PHYSICO-CHEMICAL PROPERTIES OF FRESH AND PROBIOTICATED FRUIT JUICES WITH LACTOBACILLUS CASEI PRODUCED IN NIGERIA

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ABSTRACT

The physico-chemical analysis of fresh and probioticated fruit juices with Lactobacillus casei was assessed. A total of four fruit samples from Okigwe, Nigeria consisting of mango, orange, watermelon and grape were collected and screened using standard microbiological and chemical methods. The qualitative phytochemical screening carried out with the fruits juices showed the presence of some bioactive compounds of flavoniods, tannins, carbohydrates and glycosides. Alkaloid was only present in mango fruit juice while saponin was present in orange and grape fruit juice. Physico-chemical properties of the freshly prepared fruit juices showed a pH range of 4.0 to 4.2 with percentage titrable acidity (as citric acid) range of 0.14±0.01 to 0.41±0.1. The turbidity, reducing sugar (%) and total soluble solid (%) ranged from 35.0±1.2 to 50.2±1.0, 9.0±1.0 to 17.2 ±1.2 and 12.2±1.0 to 21.0±0.6, respectively. The Physico-chemical analysis of free cell fermented various probioticated fruit juices at different time intervals (24, 48 and 72 hrs) revealed that the pH decreases from 4.2-3.2, 3.8-3.5, 3.8-3.2 and 4.8-3.2, while the titrable acidity increases from 0.49±0.01 to 0.70±0.01, 0.46±0.06 to 0.52±0.01, 0.20±0.01 to 0.40±0.01 and 0.17±0.06 to 0.28±0.01 for mango, orange watermelon and grape, respectively.

Keywords: Physico-chemical, Probiotics, Fruit Juices and Lactobacillus

INTRODUCTION

Tropical fruits have their origin in the tropics and require rather a tropical or subtropical climate; and do not tolerate frost. There are hundreds of edible tropical fruits some of which have very high export potential all over the world. Most of the tropical fruits are important source of antioxidants, vitamins, dietary fibres and minerals; and form a very healthy part of our diet (Reddy et al., 2012). Fruit juice could serve as a good medium for cultivating probiotics (Mattila-Sandholm et al., 2002). Furthermore, fruits and vegetables do not contain any allergens, as in case of dairy products, that might cause allergy in certain segments of the population (Luckow and Delahunty, 2004). Probiotics are defined as "living microorganisms, which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition" (Guarner and Schaafsma, 1998). Lactic acid bacteria (LAB), predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*, constitute a significant proportion of probiotic cultures in nutritional supplements, pharmaceuticals and functional foods (Piano et al., 2006). The LABs have been added to a variety of dairy - based products such as fermented milks and yogurts for their probiotic human health benefits (Siuta-Cruce and Goulet, 2001). As mentioned earlier, current industrial probiotic foods are basically dairy products, which may represent inconveniences due to their lactose and cholesterol content (Heenan et al., 2004). Technological advances have made it possible to alter some structural characteristics of fruit and vegetable matrices by modifying food components in a controlled way (Betoret et al., 2003). This could make them ideal

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substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers and antioxidants. There is a genuine interest in the development of fruit juice based functional beverages with probiotics, because they have taste profiles that are appealing to all age groups and also they are perceived as healthy and refreshing foods (Tuorila and Cardello, 2002; Sheehan et al., 2007). In recent years, consumer demand for nondairy-based probiotic products has increased. Generally both fresh fruits and their juices are included in our regular diet as they give health benefits to all age groups. Juices from these sources are deemed to be advantageous because of their low allergenicity, perceived health benefits and appeal to a wide segment of the population (Sheehan et al., 2007). It is envisaged to probioticate the fruit juices for certain greater benefits.

MATERIALS AND METHODS

Source of Samples

Lactic acid bacterium was isolated from milk in 10^{-4} and 10^{-5} dilution with the selective media deMann, Rogosa and Sharpe (MRS medium, Merck). The strain isolation was based on the colony appearance, at initial stages. The isolated strain was compared with that of the Standard Culture, obtained from Microbial Type Culture Collection and GeneBank, of FIIRO, Nigeria. Bacteria were proliferated for 24 hours and further subculturing was carried out after increasing the volume of the medium for the next 48 hours anaerobically at 37°C (Ayad et al., 2004).

