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Article type : Original Research

## Microbial, physico-chemical and sensory characteristics of mango juice enriched probiotic dairy drinks

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Abbreviated title: Mango flavoured probiotic dairy drink

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1471-0307.12630](https://doi.org/10.1111/1471-0307.12630)

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31 ABSTRACT

32 This study aimed to determine whether mango juice can improve the viability of probiotics in a  
33 fermented dairy-based beverage whilst maintaining its quality characteristics. Formulations  
34 containing *Lactobacillus acidophilus* La-5 culture, whole cow's milk and varying concentrations of  
35 mango juice (0%, 10%, 20%, 30% and 40% (w/w)) were produced and stored for five weeks at 4°C.  
36 Results showed that probiotic viability was enhanced with the addition of 10% mango juice.  
37 Additionally, this formulation improved probiotics tolerance when exposed to *in-vitro* gastrointestinal  
38 digestion. According to the sensory analysis, beverage sensory scores improved as levels of mango  
39 juice increased from 20% to 40%.

40 **Keywords:** Probiotics, Dairy processing, Fermented milk, Gastrointestinal health, *Lactobacillus*  
41 *acidophilus*, Sensory analysis

42

43 INTRODUCTION

44

45 Probiotics are live microorganisms that, when administered in adequate amounts, confer a health  
46 benefit on the host (Hill *et al.* 2014). The probiotic market is experiencing rapid growth in both food  
47 and nutrition supplement industries. Food manufacturers are attracted to the usage of probiotics due to  
48 the projected market growth, high margins and growing consumer interest in functional foods (Bimbo  
49 *et al.* 2017). In 2012, the probiotic market was estimated to be worth \$26 million and by 2017 it had  
50 increased to approximately \$1.7 billion (Betz *et al.* 2015). Over the last ten years, more than 500 new  
51 probiotic food and beverages have entered the marketplace (Ramos *et al.* 2018). Such growth in the  
52 probiotic market has been attributed to changing consumer needs with demand moving towards  
53 healthy, convenient and portable food and beverages to suit a fast paced lifestyle (Allgeyer, Miller and  
54 Lee 2010). There is increasing evidence supporting the use of probiotics in treating health conditions  
55 such as coeliac disease, obesity and gastrointestinal conditions including irritable bowel syndrome  
56 (Ranadheera *et al.* 2017). Probiotics have also been associated with improved digestive health,  
57 prevention of urogenital infections, enhanced immune function, anti-allergy and anti-cancer activity,  
58 reductions in blood pressure and cholesterol levels (Joseph 2018) and could be beneficial in maintain  
59 oral health (Nadelman *et al.* 2018). Fermented foods have been consumed since the beginning of  
60 modern civilisation and are considered an ideal non-invasive method of delivering beneficial probiotic  
61 bacteria to the gut. Foods are fermented for many different reasons such as to extend the shelf-life of a  
62 product, improve the sensory profile and improve the nutritional composition. Dairy based probiotic  
63 food and beverages are currently widespread in the market. This could be associated with consumers  
64 believing these products are a more credible source of active ingredients than other non-dairy  
65 functional foods (Bimbo *et al.* 2017).

66

67 Currently, the greatest difficulty for manufacturers is maintaining probiotic viability during  
68 production, storage and their eventual digestion (Thomas 2016; Champagne, da Cruz and Dang 2018).  
69 To confer a health benefit to the host a minimum therapeutic level ( $10^6$ - $10^7$  cfu/g or ml of carrier food  
70 product) capable of manipulating biological function must be maintained throughout the shelf-life of  
71 the product (Ranadheera *et al.* 2012). The dairy matrix has inherently been reported to improve  
72 probiotic tolerance during digestion when compared with non-dairy products. This is a result of  
73 reducing probiotic contact with the harsh condition of the gastrointestinal tract and providing a  
74 buffering capacity *via* the milk and milk-fat contents (Ranadheera *et al.* 2017).

