The development of micronutrient supplemented probiotic yogurt for people living with HIV: Laboratory testing and sensory evaluation

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ARTICLE INFO

Article history:
Received 20 October 2009
Accepted 20 November 2010

Editor Proof Receive Date 20 December 2010

Keywords:
Probiotic
Micronutrient
Yogurt
HIV

ABSTRACT

Many probiotic organisms, including *Lactobacillus rhamnosus* GR-1 have shown significant promise in supporting the immune function of people living with HIV. Moreover, certain micronutrients have also demonstrated the ability to improve immune function and delay disease progression. A micronutrient supplemented probiotic (*L. rhamnosus* CAN-1) yogurt was developed by first preparing a mother culture of the probiotic species and adding them to 2% milk that was supplemented with micronutrients at 25% DRI and incubating the mixture at 37°C for 5 h. A sensory evaluation was performed to assess consumer acceptance of the products as 1: 12.5% DRI and standard cultures; 2: 25% DRI and standard cultures; 3: 12.5% DRI and probiotic cultures; 4: 25% DRI and probiotic cultures. Micronutrients slightly inhibited the viable counts of *L. rhamnosus* CAN-1; however, the colony forming units remained above what is considered the therapeutic level (WHO, 2001) at the end of the shelf life (21 days) Consumers preferred product 3 over the others, suggesting yogurt is a suitable carrier for *L. rhamnosus* CAN-1 and micronutrients.

Industrial Relevance: This study is highly relevant to industry as it is a new development of a functional food for use in a clinical population. While yogurt itself has increased in popularity, so has the demand for functional foods. In addition, yogurt is a relatively simple and low-maintenance technology, which can be easily transferred to diverse settings such as Sub-Saharan Africa, where the need for strategies to alleviate suffering from malnutrition and HIV are urgently needed. Yogurt has also been shown to be an inhospitable environment for pathogenic bacteria, thus this technology would be suitable for small-scale social businesses in developing countries where electricity and hygiene are more challenging than larger industry. These products were well accepted by consumers, suggesting its potential viability in North American markets, but more specifically for patient populations in hospital. Nutrition and immune function are closely linked, which suggests that other populations suffering from nutrition and immune disorders such as inflammatory bowel disease, cancer, and aging populations may also benefit from a product that combines the immunostimulatory potential of probiotics with a nutritious medium of micronutrient supplemented yogurt.

1. Introduction

Infections caused by the Human Immunodeficiency Virus (HIV) have made a profound and devastating impact on public health globally. Over 33 million people suffer from infections caused by HIV and the prevalence increased by approximately 3 million in 2008 alone (UNAIDS). In order to reconstitute immune function, which is compromised by the virus, many people living with HIV are treated with life saving High-Active Anti-Retroviral Therapy (HAART); however, ART is only initiated once immune function (CD4 t-lymphocytes) falls below 350 cells/μl (WHO, 2009). In addition, although access to HAART in the developing world has significantly improved since 2005, the coverage is not yet universal.

People infected with HIV often suffer from significant disturbances in nutritional status (Beach, Mantero-Atienza, & Shor-Posner, 1992; Kaiser et al., 2006; Tang, Graham, & Saah, 1996); such as, increased resting energy expenditure (Batterham, 2005; WHO, 2003) and impaired or altered gastrointestinal function (Kelly et al., 2009). In addition to changes in nutrition status as a result of the virus, people living with HIV experience a preferential and rapid decline of CD4 lymphocytes, which is associated with enhanced intestinal permeability (Noyer et al., 1998), which leads to systemic inflammation and could inhibit an appropriate response to the virus (Buonaguro et al., 2009; Sindhu et al., 2006). Interestingly, people living with HIV have altered gut microbiota profiles; whereby, there is a dramatic decline of lactobacilli and bifidobacteria (certain species of which are considered

