Evaluation of fresh-cut apple slices enriched with probiotic bacteria

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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 entrainment 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 10¹⁰cfu/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. 10⁸ 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.

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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; Szavedra, Bauman, Oung, Perrin & Yolken, 1994), improvement of lactose tolerance (Hove, Norgaard & Mortensen, 1999; Kim & Gilliland, 1983; Shah, 1977), improvement in inflammatory bowel disease (Lammers et al., 2003), inhibition of some cancers (Aso et al., 1995; Cross, 2002), anti-diabetic properties (Matsuoka, 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 functional foods, therefore, are 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.
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 °C 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 then again drained for 2 min packed in clear trays (15 cm×10.5 cm×3 cm; Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and stored at 2–4 °C for 10 days. Braeburn 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 °C 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-900 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 (2WA; 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 the 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.
2.9. Statistical design

The statistical design was 2 dips (control, probiotic) × 6 test days (0, 2, 4, 6, 8, 10) × 3 replicates with 35 degrees of freedom (df) followed by ANOVA ([Genstat Version 3.2]; Lawes Agricultural Trust, Rothamsted, Harpenden, UK).

3. Results and discussion

3.1. Survival of LGG in apple wedges

LGG was tested for its ability to survive on apple wedges at 2–4 °C. To indicate the location of LGG on the apple wedges various samples were taken. The number of LGG was measured in (a) dipped wedges subsequently washed with tri-sodium citrate buffer [2% (w/v)], (b) the wash off liquid, (c) counts of washed wedges+wash off liquid, subsequently washed with tri-sodium citrate buffer [2% (w/v)], (d) dipped unwashed wedges. The wedges washed off with 2% tri-sodium citrate solution for 2 min had a cfu/g count of 108 (b) the wash off liquid, (c) counts of washed wedges+wash off liquid when mounted on a microscope slide with a coverslip on top. After 5 min incubation at room temperature, images were acquired using a 488 nm argon gas laser in conjunction with a 514 nm excitation filter and emission peaks set at 560 nm and 650 nm using a Leica SP5 confocal microscope (Leica Microsystems GmBH, Mannheim, Germany). When excited by the laser, viable cells fluoresce green whilst non-viable cells fluoresce red. Duplicate samples were examined and a minimum of 5 images acquired for each sample.

2.8. Confocal scanning laser microscopy (CSLM)

Confocal scanning laser microscopy (CSLM) in conjunction with in situ viability staining was used, based on a method by Auty et al. (2001), to visualise the distribution and viability of probiotic organisms on the surface of the wedges at day 0 and day 10. Briefly, one drop of Live/Dead BacLight viability stain (Biosciences Ltd, Dun Laoghaire, Ireland) was added to probiotic-treated surface of the apple wedge, cut parallel to the treated surface to ensure a horizontal surface when mounted on a microscope slide with a coverslip on top. After 5 min incubation at room temperature, images were acquired using a Leica SP5 confocal microscope (Leica Microsystems GmBH, Mannheim, Germany). When excited by the laser, viable cells fluoresce green whilst non-viable cells fluoresce red. Duplicate samples were examined and a minimum of 5 images acquired for each sample.

3.2. Physicochemical evaluation

No significant difference in instrumental colour values (Hunter-Lab) between control apple wedges and infused apple wedges was noted (P>0.05). However, during chill storage (2–4 °C) Hunter L values increased up to day 2 (Table 1), indicating a whitening effect, while L values between day 2 and 10 showed a slight decrease for the control (from 69.3 to 66.7) and probiotic apple wedges (from 69.1 to 66.7). Even though L values were significantly different (P<0.001) over the 10 day storage period these differences were very small in practical terms. In terms of browning levels samples from both treatments were visually still acceptable after 10 days of storage at 2–4 °C indicating that the browning inhibitor Natureseal® AS1 had a similar effect on the control and the probiotic wedges. This outcome was also confirmed by Hunter a (redness) values which decreased between day 0 and day 2 for the control and day 0 and 4 for probiotic apple wedges (Table 1). As the storage progressed a values increased for the control (from −3.32 to −2.52) and the probiotic apple slices (from −2.81 to −1.61) but remained negative throughout the 10 days (Table 1) indicating no development of redness an indicator for browning. There was no significant difference for Hunter b values for control apples and probiotic apple wedges and also the storage period had very little effect on the yellowness of the apple wedges (P>0.05). Natureseal® products have been studied extensively and have shown to be effective in prevention of colour deterioration (Rupasinghe, Murr, DeEll & Odumeru, 2005; Toivonen, 2008; Rößle, Gormley & Butler, 2009). There is also a possibility that the probiotic solution which is mainly citric acid sodium citrate buffer could have a positive effect on retaining the colour in apple wedges as citric acid has been reported to have an anti-browning effect (Pizzocaro, Torreggini & Gilardi, 1993; Rojas-Graü, Soliva-Fortuny & Martín-Belloso, 2008) or it could be due to low pH-induced inhibition of the enzyme responsible for browning development PPO (Garcia & Barrett, 2002).