75  
76 Dairy products such as yogurt (Kurtuldu and Ozcan 2018), cheese (Murtaza *et al.* 2017; Tomar 2019)  
77 and ice cream (Ayar *et al.* 2018) are considered as traditional food products that have been used in  
78 probiotic delivery. The addition of fruit juice and pulps to probiotic dairy products appears promising  
79 in improving probiotic viability, though results are seen to differ depending on the fruit and strain of  
80 bacteria used (Ranadheera *et al.* 2012). In some studies the addition of fruit juice and pulps is seen to  
81 modify the pH of the dairy product and introduce antimicrobial compounds such as organic acids and  
82 flavour compounds which could be harmful to the probiotic cultures (Shori 2016). On the other hand,  
83 improved viability has also been observed as the probiotics can obtain additional nutrients from the  
84 juice for cell synthesis, similar to the pathway used during fruit juice fermentation (Sarao and Arora  
85 2017). In the past, yoghurts enriched with fruit juices and pulps have been associated with increased  
86 acceptance by consumers, as the sour tastes developed during fermentation are masked (Januário *et al.*  
87 2017).

88  
89 Recently there appears to be an increased interest by food manufacturers in the production of  
90 functional fruit and milk beverages. These beverages are relatively simple to produce, have increased  
91 consumer acceptance, and the beverages are perceived as healthy and refreshing which conform with  
92 current food trends (Lima Tribst *et al.* 2009; Islam *et al.* 2018). Mango is a popular tropical fruit  
93 worldwide; their popularity may be attributed to their appealing sensory properties and nutritional  
94 composition (Kim, Lounds-singleton and Talcott 2009). As mango are a seasonal fruit, when the  
95 supply is in surplus the excess fruits are often processed into purees or juices for use in jam, yoghurt,  
96 ice cream and beverages to avoid wastage (Kaushik 2015). Mangos are a good source of vitamins (A,  
97 C and E), dietary fibre and the mineral magnesium and potassium. They also contain the  
98 phytochemicals phenolic acid, mangiferin, carotenoids and gallotannins (Burton-Freeman, Sandhu  
99 and Edirisinghe 2017), which could provide health benefits to humans beyond that of micro- and  
100 macro-nutrients (Fazilah *et al.* 2018). Phenolic acid and carotenoids have been associated with  
101 antioxidant activity which is important in extending the shelf life of dairy products by preventing lipid  
102 oxidation. However, mango antioxidant activity can be affected by the cold storage (Atallah 2015;

103 Fazilah *et al.* 2018). Phytochemicals have also been reported to improve the viability of gut bacteria  
104 and their adhesion to intestinal epithelia cells (Parkar, Trower and Stevenson 2013).

105

106 The objective of this study was to develop a novel probiotic (*L. acidophilus*) dairy drink enriched with  
107 mango juice. Hence, the viability of *L. acidophilus*, *in vitro* gastrointestinal survival of *L. acidophilus*  
108 in mango dairy drinks, the major physio-chemical properties (titratable acidity, pH, colour and  
109 viscosity) during five weeks of storage at 4°C and the sensory properties of these products were  
110 analysed.

111

## 112 MATERIALS AND METHODS

### 113 **Beverage formulation**

114 To activate the freeze-dried probiotic, 0.5 g of *Lactobacillus acidophilus* La-5 culture (Christian  
115 Hansen, Australia) was mixed with 1 L of pasteurised full cream cow milk (Coles, Australia) and  
116 incubated at 37°C for 30 minutes in order to achieve at least 10<sup>6</sup> cfu/ml. The milk was divided into  
117 five 200 ml portions. In order to maintain the consistency among the batches, canned mango slices  
118 (Coles Band, Coles Supermarkets Pvt Ltd, Australia, 13.6% sugar) were used to prepare mango juice  
119 in this study. The mango slices were drained of syrup and blended to a nectar-like consistency and  
120 pasteurised at 70°C for 20 minutes.

121

122 Five product formulations were trialled containing 0%, 10%, 20%, 30% and 40% mango juice (w/w)  
123 in milk (Table 1). A total quantity of 1400g per beverage formulation was produced per each trial and  
124 the whole experiment was repeated twice. Beverages were incubated/fermented at 37°C for 12 hours  
125 and stored at 4°C in plastic jars for five weeks for microbial and physico-chemical analysis. The  
126 samples for consumer acceptance were stored at 4°C in sterile plastic and glass bottles for three days  
127 before analysis was conducted.

128

### 129 **Probiotic viability during storage**

130 The probiotic viability of each beverage was evaluated weekly using De Man-Rogosa-Sharpe (MRS)  
131 agar (Edwards, Narellan, NSW, Australia) and spread plating techniques (*n*=3). Plates were incubated  
132 anaerobically at 37°C for 48 hours in containers with anaerobic sachets (Thermo Fisher  
133 Scientific, Scoresby, VIC, Australia), and counts were expressed as log cfu/ml as per Ranadheera *et*  
134 *al.* (2012).

