Chemical structure of fructooligosaccharides

Introduction

Modern people are increasingly interested in their personal health, and expect the foods they eat to be tasty and attractive also healthy and safe. As interest in the link between diet and health gathers pace, many people seek ways to feel well and stay healthy by eating nutritionally foods. Non-digestible carbohydrates such as oligosaccharides, dietary fibers, and resistant starch have various physiologic functions and the promotive effects of many non-digestible carbohydrates on well being, better health and reduction of the risk of diseases have been well examined. Among non-digestible carbohydrates, the functional oligosaccharides present important physicochemical and physiological properties beneficial to the health of consumers, and for this reason, their use as food ingredients has increased rapidly. The functional oligosaccharides are intermediate in nature between simple sugars and polysaccharides and are claimed to behave as dietary fibres and prebiotics. These compounds as non-absorbable food ingredients are microbial food supplements and may benefit the host by selectively stimulating salutary bacteria in the large intestine. The best known functional oligosaccharides include fructooligosaccharide, glucooligosaccharides (GOS), isomalto-oligosaccharides, soybean oligosaccharides, xylo-oligosaccharides and maltitol.

Fructooligosaccharides (FOS) are non-digestible carbohydrates that represent one of the major classes of bifidogenic oligosaccharides. They are compounds of a vegetable origin and are found in varying concentrations in many foods such as asparagus, onions, artichokes, garlic, wheat, bananas, tomatoes and honey. Their chemical structure consists of a chain of fructose

units with a terminal glucose unit linked by Î²-(2€¢1) glycosidic bonds, that means they cannot be hydrolysed by human digestive enzymes which are specific for Î²-glycosidic bonds. The length of the chain ranges from 2- 60. There are three categories of FOS, each of which is structurally distinct: inulin, has a polymerization degree of 2 about 60 monomers of fructose, with an average of 12 units; oligofructose is produced by the enzymatic hydrolysis of inulin and is defined as a fraction of oligosaccharides with degree of polymerization lower than 20, although commercial products tend to have a mean value of 9; these FOS are produced by the enzymatic hydrolysis of inulin and consists of fructosyl chains of different lengths, with glucose and fructose terminals. Finally, scFOS (short chain fructooligosaccharides) are specifically defined as mixed chains of fructosyl with a glucose terminal unit; they have a maximum of 5 units and are derived from sugar through natural fermentation processes, producing 1-kestose (GF2), nystose (GF3) and 1-fructosylnystose (GF4) in which the fructosyl units (F) are linked at the Î²-(2€¢1) position of sucrose (Figure 1).

FOS are water-soluble and their sweetness is 0. 3-0. 6 times that of sucrose, depending on the chemical structure and the degree of polymerization of the oligosaccharide. FOS are highly hygroscopic and their water holding capacity is greater than that of sucros. The viscosity of a FOS solution is higher than that of sucrose at the same concentration because the greater molecular weight of FOS. The enhanced viscosity of the gastrointestinal content may delay the rate of gastric emptying and the digestion and absorption of nutrients. Their thermal stability also is greater than of sucrose. FOS are highly stable in the normal range of food pH (4. 0-7. 0).

FOS can substitute sucrose as regards many of its properties, including solubility, freezing and fusion point and crystalline properties. It has been estimated that the caloric value of FOS ranges from 1. 5 to 2. 0 kcal/g, which represents 40-50% of that of digestible carbohydrates such as sucrose.

Fructooligosaccharides have interesting properties:

- Low sweetness intensity: this property makes them useful for various kinds of foods where the use of sucrose is restricted due to its high sweetness.

- Calorie free; i. e., the human body lacks the necessary enzymes to hydrolyze the beta bonds, so that they are not hydrolyzed by the digestive enzymes. Thus, since these substances can not be used as an energy source in the body, they are safe for diabetics and people on slimming diets.

- Non-cariogenic, since they are not used by Streptococcus mutans to form the acids and insoluble Î²-glucans that are the main causes of dental caries.

- They behave as soluble food fibre from a physiological point of view. They are non-digestible carbohydrates of a vegetable origin that reach to the large intestine, where they can be fermented by the colonic flora to promote the growth of bifidobacteria and prevent the growth of potentially pathogenic microorganisms. The bacterial degradation of FOS occurs in two stages: in the first stage, the monomers are hydrolyzed by bacterial beta-oxidases. In the second, the monomers released ferment anaerobically to produce volatile fat acids (SCFA) such as acetate, propionate and butyrate, and gases (H2, CO2, CH4).

