Fruit and vegetable juices tested as possible probiotic beverage

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ABSTRACT: Research was undertaken to test two strains of probiotic lactic acid bacteria (Lactobacillus rhamnosus IMC 501® and Lactobacillus paracasei IMC 502®) to ferment and survive in four different kinds of fruit and vegetable juices. Their viability was also tested when, once incorporated in a microgranule matrix, were added to the same juices and stored at room temperature. The probiotic bacteria grew well in all four kinds of juice during the fermentation period and were capable of surviving and maintaining high viability in the all fermented juices during storage at 4°C. The juices enriched with microgranules and stored at 22°C maintained their stability during the storage without any changes in terms of probiotic viability and pH alterations.

KEYWORDS: Fruit and vegetable juice, probiotic bacteria, Lactobacillus rhamnosus, Lactobacillus paracasei, microgranules.

INTRODUCTION

The consumption of foods and beverages containing functional microorganisms is a growing, global consumer trend (1). Fruit juice has been suggested as a novel, yet appropriate medium for fortification with probiotic cultures because it is already positioned as a healthy food product, and it is consumed frequently and loyally by a large percentage of the consumer population (2). According to FAO/WHO probiotics are defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (3). Most probiotic microorganisms are lactic acid bacteria belonging to the genera Lactobacillus as well as Bifidobacteria, however other kind of microorganisms are used as probiotics, such as Enterococcus faecalis, Lactococcus lactis, Saccharomyces boulardii (4).

The concept of functional foods includes foods or food ingredients that exert beneficial effects on host health and/or reduce the risk of chronic diseases beyond basic nutritional functions (5). Successful types of functional products that have been designed to reduce high blood pressure, cholesterol, blood sugar and osteoporosis have been introduced into the market (6). There are significant scientific evidences, based mainly on in vitro studies and on clinical trials using human and animals, suggesting the potentially beneficial effects of probiotic microorganisms. These include: alleviation of complaints due to lactose intolerance, control of gastrointestinal infections, cancer prevention, immunomodulation and/or regulation (7-12). The probiotic microorganisms should not be pathogenic, have no connection with diarrheagenic bacteria and no ability to transfer antibiotic resistance genes, as well as be able to maintain genetic stability. To be recognized as functional food components, they should demonstrate some of the following properties: acid-stability, bile-stability, resistance to digestive enzymes, adhesion to intestine surface, antagonistic activity against human pathogens, anti-carcinogenic activity, anti-mutagenic activity, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds and production of vitamins and enzymes (13). In addition to the health promoting properties probiotic microorganisms should have some important properties for technological purposes. These include a sufficient stability of viability during the processing and storage, facility of their application in food products, resistance to physico-chemical processing of the food, maintenance of their probiotic activity and desirable characteristics, such as low cost (14).

Traditionally, probiotics have been added to yogurt and fermented dairy products. In recent years, consumer demand for non-dairy-based probiotic products has increased and probiotics have been incorporated into drinks as well as marketed as supplements in the form of tablets, capsules and freeze-dried preparations (15).

The health benefits of probiotic bacteria normally depends on the viability of these bacteria. Therefore, International Dairy Federation (IDF) had suggested that a minimum of 10^9 probiotic bacterial cells should be alive at the time of consumption per gram or milliliter of the product (16).

Fruits and vegetables are healthy foods, because they are rich in functional components such as minerals, vitamins, antioxidants and dietary fibres. The latest are an example of prebiotic components, that selectively nourish the beneficial intestinal microflora, stimulate its development and reinforce its action (17). Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (18). It has been suggested that fruit and vegetable juice could serve as a good medium for cultivating probiotics (19).

The aim of this study was to test two strains of probiotic lactic acid bacteria (Lactobacillus rhamnosus IMC 501® and Lactobacillus paracasei IMC 502®) to ferment and survive in four
different kinds of fruit and vegetable juices. Moreover, the two bacterial strains, once incorporated in a microgranule matrix, were added to the same juices and stored at room temperature. Also their ability to survive in the fruit and vegetable juices without changing the sensory profiles was tested.

RESULTS AND DISCUSSION

Both of the two species of probiotic bacteria, L. rhamnosus IMC 501® and L. paracasei IMC 502®, were found capable of growing well on heat-treated apple juice, carrot juice, apple-carrot juice and pear juice. The viable counts on MRS (de Man, Rogosa, Sharpe) – medium of the two probiotic bacteria used in this experiment were reported on Table 1. L. rhamnosus IMC 501® presented a good growth in heat-treated apple juice. Before 24h the strain grew slowly, but during of 24-48h interval the growth was increased and continued even at 72h reaching 8.1x10⁹ CFU ml⁻¹. L. paracasei IMC 502® had a good growth, but slowly. It was observed that in the first 24h the strain survived, in the 24-72h interval the growth increased until became stable at 4.0x10⁸ CFU ml⁻¹. In the experiment with carrot juice L. rhamnosus IMC 501® grew slowly the first 24h and then after 72h reached a value of 1.1x10¹⁰ CFU ml⁻¹ and L. paracasei IMC 502® grew raising 2.5x10⁹ CFU ml⁻¹. In the mixed apple-carrot juice, L. rhamnosus IMC 501® grew rapidly reaching 2.1x10⁹ CFU ml⁻¹ at 72h. L. paracasei IMC 502® grew slowly in the first 48h up to 4.2x10⁸ CFU ml⁻¹ and after 72h of incubation the strain reached the concentration of 4.2x10⁸ CFU ml⁻¹. Considering the pear juice, both species of probiotic bacteria grew slowly until reaching a concentration of about 10⁶ CFU ml⁻¹.