135

### 136 **Probiotic viability during *in vitro* gastrointestinal tolerance assay**

137 Simulated gastric and small intestinal juice tolerance of *L. acidophilus* La-5 in the probiotic beverages  
138 was assessed at one week of storage as per Ranadheera *et al.* (2014). In brief, gastric juices were

139 prepared by suspending pepsin (Thermo Fisher Scientific, Scoresby, VIC, Australia) (3g/L) in 0.5%  
140 (w/v) NaCl solution. The pH was adjusted to 2.0 using concentrated HCl. Intestinal juices were  
141 prepared by suspending pancreatin (Thermo Fisher Scientific, Scoresby, VIC, Australia) (1 g/L) in  
142 0.5% (w/v) NaCl solution with 0.3% bovine bile salts (Sigma-Aldrich, Castle Hill, NSW, Australia).  
143 The pH was adjusted to 8.0 using 0.1N NaOH.

144  
145 A 1 ml beverage sample was transferred into screw cap tubes (Sarstedt Australia Pty Ltd., Mawson  
146 Lakes, SA, Australia) containing either 9 ml of gastric or small intestinal juices. The mixture was  
147 homogenized well for one minute using a vortex mixer (Scientific Equipment Manufacturers, SA,  
148 Australia). Gastric juice samples were incubated at 37°C for a total of 180 minutes and aliquots of 1  
149 ml were removed from the tubes at 0, 60 and 180 minutes and plated onto MRS agar using the serial  
150 dilution and spread plating techniques. Intestinal juice samples were incubated at 37°C for a total of  
151 240 minutes and aliquots of 1ml were removed from tubes at 0, 60 and 240 minutes and plated as  
152 above. Plates were incubated anaerobically at 37 °C for 48 hours in containers with anaerobic sachets  
153 and counts were expressed as log cfu/mL.

### 155 **Physico-chemical analysis**

156 All physico-chemical measures were performed weekly during the study period in duplicates. The pH  
157 was measured using a HANNA Instruments digital pH meter (HANNA Instruments, Smithfield,  
158 USA). Viscosity was determined using a Brookfield viscometer at room temperature (Brookfield,  
159 Middleboro, USA). A size 2 spindle was used for all samples, spindle speed varied between samples.  
160 Colour was assessed using a Konica Minolta colorimeter (Konica Minolta CR-410 Chroma Meter,  
161 Japan), and L\*, a\* and b\* values were recorded. As per the manufacturer, the L value indicated the  
162 brightness (100 lighter and 0 darker) of the beverage, whilst the a\* value indicated the red/green  
163 (positive being redder and negative being greener) and the b\* value indicated the yellow/blue  
164 (positive being yellower and negative being bluer). Titratable acidity (TA) was analysed as per  
165 Ranadheera *et al.* (2016), by titrating 9 g of sample with 0.1N NaOH solution using phenolphthalein  
166 (Chem-supply, Gillman, SA, Australia) as the indicator.

### 168 **Sensory analysis**

169 The sensory evaluation was conducted following approval by the Veterinary and Agricultural  
170 Sciences Human Ethics Advisory Committee of the University of Melbourne, Australia (Ethics ID:  
171 1852110.1).

172 Sensory analysis was performed within one week of production. The sensory panel included 63  
173 healthy consumers (36 female, 25 male and 2 not stated) within 20-47 of age, were recruited by  
174 advertising at the University of Melbourne online notice board, who were not allergic to milk.

175 Participants were asked to evaluate the five beverage formulations based on overall liking, colour,  
176 aroma, appearance, flavour and mouthfeel/texture using a 9 point hedonic scale; dislike extremely = 1,  
177 dislike very much = 2, dislike moderately = 3, dislike slightly = 4, neither like nor dislike = 5, like  
178 slightly = 6, like moderately = 7, like very much = 8 and like extremely = 9 (Ranadheera *et al.* 2012).

179

## 180 **Statistical analysis**

181 Data analysis was performed using Minitab (version 17) statistical software (Minitab Inc., USA).  
182 Probiotic viability and physico-chemical data were analysed using two-way repeated measure  
183 ANOVA with storage time and mango juice levels as factors. Sensory data was analysed using the  
184 ANOVA general linear model and Bonferroni post-hoc test. All tests were conducted at a 95%  
185 confidence level.

