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Pulse ingredients supplementation affects kefir quality and antioxidant capacity during storage

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ABSTRACT

Changes in kefir storage (4 °C, 28 days) were evaluated every week in response to pulse (whole faba bean [*Vicia faba* L. *minor*] and its dehulled fractions – hulls and cotyledon; whole chickpea [*Cicer arietinum* L.] and its crude mucilage) supplementation. Each supplement offered different profile of microbial count that was optimal at 14 days refrigerated storage. Bacterial growth was insignificant for faba bean hull (8.26–8.45 UFC/ml) and cotyledon (8.54–8.51UFC/ml) supplemented kefirs between 7 and 21 days storage. Titratable acidity (TTA) of kefirs decreased for the first week then increased with storage time at different rates for each supplement. Kefir pH decreased linearly with storage time differing significantly among samples after 14 days storage. Inulin and other supplementations improved lactate production and increased proteolytic activity with fermentation time. Antioxidant activity of kefir depended solely on the phenolic content and antioxidant activity of the supplements independent of storage time. The high antioxidant activity of the faba bean hull (13.03 μmol trolox eq/g kefir) supplemented kefir probably reflects its high phenolic content (57.53 mg gallic acid/g sample). Moreover, pulse supplements were superior to commercial inulin in maintaining kefir stability during refrigerated storage.

1. Introduction

The numerous health benefits of kefir is attributed to its prominent probiotic effects especially on gut health. For example, kefir can improve cardiovascular disease risk profile of young adults (18–24 yr) by attenuating C-reactive protein increase due to enhanced kefir digestibility resulting from lactose reduction by fermentation (O'Brien et al., 2015). Probiotic treatment also rescued neurogenesis and cognitive function in antibiotic treated mice by predominantly promoting progenitor cell survival in the brain (Möhle et al., 2016). These developments have spurred the food industry to invest in kefir beverages as the second generation of probiotic products. Thus, recent novelty in kefir is the new line of protein kefir drinks (20 g protein/8 oz serving) with reduced fat containing a combination of inulin and pectin as fat mimetics to bulk viscosity and increase satiety (Shelke, 2016). The veggie (beets, cucumber or tomato purees) kefir line took advantage of the vegetable's cellular matrices to reduce the amount of added sugar. Various protein-and polysaccharide-based ingredients have been developed to replace the physicochemical and sensory properties

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provided by fats. These ingredients, typically made of indigestible dietary fibers with relatively low-calorie contents can provide added health benefits and some are believed to induce greater satiety than fats (Shelke, 2016).

Pulses play important role in food and nutrition because of their numerous health benefits and are being incorporated into many popular food categories. This promotes domestic demand of pulses as a strategy to contain the soaring healthcare costs, enhance long-term health outcomes and accelerate the nutritional improvements of industrial food products. Pulses have yet to make inroads into the probiotic food category due to limited research. For example, lactic acid fermentation has been successfully applied to pulse flours including faba bean and chickpea, resulting in reduced antinutritional compounds, increased free essential amino acids and improved *in vitro* protein digestibility (Coda et al., 2015). In yogurt production, pulse ingredients including chickpea flour favored acidification by probiotic bacteria by improving lactobacilli growth (Zare, Champagne, Simpson, Orsat, & Boye, 2012).

Kefir is an excellent vehicle to deliver pulse ingredients to consumers; however, viability of probiotic organisms must be maintained within an appropriate shelf-life to be beneficial to health. Thus, faba bean flour supplementation (4%) stimulated bifidogenic microbial growth, increased titratable acidity linearly from day 1–21, and reduced pH during kefir storage for 28 days (Boudjou, Zaidi, Hosseinian, & Oomah, 2014). Subsequent studies with air-classified faba bean fractions demonstrated more efficient *Lactobacillus plantarum* growth in the starch rich than in the fiber fraction; protein enriched fraction exerted the highest lactic acid and acetic acid production and titratable acidity (TTA) indicating strong buffering capacity (Coda et al., 2015). Our investigation therefore aimed at evaluating the effects of supplementing whole faba bean flour, its cotyledon and hull fractions, chickpea flour and its mucilage on kefir stability during refrigerated storage for 28 days. Chickpea mucilage was included in the study because water-soluble polysaccharide extracted from chickpea flour has been reported to display good anti-hypertensive activities and can be used as a thickening or functional agent in food systems (Mokni Ghribi et al., 2015). Inulin was also included in our investigation since it has been extensively studied, granted blood glucose claim in Europe and provides the best evidence of prebiotic effects in human (Crane, 2016). The development of pulse-based kefir is contingent on demonstrating the prebiotic effects of the pulse ingredient/s relative to commercially available prebiotic such as inulin, the capacity of these ingredients to maintain their prebiotic effect during storage and enhance other bioactivities that can confer additional human health benefits.