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probiotic) and there is a higher concentration of pathogenic species such as *Candida albicans* and *Pseudomonas aeruginosa* (Gori et al., 2008; Wolf, Wheeler, Ataya, & Garleb, 1998). A recent review of micronutrients, N-acetyl cysteine (NAC), probiotics and prebiotics on the immune function and morbidity of people living with HIV, outlined the clear evidence for the provision of certain dietary components; such as, vitamins B, C, E, and folic acid and preliminary evidence for selenium, NAC, whey protein, prebiotics and probiotics (Hummelen, Hemsworth, & Reid, 2010). The overall conclusion was that a comprehensive nutritional supplement may be the most effective approach to improve health outcomes in people living with HIV (Hummelen et al., 2010). Thus, a product that combines the evidence from various dietary components and is intended to support adequate nutrition and health status of people living with HIV in order to delay the progression of immune function towards severe immune deficiency (or the initiation of ART) is urgently needed. Moreover, such a product could also act adjunctively in improving immune function for patients who have already initiated HAART.

Broad-spectrum micronutrient interventions, normally in capsule form, have been associated with delayed progression of HIV to AIDS, improved immune function by increased CD4 and delayed HIV-related mortality (Fawzi et al., 2004; Jiampton et al., 2003; Kaiser et al., 2006). Moreover, other dietary-based interventions mentioned in the review included probiotics and prebiotics. Certain strains of probiotics, or "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (WHO/FAO, 2001) may help to improve immune system by reducing gut epithelial permeability (Isolauri et al., 1993; Madsen et al., 2001; Rayes et al., 2005; Ukena et al., 2007) (thus helping to reconstitute depleted levels of CD4 lymphocytes) to down-regulate systemic and mucosal inflammation (Braat et al., 2004; Furrie et al., 2005; O'Mahony et al., 2005).

Probiotic yogurt is maintaining its popularity as a functional food (Hekmat & Koba, 2006). Many studies have proven the successful incorporation of probiotics into yogurt as well as other fermented milk products; such as, soy yogurt (Farnworth et al., 2007), ice cream (Akin, Akin, & Kirmaci, 2007; Davidson, Duncan, Hackney, Eigel, & Boling, 2000; Hekmat & McMahon, 1992) and cheese (Sharp, McMahon, & Broadbent, 2008). Cow's milk in the form of yogurt is proven to be a suitable carrier for probiotics as the pH drops more slowly than other media such as soy, which creates a buffered environment for lactic acid bacteria to maintain viable organisms (Farnworth et al., 2007). More specifically, a recent study by Hekmat, Soltani, and Reid (2009) showed that the yogurt can maintain viable microorganisms (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14) at one month of storage using a variety of prebiotic agents in starter culture (Hekmat et al., 2009).

Yogurt has also shown to be a suitable medium for micronutrient fortification; including, iron, manganese, zinc, molybdenum, chromium, and selenium up to 25% of the Dietary Reference Intake (DRI) (Achanta, Aryana, & Boeneke, 2006; Hekmat & Donald, 1997; Hekmat et al., 2009; Institute of Medicine, 2006). In these studies it was shown that it can be achieved without a significant loss in flavour, appearance or ratings for sensory attributes. Fortification of foods (especially dairy products) with bioavailable iron has proven to be difficult in order to maintain an acceptable flavour profile (Zhu, Miller, Nelson, & Glahn, 2008), but other studies have proven that it is possible to develop an acceptable product fortified with a bioavailable source of iron (Hekmat & Donald, 1997). Interestingly, plain yogurt itself (even without fortification with additional zinc) has been shown to improve bioavailability of zinc in people who have highly plant-based (phytates) diets (Rosado et al., 2005), for example in low-income countries.

To our knowledge, a yogurt product with both probiotics (*L. rhamnosus* CAN-1) and fortification with micronutrients has never before been developed.

Thus, we present the results from the development of a micronutrient and probiotic supplemented yogurt, including the testing of viable microorganisms over a typical shelf-life period, and a sensory evaluation trial to access its acceptability before introducing this product to our target population.

2. Materials and methods

2.1. Development of micronutrient fortification blend

The composition of micronutrients was initially based on a previously published formula by Kaiser, et al. (Kaiser et al., 2006). This blend is specifically for people living with HIV taking highly active antiretroviral therapy (HAART) and contained >100% of the DRI for most nutrients. Table 1 describes the content of the nutrient premix, which we prepared to deliver 25% of the DRI per respective nutrient per 175 g of yogurt product. The premix was supplied by Fortitech Strategic Nutrition, Schenectady, NY.