186

187

## 188 **RESULTS AND DISCUSSION**

### 189 **Probiotic viability during storage**

190 As predicted, there was a decreasing trending in *L. acidophilus* viability over the five-week storage  
191 period at 4°C (Figure 1). The beverage containing 10% mango juice was found to maintain the  
192 highest viability during the storage period. The beverage still contained sufficient counts of *L.*  
193 *acidophilus* (7.72 log cfu/mL) to be termed a probiotic product after five weeks of storage. On the  
194 contrary, the addition of 40% mango appeared to have a significant ( $p < 0.05$ ) negative influence on  
195 probiotic viability. At the end of storage period, probiotic viability in 40% mango drink (4.08 log  
196 cfu/ml) were no longer at the recommended therapeutic levels (Ranadheera *et al.* 2017). Similar  
197 trends were observed for the formulations containing 0%, 20% and 30% mango juice at the end of  
198 storage and no longer contained sufficient probiotic counts.

199

200 In order to confer a health benefit to the host, probiotic viability must be maintained at  $10^6 - 10^7$   
201 cfu/mL until the beverage expiry date (Ranadheera *et al.* 2017). The results of this study appear to  
202 agree with current literature, whereby the addition of fruit juice to a probiotic beverage can enhance or  
203 reduce probiotic viability depending on the formulations of beverages (Shori 2016; Sarao and Arora  
204 2017; Islam *et al.* 2018). Results revealed that probiotic viability across five weeks of storage was  
205 enhanced with the addition of 10% mango juice compared with the control (0% mango). Milk  
206 naturally contains the essential growth factors required for probiotic survival, though concentrations  
207 may not be optimal. Therefore, the addition of mango juice may provide additional nutrients to  
208 support cell synthesis and enhance growth (Shori 2016; Sarao and Arora 2017). However, when 20%  
209 or more mango juice was added to the beverage, probiotic viability during storage was reduced which  
210 may be a result of the decrease in pH or the addition of antimicrobial compounds present in the

211 mango juice or both (Shori 2016). Therefore, in terms of probiotic viability maintaining, there may be  
212 an optimum fruit juice level and more research of the sort is needed to establish such guidelines. Since  
213 consumers are more likely to consume dairy products with high fruit juice levels (Ranadheera *et al.*  
214 2012), it would be interesting to investigate various strategies such as microencapsulation of probiotic  
215 cells to improve their viability in dairy food formulations with higher levels of fruit juice  
216 concentrations. If viability can be maintained, the natural fruit concentrations can be increased in such  
217 formulations providing consumers with a product which brings them close to meeting the  
218 recommended therapeutic guidelines (Ranadheera *et al.* 2017).

219

### 220 **Probiotic viability during *in vitro* gastrointestinal digestion**

221 Probiotic viability during *in vitro* gastric transit phase was significantly reduced ( $p < 0.05$ ) in all  
222 beverages with or without added mango juice (Table 2). The addition of 10% mango juice resulted in  
223 greatest survival rate of *L. acidophilus* amongst the beverage formulations with a count of 4.15 log  
224 cfu/mL at the end of 180 min. Alternatively, the addition of 20% or more mango juice resulted in poor  
225 acid tolerance and reduced probiotic viability.

226

227 As seen in Table 3, within one minute of exposure to small intestinal juice *L. acidophilus* counts  
228 decreased in all samples by approximately 1-2 log cycles. A significant reduction, almost 50% loss in  
229 viability was apparent within one minute in the 40% mango juice formulations ( $p < 0.05$ ). At the end of  
230 the *in vitro* intestinal transit (after 240 minutes), formulations containing 0% and 10% mango juice  
231 still contained sufficient counts of probiotics. The beverage formulation containing 20% mango juice  
232 or more did not contain satisfactory numbers of probiotics at the end of 240 minutes.

233

234 In order to provide the health benefits for the consumer, probiotics should survive the gastrointestinal  
235 transit and colonize in the gut. When exposed to gastric and small intestinal juice *in vitro* the addition  
236 of 10% mango was observed to have a protective influence on viability. This may be due to its ability  
237 in maintaining higher viability levels during beverage formulations and refrigerated storage. The  
238 decrease in viability experienced by formulations containing 20% or more mango may be a result of  
239 the lower milk concentration within these beverages. Dairy products have been found to exert a  
240 protective effect on probiotic viability during digestion by reducing bacterial exposure to the  
241 gastrointestinal environment (Ranadheera *et al.* 2017). High fat dairy foods have also been found to  
242 enhance probiotic viability and bile acid tolerance. Adding mango at concentrations  $>10\%$  may  
243 generate diluting effect on fat contents and thus the protective effect of milk fat is reduced  
244 (Ranadheera *et al.* 2017). The lower viability in higher mango formulations may also due to its ability  
245 in lowering the probiotic tolerance to environmental stress. Probiotic viability in dairy beverages has  
246 been associated with the probiotic strain used, the presence of dissolved oxygen and hydrogen