In all experiments the pH values showed a slight decrease reflecting the growth of bacteria and also a slight increase of the juice acidity measured as percent of citric acid, shown in Figures 1 and 2. These data let us hypothesize a good fermentative action of the strains. Using the proper probiotic strain with the more suitable fruit or vegetable juice it may be obtained a desiderable fermented vegetable or fruit juice with the beneficial properties for the human health. It is known that the lactic acid fermentation enhances protein solubility, improves the availability of several vitamins, increases the utilization of iron, may reduce the serum cholesterol concentration by reducing the intestinal absorption of dietary and endogenous cholesterol (20).

Figure 3 illustrates the effect of cold storage on the viability of the two species of probiotic bacteria in fermented apple juice, carrot juice, apple-carrot juice and pear juice. L. rhamnosus IMC 501® and L. paracasei IMC 502® were capable of surviving in all fermented juices at the temperature of 4°C for four weeks showing a different level of viability. In the apple juice the viable cell counts of L. rhamnosus IMC 501® after four weeks of storage at 4°C were still 9.1x10⁵ CFU ml⁻¹, in the carrot juice the viable cell counts were 4.8x10⁸ CFU ml⁻¹, in the fermented apple-carrot juice the viable cell counts were 1.1x10⁸ CFU ml⁻¹ and in the fermented pear juice the viable cell counts had a value of 5.4x10⁷ CFU ml⁻¹ (Figure 3). After four weeks of storage at 4°C, the viable cell counts of L. paracasei IMC 502® in the apple juice were still 9.5x10⁶ CFU ml⁻¹, in the carrot juice the viable cell counts were 8.0x10⁸ CFU ml⁻¹, in the fermented apple-carrot juice the viable cell counts showed a value of 5.9x10⁶ CFU ml⁻¹ and in the fermented pear juice the viable cell counts were 9.9x10⁵ CFU ml⁻¹.

Both of the used probiotic strains were capable of surviving the low temperature during cold storage at 4°C for four weeks. They never decreased at values less than 10⁶ CFU ml⁻¹ during the whole studied period. Generally it has been suggested that for optimum health benefit, the minimum number of probiotic organisms in a food product should be 10⁶ CFU ml⁻¹, so it is recommended a drink ration of 100ml probiotic juice that give a minimum of 10⁶ CFU (15). The viability of the lactic cultures is the most important factor during refrigerated or frozen storage. The viability of probiotic organisms is dependent on the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (21). The viability of probiotic bacteria is also affected by inhibitory substances such as lactic acid produced during preparation and cold storage. New methods to protect probiotic bacteria improving their viability in functional foods have been developed (22). The viability results of probiotic incorporated into microgranules and added to the juices, stored at room temperature are reported in Figure 4. The microgranules maintained their stability during the four weeks without any changes in terms of probiotic viability.
The incorporation of probiotics in a new and innovative matrix (low vegetable fats) determined an increased probiotic and beverage stability. From the results of the assessment of all juice samples it was possible to obtain a sensorial profile with the 13 attributes present on the evaluation list used by the assessors panel. Figure 5 illustrates the sensorial profile of all the probiotic-enriched juices obtained with the addition of the probiotic bacteria after 4 weeks at 4°C. The probiotic-enriched juices maintain the original sensorial profile for all of 13 attributes excepted for the “sweet flavour”. In fact both probiotic strains seem to affect the “sweet flavour”, as regard carrot juice, apple-carrot juice, pear juice, but the values are always lower than 4 in a scale of 5. Moreover using the probiotic microgranules no significant sensory changes were observed but only light differences in the limpidity of the apple juice (data not shown).

CONCLUSION

Scientific knowledge and technological progress are used to improve the production of innovative foods with specific positive characteristics which may prevent several human diseases. From the results of this study, it is concluded that the two probiotic strains, L. rhamnosus IMC501® and L. paracasei IMC502®, as lyophilized powder or microgranules, could be used as probiotic cultures for production of a healthy beverage from apple juice, carrot juice, apple-carrot juice and pear juice. The use of the probiotic microgranules can be possible for fresh and long shelf-life juices. This result suggests that the microgranules technology could represent a quite good alternative to the use of probiotic in form of lyophilized powder for the preparation of new functional foods such as beverages. Nowadays this represents an answer to the problems of probiotic and product efficacy and shelf-life, and could be a concrete solution for manufacturer and consumer demand. This type of beverage can also be produced for particular consumers, like consumers which are intolerant to lactose present in probiotic dairy-products and for the vegetarian consumers. This will be a way for giving them a more wide range of probiotic foods to be consumed daily. At the same time this probiotic beverage encloses a combination of effects derived from the fermentation, the probiotic strains and the probiotic components from fruit and vegetable.

