in a 20-ml volumetric flask. Finally, the solvent was separated from the extract using a rotary evaporator (Heidolph, Germany) [15].

2.3. Preparation of yogurt samples

The yogurt samples were produced using skimmed milk in Pak Dairy Co. (Tehran, Iran). Firstly, the total solid content of the milk was adjusted to 11%. Then, the milk was pasteurized at 85°C for 15 min. After cooling down until 40°C, DVS probiotic starters (*B. lactis* and *L. acidophilus*) were added to the milk. Both probiotic strains (10⁸ CFU g⁻¹ of each strain) were inoculated simultaneously with the yogurt starter. Broccoli sprout extract (BSE) was firstly dissolved in glycerin (Merck, Germany) and then added. Control sample (without BSE) was also prepared. The prepared samples were poured into yogurt

containers and incubated at 40°C until reaching the pH value of 4.6. They were, subsequently, cooled down until 4°C. Eventually, the produced samples were stored at 4°C for 21 days and tested on the 1st, 11th and 21st days of storage.

2.4. Physicochemical analyses

Titrate acidity was determined as described by Momtaheni et al., and syneresis was measured as described by Sahan et al. [16,17].

2.5. Probiotic bacterial count

Counting the probiotic bacteria was conducted using MRS-bile agar (Merck, Germany). The media were anaerobically incubated at 37°C for 48 h [18].

2.6. Determination of anti-*H. pylori* effect

H. pylori, obtained through biopsy from the people suffering from gastrointestinal disorders, was suspended in 2-3 drops of sterile saline and cultured with the method of streak plate on Campylobacter selective agar (Merck, Germany) together with sheep blood 5-7%, bovine serum 7%, 2 mg l⁻¹ Amphotricin B, 10 mg l⁻¹ Vancomycin, 5 mg l⁻¹ trimethoprim, and 25.0 mg l⁻¹ polymixin B in order to create a specific medium for *H. pylori*. The medium was incubated for 72 h. In the next stage, it was cultured through the pour plate method in Brucella agar (Merck, Germany) which was free of antibiotics, and some wells had been created on it using a sterile pipette. The microbial population of each plate was adjusted to 10⁸ CFU ml⁻¹. The yogurt samples were prepared, and then 150 µl of each prepared concentration was poured into each well using a micropipette. Finally, the plates were incubated under the microaerophilic conditions at 37°C for 3-5 days. The diameter of the inhibition zone surrounding each well was measured using a digital ruler [19].

2.7. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) by SPSS 16. Duncans multiple range test was used to compare the means at p≤0.05 level. All experiments were performed in triplicate.

3. Results and Discussion

3.1. Physicochemical characteristics of the probiotic yogurt samples

Based on Table 1, on the first day of storage, the lowest acidity belonged to the yogurt sample containing 180 mg ml⁻¹ BSE. The highest acidity was related to the control sample. There was no significant difference (p>0.05) in acidity between the control and the sample containing 22.5 mg ml⁻¹ BSE. In addition, the samples containing 45 and 22.5 mg ml⁻¹ BSE were in the same group and had no significant difference (p>0.05) in acidity. The sample containing 180 mg ml⁻¹ was significantly (p≤0.05) different from all the other ones and the samples containing 90 and

45 mg ml⁻¹ BSE did not differ significantly ($p>0.05$) in terms of acidity. As BSE increased in the yogurt, acidity decreased significantly ($p\leq 0.05$) as compared to the control sample. Broccoli, because of containing anti-acid compounds, prevented the yogurt's acidity from increasing, consequently, it increased the yogurt's shelf-life due to influence of BSE compounds on the activity of lactic acid bacteria, and hence, inhibiting the acidity from increasing. Similarly, other researchers studying the enrichment of probiotic yogurt with the green tea extract at the concentrations of 0.3, 0.6 and 0.9%, reported that as the green tea extract increased, the initial acidity increased, too [20]. In another study to assess the effect of cinnamon and licorice on milk fermentation (cinnamon and licorice were added to the milk before fermentation), it was reported that the occurrence of these herbal compounds did not create a significant difference between the enriched yogurt and the control in terms of pH and titratable acidity during fermentation and storage [21].

