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Raspberry and Strawberry Addition Improves Probiotic Viability in Yogurt and Possess Antioxidant Activity

Aynur Gunenc[1], Susanna Fang[1] & Farah Hosseinian[1, 2]

[1] Food Science and Nutrition Division of Chemistry Department, Carleton University, Ottawa, Ontario, Canada

[2] Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada

Correspondence: Farah Hosseinian, Food Science and Nutrition Division of Chemistry Department, Carleton University, Ottawa, Ontario, K1S5B6, Canada. Tel: 1-613-520-2600 ext 2048. E-mail: farah_hosseinian@carleton.ca

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Abstract

This study aimed to i) investigate probiotic potentials of raspberry and strawberry addition in yogurts, ii) explore antioxidant activity of berries extracted by microwave using oxygen radical absorbance (ORAC), and 2,2-diphenyl-1-picryhydrazyl radical (DPPH) as well as iii) determine the total phenolic content (TPC) of the berries. The probiotic potentials of those berry additions into yogurts containing different probiotics were determined by subsequent viable microorganism counts in each yogurt trial using selective media, pH and total titratable acidity (TTA) during 28 days of cold storage at 4ºC. Viable microbial counts in yogurt trials containing probiotic *Lactobacillus acidophilus* and raspberry increased ($P<0.05$) for 21 consecutive days of cold storage. The pH levels decreased ($P<0.05$) as the TTA increased over 28 days of cold storage in all yogurts containing the berries. ORAC results showed that raspberry had higher antioxidant activity (505.72 µmol TE/100g of fruit) than strawberry (495 µmol TE/100g of fruit). Also, DPPH scavenging activity results showed that raspberry (86.11%) had higher antioxidant activity than strawberry (85.69%). There was not a significant ($P<0.05$) difference in TPC values of raspberry (0.20 g GAE/kg) and strawberry (0.18 g GAE/kg). This study suggests that both berries have potential as a source of prebiotics with antioxidant activity for future functional foods and nutraceutical applications.

Keywords: microwave extraction, probiotic, raspberry, selective media, strawberry, ORAC, yogurt

1. Introduction

Berries like strawberry (*Fragaria* × *ananassa* Duch.) and raspberry (*Rubus idaeus* L.) are traditionally known as part of Nordic diet (Willett et al., 1995). They are an important source of fiber and bioactive compounds like polyphenolics and well recognized because of their positive health effects on human health, especially in the prevention of various oxidative stress associated diseases like cancer (Del Rio et al., 2013).

Prebiotics are nondigestible food carbohydrates such as fructooligosaccharides (FOS) and inulin that improve host health by stimulating the growth and activity of bacteria present in the colon (Gibson & Roberfroid, 1995). Those bacteria are known as probiotics (Kailasapathy & Chin, 2000). The most common probiotics are lactic acid bacteria (LAB) and bifidobacteria (Huebner, Wehling, & Hutkins, 2007; Khurana & Kanawjia, 2007; Ötles, Çagındı, & Akçiçek, 2003). The combination of prebiotics and probiotics which is called synbiotics can be used to manage the microflora in the gut, and enhance survival of probiotics by stimulating growth/activity of bacteria in the colon by prebiotics (Gibson & Roberfroid, 1995; Khurana & Kanawjia, 2007). Examples of synbiotics are bifidobacteria (probiotics) and FOS (prebiotics) as well as lactobacilli and lactitol (Collins & Gibson, 1999). Both prebiotics and probiotics are mostly used in fermented dairy products like functional food product worldwide. Therefore, more research is needed to develop new high value bio products and increase their potential use in functional foods or nutaceuticals (Figueroa-Gonzzalez, Quijano, Ramirez, & Cruz-Guerrero, 2010).

The objectives of this study were to i) evaluate probiotic potentials of raspberry and strawberry addition in yogurts, ii) investigate antioxidant activity of microwave extracts of the both fruits using ORAC, DPPH, and iii) determine the TPC values of berries. The probiotic potentials of those fruit addition into yogurts containing

different probiotics were determined by viable microbe count in each yogurt treatment using selective media, pH and total titratable acidity (TTA) during 28 days at 4 ºC.

2. Materials and Methods

2.1 Materials

Solvents methanol, acetone, HCL, ethyl alcohol, was analytical grade and purchased from Caledon Laboratories LTC (Georgetown, ON, Canada). Monobasic and dibasic potassium phosphate, fluorescein, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), rutin, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), Folin-Ciocalteu (FC) reagent, and 2,2-diphenyl-1-picryhydrazyl radical (DPPH), NaOH Gallic acid standard (99% purity), Tween® 80 and phenolphthalein were obtained from Sigma (Oakville, ON, Canada). Sodium carbonate, sodium propionate was obtained from Church and Dwight Canada Corp. (Mississauga, ON, Canada). Peptone, yeast extract powder (pH 7.0 ± 0.5), sodium acetate anhydrous, ammonium citrate (dibasic), magnesium sulfate, manganese sulfate, agar, lithium chloride, sodium hydroxide micro pearls were purchased from BioShop® Canada Inc. (Burlington, ON). D-(+) - Trehalose dehydrate, meat extract was obtained from EMD Chemicals Inc. (Gibbstown, NJ). Difco ™ Lactobacilli MRS Agar was obtained from Becton, Dickinson and Company (Sparks, MD). M17 agar, lactose bacteriological grade, MRS broth (de man, Rogosa, Sharpe), M17 broth, anaerobic indicator, Anaerogen™, the starter cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* (B-548; USDA) and *Streptococcus salivarius* subsp. *thermophilus* (14485; ATCC) and the probiotics of *Lactobacillus acidophilus* (B-4495; USDA) and *Bifidobacterium lactis* (41405; USDA) were purchased from Oxoid Ltd. (Basingstoke, UK).

