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Lentils enhance probiotic growth in yogurt and provide added benefit of antioxidant protection

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ABSTRACT

We evaluated the antioxidant potential of lentil polysaccharides in stimulating the growth of probiotic bacteria in yogurt. Microbial counts, pH and total titratable acidity (TTA) were measured in yogurt samples containing starter cultures with or without probiotic bacteria, supplemented with whole ground green lentils. Additionally, the antioxidant potential of polysaccharides extracted from green, red, and dehulled red lentils were measured by oxygen radical absorbance capacity (ORAC) assay. Results demonstrate that green lentils selectively enhanced the number of probiotic bacteria in yogurt in the initial stages of storage and maintained overall microbial counts (starter cultures and probiotics) over a 28-day storage period. Furthermore, the overall reduction in pH and increase in TTA in lentil containing yogurt samples after the 28-day storage period at 4 °C, suggest that starter cultures preferentially utilize lentils toward the end of this storage period. Polysaccharide extracts exhibited strong antioxidant potential no significant difference between extracts from red and green whole lentil extracts (46.1 ± 2.8 and 43.1 ± 4.2 μmol trolox equivalents (TE)/g of lentil respectively). However, the antioxidant capacity of dehulled red lentil extracts was significantly lower at 24.2 ± 1.7 μmol TE/g, suggesting that the hull has bioactive components, which play an important role in free radical scavenging.

1. Introduction

Lentil (*Lens culinaris*) is a very important pulse crop in Canada, the 2nd leading producer of lentils in the world; the two main market classes are green and red lentils (Chung et al., 2008). They are nutrient rich legumes produced worldwide and serve as a staple food in various global regions such as India, Africa, and Latin America (Schneider, 2002). Lentils are an inexpensive and rich source of protein (26–30%), vitamins (folate, vitamin B1), minerals (potassium, calcium, phosphorus, magnesium, iron and zinc) and complex carbohydrates (dietary fiber, resistant starch, oligosaccharides), while also being low in fat (<1%) (Wang & Toews, 2011; Zare, Champagne, Simpson, Orsat, & Boye, 2012). Consumption of this legume has been associated with a variety of health benefits, such as a reduced risk of diabetes, colon cancer, and cardiovascular disease (Letermea, 2002; Patterson, Maskus, & Dupasquier, 2009).

Fructooligosaccharides (FOS), inulin, galactooligosaccharides as well as other related carbohydrates, are known as prebiotics. A prebiotic is defined as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Roberfroid, 2007). Current sources of prebiotics include wheat and barley, soybean, chicory, and some fruits and vegetables (Thammarutwasik et al., 2009). The addition of prebiotic sources such as inulin has also been shown to improve the stability and sensory characterization of yogurt products (Khalifa, Elgasim, Zaghloul, & Mahfouz, 2011). To date, research on the prebiotic potential of lentils is very limited (Zare, Boye, Orsat, Champagne, & Simpson, 2011; Zare et al., 2012) and none have investigated their effects on the growth rate of probiotics, *Lactobacillus acidophilus* and *Bifidobacterium lactis*, when incorporated in yogurt.

In addition to the prebiotic potential, the oligosaccharides exhibit antioxidant activity probably due to the presence of conjugated phenolics (Rao & Muralikrishna, 2006). Although the antioxidant activity of bioactive constituents such as phenolics in lentils has been previously reported (Oomah, Caspar, Malcolmson, & Bellido, 2011; Zou, Chang, Gu, & Qian, 2011), the antioxidant capacity of lentil polysaccharides has yet to be investigated.

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The overall aims of this study were to (i) evaluate the optimum concentration of ground green lentil powder that can be incorporated in yogurt, (ii) evaluate the effects of lentils on lactic acid and probiotic bacteria growth, pH, and titratable acidity in yogurt and (iii) measure and compare the antioxidant activity of polysaccharides contained in green, red, and dehulled red lentils.

2. Materials and methods

2.1. Sample preparation

Whole and dehulled red lentils (variety CDC Redcliff) and whole green lentils (variety CDC Impower) were obtained from Simpson Seed Inc. (Moose Jaw, Saskatchewan, Canada) and ground to a particle size of 1.0–2.0 mm using a Thomas Wiley Mill (Philadelphia, PA).

2.2. Probiotic activity

2.2.1. Milk preparation and sample concentration

Pasteurized, homogenized (3.25% fat) milk (commercial source in Ottawa, ON) was stirred at 85 °C for 15 min, portioned into sterile conical tubes (50 mL), and cooled to 42 °C (Espírito Santo et al., 2010). Concentrations of 0, 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.0, and 3.0 g of lentils (0–6%, w/v) were added to test tubes containing pasteurized milk and starter cultures, to determine the maximum amount of whole ground lentils that could be added to milk without disrupting fermentation. Yogurt samples of varying test concentrations were prepared in triplicate.