Table 1. The zcity (percent of lactic acid) of the probiotic yogurt containing *B. bifidum* and *L. acidophilus* during storage

Sample	1 st day	11 th day	21 st day
Control	0.02 ^{Aa±} 0.89	0.02 ^{Aa±} 0.92	0.02 ^{Aa±} 0.93
22.5 mg ml ⁻¹ BSE	0.05 ^{Aab±} 0.76	0.04 ^{Ab±} 0.80	0.04 ^{Ab±} 0.82
45 mg ml ⁻¹ BSE	0.03 ^{Ab±} 0.69	0.10 ^{Ab±} 0.74	0.02 ^{Ab±} 0.79
90 mg ml ⁻¹ BSE	0.04 ^{Ab±} 0.63	0.03 ^{ABb±} 0.70	0.03 ^{Bb±} 0.75
180 mg ml ⁻¹ BSE	0.21 ^{Ac±} 0.44	0.07 ^{Ac±} 0.56	0.07 ^{Ac±} 0.66

The results are expressed as mean \pm standard deviation. The different small letters show the significant differences in each column ($p\leq 0.05$). The different capital letters show the significant differences in each row ($p\leq 0.05$).

As observed in Table 2, syneresis increased significantly ($p\leq 0.05$) with the rise in the BSE concentration. The sample containing 180 mg ml⁻¹ had the highest amount of syneresis followed by the ones containing 90, 45 and 22.5 mg ml⁻¹ BSE, and the control, respectively. The higher syneresis of the BSE-containing samples compared with the control was expectable. Since the reduction in the dry matter content is one of the effective factors on syneresis, in the present work, the serum phase increased after the addition of BSE and the gel network was not able to retain the whole serum phase as with the rise in the storage time, the serum more diffused out of the gel [22]. A similar study revealed that the increase in the concentration of Kombucha extract led to an increase in the syneresis of milk fermented beverages containing 10, 15 and 20% Kombucha extract [23]. According to the results obtained in the present study, the syneresis of the produced yogurt samples did not change significantly ($p\leq 0.05$) during storage at 4°C. It has been reported that syneresis increases to a lesser extent in the probiotic yogurts containing the prebiotic compounds such as inulin, lactulose and oligofructose [24]. The increase in the amount of syneresis during storage is usually due to an intensive rearrangement in the casein network structure leading to the water loss [25].

Table 2. The amounts of syneresis (%) of the yogurt containing *B. bifidum* and *L. acidophilus* during storage

Sample	1 st day	11 th day	21 st day
Control	0.06 ^{Aa±} 2.69	0.05 ^{Ba±} 1.65	0.32 ^{Aa±} 2.46
22.5 mg ml ⁻¹ BSE	0.20 ^{Ab±} 2.90	0.56 ^{Ab±} 3.33	0.55 ^{Ab±} 3.33
45 mg ml ⁻¹ BSE	0.36 ^{Ab±} 4.70	0.26 ^{Ac±} 4.20	0.41 ^{Ac±} 4.56
90 mg ml ⁻¹ BSE	0.47 ^{Ac±} 5.56	0.36 ^{Ad±} 5.50	0.32 ^{Ad±} 5.93
180 mg ml ⁻¹ BSE	0.30 ^{Ad±} 7.80	0.40 ^{Ae±} 7.96	0.45 ^{Ae±} 8.20

The results are expressed as mean \pm standard deviation. The different small letters show the significant differences in each column ($p\leq 0.05$). The different capital letters show the significant differences in each row ($p\leq 0.05$).

3.2. The inhibition zone diameter of *H. pylori* in the probiotic yogurt samples

According to Table 3, as the BSE concentration increased in the probiotic yogurt, the diameter of the inhibition zone increased significantly ($p\leq 0.05$) in samples because of the severe antibacterial activity of BSE against *H. pylori*. On the 21st day, there was no significant difference ($p>0.05$) between the diameter of the inhibition zone of the samples containing 180 and 90 mg/ml BSE, demonstrating that doubling the BSE concentration did not affect the extent of *H. pylori* inhibition. The inhibitory effect increased until the 11th day; nevertheless, the inhibitory effect of the extract on *H. pylori* was not significant ($p>0.05$) from the 11th day until the 21st day. The inhibitory effect of BSE on *H. pylori* increased as time went by, probably due to the activity of probiotic bacteria and the production of antimicrobial compounds and their synergistic effect on herbal extracts. In a similar study on the effect of some extracts of medicinal plants on *H. pylori*, it was reported that Punicagranatum extract had the most effect [26].