2.2 Probiotic Activity

2.2.1 Selecting Berry Concentration

Raspberry and strawberries were provided by Dentz Orchards in Ottawa, Canada. For determining appropriate berry concentrations of raspberry and strawberry that could be added into yogurt without causing syneresis was determined by preliminary testing. Whole fruits were crushed and homogenized in a fruit blender (Black & Decker, WI, USA). Individual concentrations of 3, 4 and 5% of raspberry (Figure 1A) and strawberry (Figure 1B) were added to 50 mL of pasteurized milk with starter cultures. The tubes were incubated at 42°C until yogurt was formed (Espírito Santo et al., 2010). All yogurt treatments of varying berry concentrations were carried out in triplicate.

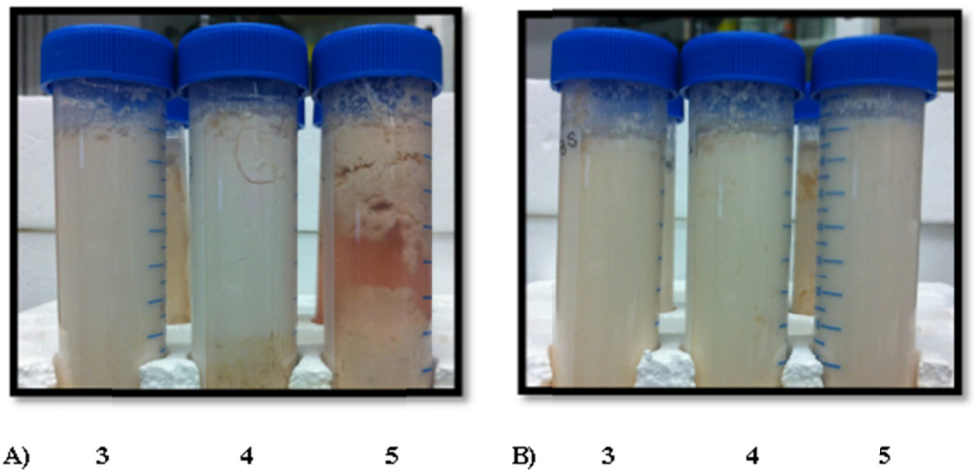

Figure 1. Yogurts with varying concentrations (3%, 4% and 5%) of A) raspberry and B) strawberry

2.2.2 Microbial Cultures

MRS broth was used to grow *Lactobacillus delbrueckii* subsp. *Bulgaricus* (yogurt starter), and *Lactobacillus acidophilus* (probiotic 1) as well as *Bifidobacterium lactis* (probiotic 2). M17 broth was used to grow *Streptococcus salivarius* subsp. *Thermophiles* (yogurt starter). For each test tube, 200 µL of bacterial culture and 10 mL of corresponding broth was added and incubated at 37 °C overnight in a shaker. The test tubes were centrifuged at 4000 rpm for 20 minutes at room temperature (23 °C) and the supernatant was decanted and 10 mL of sterile water was added to rinse the bacteria. After repeating the same steps twice, the supernatant was

decanted and 5 mL of sterile water was added. A hemacytometer was used to count the bacteria until a concentration of about 6.5 log cfu/mL was reached.

2.2.3 Yogurt Preparations

Homogenized (3.25%) milk (commercial source in Ottawa, ON) was heated until the temperature reached 85 °C for 15 minutes. Then, the pasteurized milk was cooled in a water bath and kept at 42 °C (Espírito Santo et al., 2010). Twelve different yogurt treatments were prepared; 4 with raspberry, 4 with strawberry and 4 without berry (control) as shown in Table 1. For each test tube, 50 mL of pasteurized milk and 1 mL of starter culture (microorganism diluted with milk) was added. The probiotics (1 mL) were added to the respective yogurt treatments as presented in Table 1. The treatments were incubated at 42 °C until yogurt was formed, once formed the tubes were stored at 4 °C in the fridge. All analyses were made in triplicate.

Table 1. The experimental design used to evaluate the effects of raspberry and strawberry addition on probiotic viability in different yogurt trials

Yogurt trials*	Sample coding
Y	1
Y + Pro 1	2
Y + Pro 2	3
Y + Pro 1 + Pro 2	4
Y + R	1R
Y + R + Pro 1	2R
Y + R + Pro 2	3R
Y + R + Pro 1 + Pro 2	4R
Y + S	1S
Y + S + Pro 1	2S
Y + S + Pro 2	3S
Y + S + Pro 1 + Pro 2	4S

*Y=standard yogurts containing only starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Pro1= probiotic *Lactobacillus acidophilus,* Pro 2=probiotic *Bifidobacterium lactis,* R=raspberry, S=strawberry.

2.2.4 Microbiological Analyses

Viable bacteria counts were performed on day 1, 7, 14, 21 and 28 in triplicate by following the study of Espírito Santo et al. (2010). Serial dilutions (10^{-1} to 10^{-5}) were made for each yogurt treatment using a 1: 9 ratio. For each dilution, 10 μL was plated onto agar dishes using the spread plate method.

The starter cultures and probiotics were enumerated on selective media according to the method by Vinderola et al. (1999). *L. delbrueckiii* subsp *bulgaricus* was enumerated on MRS (pH 5.4) agar and grown under aerobic conditions for 72 hours at 42°C (Gonçalves, Freitas, Nero, & Carvalho, 2009; Vinderola & Reinheimer, 1999). S. *thermophilus* was enumerated on M17 agar and grown under aerobic conditions for 24 hours at 37 °C (Vinderola & Reinheimer, 1999). *L. acidophilus* was enumerated on T-MRS agar and grown under aerobic conditions for 48 hours at 37 °C (Vinderola & Reinheimer, 1999). *B. lactis* was enumerated on LP-MRS agar and grown under anaerobic conditions in BBL GasPak™ System (GasPak System-Oxoid, Basingstoke, UK) for 48 hours at 37 °C (Vinderola & Reinheimer, 1999). The number of colonies was counted and the number of cells was converted into log cfu (colony forming units) per mL.

