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Research Article

Probiotic Yoghurts with Addition of Broccoli :Microbiological; Chemical Properties and Sensory Evaluation

Hoda Mahrous[1] and Heba Hussien[2]

[1]Industrial Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt.
[2]Department of Food Hygiene & Control, Faculty of Veterinary Medicine, Sadat City University, Egypt.
*Corresponding author: *hmahrous7@yahoo.com*

Abstract

Yoghurt is the most popular fermented milk products consumed in the all world due to its favorite sensory properties; nutritive and therapeutic values. Broccoli is one of the world's most important vegetables. The aim of the present work was to determine the microbial content, chemical and sensory quality of probiotic yoghurts supplemented with broccoli (boiled and powder) during 4-week refrigerated storage. The probiotic yoghurt supplemented with powder broccoli had significant effect on the pH, titratable acidity and total solids. Concerning the microbiological content of the probiotic yoghurt supplemented with broccoli, there is significally high ability of probiotic bacteria (*Lactobacillus acidophilus* P110) to grow than the probiotic yoghurt without broccoli and significally reduced in the count of yeast & mould, Coliforms and Aerobic spore forming bacteria in probiotic yoghurt supplemented with powder broccoli with highest notes in sensory evaluation of probiotic yoghurt supplemented with powder broccoli.

Keywords: probiotics, *Lactobacillus acidophilus*, yoghurt, broccoli.

1.Introduction

Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut. Probiotics can be used as complementary and alternative medicine (CAM) and they are available to consumers in the form of dietary supplements and foods. The addition of probiotics as lactic acid bacteria to milk used in the production of yoghurt enhances the digestibility and nutrient value of milk (De Vrese and Schrezenmeir, 2008). The probiotic (Lactic acid bacteria) breakdown carbohydrates present in milk, this process lowered PH of the milk to a point where proliferation of pathogenic bacteria such as, *Escherichia coli* and coliform bacteria were effectively inhibited (Duggan *et al*., 2002 and Hekmat& Koba, 2006)

The efficiency of probiotics can be enhanced by the addition of prebiotics, "selectively fermentable fibers which promote specific changes in the composition and/or activity of the gastrointestinal microflora, conferring benefits upon host well being and health" (Roberfroid, 2007).

Prebiotics are a type of fiber. Fiber is the part of a plant that the body can't digest it. Fibers have an effect in lowering cholesterol, control blood sugars and keep bowels regular. It also exhibit antibacterial activity against pathogenic bacteria and antifungal activity against pathogenic fungi (El-Arab *et al*., 2006). Eating foods with prebiotics helps the digestive tract by encouraging good bacteria to grow.

Broccoli is one of the most commonly consumed green vegetables. Broccoli ranks 19th out of 23 vegetables in consumption per world capita (Sun *et al*., 2007). Like other species of the Brassica family, broccoli is a source of health-promoting phytochemicals. Broccoli is known mainly for its wide range of bioactive compounds and is rich in both nutritional and non-nutritional antioxidants, including

vitamin C, vitamin E, and phenolic compounds including flavonoids, carotenoids, and glucosinolates (Lin and Chang, 2005).

Broccoli is vegetables with high antioxidant activity. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases (Verlangieri *et al*., 1985). The antioxidant activity of broccoli was ranked as the second in 10 common vegetables (Chu *et al*., 2002) and the sixth in 22 vegetables (Cao *et al*, 1996).

Consumer interest in the relationship between diet and health has increased the demand for information about functional foods. New food products are being developed to include beneficial components. The objective of this study was to include broccoli (boiled or powder) in probiotic yoghurt to enhance the organoleptic properties, improve microbiological quality of plain yoghurt and improve consumer acceptability.

2. Materials and Methods

2.1. Preparation of Microorganism

The probiotic mother culture containing *Lactobacillus acidophilus* P110 isolated and tested for its probiotics properties by Mahrous (2010) was added to sterile MRS broth then anaerobically incubated using BBL gas packs at 37°C for 16 hours. Strain was stored at –80°C in MRS broth supplemented with 25% (v/v) glycerol. For routine analysis, the strains were subculture twice in MRS broth at 37°C for 24 h.

2.2. Preparation of broccoli

Broccoli was cut into small pieces and macerated in a food processor. The plants were separated into two groups, the first group was boiled and the second group was dried in oven at 50^{o}C after that ground into a fine powder and stored at -80°C for using. The dosage of broccoli preparation (7%) was chosen from three different concentrations tested 5, 7, 10 %for sensory acceptance in the preliminary study.