Table 3. Variations in the inhibition zone diameter (mm) of *H. pylori* in the yogurt containing *B. bifidum* and *L. acidophilus* during storage

Sample	1 st day	11 th day	21 st day
Control	1.00 ^{Aa±} 13.00	1.15 ^{Ba±} 22.66	4.04 ^{Ca±} 33.66
22.5 mg ml ⁻¹ BSE	1.52 ^{Ab±} 24.66	3.21 ^{ABa±} 26.33	3.05 ^{Ba±} 31.66
45 mg ml ⁻¹ BSE	4.93 ^{Ac±} 30.33	5.56 ^{ABb±} 36.00	5.50 ^{Bb±} 45.66
90 mg ml ⁻¹ BSE	4.16 ^{Ad±} 63.33	4.72 ^{Bc±} 73.33	1.73 ^{Bc±} 79.00
180 mg ml ⁻¹ BSE	0.00 ^{Ae±} 80.00	0.00 ^{Ad±} 80.00	0.00 ^{Ac±} 80.00

The results are expressed as mean \pm standard deviation. The different small letters show the significant differences in each column ($p\leq 0.05$). The different capital letters show the significant differences in each row ($p\leq 0.05$).

3.3. Probiotic bacterial count of BSE-containing probiotic yogurt samples

As shown in Table 4, after the incorporation of BSE, the probiotic bacterial count of the samples increased as compared with the control except those samples containing 22.5 mg ml⁻¹ on days 1 and 11. At the end of the storage period, the sample containing 180 mg ml⁻¹ BSE had the highest number of probiotic bacteria on the 21st day. The presence of prebiotic compounds because of the stimulation of the growth and activity of probiotics is one

of the most important reasons behind the viability of most bacteria [27]. Production of large amounts of acid by yogurt starter bacteria and lack of growth stimulating compounds such as prebiotics were of the reasons for the significant reduction in the number of probiotic bacteria in the control sample [28]. In a similar study, some research-

ers reported that the growth of *B. bifidum* and *L. acidophilus* increased with the rise of green tea extract in probiotic yogurt due to the presence of polyphenolic compounds in green tea extract. These compounds are known to serve as an oxygen scavenger and to reduce the redox potential of the growth media, as probiotic bacteria grow better in the absence of oxygen [20].

Table 4. The number of probiotics *B. bifidum* and *L. acidophilus* (CFU g⁻¹) in the probiotic yogurt samples during storage

Sample	1 st day	11 th day	21 st day
Control	10 ^{5Aa} ×0.05±10 ⁶ ×6.63	10 ^{5ABa} ×0.24±10 ⁶ ×6.36	10 ^{5Ba} ×0.08±10 ⁵ ×6.32
22.5 mg ml ⁻¹ BSE	10 ^{5Ac} ×0.06±10 ⁶ ×6.31	10 ^{5Ba} ×0.17±10 ⁶ ×6.07	10 ^{5Ab} ×0.05±10 ⁶ ×6.52
45 mg ml ⁻¹ BSE	10 ^{5Ab} ×0.05±10 ⁶ ×6.52	10 ^{5Ab} ×0.02±10 ⁶ ×6.50	10 ^{5Ab} ×0.04±10 ⁶ ×6.52
90 mg ml ⁻¹ BSE	10 ^{5Ab} ×0.03±10 ⁶ ×6.48	10 ^{5Ab} ×0.01±10 ⁶ ×6.47	10 ^{5Ab} ×0.04±10 ⁶ ×6.79
180 mg ml ⁻¹ BSE	10 ^{5Ab} ×0.05±10 ⁶ ×6.54	10 ^{5Ab} ×0.07±10 ⁶ ×6.56	10 ^{5Aa} ×0.08±10 ⁶ ×6.32

The results are expressed as mean ± standard deviation.

The different small letters show the significant differences in each column (p≤0.05).

The different capital letters show the significant differences in each row (p≤0.05).

4. Conclusion

The present study revealed that broccoli sprout extract had no inhibitory effect on *B. bifidum* and *L. acidophilus*. As the BSE concentration increased, the syneresis of the product increased as compared to the control sample, and as BSE increased in the yogurt, the acidity decreased comparing to the control. Broccoli extract prevented the increase of yogurt acidity and consequently, it increased the yogurt's shelf-life. The antimicrobial effect of BSE was shown in this study. In addition, as the BSE concentration increased, the number of probiotic bacteria increased in the produced samples. The findings suggest the synergistic effect of BSE and probiotic bacteria on the growth inhibition of *H. pylori*.

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

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