These properties, together with their other beneficial physiological effects (low carcinogenicity, prebiotic effect, improved mineral absorption, and decreased serum cholesterol, phospholipid and triacylglycerol levels) defend the addition of FOS to foods as infant formulas which, in any case, have only very low quantities of these nutrients.

Experimental 1

Impact of a jelly containing short-chain fructo-oligosaccharides and Sideritis euboea extract on human faecal microbiota. (Mitsou et al., 2009)

1. Materials & Methods

1. 1 Subjects

Sixty-four healthy adult volunteers (26 men and 38 women) aged 22-51 years (mean age: 33 years) enrolled. Elimination criteria were a history of gastrointestinal disease and chronic diseases (i. e., diabetes, hyperlipidemia, autoimmune disorders, cardiovascular diseases), a history of extreme dietary behaviors, epileptic seizures, consumption of antibiotics and other medication 2 months prior and during the investigation period. Smoking patterns were recorded prior to the study.

1. 2 Feeding regime

Placebo food was a commercial dessert (jelly, lemon flavored) in powder form containing 86 g sugar, 10 g gelatin, 2. 20 g citric acid, 1 g sodium citrate, 0. 5 g flavors and 0. 3 g colors per 100 g of product. Experimental functional food (jelly) provided additionally 15 g sc-FOS and 0. 9 g S. euboea extract per 100 g of product (respectively, 5 g and 0. 3 g per jelly portion

daily). Powder from aqueous extract of S. euboea was produced using a spray drier. The sc-FOS tested was Actilight® 950P, a mixture of FOS comprising 37% 1-kestose (GF2), 53% nystose (GF3) and 10% 1F-Î²-fructofuranosyl nystose (GF4) (Béghin Meiji Industries, Neuilly sur Seine, France). Jotis S. A. Food Industry provided both the placebo and experimental food product.

The experimental and placebo food were supplied in a powder form (100-g packages) and volunteers were asked to prepare 3 portions of jelly per 100-g package according to manufacturer’s instructions. Subjects were free to eat one portion of the jelly at any time of day.

1. 3 Experimental design

Subjects were instructed to preserve their usual diet and fluid intake during the study with the exception of additional prebiotics and probiotic supplements. Volunteers were assessed for restriction of probiotic and prebiotic consumption during a period of two weeksprior to the intervention. One pre-treatment faecal sample was taken before treatment period begun (day 0).

During the intervention, subjects were randomly assigned to two groups according to feeding regime (placebo group, sc-FOS+extract group) and consumed, respectively, one portion of placebo or experimental jelly daily for 30 d. Neither the subjects nor the researchers were informed about the type of jelly ingested (doubleblinded). Faecal samples were obtained after 2 weeks (day 15) and 4 weeks (day 30) of the treatment period. Stool sampling

took place also at the end of the follow-up period, 2 weeks after the dietary intervention (day 45).

1. 4 Gastrointestinal symptoms

Gastrointestinal side effects were evaluated during the treatment period (day 1-15 and day 16-30) using a daily questionnaire in which symptoms (i. e. abdominal pain, bloating, flatulence) were marked from 0 (no symptoms) to 3 (severe symptoms). The 15-d symptom score (sum of symptom intensity during a 15-d period) was graded as 0 = no symptoms, 1-15 = mild symptoms, 16-30 = moderate symptoms and 31-45 = severe symptoms with possible range for each 15-d symptom score estimated at 0-45 and for total symptom score at 0-135.

1. 5 Sample collection

Faecal specimens were collected rapidly into sterile plastic containers and transferred under anaerobic conditions (GÎ•Î bag anaer, 45534 Biomérieux® SA, Marcy-l'Etoile, France), to a laboratory for microbiological analysis.