2.2.5 pH and TTA

Both pH and TTA values of each yogurt treatment (Table 1) was measured on day 1, 7, 14, 21 and 28 according to the method by Espírito Santo et al. (2010). The pH was measured using the Denver Instrument UB-5 pH meter. TTA value was determined by mixing of 1 mL of yogurt with 9 mL of sterile water (1: 9) which was titrated with 0.1 M NaOH and 0.1% phenolphthalein colour indicator (Behrad, Yusof, Goh, & Baba, 2009). The amount of acid produced during fermentation was expressed as TTA%. All analyses were made in triplicate.

2.3 Antioxidant Activity Analysis

2.3.1 Microwave Extraction of Water Extractable Material (WEM)

Raspberries and strawberries were crushed using a juice processor and stored in Ziploc bags in the freezer at -20 °C prior to analysis. WEM was extracted by using microwave. The samples (5 g of each berry samples) and 50 mL of distilled water were added into a quartz vessel and placed into the CEM STAR System 2 microwave digestion system (CEM Corporation, Matthews, NC, USA) at 90 °C for 30 minutes. The mixture was then cooled and centrifuged at 4000 rpm for 20 minutes at room temperature (23 °C). The supernatant was collected and stored at -20 °C in the freezer until further analysis (Liazid, Palma, Brigui, & Barroso, 2007). All analyses were made in triplicate.

2.3.2 ORAC

The antioxidant activity of raspberry and strawberry WEM was measured using ORAC assay by following the procedures of Huang et al. (2002) and Hosseinian et al. (2007). The FLx800^{TM} Multi-Detection Microplate Reader with Gen5^{TM} software by BioTek Instruments was used to carry out the assay. Basically it consists of using 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) as the free radical generator, Trolox (water-soluble α-tocopheral (Vitamin E) analogue) as the standard and fluorescein working solution as the fluorescent probe (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002; Wang & Lin, 2000). Five different concentrations of Trolox standard (6.25, 12.5, 25, 50 and 100 μM) and two concentrations (200 and 100 μM) of rutin control were prepared. For each ORAC run, a 96 micro-well plate was prepared; including 20 μL of buffer (blank), Trolox (standards), sample, and rutin was loaded into designated wells. In each well, 120 μL of fluorescein working solution was added to incubate at 37 °C for 20 min. Then, 60 μL of AAPH peroxyl radical generator was added into each well to make a total well volume of 200 μL. The fluorometric microplate reader was used at an excitation wavelength and emission wavelength of 485 nm and 528 nm, respectively. The ORAC value was calculated by using the area under the curve (Net AUC) of the sample and the equation of the line from the Trolox standard curve. ORAC values were expressed as micromole Trolox Equivalents/100 g berry. All analyses were carried out in triplicate.

2.3.3 DPPH

The antioxidant activity of raspberry and strawberry WEM was determined using the DPPH scavenging activity assay (Li, Hydamaka, Lowry, & Beta, 2009). Briefly, 200 μL of sample was mixed with 3.8 mL of DPPH solution (60 μM). The absorbance (A) of the mixture was measured at a wavelength of 515 nm at 0, 5, 10, 15, 20, 30, 40, 50, 60 minutes using the UV-Visible SpectraMax Plus384 spectrophotometer in triplicate. Absorbance was measured against a blank of methanol. The antioxidant activity was calculated as percent discoloration as shown in the following equation. All analyses were made in triplicate.

$$\%\, DPPH = \left(1 - \left[\frac{A_{sample}}{A_{control\ t=0}}\right]\right) \times 100 \quad (1)$$

2.3.4 Extraction of Phenolics and TPC Analysis

Extraction: Phenolic compounds from raspberry and strawberry were extracted according to the method of Li et al. (2009). Each berry sample (1.0 g) was mixed with 15 mL of ethanol (95%)/1N HCl (85:15, v/v) solution and the mixture was stirred for 6 hours at room temperature (23°C). Then the mixture was centrifuged at 4,000 rpm for 15 minutes at 5°C. The supernatant was collected and stored at -20°C in the freezer under further analysis.

TPC analysis: 200 μL of each berry extract was mixed with 1.9 mL of 10 fold diluted FC reagent. After 5 minutes at room temperature (23°C), 1.9 mL of a 60 g/L sodium carbonate solution was added. The absorbance was measured after 120 minutes of incubation at room temperature (23°C) at 725 nm against a blank of distilled water using the UV-Visible SpectraMax Plus384 spectrophotometer. The absorbance was measured in triplicate and the results were expressed as gallic acid equivalents per gram of sample.

2.4 Statistical Analyses

All experiments were conducted in triplicates by means of Analysis of variance (ANOVA) with Statistical Analysis System (SAS, version 9.2, SAS Institute Inc., Cary, NC). Duncan's Multiple Range test was used when significant ($P<0.05$) mean comparison was performed.

3. Results and Discussion

3.1 Probiotic Activity

3.1.1 Berry Concentrations

The best raspberry and strawberry concentration that could be added into milk was determined by adding different amount of berry to milk and maintain a stable yogurt product that would be acceptable to the consumers as palatable yogurt (Espírito Santo et al., 2010). Therefore, 3% berry addition in yogurt was determined as the best concentration to be employed for the all yogurt trials (Table 1) since there was no sign of syneresis (separation of water from gel) (Figure 1A and B).