2. Materials and method

Faba bean (*Vicia faba* L.) subspecies *minor* and chickpea (*Cicer arietinum* L.) samples were from Skikda and Oeud Amizour Wilaya of Bejaia, Algeria, respectively. Faba bean seeds were cleaned, air dried, and manually separated into hulls and cotyledons. All samples were initially crushed in a traditional stone mill followed by an electric coffee mill (Moulinex, France) then sieved (Tap sieve shaker AS 200; Retsch GmbH, Haan, Germany) to pass a 500 μm screen. The powders were stored in the fridge in sealed plastic bags until analysis.

2.1. Chickpea crude mucilage extraction

Ground chickpea was extracted according to HadiNezhad, Duc, Han, and Hosseinian (2013) with distilled water (1:40, w/v), stirred for 3 h at 60 °C; the extracts allowed to cool to room temperature then centrifuged (4000×*g*, 20 min; Sorvall Legend XTR, Thermo Scientific, Ashville, NC, USA). The supernatant was considered as the crude mucilage and used for further analysis.

2.2. Phenolic extraction and analysis

Phenolics were extracted with 95% acidified (1 N HCl) methanol as described previously (Hosseinian & Mazza, 2009). Briefly, defatted samples (1 g) were extracted with methanol (20 ml) by magnetic stirring for 6 h at room temperature. The extract was centrifuged (4000×*g*, 15 min; Sorvall Legend XTR, Thermo Scientific, Ashville, NC, USA), the supernatant recovered and stored in the fridge until analysis.

Total phenolics of the methanol extracts were determined by the Folin-Ciocalteau method (Singleton & Rossi, 1965). Absorbance of samples and gallic acid standards (0–0.9 mg/ml prepared in 80% ethanol) was monitored at 725 nm (Cary 50 Bio UV-visible Spectrophotometer, Varian, Mulgrave, Australia). Samples were analyzed in triplicates and results expressed in mg gallic acid equivalent (GAE)/g sample.

The $AlCl_3$ method (Lamaison & Carnet, 1990) was used for determination of total flavonoid content of the methanol extracts. Aliquots (2 ml) of extracts were added to equal volumes of a solution of 2% $AlCl_3.6H_2O$ (2 g/100 ml methanol). The mixture was vigorously shaken, and absorbance was monitored at 430 nm after 15 min incubation using quercetin (0–0.013 mg/ml in 80% ethanol) as standard. Flavonoid content was expressed in mg quercetin equivalent (QE)/g sample.

2.3. HPLC analysis of phenolic compounds

Chemicals (acetonitrile, formic acid) used for high-performance liquid chromatography (HPLC) were of chromatographic grade (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Analysis of phenolic acids in the methanol-HCl extracts of legume powders was carried out on an HPLC (Alliance Waters 2695, Canada) system equipped with photodiode array detector (PDA, Waters 2998), Empower software, and auto sampler (Waters Corp., Milford, MA,USA). The separation was carried out with an Atlantis RT3 column (150 mm × 4.6 mm, 5 μm particle size; Waters, Milford, MA, USA). Chromatographic separation was carried out with 10 μl extract using two solvent systems: (A) water: formic acid (99.99:0.01, v/v) and (B) acetonitrile 100% at 1.23 ml/min and 30 °C. The gradient conditions were as follows: solvent B: 0 min, 10%; 35 min, 50%; 40 min, 90%. The chromatograms were recorded at 254, 280, 320 and 520 nm for phenolic acids and flavonoids, respectively. Phenolics were quantified using authentic commercial compounds supplied by Sigma Aldrich Chemicals (St.Louis, MO, USA). Concentration of phenolic compounds were determined from the average of three replicate chromatograms and expressed in mg/g sample.

2.4. Oxygen radical absorbance capacity (ORAC)

Antioxidant activity was measured using the radical absorbance capacity ($ORAC_{FL}$) described previously (Agil & Hosseinian, 2012), according to established procedure (Prior et al., 2003). A multi-detection microplate fluorescence reader (BioTek Instruments, Ottawa, ON, Canada) was used with excitation and emission wavelengths at 485 and 525 nm, respectively. Sample extracts and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) standards were diluted with 75 mM phosphate buffer (pH 7.4) prior to transfer into a 96-well microplate (Fluotrac 200, Greiner Bio-One Inc., Longwood, FL, USA). A peroxyl radical was generated by AAPH [2,2′-azobis (2-methylpropionamide) dichloride] (Sigma-Aldrich, St.