Mango, orange, watermelon and grape fruits were purchased from Okigwe market, Imo State. After collection, the fruits were allowed to ripen for a period of 5 -7 days. After that, the fruits were washed with tap water followed by sterile water. Then, fruits were peeled with the help of sharp knife and cut into small pieces and then pulped with the help of laboratory blender and added with 0.1g/L of potassium metabisulphite as a preservative and kept in a refrigerator at 0°C until further use.

Extraction of fruit juices by enzyme treatment: The fruit pulp samples obtained from various fruits were treated with 0.1% (w⁄v) of commercial pectinase enzyme and incubated at 40ºC for 2 h. The activity of enzyme reaction was stopped by heating at 80ºC for 5 min. The juice was extracted by passing through a cheese cloth (Kumar et al., 2009). The juice samples obtained in this manner were then subjected to analysis of phytochemicals, reducing sugars, total acidity, pH, turbidity and soluble solid contents and stored at 4ºC prior to probiotication using *L. casei*.

Phyto-chemical Analysis

The analysis were carried out by using the standard methods as described by Harborne (1992) and Ekeleme et al. (2013). Probiotication of various fruit juices. Probiotication experiments were conducted in 150 ml conical flasks each containing 100 ml of various pasteurized fruit juices which were inoculated with a culture of *L. casei* in MRS broth (10^5CFU/ml) for free fermentation. The flasks were incubated at 37ºC for 72 h. Also, the immobilized cell culture was inoculated in the same set of substrates for immobilized cell fermentation. 100 ml juice(s) were mixed with immobilized cells following removal of the MRS broth by decantation. Inoculated juice was incubated at 37ºC for 72 h. Bacterial counts were immediately taken following the separation of immobilized cells in the fermented products on a Probiotication of various fruit juices Probiotication experiments were conducted in 150 ml conical flasks each containing 100 ml of various pasteurized fruit

juices which were inoculated with a culture of *L. casei* in MRS broth (10^5CFU/ml) for free fermentation. The flasks were incubated at 37°C for 72h. Also, the immobilized cell culture was inoculated in the same set of substrates for immobilized cell fermentation. 100 ml juice(s) were mixed with immobilized cells following removal of the MRS broth by decantation. Inoculated juice was incubated at 37ºC for 72 h. Bacterial counts were immediately taken following the separation of immobilized cells in the fermented products on a transmittance and absorbance (turbidity) of the fruit substrates were measured using spectrophotometer (Model using UV U-2900/2910, Hitachi High tech, Japan) at 540nm Total acidity in juices was determined by titrating with 0.1N NaOH previously standardized using standard oxalic acid and the values were expressed as tartaric acid equivalents. Total soluble solids (TSS) were determined using a hand refractometer (Erma, Japan) interms of °Bx (°Brix) (Varakumar et al., 2011). Reducing sugars were determined using a glucose-oxidase spectrophotometric method (EI, England).

Statistical Evaluation

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. The results are expressed as mean ± S.D (standard deviation). The SPSS statistical computer package was used to analyze the experimental data, charts and tables were also used to represent data.

RESULTS

The qualitative phytochemical screening carried out with mango, orange, watermelon and grape fruit juices showed the presence of some bioactive compounds of flavoniods, fannins, carbohydrates and glycosides. Alkaloids was only present in mango fruit juice while saponins was present in orange and grape fruit juice (Table 1).

Physico-chemical properties of the freshly prepared fruit juices is shown in Table 2. The juices yielded 400 to 550 mg/kg. The pH were 4.2, 4.0,4.0 and 4.2 for mango, orange, watermelon and grape, respectively, with percentage titrable acidity (as citric acid) range of 0.1±0.01 to 0.4±0.1. The turbidity ranging from 35.0±1.2 to 50±1.0. The reducing sugar (%) and total soluble solid (%) ranged from 9.0±1.0 to 17.2 ±1.3 and 12.2±1.0 to 21.0±0.8, respectively.

Table 1. Qualitative phytochemical constituent of various fruit juices.