247 peroxide, pH, the concentration of metabolites (lactic acid), storage temperature, other ingredients in  
248 the formulation and buffering capacity (Shori 2016). However, *in vivo* and clinical studies are needed  
249 (Martins *et al.* 2018) to fully elucidate gastrointestinal survival of *L. acidophilus* in this product.

250

### 251 **Physico-chemical analysis**

252 The beverage formulations *via* adding mango juice were detected to inherently vary in pH. Data  
253 showed a positive correlation between the amounts of added mango juice and the decline in pH value  
254 (Figure 2). Storage time was only found to have a significant influence on the pH of the formulation  
255 containing 0% mango ( $p<0.05$ ). As shown in Figure 3, titratable acidity was significantly influenced  
256 by beverage formulation ( $p<0.05$ ). In terms of TA, the formulations containing 0%, 10% and 20%  
257 mango were found to be significantly influenced by storage time ( $p<0.05$ ). The beverages containing  
258 30% and 40% mango maintained a relatively stable TA over the storage period.

259

260 Several studies have reported an association of probiotic viability with the intrinsic characteristics of  
261 the beverages such as acidity (Pereira *et al.* 2018). Milk is known to have a pH of approximately 6.5-  
262 6.7, whereas mango has a pH of 3.5-4.0 (Kaushik, Nadella and Rao 2015). As predicted, when  
263 formulations contained additional mango the pH appeared to decrease. This was associated with the  
264 lower viability of probiotics in beverages with higher levels of mango juice in the present study  
265 (Figure 1). Beverage pH was also decreased gradually over the study period as a result of the  
266 production of lactic acid by *L. acidophilus* which is known as post-fermentation. Similar trends in pH  
267 were reported by Ertem and Cakmakci (2018) in a fermented yoghurt containing *L. acidophilus*. Post-  
268 fermentation acidity development is detrimental for probiotics and was also observed by Ranadheera  
269 *et al.* (2012) in stirred fruit yoghurt containing ABY-1 culture (*Streptococcus thermophilus*, *L.*  
270 *delbrueckii subsp. bulgaricus*, *L. acidophilus* LA-5 and *B. animalis subsp. lactis* BB-12).

271

272 The decrease in pH during storage appeared to be positively correlated with the increasing TA;  
273 whereby as lactic acid is produced the pH decreases and the TA increases. Beverage formulations  
274 containing 0% and 10% mango appeared to experience a greater increase in TA than formulations  
275 containing 20%, 30% and 40% mango juice. This could be a result of maintaining probiotic viability  
276 in relatively higher numbers in formulations containing 0% and 10% mango and consequentially an  
277 increase in rate of lactic acid production during storage. Higher levels of initial TA and lower pH in  
278 mango containing beverages in this study could also be associated with the presence of malic and  
279 citric acid within the mango juice (Medlicott and Thompson 1985).

280

281 There was a significant positive relationship between viscosity and the amount of added mango juice  
282 ( $p<0.05$ ) (Figure 4). A significant difference was noted between the formulation containing 40%

283 mango with the rest of the beverages and the viscosity of the formulation containing 30% mango was  
284 also significantly different to the 0%, 10% and 20% mango juice containing beverages.

285

286 The beverage containing 40% mango measured 6560 mPas initially whilst formulations containing  
287 0%-30% mango measured 11-615 mPas. The inherently thick viscosity of the 40% formulation may  
288 be a result of the total soluble solids content of mango juice (Islam *et al.* 2018). In yoghurt, casein,  
289 calcium and phosphorus within milk is known to produce the gel-like structure (Kanurić *et al.* 2018)  
290 and addition of mango juice may have supported to strengthen the structure. Similar viscosity trends  
291 were observed by Islam et al (2018) during the development of a probiotic (*Lactobacillus* spp.) dairy  
292 beverage enriched with 5% and 10% mango juice.

293

294 As per the manufacturer's instructions, colour change was determined through the differences in L\*,  
295 a\* and b\* values (Konica Minolta CR-410 Chroma Meter, Japan). Beverage formulation had a  
296 significant influence on the beverage L\*, a\* and b\* values (p=0.000) (Table 4). L\* and b\* values  
297 were seen to decrease as formulated mango concentration increased, whilst the a\* values increased.  
298 Storage time was only found to be significant for the a\* value of formulations containing 20% and  
299 30% mango (p<0.05).