1. 6 Bacterial enumeration

Approximately 1 g of the specimenwasweighed and diluted in 9-ml pre-reduced peptone physiological saline (PPS), containing 0. 1% bacteriological peptone (OXOID Basingstoke, Hamshire, England) and 0. 85%NaCl. After homogenization, serial 10-fold dilutions of the homogenateswere performed in PPS under anaerobic environment (BACTRON™ 1. 5 Anaerobic Environmental Chamber, SHELLAB, Cornelius, Oregon). Columbia blood agar

was used for the enumeration of the total mesophilic aerobic and anaerobic microflora (incubation under aerobic and anaerobic conditions at 37 °C for 48 h). Enumeration of total coliforms and E. coli was performed on Chromocult® Coliform agar (Merck KGaA, Darmstadt, Germany) and bacterial counts of enterococci were performed on Slanetz and Bartley medium (LabM Limited, Lancashire, UK) after aerobic incubation at 37 °C for 24 and 48 h, respectively. Rogosa agar (Merck KGaA) and Wilkins-Chalgren anaerobe agar (OXOID), supplemented with 5% (v/v) defibrinated horse blood and G-N anaerobe selective supplement (OXOID), were used for the enumeration of Lactobacillus spp. and Bacteroides spp. respectively, after anaerobic incubation at 37°C for 48 h. Clostridium perfringens was enumerated on Perfringens agar (LabM Limited) supplemented with D-cycloserine (400 mg/L) (LabM Limited) after 24-h anaerobic incubation at 37°C. Finally, Beerens' agar was used for the enumeration of Bifidobacterium spp. (anaerobic incubation at 37°C for 72 h).

Bacteria were characterized on the basis of colony appearance, Gram's stain, catalase reaction and cell morphology. Since Rogosa and Beerens' agars are likely to support growth of non-Lactobacillus and non-Bifidobacterium species respectively one representative isolate from each colony phenotype in these media was further identified to the genus level by molecular methods as described previously. Colony counts were obtained and expressed as a log10 of the colony forming units (CFUs)/g fresh faeces.

1. 7 Statistics

Bacterial counts between the feeding groups at each sampling time (day 0, 15, 30 and 45) prospectively were compared using repeated measures ANOVA (RM-ANOVA) for parametric and the Friedman test for non-parametric data, after age adjustment and Bonferroni’s adjustment for multiplicity. Bacterial counts into each group were compared prospectively using paired-samples t test for parametric and the Wilcoxon signed ranks test for non-parametric data. Comparison of colonization levels was based on log10 transformation of viable bacterial counts. Correlations between initial bifidobacterial levels and increases in bifidobacteria counts in sc-FOS+extract group at day 15 and 30 were tested by the Spearman correlation and a linear regression analysis was performed for the best prediction of the dependent variable.

Digestive symptom intensity was expressed as a 15-d score (day 1-15 and day 16-30) as well as the number of evacuations, watery stools and diarrheic days. Comparisons between study groups and intragroup analysis were performed by the Friedman test for nonparametric data, after age adjustment and Bonferroni’s adjustment for multiplicity. The statistical analysis of the results was performed by the software program SPSS® for Windows Release 11. 5 and the significance threshold was set at 5% (P <0. 05).

2. Results

Fifty-two volunteers (23 men and 29 women) aged 23-50 years (mean age: 34 years) managed to complete the study. Dropoutwas due to antibiotic consumption during the investigation period. According to randomized design of the study, 23 volunteers ingested the placebo and 29 volunteers

consumed the experimental jelly. No significant differences were detected between the placebo and the sc-FOS+extract groups in terms of age (mean age: 33. 78 years vs. 34. 28 years), sex distribution (10 men and 13 women vs. 13 men and 16 women) or smoking patterns (13 non smokers and 10 smokers vs. 20 non smokers

and 9 smokers), respectively.

2. 1 Bacterial populations

In thewhole study population, no differences in intestinalmicroflora were observed between smokers and non smokers, while genderspecific comparisons revealed higher initial total anaerobe bacterial (9. 56±0. 46 vs. 9. 26±0. 61 log10CFU/g faeces, P = 0. 047) and bifidobacterial levels (8. 87±1. 37 vs. 8. 19±1. 83 log10CFU/g faeces, P = 0. 007) in females than males.