3.1.2 Viable Microorganism Counts With Selective Media

The viable microorganism counts of *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* in all yogurt trials (Table 1) are shown in Table 2 as log cfu mL^{-1}. Both counts showed no significant ($P<0.05$) difference among treatments (Table 2) after 1 day of cold storage and varied from 7.18 to 7.82 log cfu mL^{-1}.

Table 2. *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* counts (log cfu mL^{-1}) in control (1-4), raspberry (1R-4R) and strawberry (1S-4S) yogurts

Samples*	Day 1	Day 7	Day 14	Day 21	Day 28
S.thermophilus					
1	7.73^{a}	7.96^{a}	7.57^{ab}	8.17^{abc}	7.88^{ab}
2	7.68^{a}	7.84^{ab}	7.49^{ab}	7.85^{cd}	8.03^{a}
3	7.38^{a}	7.49^{bcd}	7.51^{ab}	8.00^{bcd}	7.99^{a}
4	7.18^{a}	7.64^{abc}	7.69^{ab}	8.15^{abc}	7.95^{a}
1R	7.29^{a}	6.73^{f}	8.13^{a}	8.51^{a}	8.08^{a}
2R	7.39^{a}	7.43^{cd}	7.59^{ab}	8.38^{ab}	8.22^{a}
3R	7.67^{a}	7.23^{de}	7.44^{b}	8.55^{a}	8.14^{a}
4R	7.68^{a}	7.63^{abc}	7.75^{ab}	8.41^{ab}	8.05^{a}
1S	7.45^{a}	7.01^{ef}	7.44^{b}	7.65^{de}	8.13^{a}
2S	7.55^{a}	7.32^{cde}	7.52^{ab}	7.42^{ef}	7.60^{bc}
3S	7.59^{a}	7.54^{bcd}	7.49^{ab}	7.61^{def}	8.04^{a}
4S	7.82^{a}	7.16^{de}	7.41^{b}	7.23^{f}	7.31^{c}
L.bulgaricus					
1	7.60^{a}	7.70^{b}	7.04^{d}	7.50^{abc}	7.66^{ab}
2	7.18^{a}	7.36^{b}	8.14^{bc}	7.75^{abc}	8.18^{a}
3	7.28^{a}	7.40^{b}	7.95^{c}	7.66^{abc}	7.73^{ab}
4	7.18^{a}	7.58^{b}	8.18^{bc}	8.10^{a}	7.80^{ab}
1R	7.29^{a}	8.37^{a}	8.90^{a}	7.98^{ab}	8.03^{ab}
2R	7.39^{a}	8.41^{a}	8.71^{ab}	7.94^{ab}	7.61^{ab}
3R	7.67^{a}	8.44^{a}	8.52^{abc}	8.06^{ab}	7.73^{ab}
4R	7.68^{a}	8.49^{a}	8.40^{abc}	7.96^{ab}	7.94^{ab}
1S	7.45^{a}	8.62^{a}	8.20^{bc}	7.73^{abc}	7.90^{ab}
2S	7.75^{a}	8.40^{a}	7.85^{c}	7.30^{c}	7.43^{b}
3S	7.49^{a}	8.51^{a}	8.07^{bc}	7.53^{abc}	7.66^{ab}
4S	7.82^{a}	8.40^{a}	7.86^{c}	7.43^{bc}	7.62^{ab}

*1-4=control yogurts containing no berry, 1R-4R=yogurts containing raspberry, 1S-4S=yogurts containing strawberry. *Lactobacillus acidophilus* (probiotic 1) added yogurts (2, 4, 2R, 2S, 4R, 4S), and *Bifidobacterium*

lactis (probiotic 2) added yogurts (3, 4, 3R, 3S, 4R, 4S). cfu =colony forming units. Different letters in columns in the same day are significantly different (P<0.05) in Duncan's multiple range tests.

At day 7, *L.bugaricus* counts in both berry containing yogurts were significantly (P < 0.05) higher than the control yogurts just to the opposite of *S.thermophilus* counts. At day 14, *L.bulgaricus* counts in yogurts containing raspberry (1R), raspberry with *L. acidophilus* (2R) and strawberry (1S) were significantly higher (P<0.05) than the control. At day 21, *S.thermophilus* counts were significantly higher (P<0.05) in treatments that contained raspberry and *L. acidophilus* (2R) and raspberry and *B. lactis* (3R). Meanwhile, *L.bulgaricus* count on the same day for strawberry yogurts with the exception of strawberry and *B. lactis* (3S) were significantly lower (P < 0.05) than the control and there was not a significant difference (P<0.05) in treatments containing raspberry. The treatment containing strawberry and both probiotics (4S) was significantly lower than the control. At day 28, the counts of *S. thermophilus* ranged from 8.05 to 8.22 log cfu mL^{-1} in raspberry yogurts, 7.31 to 8.13 log cfu mL^{-1} in strawberry yogurts and 7.88 to 8.04 log cfu mL^{-1} in the controls. There was not a significant difference (P < 0.05) in treatments that contained raspberry. Treatments that contained strawberry and *L. acidophilus* (2S) and strawberry with both probiotics (4S) were significantly lower (P <0.05) than the control.