300

### 301 **Sensory analysis of beverages**

302 The participants originated from 25 different countries, with majority stated their nationality as  
303 Australian (n=10), Chinese (n=13) and Indian (n=8). It was found that 79% of participants consumed  
304 dairy products daily (n=50). A total of 6% of respondents consumed probiotics at least daily, 41%  
305 weekly, 13% monthly, 17% yearly and 22% stated they had never consumed probiotic beverages.  
306 Additionally, it was found that none of the respondents consumed mango or mango flavoured  
307 beverages daily, 14% consumed them weekly, 49% monthly, 30% yearly and 6% stated they never  
308 consumed these types of products.

309

310 As seen in Table 5, beverage formulation was found to have a significant impact on consumer liking  
311 in all categories (overall liking, colour, aroma, appearance, flavour and mouthfeel) (p<0.05). The  
312 sensory scores also indicate that the addition of mango juice was positively associated with increased  
313 consumer liking across all categories, as 40% mango achieved the highest average score across all  
314 categories. The 10% mango formulation surprisingly received the lowest mean score in the overall  
315 liking, flavour and mouthfeel categories (although did not differ significantly from the 0%  
316 formulation), otherwise there was generally a correlation between increased liking and mango  
317 concentration.

318

319 Sensory flavour scores were generally increased with a higher mango juice concentration. The  
320 improved scores are likely to be a result of the mango providing sweetness and fruity flavours which  
321 mask the sour notes developed during fermentation. A study by Thompson *et al.* (2007) found that  
322 natural strawberry flavour and aroma, and sweet taste were key factors influencing consumer liking of  
323 strawberry drinkable yoghurts which corresponds with the finding of this study. Another study by  
324 Allgeyer, Miller and Lee (2010) found that consumers preferred a yoghurt drink which was neither  
325 too sweet or too sour. The lower sensory scores achieved by the 10% mango formulation in overall,  
326 mouthfeel and flavour categories may be a result of mango being too faint to be appealing. The  
327 addition of mango juice also introduced a stringy pulp which appears to be acceptable in the high  
328 mango formulation, but unappealing at low concentrations (hence the mouthfeel scores). The 40%  
329 mango formulation also achieved the highest mouthfeel and appearance scores by the consumers. This  
330 formulation was the most viscous which appeared to appeal to the consumers, which may be due to  
331 the beverage texture being likened to a smoothie.

332  
333 The colour of the beverage is thought to be a result of the instinct characteristics of the mango and  
334 milk when combined in different concentrations. When colour measurements (Table 4) were viewed  
335 in conjunction with the colour sensory scores (Table 5,) it appeared that liking was correlated with a  
336 lower L\* value (darker colour), and higher a\* (redder) and b\* (more yellow) values, resulting in the  
337 intense colour of the 40% formulation which received the highest sensory acceptability for colour.  
338 Alternatively, 0% mango had the highest L\* value indicating a lighter sample, and the lowest a\* and b  
339 values. This sample received lowest sensory scores for the colour attribute. Thick consistency of the  
340 40% formulation as indicated by viscosity data (Figure 4) may be more appealing for the consumers  
341 and this could be a reason to achieve highest mouthfeel values for the 40% formulation. Apparently,  
342 acidic and sour nature in the same formulation (Figures 2 and 3) may have also contributed to higher  
343 consumer acceptability of tested sensory attributes. However, sensory evaluations with the help of  
344 trained panellists would be highly beneficial (Silva *et al.* 2018) in the process of commercialization of  
345 this product.

346

## 347 CONCLUSIONS

348 The addition of mango juice to a fermented probiotic beverage has potential in improving the quality  
349 characteristics of the final product. The addition of 10% mango juice appeared to improve viability  
350 over storage, though when added in higher concentrations a reduction in viability was evident. Similar  
351 trend was observed for the *in vitro* gastrointestinal survival of probiotics. However, a higher  
352 concentration of mango juice (40%) was preferred by consumers than at the lower levels. Results  
353 suggest that commercialisation of such a product must find a balance between consumer liking and  
354 maintaining therapeutic amounts of probiotics in these types of products.

