Innovative Food Science and Emerging Technologies 11 (2010) 203–209

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Evaluation of fresh-cut apple slices enriched with probiotic bacteria

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ARTICLE INFO

Article history:
Received 20 May 2009
Accepted 28 August 2009

Editor Proof Receive Date 10 September 2009

Keywords:
Probiotic
Lactobacillus
LGG
Fresh-cut
Apple
Non-dairy

ABSTRACT

The aim of this study was to apply a probiotic microorganism (*Lactobacillus rhamnosus* GG; LGG) to fresh-cut apple wedges (cultivar *Braeburn*) and measure entrapment and stability of the microorganism. Instrumental eating quality parameters (Colour Lab, texture, soluble solids, titratable acidity and pH) and sensory acceptability were also monitored to investigate if application of the probiotic significantly influenced eating quality. Apple samples were cut into skin-on wedges and were dipped in an edible buffer solution containing approximately 10^{10} cfu/ml of LGG. LGG were enumerated on each test day (0, 2, 4, 6, 8 and 10) on whole wedges, on wedges flushed with a buffer solution (2% tri-sodium citrate), and on the flush-off liquid itself. All three samples sets contained ca. 10^8 cfu/g over the test period, which is sufficient for a probiotic effect, and is comparable to counts of probiotic bacteria in commercially available dairy products. This included the sample set of wedges which had been flushed with buffer solution indicating good adherence of the bacteria over the test period. Physicochemical properties of the apple wedges containing LGG compared to the control remained stable over the 10 day period. Cryo scanning electron microscopy and confocal scanning laser microscopy demonstrated good adherence of LGG to the surface of apple wedges.

Industrial relevance: Probiotic dairy foods, e.g. yoghurts, are well recognised by most consumers and command a significant market share. However, many people are allergic or intolerant to dairy products and an alternative option is desirable. Minimally processed freshly prepared fruits are a popular item and are perceived as healthy by consumers. They are therefore an ideal vehicle for incorporation of other functional components such as probiotics. Therefore, a probiotic bacterium was applied to fresh-cut apple wedges. This will provide an alternative probiotic food choice for consumers and could be particularly appealing to children. The process for making this product is relatively simple and the product would retail from the conventional chill counters of supermarket stores. It is likely that its price would be competitive with existing probiotic dairy products.

1. Introduction

In the early years bacteria were generally regarded as undesirable and the cause of many diseases. More recently scientific research has done much to reduce their negative image. In particular, much research has been aimed at searching for healthy bacteria and components which can beneficially affect health conferring bacteria. Probiotics belong to the former category and are classically defined as 'live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance' (Fuller, 1989). Several scientific studies have shown that microbial cells have a beneficial effect on the health and well-being of the human host (Salminen et al., 1998a,b) if directed in the right amounts (Dave and Shah, 1997; Kailasapathy & Rybka, 1997; Brown & Valiere, 2004; Prado, Parada, Pandey & Soccol, 2008). Some studies have indicated that regular consumption of viable probiotics can confer a number of health benefits such as a reduction of cholesterol (Anderson & Gilliland, 1999; Nguyen, Kang & Lee, 2007), control of gastrointestinal infections (McFarland et al., 1995; Saavedra, Bauman, Oung, Perman & Yolken, 1994), improvement of lactose tolerance (Hove, Norgaard & Mortensen, 1999; Kim & Gilliland, 1983; Shah, 2007), improvement in inflammatory bowel disease (Lammers et al., 2003) , inhibition of some cancers (Aso et al., 1995; Cross, 2002), anti-diabetic properties (Matsuzaki, Yamazaki, Hashimoto & Yokokura, 1997; Yadav, Jain & Sinha, 2007), anti-diarrhoeal effects (Nomoto, 2005), and immune system stimulation (De Moreno de LeBlanc et al., 2008; Cross, 2002).

Over the last years the minimally processed foods market has been extended especially due to an increase of the fresh-cut fruits market (Buckley, Cowan & McCarthy, 2007; Gorny, 2003). Development of health promoting foods is one of the key drivers for the food industry due to an increasing demand for foods enriched with physiologically active components such as probiotics (Mark-Herbert, 2004). Probiotic

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doi:10.1016/j.ifset.2009.08.016

dairy foods are a well established product and are recognised by most consumers as a healthy product. However, many people are allergic or intolerant to dairy products and an alternative option such as fruits would be desirable (Rivera-Espinoza & Gallardo-Navarro, 2008). Therefore there is a need for non-dairy products enhanced with probiotic bacteria (Betoret et al., 2003; Heenan, Adams, Hosken & Fleet, 2004; Yoon, Woodams & Hang, 2006). The aim of this study was, therefore, to apply a probiotic microorganism (*Lactobacillus rhamnosus* GG; LGG) to fresh-cut apple wedges thereby producing a doubly functional food product, i.e. the inherent functionality of the apple wedges plus the added functionality of LGG.

2. Materials and methods

2.1. Culture

The commercial strain *L. rhamnosus* GG (LGG) was obtained from Moorepark Food Research Centre (Teagasc; Fermoy, Co. Cork, Ireland).

2.2. Preparation of probiotic solution

Lyophilized cultures were grown in MRS (Oxoid Ltd., Hampshire, UK) broth overnight at 37 ºC for approximately 15 h. The cells were then washed with citric acid-sodium: citrate buffer (pH 3.8) by centrifugation (5810R; Eppendorf AG, Hamburg, Germany) at 7000 rpm for 15 min. This was repeated until the supernatant was clear (approximately 3–4 times). Washed cells were then re-suspended in citric acid: sodium citrate buffer [pH 3.8; 1:10 (w/v)].