2.3. Manufacturing of Probiotic Yoghurt

Standardized milk with 3.5% fat was heated to 85°C for 15 minutes, cooled to 40°C, and then a 2% by weight sample of the standard yoghurt cultures *Lactobacillus del-brueckii* subsp. *bulgaricus* and *Steptococcus thermophilus* was used to inoculate the milk .Then the inoculated milk separation into 4 jars as: A) Natural (control) yoghurt ; B) inoculated with 2% by weight sample of the prepared probiotic strain *Lactobacillus acidophilus* P110;C) inoculated with 2% by weight sample of the prepared probiotic strain *Lactobacillus acidophilus* p110 and 7% boiled broccoli was added and D) inoculated with 2% by weight sample of the prepared probiotic strain *Lactobacillus acidophilus* P110 and 7% dried broccoli was added then the total mixture of all 4 groups were incubated at 37°C for six hours (to the 4.7 pH). The yoghurts were kept at 4°C and analyzed after 1, 7, 14 and 21 days of cold storage.

2.4. Microbiological and chemical analysis

2.4.1. Preparation of samples:

Yoghurt samples were thoroughly mixed in their plastic containers by a sterile stirrer before being examined.

2.4.2. Chemical examination

2.4.2.1. Determination of titratable acidity (Chandan 2006)

measurement of 9 g of sample into a beaker ,addition of two volumes of water plus 0.5 ml of the pH indicator phenolphthalein and titration to the first permanent shade of pink produced by the indicator . This color appear at pH of approximately 8.3 a pH at which the buffering capacity of milk is quite low ,the titran ,0.1000 sodium hydroxide ,is added from a calibrated buret .

2.4.2.2. Determination of fat by Gerber method (BSI 696 1955)

Weighting 11.3 g of the sample in the butyrometer then Measure 10 ml of sulphuric acid into a butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube and add 1 ml of Amyl alcohol, close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath for 5 min. at 65 $\pm$ 2 ^{0}C taking care to have casein particles if any to dissolve fully, and centrifuge for 4 min. at 1100 rpm. The tubes should be put in centrifuge, so as to

conform to radial symmetry, and as evenly spaced as Possible, in order to protect bearings of the centrifuge. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in water bath for 5 min. at 65 ± 2 ^{0}C. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid. When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.

2.4.2.3. Determination of total solids (IDF Anonymous, 1991)

Adding 25 g of clean sand and a small stirring rod to dry moisture dish then drying this in vacuum oven at 102 ^{o}C for at least 1 hr then 3 ± 0.5 g of sample is weighed into the center of the cooled pan ,the sample is mixed into the sand with the stirring rod and is covered with dried fiberglass cover .the entire unit is then placed in the vacuum oven to dry for 2 hrs at 102 ^{o}C and at a minimum vacuum of -86kPa. After cooling the dish in a desiccators at room temperature for 45 minutes, the dish is weighed on an analytical balance .the percent moisture is calculated by dividing the loss in weight by the weight of the sample and then multiplying the result by 100 .the percent solids is 100 minus the percent moisture .a small amount of dried air is permitted to pass through the oven during drying.

2.4.2.4. Determination of non fat milk solids (Chandan 2006)

When the sample is composed only of the constituents of milk, the percentage of non fat milk solids is the product of subtraction of the percent fat from the percent total solids.

2.4.3. Microbiological examination

2.4.3.1. Preparation of serial dilution (A.P.H.A., 1992)

Eleven grams of the prepared samples of yoghurt were transferred into a sterile flask contained 99 ml of sterile distilled water , then thoroughly mixed to prepare a dilution of 1/10, from which decimal dilutions were prepared using buffering peptone water.

2.4.3.2. Yeast and mould count (I.C.M.S.F, 1986).

Duplicate plates of Sabaroud's agar medium (containing 0.05 mg of chloramphenicol per ml) were inoculated each with 1 ml from the prepared serial dilutions. Inoculated plates were incubated at 25 ^{o} C for 5 days. The first examination of plates was done after 3 days incubation to determine the degree of yeast growth, and large numbers are visible. A count was made and repeated on the fifth day. The yeast and mould colonies were counted (each type of the countable plates being enumerated separately). The total yeast and mould counts per gram or ml were calculated and recorded.