Overall, no significant differences were observed in viable counts of aerobes between the two feeding groups (Table 1). A trend towards lower levels of total aerobes at day 30 (8. 13±0. 96 vs. 8. 61±0. 92

log10CFU/g faeces, P = 0. 077), which turned into a significant difference at day 45 was observed in functional food group compared to the placebo. Bacterial levels of total coliforms and E. coli were statistically different between the placebo and sc-FOS+extract groups at day 30, while group-specific analysis revealed higher levels of total coliforms and E. coli only for the placebo regime at day 30 compared to the baseline and day 15. Enterococci counts were not significantly influenced by the ingestion of the

functional jelly compared to placebo during the study period. In sc-FOS+extract group, enterococci were significantly decreased after 30 d of ingestion (6. 77±1. 29 vs. 6. 29±1. 24 log10CFU/g faeces, P = 0. 038) compared to the baseline.

No significant overall viable counts differences were detected between the study groups in the case of total anaerobe mesophilic microflora, Bacteroides spp., Lactobacillus spp. and C. perfringens (Table 1). Total anaerobes were estimated in comparable densities in the two study groups during the entire research period. Higher levels of total anaerobes were detected in the functional food group at day 15 (9. 87±0. 58 vs. 9. 38±0. 56 log10CFU/g faeces, P = 0. 001) and day 45 (9. 66±0. 62 vs. 9. 38±0. 56 log10CFU/g faeces, P = 0. 015) compared to pre-treatment counts. Bacteroides and lactobacilli did not demonstrate significant differences at any sampling time between the feeding groups. Increased Bacteroides population was detected in sc-FOS+ extract group after 15 d of consumption and two weeks after the end of the nutritional intervention compared to initial counts (8. 71±0. 54 vs. 8. 30±0. 81 log10CFU/g faeces, P = 0. 010 and 8. 80±0. 60 vs. 8. 30±0. 81 log10CFU/g faeces, P = 0. 002, respectively). Ingestion of the experimental food in comparison to placebo was related to a trend for lower levels of C. perfringens at day 15 (4. 16±1. 24 vs. 4. 78±1. 23 log10CFU/g faeces, P = 0. 065).

Analysis of bacterial counts demonstrated an overall significant effect of feeding regime in Bifidobacterium spp. levels (Table 1). The enumeration data presented a significant bifidogenic effect of the functional food preparation compared to the placebo after 15 and 30 d of consumption and

a non-significant higher level of faecal bifidobacteria in this group 2 weeks after the end of ingestion. Furthermore, faecal bifidobacteria counts were significantly higher at 15 (9. 54± 0. 83 log10CFU/g faeces, P = 0. 002) and 30 d of intervention (9. 34±1. 04 log10CFU/g faeces, P = 0. 027) and two weeks after the treatmentperiod (9. 33±0. 61 log10CFU/g faeces, P = 0. 015) in the sc-FOS+extract group, compared to pre-treatment levels (8. 79±0. 93 log10CFU/g faeces).

During the 15 and 30 d of dietary intervention, respectively 24 and 21 cases of healthy volunteers consuming the experimental jelly gave increased bifidobacterial counts, with mean increase being estimated at 1. 06 log10CFU/g faeces and 1. 14 log10CFU/g faeces for two and four weeks of intervention. Figs. 2 and 3 indicate a correlation between initial levels of bifidobacteria and positive change in these bacterial populations after 15 (R2 = 0. 747, P = 0. 000) and 30 d (R2 = 0. 712, P = 0. 000) of functional food consumption. Subjects with lower baseline bifidobacterial counts gave larger increase on ingestion of experimental jelly.

2. 2 Gastrointestinal symptoms

No significant differences were observed for gastrointestinal symptoms and characteristics of evacuation during the 30 d of dietary intervention (Table 2). During the first two weeks of the study, a trend for greater flatulence score (6. 88±6. 94 vs. 3. 57±4. 72, P = 0. 070) was observed in functional food group and five cases of moderate flatulence symptoms were reported in this group instead of none in the control group.

Table 1

Faecal bacterial countsa (log10CFU/g faeces) in sc-FOS+Sideritis euboea extract group (n = 29) and placebo group (n = 23) during the 30-d dietary intervention and 2-week posttreatment period.

aAll values are mean±S. D.; CFU, colony forming units; sc-FOS, short-chain fructo-oligosaccharides.

b-fSignificantly different from placebo: bP = 0. 042, cP = 0. 018, dP = 0. 040, eP = 0. 001, fP = 0. 027; gSignificantly different from baseline (day 0) (P <0. 05); hSignificantly different from day 15 (P <0. 05); iSignificantly different from day 30 (P <0. 05).