2.2. Preparation of probiotic mother culture

The mother culture was prepared by first streaking standard agar/MRS with 0.05% fusidic acid plates with freeze dried *L. rhamnosus* CAN-1 (Urex Biotech Inc. London, Ontario, Canada) and incubating these plates at 37 °C for 24 h in anaerobic conditions. The cell morphology was confirmed using the Gram stain technique. One to two bacterial colonies were transferred by sterile loop directly to 40 ml sterilized (autoclaved at 15 psi at 121 °C) Man, Rogosa and Sharpe (MRS) broth (BD Diagnostic Systems, Sparks, MD, USA) and incubated at 37 °C overnight in an anaerobic jar together with BBL GasPak™ sachet (BBL GasPak™, Becton Dickinson & Co., Sparks, MD, USA) to achieve anaerobic conditions. Fresh milk was separated into several 400 ml Pyrex™ jars and autoclaved at 15 psi at 121 °C. Yeast extract (0.3% wt/vol) was added to autoclaved and cooled 2% M.F. milk and then inoculated with 1% probiotic broth culture and incubated in anaerobic conditions at 37 °C overnight. This process was repeated for every new yogurt manufacture.

2.3. Manufacture of micronutrient supplemented probiotic yogurt

The micronutrients were prepared for addition to the milk by first making a thin paste with the dry nutrient premix. Twenty grams of plain yogurt and 20 g of regular 2% milk were added in a polyethylene weighing dish to 9.66 g of nutrient premix (0.42 g per 175 g serving of yogurt). This was intended to improve the solubility and prevent clumping of the micronutrients in the yogurt product. Standard 2% M.F. milk was combined with 5% (wt/vol) sugar, 3% (wt/vol) milk solids non fat, 0.4% (wt/vol) gelatine, and the nutrient blend. The yogurt blend was heat treated to 85 °C for 30 min and cooled to 37 °C before

Table 1
Micronutrient blend used to supplement the probiotic yogurt. This specific blend was developed to meet the unique nutrient needs of an HIV-infected population. It was prepared in powder form and added to milk before heat treatment. The amount delivered per serving of yogurt (175 g) is depicted.

Ingredients micronutrient-probiotic yogurt	Amount per 175 g yogurt serving
Vitamin A (as beta carotene and palmitate)	1500 IU
Vitamin E (as acetate)	5.7 IU
Niacinamide	3.8 mg
Vitamin B1 (thiamin)	0.3 mg
Vitamin B12 (cyanocobalamin)	0.6 μg
Vitamin B6 (pyroxine)	0.3 mg
Vitamin C (ascorbic acid)	21 mg
Iron (as ferric pyrophosphate)	3.3 mg
Selenium (sodium selenite)	13.8 μg
Zinc (zinc sulphate)	2.4 mg
DHA (omega-3 fatty acid from fish oil)	13 mg

being inoculated with 2% (wt/vol) standard yogurt cultures (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*) and 4% (wt/vol) *L. rhamnosus* CAN-1. The inoculated milk was incubated at 37 °C for 5 h. The yogurt was placed in a cold room (4 °C) overnight. After the yogurt was cooled overnight, 12% (wt/vol) strawberry flavouring (Sensient Flavours Canada, Rexdale, ON) was added to improve the flavour. The yogurt was kept in refrigeration temperatures (4 °C) for up to 21 days.

2.4. Microbial analysis

The viable counts of *L. rhamnosus* CAN-1 in yogurt were tested at days 1, 7, 14, and 21 of storage at 4 °C. *L. rhamnosus* CAN-1 was counted on selective MRS (EMD Laboratories) and agar plates with 15 μg/ml fusidic acid (Sigma Laboratories). Four biological replicates were done for each of the two treatments (1. micronutrient supplementation at a rate of 25% DRI; 2. standard yogurt) at each time point (days 1, 7, 14, and 21). Thus, there are a total of 32 data points. Cell density was determined using the serial dilution and drop plate method. The plates were inoculated with either plain probiotic yogurt or 25% DRI micronutrient supplemented probiotic yogurt (the highest supplementation rate). Each plate was incubated at 37 °C for 48 h in anaerobic conditions.