2.4.3. 3. Coliform content "MPN/ml or g." (A.P.H.A., 1985)

A series of 3 fermentation tubes containing MacConkey broth and supplemented with inverted Durham's tubes were inoculated with one ml from each of previously prepared decimal dilutions. Inoculated and control tubes were incubated at 37 ^{o}C for 48 hrs. Tubes showing gas production were considered positive. From the results indicating the number of positive tubes, the Coliform counts (MPN/ml) were calculated and recorded.

2.5. Sensory evaluation

Ten panelists participated in a sensory evaluation of the yoghurt on the basis of appearance, flavour, texture, and overall acceptability. The panelists rated these characteristics according to the hedonic. Panelists were asked to assess the sensory characteristics of the four gropes of yoghurt: yoghurt A = plain (non-probiotic) yoghurt, yoghurt B = probiotic yoghurt, yoghurt C=probiotic yoghurt with 7% boiled broccoli and yoghurt D= probiotic yoghurt with 7% dried broccoli All participants gave signed consent before participating in the study. Panelists were then given four samples at a time at storage temperature (4 °C), a pencil, and a glass of cold water to rinse their mouths between samples.

2.6. Statistical Analysis

Results from the facial hedonic scale record sheets were collated and input into a SPSS version 15 database, mean, standard deviations and p-values were calculated for each sample. P-values less than 0.05 were considered statistically significant.

3. Results and Discussions

3.1. Microbiological counts

3.1.1. Probiotic count

Probiotics have positive effects on health, especially on gastrointestinal and immune systems, according to the National Center for Complementary and Alternative Medicine. Prebiotics refer to the compounds that nourish these probiotics and other friendly bacteria living in gut. Broccoli is a very good source of prebiotics. Prebiotics and probiotics work in synergy, and are more efficient when present together. Table 1 showed the changing in the count of *Lactobacillus acidophilus* P110 in the yoghurts during storage. Results showed that there were significantly ($P<0.05$) effect on the count of probiotic strain between the yoghurt with broccoli (boiled or powder) and without broccoli especially after 14 days from the production on the refrigerator storage which the results were $1.4\pm0.62\times 10^{9d;}$ $1.2\pm0.56\times 10^{9d}$ with broccoli boiled and dried respectively, and $8.9\pm0.45\times 10^{8b}$ for yoghurt without broccoli. On the other hand there were no significantly ($P<0.05$) effect on the yoghurt produced by broccoli either boiled or powder on the count of probiotic strain. These results were agreement with Bielecka and others (2002) have confirmed the appropriateness of combining prebiotics and probiotics, demonstrating greater effectiveness compared with probiotics alone. Fermented milks are the best sources of probiotics, live microorganisms that are either the same or similar to the ones living in gut.

Table 1: probiotic content

Days	Group B	Group C	Group D
Zero	$8.0\pm0.11\times 10^{8b}$	$8.2\pm0.41\times 10^{8c}$	$8.5\pm0.31\times 10^{8c}$
7	$8.3\pm0.01\times 10^{8b}$	$9.1\pm0.26\times 10^{8c}$	$9.4\pm0.41\times 10^{8c}$
14	$8.9\pm0.45\times 10^{8b}$	$1.2\pm0.56\times 10^{9d}$	$1.4\pm0.62\times 10^{9d}$
21	$8.5\pm0.21\times 10^{8d}$	$9.6\pm0.11\times 10^{8a}$	$9.9\pm0.01\times 10^{8a}$

Means that bearing different superscripts are significantly at ($P<0.05$)

Group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% dried broccoli.

3.1.2. Other microbial counts

Table 2 & 3 showed the development of yeast & molds (table 2) and coliform bacteria (table 3) counts on the different groups of yoghurt group A with no probiotic, group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% dried broccoli. Results indicated that the yoghurt with probiotic strain with and without broccoli (groups B; C & D) showed inhibition effect on the count of yeast & molds and coliform bacteria comparing with the control (group A).The same observation was recorded in the count of aerobic spore forming bacteria Table 4 compared with the control group. Many reports have shown that probiotic bacteria have an advantage of inhibiting growth of undesirable organisms (Rubin *et al*., 1982; Giraffa *et al*., 1994). Mechanisms responsible for the inhibition of pathogens include competition for nutrients, adhesion of sites, production of inhibitory metabolites such as organic acids and hydrogen peroxide and the stimulation of the immune system (O' Sullivan *et al*., 1992). On other hand groups with probiotic and broccoli especially dried broccoli show good inhibition activity against yeast& mould, Coliformis and aerobic spore forming bacteria comparing with yoghurt with probiotic only. This result was explaining the synergistic action between the probiotic and prebiotics.