355

356 CONFLICT OF INTEREST

357 There is no conflict of interest.

358

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457 **Figure 1.** Viable counts of *L. acidophilus* in fermented dairy drinks enriched with various  
458 concentrations of mango juice during 5 weeks of storage at 4 °C (Mean±SD)

459 **Figure 2.** pH of mango juice enriched probiotic dairy drink over 5 weeks of storage at 4 °C (n=4)  
460 Mean±SD

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462 **Figure 3.** TA of mango juice enriched probiotic dairy drink over 5 weeks of storage at 4°C (n=4)  
463 Mean±SD

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465 **Figure 4.** Viscosity of mango juice enriched probiotic dairy drink over 5 weeks of storage at 4°C

466 (n=4) Mean±SD

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**Table 1.** Mango juice enriched probiotic dairy drink formulations

Mango (%)	Cultured milk (g)	Uncultured milk (g)	Mango juice (g)
0	200	1200	0
10	200	1060	140
20	200	920	280
30	200	780	420
40	200	640	560

**Table 2.** Effect of simulated gastric juice (pH 2.0) on the viability of *L. acidophilus* in mango juice enriched probiotic dairy drink (viable counts shown as log cfu/mL)

Formulation	0 min	1 min	60 min	180 min
0%	7.22 ± 0.14 <sup>A</sup>	6.05 ± 0.18 <sup>aA</sup>	4.88 ± 0.17 <sup>aA</sup>	<1
10%	8.02 ± 0.03 <sup>B</sup>	6.09 ± 1.54 <sup>aA</sup>	6.07 ± 0.23 <sup>aB</sup>	4.15 ± 0.21 <sup>aA</sup>
20%	7.47 ± 0.02 <sup>A</sup>	6.04 ± 0.30 <sup>aA</sup>	3.06 ± 0.23 <sup>aC</sup>	<1
30%	7.27 ± 0.08 <sup>A</sup>	6.60 ± 0.00 <sup>aB</sup>	<1	<1
40%	7.60 ± 0.33 <sup>A</sup>	6.84 ± 0.00 <sup>aB</sup>	2.00 ± 0.00 <sup>aD</sup>	<1

Mean values (±SD).

<sup>A,B,C,D</sup> Values in the same row having different superscripts differ significantly.

<sup>a</sup> Indicates a significant difference (p<0.05) in viable counts compared with the control (0 minutes/before the gastric tolerance assay).

**Table 3.** Effect of simulated small intestinal juice (pH 8.0) on the viability of *L. acidophilus* in mango juice enriched probiotic dairy drink (viable counts shown as log cfu/mL)

Formulation	0 min	1 min	60 min	240 min
0%	7.22 ± 0.14 <sup>A</sup>	6.32 ± 0.56 <sup>aA</sup>	6.27 ± 0.27 <sup>aA</sup>	6.42 ± 0.15 <sup>aA</sup>
10%	8.02 ± 0.03 <sup>B</sup>	6.91 ± 0.09 <sup>aA</sup>	7.00 ± 0.00 <sup>aB</sup>	6.50 ± 0.14 <sup>aA</sup>
20%	7.47 ± 0.02 <sup>A</sup>	5.94 ± 0.06 <sup>aB</sup>	6.11 ± 0.24 <sup>aA</sup>	4.45 ± 0.64 <sup>aB</sup>
30%	7.27 ± 0.08 <sup>A</sup>	5.16 ± 0.34 <sup>aC</sup>	<1	<1
40%	7.60 ± 0.33 <sup>A</sup>	4.29 ± 0.02 <sup>aD</sup>	<1	<1

Mean values (±SD).

<sup>A,B,C,D</sup> Values in the same row having different superscripts differ significantly.

<sup>a</sup> Indicates a significant difference ( $p < 0.05$ ) in viable counts compared with the control (0 minutes/before the intestinal tolerance assay).