Shear values for control apple wedges and probiotic apple wedges showed no significant difference (P>0.05, Table 1). However, shear values changed significantly (P<0.001) over the 10 day storage period. Between day 0 and 2 shear values increased which could be due to the AS1 browning inhibitor. As Natureseal® AS1 has calcium content of 90 mg 100 g−1 (Rößle et al., 2009) the firming effect could be due to cross-linking of both cell wall and middle lamella pectin by calcium ions (Rico et al., 2007). Control (from 38.6 N to 36.1) and probiotic apple (from 36.3 to 31.8) lost their firmness between day 2 and 10 but the shear values at day 10 were similar to the firmness measured on day 0 (Table 1). Natureseal® AS1 had less firming effect on the probiotic wedges which could be due to the fact that the wedges were previously dipped for 10 min in probiotic solution which could induce tissue softening.

Soluble solid contents (SSC) were not influenced by dipping apple wedges in probiotic solution as indicated in Table 1 (P>0.05). SSC ranged from 11.8 to 12.2% for both control and apple wedges containing LGG.

Values for total titratable acidity (TTA) for control apple wedges and infused apple wedges showed no difference as shown in Table 1.
P > 0.05. TA ranged from 0.53 to 0.59% malic acid with the highest value for control apples. The stability of TTA of probiotic apple wedges was expected as the pH (3.8) of the citric acid sodium citrate buffer was adjusted according to the inherent pH of the apple batch. Prior to treatment 5 apples were chosen at random to determine their pH. This is very important as large differences in the inherent pH can be a risk in terms of microbial growth for minimally processed foods (Ahvenainen, 1996; Soliva-Fortuny, Elez-Martínez & Martín-Belloso, 2004) and therefore result in a shorter shelf-life. However, the TTA and pH values for control and probiotic apple wedges showed similar patterns over the 10 days storage period but they did not correlate very well (R² = 0.66).

Overall physicochemical properties were not strongly influenced by dipping apple wedges into probiotic solution containing LGG (Table 1). However, any negative effect could have been also inhibited by post dipping wedges into browning inhibitor AS1.

### 3.3. Sensory evaluation

Sensory evaluation was conducted on day 0 to indicate the differences between sample sets of control and probiotic apple wedges (Table 1). Panellists did not express a preference for apple wedges containing LGG over control apples. 13 tasters preferred control wedges while 12 preferred probiotic apple wedges. Similarly,

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### Table 1: Physicochemical and sensory properties of apple wedges containing Lactobacillus rhamnosus GG (probiotic) and control apple wedges (dipped in AS1) stored for 10 days at 2–4 °C.

<table>
<thead>
<tr>
<th>Day</th>
<th>LSSC (%)</th>
<th>TTA (%)</th>
<th>pH</th>
<th>Shear (N)</th>
<th>Sensorya</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>68.5 ± 0.8</td>
<td>−2.22 ± 0.3</td>
<td>12.3 ± 0.1</td>
<td>0.58 ± 0.02</td>
<td>3.77 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>69.3 ± 0.8</td>
<td>−3.32 ± 0.1</td>
<td>11.9 ± 0.1</td>
<td>0.59 ± 0.02</td>
<td>3.74 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>68.7 ± 1.4</td>
<td>−2.85 ± 0.5</td>
<td>11.8 ± 0.3</td>
<td>0.53 ± 0.01</td>
<td>3.79 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>68.3 ± 0.5</td>
<td>−3.25 ± 0.3</td>
<td>18.0 ± 1.6</td>
<td>0.56 ± 0.01</td>
<td>3.75 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>67.2 ± 0.5</td>
<td>−2.09 ± 0.2</td>
<td>12.1 ± 0.1</td>
<td>0.56 ± 0.01</td>
<td>3.76 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>66.7 ± 0.5</td>
<td>−2.52 ± 0.1</td>
<td>17.0 ± 0.5</td>
<td>0.55 ± 0.02</td>
<td>3.76 ± 0.03</td>
</tr>
<tr>
<td>Probiotic</td>
<td>68.7 ± 0.4</td>
<td>−2.34 ± 0.3</td>
<td>12.0 ± 0.2</td>
<td>0.56 ± 0.02</td>
<td>3.76 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>69.1 ± 0.3</td>
<td>−2.62 ± 0.1</td>
<td>16.3 ± 0.1</td>
<td>0.55 ± 0.02</td>
<td>3.78 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>68.6 ± 1.8</td>
<td>−2.81 ± 0.2</td>
<td>17.7 ± 1.0</td>
<td>0.55 ± 0.02</td>
<td>3.76 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>68.3 ± 1.0</td>
<td>−2.27 ± 0.1</td>
<td>16.9 ± 1.1</td>
<td>0.58 ± 0.03</td>
<td>3.75 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>65.5 ± 0.7</td>
<td>−1.84 ± 0.3</td>
<td>16.1 ± 0.4</td>
<td>0.55 ± 0.02</td>
<td>3.76 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>66.7 ± 1.1</td>
<td>−1.61 ± 0.2</td>
<td>16.2 ± 0.5</td>
<td>0.58 ± 0.01</td>
<td>3.75 ± 0.01</td>
</tr>
</tbody>
</table>