The viable microbial counts of each probiotic-1 (2, 2R, 2S) and probiotic-2 (3, 3R, 3S) and both probiotics (4, 4R, 4S) in yogurt trials during 28 days of cold storage were shown in Figure 2 and Figure 3. On day 1, for yogurts containing raspberry and strawberry, there was not a significant difference (P<0.05) on the growth of probiotic-1 compared to the control yogurts, the counts of *L. acidophilus* ranged from 6.37 to 6.39 log cfu mL^{-1} in yogurts containing raspberry, and 6.33 to 6.40 log cfu mL^{-1} in yogurts containing strawberry and 5.78 to 6.30 log cfu mL^{-1} in the control (Figure 2). The counts of *B. lactis* ranged from 6.30 to 6.54 log cfu mL^{-1} in yogurts containing raspberry, 6.20 to 6.56 log cfu mL^{-1} in yogurts containing strawberry and 6.58 to 6.68 log cfu mL^{-1} in the control (Figure 3). The counts in yogurt containing strawberry and *B. lactis* (3S) was significantly lower (P<0.05) than the control. There was not a significant difference (P < 0.05) in treatments containing berry and both probiotics compared to the control. At day 7, the counts of *L. acidophilus* in yogurt containing raspberry and strawberry and *L. acidophilus* (2R and 2S) and in yogurts containing berry with both probiotics (4R and 4S) were significantly higher (P<0.05) than the control. The counts of *B. lactis* in yogurts containing raspberry and strawberry and *B. lactis* (3R and 3S) were not significantly different (P<0.05) from the control. *B. lactis* counts in yogurt containing berry with both probiotics (4R and 4S) were significantly higher (P<0.05) than the control. At day 14, the counts of *L. acidophilus* in yogurt containing raspberry and *L. acidophilus* (2R) was significantly higher (P<0.05) compared to the control. There was not a significant difference (P<0.05) in *L. acidophilus* counts in yogurt containing berry with both probiotics (4R and 4S) compared to the control. The counts of *B. lactis* in yogurt containing raspberry and *B. lactis* (3R) was significantly higher (P<0.05) than the control. *B. lactis* counts in yogurt containing berry with both probiotics (4R and 4S) was not significantly different (P<0.05) compared to the control.

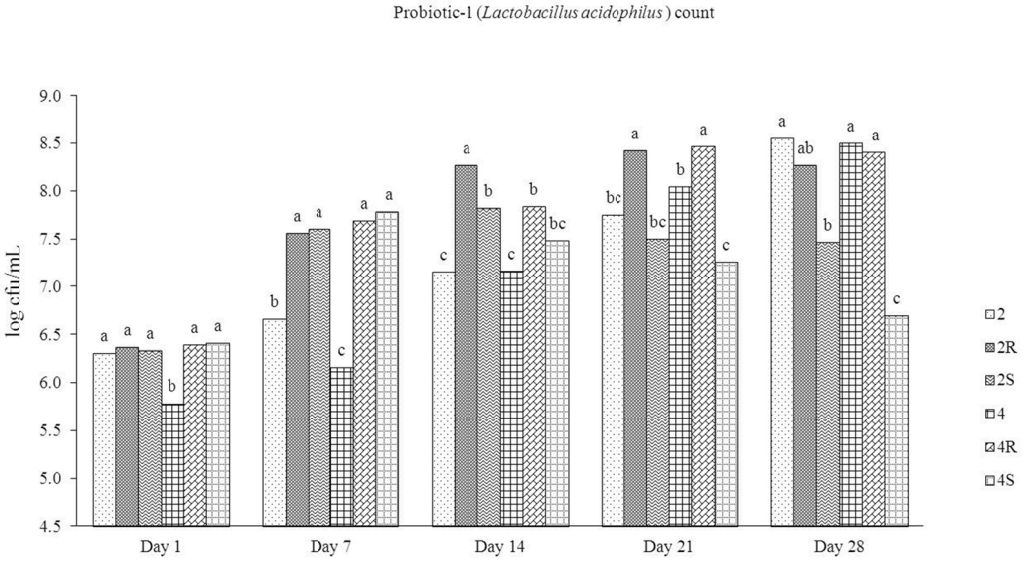

Figure 2. Probiotic 1-*Lactobacillus acidophilus* counts in control (2, 4), raspberry (2R, 4R) and strawberry (2S, 4S) yogurts over 28 days of cold storage at 4 °C

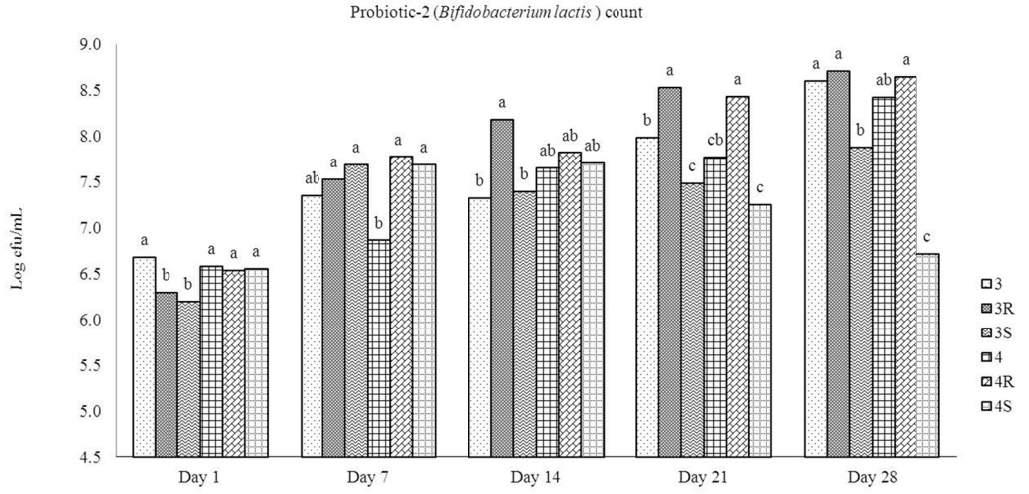

Figure 3. Probiotic 2-*Bifidobacterium lactis* counts in control (3, 4), raspberry (3R, 4R) and (3S, 4S) yogurts over 28 days of cold storage at 4 °C