2.2.2. Microbial cultures

Microbial cultures consisted of yogurt starters *Lactobacillus delbrueckii* ssp. *bulgaricus* (B-548; USDA) and *Streptococcus salivarius* ssp. *thermophilus* (14485; ATCC) as well as probiotic bacteria, *L. acidophilus* (B-4495; USDA) and *B. lactis* (41405; USDA). All microbial cultures were grown in tryptic soy broth (TSA; Becton Dickinson, Sparks, MD) at 37 °C for 24 h. The bacterial cells were washed with sterile water and adjusted to a concentration of 6.5 ± 0.1 log cell/mL.

2.2.3. Yogurt preparation and sample treatments

Whole ground green lentils were incorporated at 2 g/50 mL milk (4%). Yogurts were prepared with or without lentil powder and with or without 0.5 mL of one or both probiotic bacteria as described in Table 1. All yogurt samples consisted of both starter cultures and final cell counts were as follows: 6.8 log cell/mL, 7.0 log cell/mL and 7.1 log cell/mL, in yogurts with zero, one, and both probiotics added respectively. The inoculated milk samples were prepared in triplicate and incubated at 42 °C until completion of fermentation at pH 4.5 (Behrad, Yusof, Goh, & Baba, 2009).

Table 1
Experimental design used to determine the effects of green lentils (GL) on probiotic activity in yogurt.

Microorganisms	Green lentils	Sample coding
Y	–	1
Y + Pro1	–	2
Y + Pro2	–	3
Y + Pro1 and Pro2	–	4
Y	+	1 GL
Y + Pro1	+	2 GL
Y + Pro2	+	3 GL
Y + Pro1 and Pro2	+	4 GL

Abbreviations: without GL (–); with GL (+); standard yogurt containing starter cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Y); probiotic *Lactobacillus acidophilus* (Pro1); probiotic *Bifidobacterium lactis* (Pro2).

2.2.4. Microbiological analyses

Once fermentation was complete, yogurt samples were stored at 4 °C, and bacterial enumerations were carried out once a week for a total of 4 weeks. Samples were plated in triplicate on MRS agar (Sigma–Aldrich Canada Ltd., Oakville, Ontario) following serial dilution as described previously (Espírito Santo et al., 2010) and colony counts were converted to log CFU/mL.

2.2.5. pH and titratable acidity

The pH and total titratable acidity (TTA) of yogurt samples were measured once a week for the 4-week storage period. To determine TTA, a mixture of yogurt and sterile water (1:9 v/v) was titrated with 0.1 N NaOH using 0.1% phenolphthalein indicator.

2.2.6. Statistical analyses

The experiment was conducted according to a completely randomized design with 3 replicates. Analysis of variance (ANOVA) was determined using the GLM procedure of SAS (SAS Institute, Cary, NC) and when significant ($P \leq 0.05$) mean comparison was performed using Fisher's protected Least Significant Difference test (α level = 0.05).

2.3. Antioxidant activity

2.3.1. Polysaccharide extraction

Sequential extraction of water extractable polysaccharides (WEP) from lentils was carried out in triplicate following previously described methods (Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011; Maes & Delcour, 2002). Unless otherwise indicated, all chemicals for WEP extraction and antioxidant activity analyses were purchased from Sigma–Aldrich Canada Ltd., Oakville, Ontario. Green, red or dehulled red lentils (10 g) were soaked in water overnight (16 h) using a 1:10 (w/v) ratio. The mixture was centrifuged at 5000 rpm for 20 min in a Thermo Sorval centrifuge, Legend XT Series (Fisher Scientific Canada, Nepean, ON) and the water insoluble precipitate was discarded. Enzymes, α-amylase and protease from *Bacillus lichenformisis* (Type XII-A) were added to the supernatant solution (20 μL/100 mL) to remove starch and proteins/peptides and stirred at 37 °C for 24 h. The supernatant was then heated at 95 °C for 5 min to inactivate the enzymes, cooled to room temperature and centrifuged as described previously. The supernatant solution was dialyzed against double deionized water at room temperature (23 °C) for 48 h using membrane tubing (Spectra/Por® dialysis membrane wet in 0.1% sodium azide, molecular weight cut-off: 3500 Da). Extract solutions were stored at −20 °C prior to analysis.

2.3.2. Oxygen radical absorbance capacity (ORAC) assay

The antioxidant activity of lentils (green, red, and dehulled red) was measured using an ORAC assay according to Agil and Hosseinian (2012). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analog, and *trans*-cinnamic acid were used in this assay to establish a reference range. Peroxyl radicals were generated using 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), which was prepared fresh for each run. Fluorescein was used as a fluorescence probe and rutin trihydrate served as a positive control. Fluorescence was measured at an excitation and emission wavelength of 360 and 520 nm, respectively, using an automated plate reader (FLx800, BioTek Instruments, Winooski, VT) using Gen5™ software. All solutions were prepared in phosphate buffer pH 7.4, which also served as the blank. Assays were run at 37 °C and analyses carried out in triplicate. The regression