Figure 2. Correlation between the initial levels of bifidobacteria and increase in bifidobacteria after consumption of a jelly containing sc-FOS+Sideritis euboea extract for 15 d. Bacterial counts are expressed as log10CFU/g faeces; CFU, colony forming units; sc-FOS, short-chain fructo-oligosaccharides.

Figure 3. Correlation between the initial levels of bifidobacteria and increase in bifidobacteria after consumption of a jelly containing sc-FOS+Sideritis euboea extract for 30 d. Bacterial counts are expressed as log10CFU/g faeces; CFU, colony forming units; sc-FOS, short-chain fructo-oligosaccharides.

Table 2

Gastrointestinal symptoms and characteristics of evacuations in sc-FOS+Sideritis euboea extract group (n = 29) and placebo group (n = 23) during the study (0-15 and 16-30 d). a

Symptom intensity was graded as 0 = no symptoms, 1-15 = mild symptoms, 16-30 = moderate symptoms and 31-45 = severe symptoms. The possible range for each 15-d symptom score is 0-15 and for total symptom score 0-135.

a All values are mean±S. D.; sc-FOS, short-chain fructo-oligosaccharides.

3. Discussion

Results indicated a significant bifidogenic effect of the experimental jelly during the intervention. Differences in levels of total coliforms/E. coli and total aerobes were detected between the two feeding groups at day 30 and 45, respectively. Total anaerobes, lactobacilli, Bacteroides spp. C. perfringens and enterococci were not significantly influenced by the ingestion of the functional food compared to placebo during the study period.

The present study demonstrated a strong and selective stimulation of bifidogenesis in healthy volunteers after 2- and 4-week consumption of an experimental jelly compared to the placebo. In our study, high levels of bifidobacteria persisted within the sc-FOS+extract group two weeks after the end of the intervention, a finding that proposes an extended prebiotic effect of sc-FOS.

Another results indicated a decrease in total aerobes in sc-FOS+ extract group compared to the control group two weeks after the end of intervention and no significant differences in enterococci counts

throughout the entire study period. Previous data from studies that used culture-based enumeration techniques proposed no significant effects of both inulin and oligofructose consumption on total viable

counts of aerobes and an unexplained transient increase in aerobic microflora after ingestion of 4 g sc-FOS. A significant difference in enterobacterial counts between the two dietary groups was detected at the cessation of the 30-d intervention, which could be attributed rather to the significant increase of total

coliforms and E. coli densities in the control group. Bacterial counts for Enterobacteriaceae were not significantly affected by the ingestion of sc-FOS, such as Actilight and Neosugar.

Analysis of digestive symptoms indicated that, compared to placebo, consumption of the experimental functional jelly related only with a trend for greater flatulence during the first two weeks of

dietary intervention. A 7-d ingestion of sc-FOS correlated with minor bloating at doses from 2. 5-10 g/d and with excess flatus at 20 g/d In general, excess flatus and/or bloating are the most common gastrointestinal symptoms during sc-FOS ingestion, but they are usually characterized as limited and very mild.

In conclusion, this study demonstrated the prebiotic potential of a jelly containing sc-FOS and S. euboea extract in healthy volunteers. The product was well-tolerated, with no severe gastrointestinal side

effects. Future applications of the experimental food could be focused on people with abnormal intestinal microbiota.

Experimental 2

Fructooligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics (Renuka et al., 2009)

1. Materials & Methods

1. 1 Preparation of FOS syrup

FOS was produced by the transfructosylation of sucrose using FTase enzyme obtained by submerged fermentation using Aspergillus oryzae MTCC 5154

1. 2 Preparation of fruit juice beverages

Ripe pineapple, mango and orange fruits were procured from the local fruit market. The fruits were washed, peeled, crushed and passed through pulper to obtain pulp. In case of oranges, the fruits were peeled and passed through a screw type juice extractor to obtain orange juice. Based on the initial sucrose content of each of the three fruit pulp/juice, sugar syrups were prepared by mixing 135, 35, and 195 g of sucrose in 5847, 5947, and 5947 g of water for pineapple, mango, and orange fruit juices respectively to achieve uniform sweetness. To each of the sugar syrups prepared, citric acid (18 g), FOS syrup (2000 g) and respective fruit pulp/juice (2000 g) were

added. The prepared fruit juice beverages were heated to 90 and hot filled into presterilized bottles and were allowed to cool. Another set of fruit juice beverages containing only sucrose without any added FOS was prepared and used as control.