Table 2: Yeasts and molds

Days	Group A	Group B	Group C	Group D
Zero	$7\pm0.12\times 10^{4a}$	$6.9\pm0.21\times 10^{4b}$	$6.6\pm0.21\times 10^{4c}$	$6.5\pm0.21\times 10^{4c}$
7	$9\pm0.21\times 10^{4a}$	$8\pm0.21\times 10^{4b}$	$7.6\pm0.21\times 10^{4c}$	$6\pm0.21\times 10^{4d}$
14	$9.8\pm0.21\times 10^{4a}$	$9.3\pm0.21\times 10^{4b}$	$7.6\pm0.21\times 10^{4c}$	$7.1\pm0.21\times 10^{4d}$
21	$8.5\pm0.21\times 10^{4c}$	$7.8\pm0.21\times 10^{4d}$	$7.0\pm0.21\times 10^{4a}$	$3.6\pm0.21\times 10^{4b}$

Means that bearing different superscripts are significantly at (P<0.05)

group A with no probiotics, group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% dried broccoli

Table 3: Coliforms

Days	Group A	Group B	Group C	Group D
Zero	1100±122	1100±122	±1221100	1100±122
7	1500 ± 122^{a}	600 ± 12.2^{b}	210 ± 12.2^{c}	$\pm12.2^{c}200$
14	$\pm122^{a}11000$	210 ± 12.2^{b}	150 ± 12.2^{b}	$\pm12.2^{b}120$
21	21000 ± 122^{a}	$\pm12.2^{b}200$	23 ± 1.22^{c}	$\pm1.22^{c}20$

Means that bearing different superscripts are significantly at (P<0.05)

group A with no probiotics, group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% dried broccoli

Table 4: Aerobic spore forming bacteria

Days	Group D	Group C	Group B	Group A
Zero	-	-	-	-
7	$5\pm1.22\times 10^{1}$	-	-	-
14	$3\pm1.22\times 10^{2}$	-	-	-
21	$10\pm1.22\times 10^{3a}$	$2\pm1.22\times 10^{2b}$	-	-

Means that bearing different superscripts are significantly at (P<0.05)

group A with no probiotics, group B containing 2%w/w of Lactobacillus acidophilus P110, group C containing 2%w/w of probiotic strain (Lactobacillus acidophilus 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (Lactobacillus acidophilus 110); with 7% dried broccoli

3.2. Chemical analysis

Broccoli is a high-fiber cruciferous superfood that contains a powerful cancer-fighting agent called sulforaphane. Researchers at the University of Illinois discovered that probiotics work a crucial magic on digesting broccoli, helping to release and absorb sulforaphane into the body. Table 5 showed that the yoghurt enrichment with broccoli (boiled or powder) had no significant differences detected ($P > 0.05$) on the pH; titratable acidity fat and moisture. The pH level and fat content decreased and titratable acidity increased throughout the storage. But there were significant effect on the Total solid% ether in group C or D that may be due to the highest effect of probiotic with prebiotic on the enzymes contenting. During lactic acid fermentation, yoghurt bacteria (*L. bulgaricus and Streptococcus thermophilus*) metabolize lactose to produce lactic acid (Tamime and Robinson, 1985). Furthermore, probiotic bacteria were also found to produce both lactic acid and acetic acid (Samona *et al*., 1996).