At day 21, the counts of *L. acidophilus* in yogurt containing raspberry and *L. acidophilus* and (2R) was significantly higher (P<0.05) than the control. *L. acidophilus* counts were significantly lower (P<0.05) in yogurt containing strawberry and both probiotics (4S). The counts of *B. lactis* in yogurt containing raspberry and *B. lactis* (3R) and raspberry with both probiotics (4R) was significantly higher (P<0.05) than the control. At day 28, the counts of *L. acidophilus* ranged from 8.27 to 8.40 log cfu mL^{-1} in yogurts containing raspberry, 6.69 to 7.46 log cfu mL^{-1} in yogurts containing strawberry and 8.50 to 8.55 log cfu mL^{-1} in the control. Counts were significantly lower (P<0.05) in yogurt containing strawberry and *L. acidophilus* (2S) and strawberry and both probiotics (4S). The counts of *B. lactis* ranged from 8.65 to 8.72 log cfu mL^{-1} in yogurts containing raspberry, 6.72 to 7.88 log cfu mL^{-1} in yogurts containing strawberry and 8.43 to 8.60 log cfu mL^{-1} in the control. Yogurt containing strawberry and *B. lactis* (3S) and strawberry and both probiotics (4S) were significantly lower (P<0.05) than the control. From day 21 to 28, the counts of probiotics in treatments containing strawberry were lower than the control. At the end of 28 days of cold storage, the counts of probiotics in the control and yogurts containing berry were above 6 log cfu mL^{-1}. The counts of probiotics should range from 6 to 8 cfu mL^{-1} which is the recommended amount that should remain at the end of cold storage (Vasiljevic & Shah, 2008). Compounds such as the nutrients in the berries may act as prebiotic substrates and possibly stimulate the growth of microorganisms and their viability. The counts of *L. acidiphulis* were positively correlated (r = 0.89, Figures 2 and 3) with *B. lactis* from day 7 to 28. Moreover, the starter of *Lactobacillus delbruekii* subsp. *bulgaricus* was positively correlated (r = 0.72, Table 2, Figures 2) with probiotic 1 (*L. acidiphulis).*

There are studies which show that the addition of fruit pulp increase the counts of probiotic bacteria. In a study by Espírito Santo et al. (2010), the effect of açai addition into yogurts containing different probiotic bacteria; *Lactobacillus acidophilus* L10, *Bifidobacterium animalis* ssp. *lactis* B104 and *Bifidobacterium longum* B105 and *Bidfidobacterium anaimalis* ssp. *lactis* B94 were investigated. The addition of açai favoured the increase of *Lactobacillus acidophilus* L10, *Bifidobacterium animalis* ssp. *lactis* B104 and *Bifidobacterium longum* B105 with values of 7.65, 9.36 and 5.42 log cfu mL^{-1} respectively (Espírito Santo et al., 2010). In another study, the addition of dietary fibers from apple, banana and passion fruit into yogurt containing different probiotic bacteria; *Lactobacillus acidophilus* L10 and *Bifidobacterium animalis* subsp. *lactis* Bl04, HN019 and B94 were investigated (Espírito Santo et al., 2011). Probiotic viability increased in *B. animalis* subsp. *lactis* Bl04, HN019 and B94 as well as *Lactobacillus acidophilus* L10 with apple and banana fiber addition (Espírito Santo et al., 2010). In a study by Kailasapathy et al. (2008), the effect of mango, mixed berry, passion fruit and strawberry addition into yogurt containing the probiotic bacteria *Lactobacillus acidophilus* L10 and *Bifidobacterium animalis* ssp. *lactis* B94 were investigated. The addition of 10 g/100 g of passion fruit or mixed berry had an effect on *L. acidophilus* L10 (Kailasapathy, Harmstorf, & Phillips, 2008).

There are various factors that affect the viability of probiotic bacteria in yogurt which include the inoculum level, temperature, length of fermentation as well as the strains of microorganisms used (Kailasapathy & Chin, 2000). *L. acidophilus* and *Bifidobacterium* are affected by oxygen; these microorganisms do not have an

electron-transport chain which means that oxygen is not going to be reduced properly and exposure to oxygen can lead to cell death due to metabolite accumulation (Talwalkar & Kailasapathy, 2004). The growth of bifidobacteria can be affected by other microorganisms that are present within the yogurt which can possible restrict its growth (Kailasapathy & Chin, 2000). The decrease could also be due to a reduction in sugars present in the yogurt which in turn provides fewer nutrients for the bacteria to consume (Agil & Hosseinian, 2012).

3.1.3 pH and TTA

Probiotic bacteria produce various acids such as butyric, lactic and propionic acid with lactic acid being the most prevalent (Agil & Hosseinian, 2012). On day 1, the pH among all yogurt treatments ranged from 6.16 to 6.45 (Table 3). Yogurt containing raspberry and *B. lactis* (3R), strawberry and *L. acidophilus* (2S), strawberry and *B. lactis* (3S), strawberry and both probiotics (4S) were significantly lower ($P<0.05$) than the control. The TTA% among all yogurt treatments ranged from 0.13 to 0.20 (Table 4). In yogurt treatments containing raspberry (1R-4R) and strawberry (1S-4S), the lactic acid content was significantly higher ($P<0.05$) compared to the control.

On day 7, the pH among all yogurt treatments ranged from 6.06 to 6.50 (Table 3). There was not a significant difference ($P<0.05$) in pH among treatments containing berry compared to the control. The TTA% among all yogurt treatments ranged from 0.16 to 0.25 (Table 4). Yogurt treatments containing raspberry (1R-4R) were significantly higher ($P<0.05$) than the control. Yogurt containing strawberry with *L. acidophilus* (2S) and strawberry with both probiotics (4S) were significantly higher ($P<0.05$) than the control.