**Table 4.** Weekly colour difference expressed as L\*, a\* and b\* values over 5 weeks of storage (n=4).

Formulation		WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
0%	L*	57.10 ± 1.45	58.79 ± 0.35	58.13 ± 0.46	57.64 ± 0.20	54.77 ± 1.29	53.22 ± 2.30
	a*	-1.87 ± 0.18	-1.98 ± 0.07	-1.83 ± 0.05	-1.75 ± 0.12	-1.88 ± 0.06	-1.82 ± 0.13
	b*	3.92 ± 0.25	4.53 ± 0.63	4.84 ± 0.67	4.70 ± 0.51	4.82 ± 0.61	4.23 ± 0.29
10%	L*	55.46 ± 1.41	55.83 ± 1.59	56.13 ± 1.42	56.06 ± 0.58	56.23 ± 0.56	56.31 ± 0.90
	a*	-1.63 ± 0.11	-1.72 ± 0.03	-1.64 ± 0.02	-1.65 ± 0.11	-1.59 ± 0.08	-1.65 ± 0.04
	b*	10.48 ± 1.18	8.55 ± 0.68	11.21 ± 0.36	10.68 ± 0.45	10.07 ± 0.89	9.91 ± 0.76
20%	L*	53.03 ± 1.28	54.62 ± 1.28	54.95 ± 1.00	54.29 ± 0.43	54.46 ± 0.46	54.20 ± 0.58
	a*	-1.60 ± 0.13	-1.54 ± 0.01	-1.43 ± 0.01	-1.42 ± 0.03	-1.36 ± 0.01	-1.25 ± 0.03
	b*	13.70 ± 1.71	14.69 ± 0.03	14.45 ± 0.12	13.87 ± 0.19	14.07 ± 0.03	14.29 ± 0.18
30%	L*	50.59 ± 0.94	52.47 ± 1.08	53.37 ± 1.08	53.71 ± 0.63	52.99 ± 0.63	52.34 ± 0.28
	a*	-1.29 ± 0.12	-1.19 ± 0.02	-1.16 ± 0.05	-1.19 ± 0.04	-1.03 ± 0.01	-0.95 ± 0.01
	b*	15.98 ± 1.98	16.98 ± 0.41	16.61 ± 0.14	16.64 ± 0.35	16.47 ± 0.42	15.90 ± 0.59
40%	L*	48.90 ± 1.26	51.78 ± 1.03	51.87 ± 1.03	51.84 ± 0.59	51.15 ± 0.85	50.98 ± 0.33
	a*	-0.81 ± 0.02	-0.69 ± 0.15	-0.70 ± 0.12	-0.69 ± 0.05	-0.58 ± 0.02	-0.52 ± 0.08
	b*	16.97 ± 2.35	18.58 ± 0.56	17.96 ± 0.45	17.56 ± 0.73	17.10 ± 0.62	16.51 ± 0.88

Mean (±SE)

The L value indicated the brightness (100 lighter and 0 darker) of the beverage, whilst the a\* value indicated the red/green (positive being redder and negative being greener) and the b\* value indicated the yellow/blue (positive being yellower and negative being bluer).

**Table 5.** Mean scores of tasting panellists (n=63) for sensory properties of mango juice enriched dairy drink (0%, 10%, 20%, 30% and 40%) after one week of storage.

Formulation	Overall	Colour	Aroma	Appearance	Flavour	Mouthfeel
0%	4.63 ± 0.25 <sup>BC</sup>	5.27 ± 0.25 <sup>C</sup>	4.89 ± 0.20 <sup>C</sup>	5.24 ± 0.24 <sup>D</sup>	4.54 ± 0.25 <sup>BC</sup>	4.89 ± 0.26 <sup>BC</sup>
10%	4.55 ± 0.20 <sup>C</sup>	5.43 ± 0.22 <sup>BC</sup>	5.16 ± 0.17 <sup>C</sup>	5.60 ± 0.18 <sup>CD</sup>	4.08 ± 0.22 <sup>C</sup>	4.59 ± 0.22 <sup>C</sup>
20%	5.37 ± 0.20 <sup>B</sup>	5.98 ± 0.17 <sup>B</sup>	5.44 ± 0.14 <sup>BC</sup>	6.06 ± 0.18 <sup>BC</sup>	5.08 ± 0.22 <sup>B</sup>	5.38 ± 0.22 <sup>B</sup>
30%	6.19 ± 0.20 <sup>A</sup>	6.89 ± 0.13 <sup>A</sup>	5.98 ± 0.18 <sup>AB</sup>	6.41 ± 0.19 <sup>B</sup>	6.00 ± 0.21 <sup>A</sup>	6.16 ± 0.20 <sup>A</sup>
40%	6.66 ± 0.20 <sup>A</sup>	7.48 ± 0.14 <sup>A</sup>	6.25 ± 0.18 <sup>A</sup>	7.11 ± 0.16 <sup>A</sup>	6.70 ± 0.22 <sup>A</sup>	6.49 ± 0.21 <sup>A</sup>

Mean (±SE)

<sup>A,B,C,D</sup> Values in the same column indicate significantly difference (p < 0.05)

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