2.5. Sensory evaluation

At Brescia University College Sensory Testing Centre, one hundred and four untrained panellists participated in a sensory evaluation of the yogurt on the basis of appearance, flavour, texture, and overall acceptability. The panellists rated these characteristics according to the hedonic scale where 1 corresponds with "dislike extremely" and 9 corresponds with "like extremely". Panellists were asked to assess the sensory characteristics of the four different types of yogurt: yogurt 1 = plain (non-probiotic) yogurt fortified with micronutrients at 12.5% DRI (half the rate described in the previously mentioned methods), yogurt 2 = plain (non-probiotic) yogurt fortified with micronutrients at 25% DRI (full rate described in the previously mentioned methods), yogurt 3 = probiotic yogurt with 12.5% DRI, and yogurt 4 = probiotic yogurt with 25% DRI. All yogurt varieties had equal levels of sugar, milk solids, gelatine, and strawberry flavouring. All participants received a letter of information and consent document, and gave signed consent before participating in the study. Panellists were then given four samples at a time at storage temperature (4 °C), a pencil, and a glass of cold water to rinse their mouths between samples. The sensory evaluation study protocol was submitted to and approved by the Brescia University College Research Ethics Board.

2.6. Statistical analysis

Statistical analysis of the treatment and shelf-life effect on the viable microorganisms over time were performed using a one-way repeated measures analysis of variance (ANOVA) in STATA 10 (College Station, Texas, USA), using fixed effects as time, treatment and treatment × time. Assumptions of power was $\alpha = 0.05$. Prior to statistical analysis, the data was log-transformed and was transformed back for graphing and presentation of the results.

To evaluate the differences between the yogurts with respect to sensory attributes, SPSS Statistical Package Version 17.0, a one-way analysis of variance was used (yogurt 1 = 12.5% DRI + probiotic; yogurt 2 = 25% DRI + non-probiotic; yogurt 3 = 12.5% DRI + probiotic; yogurt 4 = 25% DRI + non-probiotic) × sensory attribute interaction (appearance, flavour, texture, and overall acceptability). Fisher's protected test was used to calculate the Least Significant Difference (LSD) between the mean scores of each treatment for each sensory attribute.

3. Results

3.1. Viability of *L. rhamnosus* CAN-1 in micronutrient supplemented probiotic yogurt

The mean viable colony forming units (CFUs) at the end of shelf-life (day 21) in the standard probiotic yogurt was 1.1×10^9 CFU/ml, while the CFUs at the end of shelf-life in the micronutrient supplemented probiotic yogurt was 5.0×10^7 CFU/ml (Table 2). The effect of time (shelf life period) on the viable counts of *L.rhamnosus* CAN-1 was not significant ($p = 0.57$). However, the effect of the treatment and the interaction of time and treatment (treatment × time) were statistically significant ($p = 0.00$ and 0.00, respectively) (Table 2). Fig. 1 depicts graphically the viability of *L. rhamosus* CAN-1 over the 21 day period.

3.2. Sensory characteristics

3.2.1. Appearance

The results for the mean appearance scores for the different supplemented yogurts can be found in Fig. 2. The micronutrient supplemented yogurt had a soft yellow colour, imparted by the beta carotene as part of the fortification mix. The high rate of nutrient fortification (25% DRI) resulted in a soft yellow colour, and the low rate (12.5% DRI) resulted in a pale off-white colour. The mean scores for yogurt 1 (12.5% DRI / standard yogurt cultures) was 6.9 ± 1.5, yogurt 2 (25% DRI/standard) was 6.3 ± 1.6, yogurt 3 (12.5% DRI/probiotic) 7.3 ± 1.2 and yogurt 4 (25% DRI/probiotic) 6.5 ± 1.4. There was a statistically significant difference in appearance scores between the products ($p<0.05$).