EXPERIMENTAL SECTION

Probiotic lactic acid bacteria
The probiotic strains, L. rhamnosus IMC501® and L. paracasei IMC502® (23, 24), were kindly supplied by Symbiotec S.r.l., Camerino, Italy. The cultures of the two strains were grown at the temperature of 37°C for 24h in De Man, Rogosa and Sharpe agar (MRS) (OXOID, Hampshire, England) at aerobic conditions.

Preparation of fruit and vegetable juices
Apple, carrot and pear fruits were provided from the local fruit market. The fruit were washed to remove any surface dirt and microbial flora, peeled, crushed and centrifuged (by home centrifuge) to obtain the juice. Each juice was added with 5 percent saccharose (w/v) (J.T. Baker B.V., Deventer, Holland). All the juices were heat-treated for 15 min at the temperature of 100°C.

Fermentation of juices by probiotic bacteria
Four different fermentation experiments were conducted in Erlenmeyer flasks, each containing 50ml of heat-treated apple juice, carrot juice, apple-carrot juice (50 % v/v) and pear juice. All samples were inoculated with 2.5ml of 24-h-old culture of each Lactobacillus (12x10⁶ CFU ml⁻¹ correspondent to n.4 of McFarland standard scale) and incubated at 37°C for 72h in aerobic conditions. Samples were taken at 0, 24, 48 and 72h for microbiological analyses. All the experiments were performed in triplicate.

Effect of cold storage on cell viability in probiotic juices
After 72h of fermentation at 37°C, the fermented juices were stored at the temperature of 4°C for four weeks. Samples were taken at weekly intervals and the viability of probiotic bacteria in juices was determined and expressed as colony forming units (CFU ml⁻¹).

Preparation of fruit and vegetable juices added with probiotic microgranules
The same juices prepared as described in a previous paragraph were treated with...
ascorbic acid at a concentration of 1g l⁻¹ (Sigma-Aldrich, Milano, Italy) and then enriched with microgranules (1g per litre of juices) containing the two probiotic strains, L. rhamnosus IMC 501® and L. paracasei IMC 502® to obtain a final concentration of probiotics around 10⁷ CFU ml⁻¹. The microgranules were prepared applying the spray-chilling technique. A mixture of two vegetable oils (palm and sunflower oils) were heated at 50°C and the lyophilized probiotic strains (1:1) were added to the oils. After two minutes the preparation was sprayed and chilled in a refrigerated room to obtain microgranules. The microgranules contained a concentration of 10¹⁰ probiotic cells g⁻¹. The microgranules with a diameter lower than 80μm were sifted and utilized. The juices were stored at room temperature (20±2°C) for four weeks and analyzed.

Microbiological analyses
Viable cell counts (CFU ml⁻¹) of L. rhamnosus IMC 501® and L. paracasei IMC 502® were determined by the standard count method using MRS with the addition of vancomycin (12mg l⁻¹) agar plates [24]. The counts were made after 72h of incubation at the temperature of 37°C at aerobic conditions. The same method was used for counting the probiotic viability during storage at 4°C and 22°C.

Chemical analysis
A pH meter was used to measure the pH of juices containing probiotic bacteria during the fermentation period and weekly intervals during the storage period of four weeks. An increase in the overall acidity of the four kinds of fermented juices was assayed by the titrimetric method. Juice samples were titrated with 0.1N NaOH until pH 8.3 was obtained and the results was given in percent of citric acid.

Sensory analysis
The sensory profiling technique is a very effective method measuring the sensory characteristics of a product to investigate. Ten assessors (8 female and 2 male) were selected for participation of this study. During an instructive session, a group discussion was held with the panelists, in order to develop a consensus vocabulary for appearance, aroma, texture and flavour attributes for all juices to examine. A list of 13 attributes was developed to perform the descriptive analysis of the juice samples: appearance attributes (natural colour, particle presence), aroma attributes (natural, artificial and specific aroma), texture attributes (consistence, limpidity), flavour attributes (sweet, bitter, acid, astringent and fruity taste) and after-taste. The sensory analysis was applied on both type of juices, fermented and enriched with microgranules, at the end of the storage period.

Statistical analysis
All experiments were carried out in triplicate, and each sample was analysed in duplicate. The results were expressed as mean ± standard deviation (SD). For each type of juice, when tested, was made a negative control. The f Student’s test was used for statistical analysis.

REFERENCES AND NOTES