2.3. Sample preparation

Apples (cultivar *Braeburn*) were purchased in a local supermarket, washed in water, cored (20 mm diameter stainless-steel cork borer) and cut with a stainless-steel knife into wedges (each *ca.* 10 g). Five skin-on wedges from each of five apples (chosen randomly) were used for infusion. The wedges were then dipped for 10 min in probiotic solution at a 1:1 solution/wedge ratio containing approximately 10^{10} cfu/ml of LGG. The wedges were then drained for 2 min and dipped for 2 min in a 6% (w/v) solution at a 3:1 solution/wedge ratio of browning inhibitor Natureseal® AS1 (AgriCoat Ltd., Great Shefford, UK). Natureseal® AS1 is a commercial available anti-browning agent and is widely used for inhibition of browning in the fresh-cut fruit industry. The wedges were again drained for 2 min packed in clear trays (15 cm × 10.5 cm × 3 cm; Versatile Packaging, Silverstream, Ireland), heat sealed with a breathing film (O_2 transmission of <2 ml /24 h/23 °C; water vapour transmission of <6 g/24 h/38 °C) using a modified atmosphere packaging machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and stored at 2–4 ºC for 10 days. *Breaburn* apple wedges were dipped in Natureseal® AS1 browning inhibitor as described above and were used as a control treatment. Apple wedges with probiotics and control wedges were prepared in 3 replicates and tests were carried out on the samples on day 0, 2, 4, 6, 8 and 10.

2.4. Enumeration of LGG in apple wedges

On each test day one wedge containing LGG was removed from each tray ($n = 3$) and the surface was washed off with 2% tri-sodium citrate solution [1:1 (w/v)]. This wash off was serially diluted in maximum recovery diluent (Oxoid Ltd., Hampshire, UK). Dilutions were then plated on lactobacillus selective Rogosa Agar (Oxoid Ltd., Hampshire, UK). The previously washed off wedges were then macerated in 2% tri-sodium citrate solution [1:10 (w/v)], serially diluted in MRD and plated on Rogosa Agar. A second wedge was removed from each tray ($n = 3$) macerated in 2% tri-sodium citrate solution [1:10 (w/v)], without washing off, serially diluted in MRD and plated on Rogosa Agar. All plates were incubated aerobically for 72–96 h at 37 ºC followed by enumeration. This procedure gave the LGG content of: (a) the wash off liquid, (b) washed wedges, (c) the wash of liquid + washed wedges and (d) wedges that were not washed with buffer. All samples were serially diluted in duplicates and plated in triplicates.

2.5. Physicochemical evaluation

Physical and chemical properties were measured on day 0, 2, 4, 6, 8 and 10 using five wedges per replicate of treated and control samples. Colour and firmness were measured first as described below. Samples were then homogenized for subsequent measurements.

2.5.1. Measurement of colour

The colour of the apple wedges was measured using a HunterLab D25A DP-9000 colour meter (HunterLab, Reston, VA, USA). The colour for 5 wedges per replicate was measured and expressed as a three dimensional Lab colour solid.

2.5.2. Measurement of firmness

The firmness of apple wedges was measured on 5 wedges for each treatment using a Texture Analyzer TA-XT2i (Stable Micro Systems, Godalming, UK) fitted with a 25 kg load cell and equipped with a Warner–Bratzler Blade. The wedge was fractured by a downward motion (10 mm/min) of a steel blade with a thickness of 3 mm. The maximum force (highest value in *N*) applied to break the wedge was used to quantify the firmness.

2.5.3. Measurement of soluble solids contents

Soluble solids contents (SSC) were measured using an Abbe refractometer (2WAJ; Guru Nanak Instruments, New Delhi, India). The scale was set to zero using the refractive index of water. Apple pulp was squeezed through muslin. A drop was placed on the refractometer glass prism and the percentage soluble solids content obtained.

2.5.4. Measurement of total titratable acidity and pH

For total titratable acidity (TTA) approximately 5 g of apple pulp were diluted in 100 ml of distilled water and 3–4 drops of indicator phenolphthalein was added. The solution was then titrated with a 0.1 N NaOH solution beyond pH 8.1 (AOAC, 1995). The TTA calculated as a percentage of malic acid [(ml NaOH × 0.1 N/weight of sample titrated) × 0.067 × 100)].

pH was measured on homogenous apple pulp with an Orion pH meter (420A; Thermo Fisher Scientific Inc., Waltham, MA, USA) which was calibrated prior to each measurement with phosphate buffers at pH 4.005 and 7.

2.6. Sensory evaluation

A blind study was used to evaluate the overall acceptability of apple wedges containing LGG and control wedges by an untrained 25 member taste panel between the ages of 23 and 67 years. Each panellist was given a plate containing 2 wedges (1 probiotic, 1 control). Panellists were asked to quantify the using a scale from 0 (unacceptable) to 6 (very acceptable). A score equal to three was used as the threshold to produce acceptability. Tasting was performed in a sensorial testing room with individual booths and controlled lighting. The evaluation was performed only on day 0.

2.7. Cryo scanning electron microscopy (Cryo-SEM)

Apple wedges (control and probiotic-treated, day 0) were examined using cryo scanning electron microscopy. Both the treated apple surfaces and fracture surfaces were analysed to investigate whether probiotic bacteria had penetrated the apple tissue. For fracture surfaces, thin sections approximately 5 × 5 × 2 mm were cut