Table 5: Chemical analysis:-

Parameters	Time	Group A	Group B	Group C	Group D
pH	Zero	4.7±0.12^{a}	4.2±0.12^{c}	4.4±0.12^{b}	4.1±0.12^{c}
	7	4.7±0.12^{a}	4.5±0.12^{ab}	4.5±0.12^{ab}	4.3±0.12^{b}
	14	4.6±0.12^{b}	4.9±0.12^{a}	4.7±0.12^{a}	4.6±0.12^{b}
	21	4.4±0.12	4.3±0.12	4.4±0.12	4.3±0.12
Titratable acidity	Zero	1.15±0.12^{c}	1.1±0.12^{b}	1.05±0.12^{d}	1.3±0.12^{a}
	7	1.2±0.12^{b}	1.5±0.12^{a}	1.1±0.12^{c}	1.2±0.12^{b}
	14	2.8±0.12^{a}	2±0.12^{b}	1.3±0.12^{c}	1.1±0.12^{c}
	21	1.3±0.12^{a}	1.2±0.12^{a}	1.9±0.12^{b}0	1.35±0.12^{a}
Total solid%	Zero	19.6±0.12^{c}	19.4±0.12^{c}	20.4±0.12^{b}	21.4±0.12^{a}
	7	18.4±0.12^{b}	18.8±0.12^{ab}	18.6±0.12^{ab}	19.5±0.12^{a}
	14	17.2±0.12^{a}	16.2±0.12^{ab}	15.5±0.12^{b}	15.8±0.12^{b}
	21	15.4±0.12^{a}	14.2±0.12^{b}	8.4±0.12^{d}	9.6±0.12^{c}
Moisture %	Zero	80.4±1.22	6±1.22	79.6±1.22	78.6±1.22
	7	81.6±1.22	81.2±1.22	81.4±1.22	80.5±1.22
	14	82.8±1.22	83.8±1.22	84.5±1.22	84.2±1.22
	21	84.6±1.22^{b}	85.8±1.22^{b}	91.6±1.22^{a}	90.4±1.22^{a}
Fat%	Zero	3.5±0.12	3.4±0.12	3.5±0.12	3.5±0.12
	7	3.5±0.12	3.4±0.12	3.5±0.12	3.5±0.12
	14	3.4±0.12	3.3±0.12	3.4±0.12	3.4±0.12
	21	3.2±0.12	3.1±0.12	3.2±0.12	3.2±0.12
	Zero	16.1±0.12^{c}	±0.12^{c}16	16.9±0.12^{b}	17.9±0.12^{a}
	7	14.9±0.12^{b}	15.4±0.12^{a}	15.1±0.12^{b}	16±0.12^{a}
	14	13.8±0.12^{a}	12.9±0.12^{b}	12.1±0.12^{c}	12.4±0.12^{c}
	21	12.2±0.12^{a}	11.1±0.12^{b}	5.2±0.12^{d}	6.4±0.12^{c}

Means that bearing different superscripts are significantly at (P<0.05)

group A with no probiotics, group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% dried broccoli

3.3. Sensory evaluation

Table 6 shows mean sensory scores of overall sensory evaluation of the 4 groups of yoghurts was carried out among 10 panelists, using 9-point Hedonic Scale Method. The mean acceptability score for the control product of plain probiotic yoghurt received a mean score of 8 ± 1.01. Group B containing probiotic strain, received the best overall score (8.3 ± 0.54). Samples C and D containing boiled and dried broccoli were (8.4± 1.05 and 8.5 ± 0.54), respectively. On the other hand, addition of broccoli did not produce any defects with colour or general appearance when compared to the control yoghurt throughout storage period, therefore we can use broccoli to extend shelf life of yoghurt.

Table 6: Sensory evaluation of probiotic yoghurts

Properties	Mean scores			
	Group A	Group B	Group C	Group D
Aroma	7.85±0.2	7.95±1.2	8.02±2.02	7.85±0.2
Color	7.78±0.1	7.93±0.2	7.95±0.1	7.78±0.1
Texture	7.46±1.02	7.75±1.2	7.95±0.2	7.46±1.02
Sourness	7.43±0.04	7.96±0.02	8.02±0.3	7.43±0.04
Overall acceptability	8 ± 1.01	8.3 ± 0.54	8.4± 1.05	8.5 ± 0.54

Data are presented as mean ± SD group A with no probiotics, group B containing 2%w/w of *Lactobacillus acidophilus* P110, group C containing 2%w/w of probiotic strain (*Lactobacillus acidophilus* 110); with 7% boiled broccoli and group D containing 2%w/w of probiotic strain *(Lactobacillus acidophilus* 110); with 7% dried broccoli .

Conclusions

Yoghurt is an excellent dairy product that act as a vehicle for probiotic and prebiotic to produce their synergic effect. Probiotics may help in keeping digestive tract healthy by increasing the number of good bacteria and reduced the number of pathogenic bacteria. Prebiotic reduce the sour taste of plain yoghurt and sweet flavors appear to be the most promising means of increasing probiotic yoghurt consumption. Broccoli is special in that it is a rich source of sulforaphane, and eating less than one daily serving of broccoli is shown to be enough to have an anti-cancer effect. Thus, it is recommended that to obtain the greatest health benefits of broccoli yoghurt this product should be consumed in a short time after manufacturing. Among all examined procedures addition of the powder broccoli was the most beneficial concerning microbiological quality as well as chemicals quality and sensory properties of yoghurts.

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