On day 14, the pH among all yogurts treatments ranged from 5.77 to 6.47 (Table 3). Yogurt containing raspberry (1R) had a significantly lower ($P<0.05$) pH than the control. The TTA% among all yogurt treatments ranged from 0.21 to 0.49 (Table 4). There was a significant higher ($P<0.05$) lactic acid content in yogurt containing raspberry with the exception of yogurt with raspberry and both probiotics (4R) compared to the control.

Table 3. The pH values of control (1-4), raspberry (1R-4R), and strawberry (1S-4S) yogurts

Samples*	Day 1	Day 7	Day 14	Day 21	Day 28
1	6.40^{abc}	6.56^{a}	6.47^{a}	6.30^{ab}	6.04^{a}
2	6.30^{cd}	6.09^{b}	6.02^{bcd}	5.90^{bcd}	5.70^{b}
3	6.16^{e}	6.43^{ab}	6.36^{ab}	6.30^{ab}	6.08^{a}
4	6.23^{ed}	6.50^{ab}	6.47^{a}	6.48^{a}	6.19^{a}
1R	6.38^{abc}	6.15^{ab}	5.77^{d}	5.64^{cd}	5.12^{c}
2R	6.31^{bcd}	6.11^{b}	5.88^{cd}	5.41^{d}	5.10^{c}
3R	6.31^{bcd}	6.06^{b}	5.98^{bcd}	5.58^{cd}	5.13^{c}
4R	6.28^{cd}	6.12^{ab}	6.04^{abcd}	5.53^{cd}	5.21^{c}
1S	6.45^{a}	6.22^{ab}	6.14^{abcd}	5.84^{bcd}	5.25^{c}
2S	6.43^{ab}	6.21^{ab}	6.11^{abcd}	5.87^{bcd}	5.12^{c}
3S	6.4^{abc}	6.22^{ab}	6.15^{abcd}	6.01^{abc}	5.26^{c}
4S	6.43^{ab}	6.20^{ab}	6.24^{abc}	5.88^{bcd}	5.10^{c}

*1-4=control yogurts containing no berry, 1R-4R=yogurts containing raspberry, 1S-4S=yogurts containing strawberry. *Lactobacillus acidophilus* (probiotic-1) added yogurts (2, 4, 2R, 2S, 4R, 4S) and *Bifidobacterium lactis* (probiotic 2) added yogurts (3, 4, 3R, 3S, 4R, 4S). Different letters in columns in the same day are significantly different ($P<0.05$) in Duncan's multiple range tests.

Table 4. Total titratable acidity (TTA %) values of control (1-4), raspberry (1R-4R), and strawberry (1S-4S) yogurts

Samples*	Day 1	Day 7	Day 14	Day 21	Day 28
1	0.14^{bc}	0.17^{b}	0.21^{e}	0.41^{c}	0.57^{cd}
2	0.12^{c}	0.17^{b}	0.22^{e}	0.40^{c}	0.52^{d}
3	0.13^{c}	0.16^{b}	0.24^{de}	0.40^{c}	0.52^{d}
4	0.13^{c}	0.16^{b}	0.22^{de}	0.36^{c}	0.48^{d}
1R	0.20^{a}	0.25^{a}	0.45^{ab}	0.59^{ab}	0.68^{bc}
2R	0.20^{a}	0.24^{a}	0.38^{abc}	0.60^{a}	0.71^{bc}
3R	0.18^{a}	0.25^{a}	0.49^{a}	0.54^{ab}	0.69^{bc}
4R	0.17^{ab}	0.24^{a}	0.36^{abcd}	0.58^{ab}	0.88^{a}
1S	0.19^{a}	0.22^{ab}	0.33^{bcde}	0.58^{ab}	0.75^{ab}
2S	0.19^{a}	0.24^{a}	0.32^{bcde}	0.51^{ab}	0.69^{bc}
3S	0.19^{a}	0.20^{ab}	0.30^{cde}	0.50^{b}	0.75^{ab}
4S	0.18^{ab}	0.24^{a}	0.32^{bcde}	0.52^{ab}	0.88^{a}

*1-4=control yogurts containing no berry, 1R-4R=yogurts containing raspberry, 1S-4S=yogurts containing strawberry. *Lactobacillus acidophilus* (probiotic-1) added yogurts (2, 4, 2R, 2S, 4R, 4S) and *Bifidobacterium lactis* (probiotic 2) added yogurts (3, 4, 3R, 3S, 4R, 4S). Different letters in columns in the same day are significantly different ($P<0.05$) in Duncan's multiple range tests.

On day 21, the pH among all yogurt treatments ranged from 5.41 to 6.48 (Table 3). Yogurt that contained raspberry (1R), raspberry and *B. lactis* (3R), raspberry and both probiotics (4R) and strawberry and both probiotics (4S) had a significantly lower ($P<0.05$) pH compared to the control. The TTA% among all yogurt treatments ranged from 0.40 to 0.60 (Table 4). In all yogurts containing raspberry (1R-4R) and strawberry (1S-4S), the lactic acid content was significantly higher ($P<0.05$) than the control.

On day 28, the pH among all yogurt treatments ranged from 5.10 to 6.04 (Table 3). Yogurt containing raspberry (1R-4R) and strawberry (1S-4S) had a significantly lower ($P<0.05$) pH compared to the control. The TTA% among all yogurt treatments ranged from 0.36 to 0.60 (Table 4). All yogurt treatments containing berry had a significantly higher ($P<0.05$) lactic acid content compared to the control with the exception of yogurt containing raspberry (1R).