3.2.2. Flavour

All of the mean scores for flavour, according to the hedonic scale (1–9), were above average, which suggests additional nutrients can be added to yogurt without adversely affecting the flavour profile. (Fig. 2). The mean scores (±SD) for yogurt 1 (12.5% DRI / standard yogurt cultures) was 6.9 ± 1.6, yogurt 2 (25% DRI/standard) was 6.3 ± 1.8, yogurt 3 (12.5% DRI/probiotic) 7.3 ± 1.3 and yogurt 4 (25% DRI/probiotic) 6.7 ± 1.7. This was statistically significant ($p<0.05$), and according to multiple comparisons test LSD, yogurt 3 was statistically different from the rest. This suggests that the low level of nutrient supplementation (12.5% DRI) tested in yogurt 1 and 3 are better accepted by consumers.

Table 2
Summary statistics (mean ± SD) and results from one-way ANOVA assessing the viability of *Lactobacillus rhamnosus* CAN-1 in probiotic yogurt supplemented with micronutrients, versus standard probiotic yogurt without micronutrient over the shelf-life period. Data were log-transformed for analyses and fixed effects were time, treatment and treatment × time. Statistical significance was assumed at $p<0.05$.

	Colony forming units/ml over time (days) mean ± SD						
	1	7	14	21	Time effect	Treatment effect	Treatment × time effect
Standard	1.13E + 09 ± 6.18E + 08	1.50E + 09 ± 1.36E + 09	2.13E + 09 ± 1.64E + 09	9.50E + 08 ± 1.35E + 08	p = 0.57	p = 0.00	p = 0.00
Micronutrient	4.44E + 08 ± 4.11E + 08	3.05E + 08 ± 2.25E + 08	1.08E + 08 ± 6.22E + 07	5.75E + 07 ± 3.77E + 07			

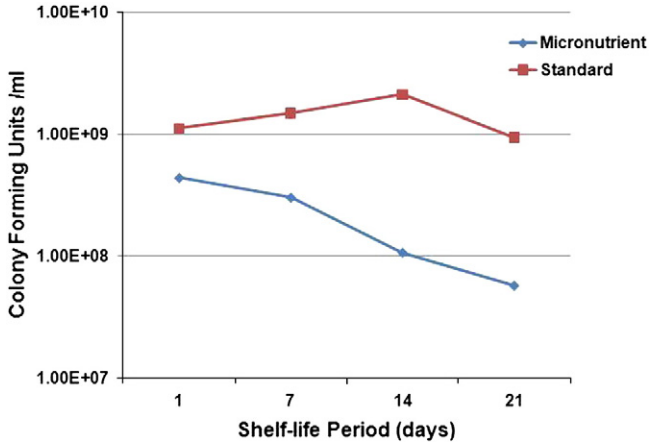

Fig. 1. Comparison of the viability of *Lactobacillus rhamnosus* CAN-1 in probiotic yogurt supplemented with micronutrients, versus standard probiotic yogurt without micronutrients.

3.2.3. Texture

Overall texture scores were comparable when assessed according to the hedonic scale (Fig. 2). The mean scores (±SD) for yogurt 1 (12.5% DRI / standard yogurt cultures) was 7.1 ± 1.6, yogurt 2 (25% DRI/standard) was 7.1 ± 1.5, yogurt 3 (12.5% DRI/probiotic) was 7.6 ± 1.1 and yogurt 4 (25% DRI/probiotic) 6.9 ± 1.6. There was a statistically significant difference between the products ($p<0.05$) with yogurt 3 significantly different from the others. All yogurts received above average or high scores, which indicates that consumers liked the product and micronutrients did not significantly affect textural properties.

3.2.4. Overall acceptability

The mean scores (±SD) for yogurt 1 (12.5% DRI / standard yogurt cultures) was 7.0 ± 1.5, yogurt 2 (25% DRI/standard) was 6.5 ± 1.6, yogurt 3 (12.5% DRI/probiotic) was 7.5 ± 1.0 and yogurt 4 (25% DRI/probiotic) was 6.8 ± 1.5 ($p<0.05$) (Fig. 2). According to the Least Significant Difference (LSD), all yogurts were significantly different from each other. Yogurt 3 was significantly different from all of the yogurts, but yogurts 1, 2 and 4 had no similarities with each other. All scores are above average, suggesting that all products were well accepted by consumers, but there was a particular preference for yogurt 3 (12.5% DRI/probiotic).