| F-testb dip NS | P < 0.05 | NS | NS | NS | NS |
| F-test day P < 0.001 | P < 0.001 | P < 0.05 | NS | NS | P < 0.001 |

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Note: Overall acceptability from 0 (unacceptable) to 6 (very acceptable).

Fischer test.

Not significant.
Overall acceptability of the samples was the same with a mean value of 4.25 for control and 4.20 for probiotic apple wedges showing that probiotic apple wedges were accepted well by the panellist ($P > 0.05$, Table 1). The softer texture of the probiotic apple wedges compared to the control apple wedges was the main reason for choosing control over probiotic wedges. The time between dipping apples in AS1 and the actual taste panel was too short to allow AS1 to have a positive effect on the textural properties of the apple wedges hence the negative comments on the texture. Also some panellist disliked the slight lactic acid odour of the wedges with LGG as they commented that it is an odour which they do not expect in context with apple wedges. However, some comments indicated that LGG gave the apple wedges a unique and pleasant odour. Taste panel tests were conducted on day 0 only for logistic reasons. This aspect of the work must, therefore, be considered preliminary and will be addressed in further trials relating to intellectual property aspects of the research findings and know-how. These results are preliminary and future work will focus on the sensory evaluation once the microbial safety status has been confirmed. The effect of LGG on the natural microflora of the apple wedges and the microbial safety of the product are currently being investigated by and ISAFruit partner.
3.4. Cryo-SEM observation

Cryo-SEM revealed the presence of numerous rod-shaped bacteria on the cut, treated surface of the apple wedges (Fig. 2). Higher magnification showed the presence of needle-like crystals associated with the bacteria (Fig. 3). The needle-like crystals were probably due to the crystallisation of buffer salts. No bacteria were seen within the apple tissue either within cells or at intercellular junctions (Fig. 4). No bacteria were seen on the surface (Fig. 5) or fracture (Fig. 6) of the control sample.

3.5. Confocal scanning laser microscopy

C LSM of apple wedge surfaces revealed large numbers of unevenly distributed viable bacteria on day 0 (Fig. 7). High numbers of viable bacteria were still present at day 10 but there appeared to be a relative decrease in the number of non-viable bacteria (Fig. 8). This reflects the results shown in Fig. 1 as there was a decrease in viable bacteria on day 10 compared to day 0 for all apple wedges containing LGG.

4. Conclusion

Physicochemical and sensory evaluation indicated that dipping apple wedges in a solution containing probiotic bacteria resulted in wedges of acceptable quality with sufficient numbers of LGG adsorbed on to the surface for a probiotic effect. To make LGG even more resistant, future work could be focused on the possibility of microencapsulating the probiotic bacteria followed by infusion. Probiotic apple slices have shown to be a good alternative for allergic or intolerant people to dairy products and future studies should focus more on these groups. To increase the possible beneficial effects of probiotic bacteria for the host, it could be combined with an appropriate concentration of probiotics.

Acknowledgments

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References


Fig. 7. Confocal scanning laser micrograph of probiotic treated apple wedge surface (Day 0). Labelled with Live/Dead BacLight viability stain. Viable bacteria are green; non-viable bacteria are red. Bar = 50 μm.

Fig. 8. Confocal scanning laser micrograph of probiotic treated apple wedge surface (Day 10). Labelled with Live/Dead BacLight viability stain. Viable bacteria are green; non-viable bacteria are red. Bar = 50 μm.