After 28 days of cold storage the pH in yogurts containing raspberry and strawberry were lower than control yogurts ($P<0.05$). The TTA% in yogurts with raspberry and strawberry were higher than control yogurts which has no berry addition. Yogurts that contain berry possibly have a greater decrease in pH due to microorganisms that are more active in the presence of berries (Kailasapathy et al., 2000). The increase in TTA indicates that during the growth of bacteria there is lactic acid production (Agil & Hosseinian, 2012).

3.2 Antioxidant Activity

3.2.1 ORAC

Raspberry had higher antioxidant activity ($P<0.05$) compared to strawberry with ORAC values of 505.72 μmol TE/100 g of fruit and 495 μmol TE/100g of fruit respectively (Table 5). In the study of Wang and Lin (2000), the antioxidant activity of fruits and leaves of different genotypes and development stages of blackberry, raspberry and strawberry fruits and was investigated. The ORAC values of fresh red and black raspberries from different cultivars ranged from 7.8 to 33.7 μmol of TE/g during different stages of maturity (Wang & Lin, 2000). The ORAC values of fresh strawberries from different cultivars ranged from 12.2 to 17.4 μmol of TE/g during different stages of maturity (Wang & Lin, 2000). The antioxidant capacity was lower in strawberry compared to blackberries and raspberries (Wang & Lin, 2000). The higher the phenolic and flavonoid content, the higher the antioxidant activity of the fruit (Liu et al., 2002).

Table 5. ORAC[1] and TPC[2] values of raspberry and strawberry

Berry	ORAC (μmol TE/100g)	TPC (GAE (g/kg)
Raspberry	505.05[a*]	0.20[a]
Strawberry	495.42[b]	0.18[a]

[1]ORAC = Oxygen radical absorbance capacity values was calculated as μmole Trolox Equivalent (TE)/100g of sample, [2]TPC = Total phenolic count of crude extract was calculated as g gallic acid equivalent (GAE)/kg of sample.* Different letters in columns are significantly different (P<0.05) in Duncan's multiple range tests.

3.2.2 DPPH

The DPPH scavenging capacity of raspberry was significantly (P<0.05) higher than strawberry with scavenging activity of 86.11% and 85.69% respectively (Figure 4). As shown in the Figure 4, it could be seen on the kinetic curve that the raspberry had a slightly higher scavenging activity during the first 20 minutes. Then, the rate of scavenging capacity of both raspberry and strawberry was almost even. The scavenging activity of six fruits was measured in a study by Li et al. (2009). The DPPH scavenging activity after 60 minutes were: chokecherry (78.86%), raspberry (51.23%), strawberry (40.33%), Saskatoon berry (36.59%), wild blueberry (34.13%) and seabuckthorn (29.97%) (Li et al., 2009). In a study by Ogawa *et al.* (2008), the anthocyanin composition and antioxidant activity of various berries were measured. After 30 minutes of incubation, the % DPPH scavenging activity of raspberry and strawberry was 46% and 25% respectively (Ogawa et al., 2008). The antioxidant activity of berries is attributed to the different antioxidants that are present (Ogawa et al., 2008).Our findings was in the agreement with those mentioned studies.

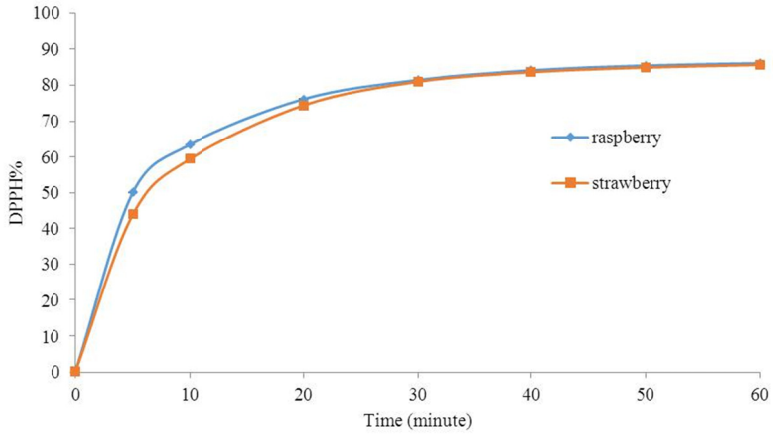

Figure 4. DPPH% scavenging activity of raspberry and strawberry extracts

3.2.3 TPC

The TPC values of raspberry and strawberry were not significantly different (P<0.05) as shown in Table 5. In a study by Wang et al. (2000), the TPC of red and black raspberries from different cultivars ranged from 0.57-3.40 g GAE/kg during different stages of maturity (Wang & Lin, 2000). The TPC of fresh strawberries from different cultivars ranged from 0.95-1.50 g GAE/g during different stages of maturity (Wang & Lin, 2000). Our findings were in agreement and in the parallel range of the mentioned study results. Moreover, in another study, the total phenolic and flavonoid contents in selected fruits and vegetables was investigated. In 0.1 mL of strawberry extract, the TPC was 0.36 g GAE/kg (Lin & Tang, 2007).

4. Conclusion

In yogurt treatments containing *L. acidophilus* and raspberry, there was an increase in microbial counts for 21 consecutive days of cold storage. After 28 days of cold storage, the pH of all yogurt treatments containing berry and probiotics decreased and showed increased TTA%. Raspberry had higher antioxidant activity than strawberry in both the ORAC and DPPH assay. This study shows that raspberries and strawberries have antioxidants activity and might act as a source of prebiotics. The presented results concerning the prebiotic effect

of raspberry and strawberry are preliminary and further study is required to investigate how polysaccharides and other bioactive compounds extracted from raspberry and strawberry will affect the growth of starter and individual pure probiotic bacteria.

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