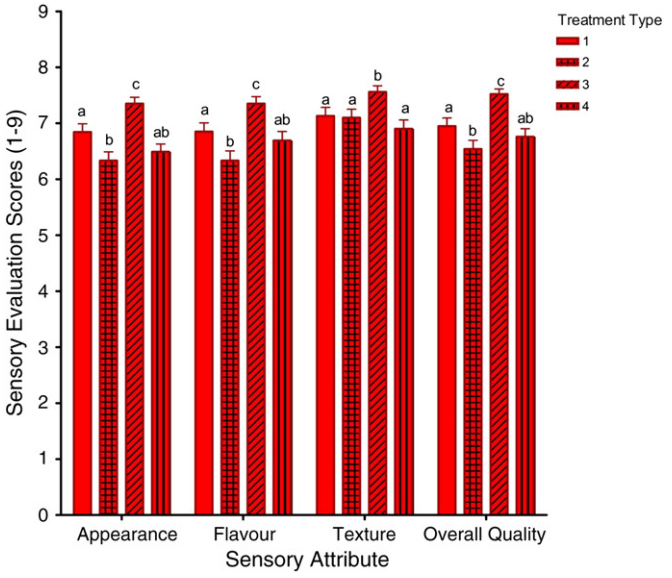

Fig. 2. The results for the sensory evaluation are depicted using the hedonic scale where 1 = dislike extremely, and 9 = like extremely. 104 untrained panellists sampled four different types of yogurt for appearance, flavour, texture, and overall acceptability. 1 = 12.5% DRI + plain, 2 = 25% DRI + plain, 3 = 12.5% DRI + probiotic and 4 = 25% + plain. Statistical significance is defined by different lettering, where a is significantly different than b at $p<0.05$.

4. Discussion

The primary outcome of this study was to assess whether adding micronutrients to a standard probiotic yogurt would affect the level of probiotic colony forming units which are believed to be necessary to confer health benefits on the host ($>10^7$ CFU/ml); (WHO/FAO, 2001). After three weeks of refrigerated storage, this micronutrient supplemented yogurt maintained high levels of *L. rhamnosus* CAN-1 at a mean concentration of 5.8×10^7 CFU/ml, which is above the supposed therapeutic level. The unsupplemented yogurt did appear to have a higher concentration of *L. rhamnosus* CAN-1 throughout the shelf-life period, suggesting the micronutrients do slightly hinder the viable counts. In another study, *L. rhamnosus* GR-1 was maintained in standard micronutrient supplemented yogurt for 28 days storage at 5×10^7 CFU/ml (Hekmat et al., 2009), which is the same level that is reported in this study in the micronutrient supplemented yogurt. In order to preserve microbial viability in the supplemented yogurt at the same level as its unsupplemented counterpart, the combined use of inulin (0.4% wt/vol) with yeast extract (0.33% wt/vol) as opposed to yeast extract alone in the starting probiotic culture may help to improve survival over the shelf-life period (Hekmat et al., 2009). This combination of prebiotic agents yielded the highest viable counts of *L. rhamnosus* GR-1 in yogurt over the 28-day shelf-life period compared to other combinations of prebiotic agents and control (Hekmat et al., 2009). One of the limitations of this study is the absence of data on the pH level of each yogurt treatment over time. Some studies report pH as a strong factor which influences viability of probiotic microorganisms in yogurt and other dairy products (Dave & Shah, 1998). It could be that the micronutrients, although most are in the form of salt, reduced the pH of the yogurt product and thus slightly inhibiting the growth of the probiotic microorganisms. It may also be that the fermentation period for the micronutrient supplemented probiotic yogurt should be longer to allow for some inhibited proteolytic activity of the bacteria caused by a change in the contents of the yogurt base (Dave & Shah, 1998). Although the counts are significantly lower in the micronutrient supplemented product, the counts are still maintained above what is defined as the therapeutic level ($>10^7$ CFU/ml) at the end of the shelf-life period.

Our secondary outcome was to assess consumer acceptance of probiotic yogurt supplemented with micronutrients. Our results for all sensory characteristics showed a significant difference between the yogurts. Appearance of the yogurt appears to be an important factor for consumer acceptability. In the present study, the lighter coloured yogurts (1 and 3–12.5% DRI) scored significantly higher than the brighter yellow samples (2 and 4–25% DRI), indicating preference of this group of consumers.