1. 3 Characterization and storage studies of fruit juice beverages

Fruit juice beverages were stored at ambient (25 ± 2 à¹C) and refrigeration (4 à¹C) temperature for 6 months and were analyzed for colour, changes in the FOS content, total soluble solids (TSS), titratable acidity, pH and sensory qualities at regular intervals of 2 months.

1. 4 FOS content

A known volume of fruit juice beverages fortified with FOS was centrifuged at 8000 rpm for 20 min. The supernatant was filtered through 0. 45 m cellulose nitrate filter (Millipore India Pvt ltd.) and

appropriately diluted with triple distilled water and analyzed by HPLC.

1. 5 Sensory evaluation

Sensory evaluation was carried out by hedonic scale consisting of 10 points (1-10), where 9-10 = excellent, 7-8 = very good, 5- 6 = good, 3-4 = fair, 1-2 = poor. An internal panel of seven expert members evaluated the products for colour, appearance, taste/flavour, mouth feel and overall acceptability.

2. Results and discussion

2. 1. Retention of FOS in the fortified fruit juice beverages

Figure. 4 presents retention of FOS in the fortified fruit juice beverages as a function of storage time. At the end of 6 months of storage, a significant amount of FOS was retained in the fruit juice beverages stored at refrigeration temperature in comparison with those stored at ambient temperatures. There was a noticeable change in the acceptable quality characteristics after 4 months storage at ambient temperature. Fruit juice beverages in general are fast moving commodity and generally do not remain unsold for more than 2-4 months. Thus, the present study clearly indicates that fruit juice beverages can successfully be fortified with FOS with existence of 4 months at ambient temperature.

Figure 4. Effect of storage period on FOS content (g/100 g) of fruit juice beverages. : Pineapple, : Mango and : Orange juices fortified with FOS.

2. 2. Characterization of fruit juice beverages during storage

The changes in pH, TSS (à¹Brix), and titratable acidity of the fruit juice beverages, when analyzed using ANOVA were not statistically significant at the 5% level between time zero (initial) and 180 days (6

months) of storage at ambient and refrigeration temperature. The pH of the fruit juice beverages fortified with FOS was in the range of 3. 23-3. 57 as against the control (3. 30-3. 82). Similar observations with respect to the changes in pH as a function of storage time and temperature have been made. TSS (à¹Brix) of the fruit juice beverages varied from 15 to 16 à¹Brix and was stable throughout the storage period (4 à¹C & 25 ± 2 à¹C). The stability of the TSS could be due to the heat treatment prior to storage.

Titratable acidity of fruit juice beverages varied from 0. 23 to 0. 35 g citric acid/100 mL juice. The acidity was fairly constant

throughout the storage. The fruit juice beverages fortified with FOS have stability over storage and the beverages kept all the good sensorial properties, as compared to control. Results also showed that there was no visible change (Pâ‰¥0. 05) in the colour during storage.

2. 3. Sensory evaluation

No significant changes were detected in the overall quality of the fruit juice beverages fortified with FOS in comparison with that of control by the panelists after 4 months of storage at ambient temperature (Table 3). Sensory qualities of the fruit juice beverages stored at ambient and refrigerated temperature was studied on the basis of the consideration that a minimally acceptable product should be equivalent to rating 5 (colour, consistency, taste, flavour and overall quality) of its sensory quality. Fruit juice beverages fortified with FOS were found to be acceptable up to 4 and 6 months based on the evaluation of overall quality at ambient and refrigeration temperature respectively.

3. Conclusion

The changes in the present day consumers' life style have led to a vital change in the marketing trends of food sector. Today's consumer being more health conscious is seeking products with greater health benefits and there is a great demand for ' health foods'. The present study showed that fruit juice beverages can fortified with FOS with existence of 4 months and 6 months at ambient and refrigeration temperature respectively. There were

no undesirable changes in the physicochemical characteristics of the fruit juice beverages fortified with FOS. Overall quality of the fruit juice beverages fortified with FOS for 4 months of storage at ambient temperature was acceptable as indicated by sensory analysis. Constant pH, TSS, TA and viscosity of fruit juice beverages clearly indicates that there is no spoilage either due to microbial or enzymatic reaction.