With respect to flavour, yogurt 3 (12.5% DRI and probiotic) was significantly different than the others. Although there were significant differences in how the four different products were scored, all of the mean hedonic scores were above average, indicating that consumers were satisfied with all the formulations. Other studies assessing the sensory properties of fortified yogurts (Achanta et al., 2006; Cueva & Aryana, 2008; Hekmat & Donald, 1997) found no significant differences between consumer's taste ratings of supplemented versus unsupplemented yogurts. Hekmat and Donald (1997) have suggested that yogurts supplemented with iron (in the form of iron chloride, casein chelated iron, and whey protein chelated iron) do not impart a metallic taste and can be comparable in flavour to standard yogurts. Other studies looking at high rates of fortification of nutrients in yogurt (25–90% of the RDA for both vitamins and minerals) did not report any statistical significance between the levels of fortification in

terms of flavour (Achanta et al., 2006; Cueva & Aryana, 2008). In this study, however, there was a difference between the products in terms of the scores for flavour. This could be remediated by the addition of a masking agent; however, this formulation using different types of masking agents was not acceptable during a preliminary tasting panel. At the micronutrient premix level, the omega 3 fatty acids (DHA) were microencapsulated in order to avoid concerns of off-flavour, which is a well described practice for incorporating nutrients into a stable edible matrix (Wegmuller, Camara, Zimmermann, Adou, & Hurrell, 2006; Wegmuller, Zimmerman, Buhr, Windhar, & Hurrell, 2006). Some probiotic strains can influence the flavour profile of yogurt, but *L. rhamnosus* CAN-1 does not, and its addition resulted in similar consumer ratings to standard yogurt in a previous study, (Hekmat & Reid, 2006), and enhanced the consumer acceptability in our study (as per yogurt 3–12.5% DRI and probiotic receiving the highest score on all of the sensory attributes).

Depending on the objectives of the clinical study or program, the nutrient density of the yogurt could be altered to meet the specific needs of the population and to improve the consumer acceptance of this product. Since the low supplementation level was preferred by healthy panellists during the sensory evaluation, the full intended level of micronutrients (25% DRI) could be spread out to two separate 175 g servings of yogurt; in other words, reduce the concentration of micronutrients, but still achieve the same daily intake level.

5. Conclusion

A micronutrient supplemented probiotic yogurt using a novel strain *L. rhamnosus* CAN-1 was successfully developed and delivered an excellent source of micronutrients and probiotic bacteria. This new yogurt may act as an important functional food and provide adjunct benefits for nutrition and immune function for people living with HIV. Yogurt is a relatively low risk food product for pathogenic contamination (Hekmat & Donald, 1997) which increases its appeal in a resource-poor context where refrigeration and other quality control measures are difficult to access and not always reliable. Thus, this type of yogurt would be suitable for use in low-income countries. The technology of manufacturing yogurt is relatively simple and has been transferred to rural community groups in several Sub-Saharan African countries. These community groups have then provided it free of charge to vulnerable people (such as children, and those living with HIV and other infections). It remains to be seen if *L. rhamnosus* CAN-1 will have a prolonged shelf-life in a sub-Saharan African setting, and if it will be able to alleviate problems associated with malnutrition and infectious diseases. But given that yogurt supplemented with *L. rhamnosus* CAN-1 and 12.5% DRI nutrient scored consistently higher in all sensory attributes, it is worth testing in a human trial.

Acknowledgements

The authors are very grateful for Hoda Soltani, Lin Yuan, Tara Koyama and Rajesh Bhayana for their instrumental role in the laboratory preparation and analyses of the samples. The authors are also grateful to Ruben Hummelen for his input to the final draft of this manuscript. We would like to thank Brescia University College for the strong support of the administration and use of the laboratory and sensory evaluation facilities. Our sincere appreciation to Fortitech Strategic Nutrition for providing the micronutrient samples. This project was supported by a grant from the Advanced Food and Materials Network (AFMnet) of Canada. AFMnet did not take part in the development of the study design, the implementation of any part of the study or in the writing of this manuscript.

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