Components	*Mango*	*Orange*	*Watermelon*	*Grape*
Carbohydrates	+	+	+	+
Flavoniods	+++	++	+	+
Tannins	+	++	+	++
Glycosides	+	+	+	+
Alkaloids	+	-	-	-
Saponins	-	+	-	+

+++ = appreciable amount, ++ = moderate amount, + = minute amount, - = not detected

The Physico-chemical analysis of free cell fermented various probioticated fruit juices at different time intervals (24, 48 and 72 hrs) revealed that the parameters analyzed decreases in values as the time increases except for the titrable acidity (%) which increased progressively.

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However, the reducing sugars were decreased due to utilization of the same for the growth of the bacteria. The pH decreases from 4.2-3.2, 3.8-3.5, 3.8-3.2 and 4.8-3.2, while the titrable acidity increases from 0.49±0.01-0.70±0.01, 0.46±0.06-0.52±0.01, 0.20±0.01-0.40±0.01 and 0.17±0.06-0.28±0.01for mango, orange watermelon and grape, respectively (Table 3).

Table 2. Physico-chemical properties of freshly prepared fruit juices

Name of the fruit	Juice yield (mg/kg)	pH	Turbidity	Titrable acidity (%)	Reducing Sugar (%)	Total soluble solids (Brix %)
Mango	400	4.2	35.0±1.2	0.4±0.1	17.2 ±1.3	21.0±0.8
Orange	500	4.0	50.0±0.5	0.2±0.1	16.0 ± 1.0	17.4±0.9
Watermelon	550	4.0	36.5±1.0	0.1±0.01	10.2±1.2	12.2±1.0
Grape	500	4.4	50.0±1.0	0.1±0.01	9.0±1.0	15.4 ±1.0

Table 3. Physico-chemical analysis of free cell fermented various probioticated fruit juices at different time intervals

Name of the fruit juice	*Time intervals (h)*	*pH*	*Turbidity*	*Titrable acidity (%)*	*Reducing Sugar (%)*	*Total soluble solids (Brix %)*
Mango	24	4.0	32.2±0.1	0.49±0.01	10.7±0.58	20.0±1.1
	48	3.6	30.0±0.1	0.66±0.07	5.3±0.10	18.0±1.0
	72	3.2	28.1±0.1	0.70±0.01	3.1±0.11	11.0±1.0
Orange	24	3.8	40.1±1.2	0.46±0.06	13.1±0.60	17.0±1.3
	48	3.7	35.2±1.1	0.48±0.01	10.3±0.75	15.1±1.0
	72	3.5	30.0±1.0	0.52±0.01	5.5±0.81	13.0±1.2
Watermelon	24	3.8	33.5±1.0	0.20±0.01	5.4±0.71	10.0±1.0
	48	3.6	30.0±1.0	0.30±0.02	4.1±0.05	8.3±1.5
	72	3.2	28.2±1.1	0.40±0.01	3.1±0.06	6.0±1.0
Grape	24	4.2	45.0±1.2	0.17±0.06	8.1±1.0	14.0±1.3
	48	4.0	43.1±1.0	0.22±0.01	7.0±0.73	12.0±1.0
	72	3.8	40.0±1.0	0.28±0.01	5.5±0.85	10.0±1.2

DISCUSSIONS AND CONCLUSION

The qualitative phytochemical constituent of the various fruit juices showed the presence of bioactive compounds in the fruit. A moderate amount of tannins was obtained from the orange and grape fruit juice, while mango and watermelon showed the presence of minute amount. Alkaloids was only obtained in minute amount from the mango fruit juice, but was not detected in the order fruit juices, the main reason that can be adduced from this is the mode of extraction. The presence of tannins suggests the ability of these fruits to play a role

as anti-diarrhoea and anti-haemorrhagic agents, the presence of alkaloids has been implicated in its detoxifying and antihypertensive properties as a result of its stimulatory effects (Dave and Shah, 1997).

Saponins though positive for orange and grape, but less frosting and was not intense in the fruit juices (minute amount) than the mango and watermelon juice (not detected), this compound has been shown to have immense significance as anti-hypercholesterol, hypotensive and cardiac depressant properties. Flavonoids was positive for the fruit juices, on this premise it will be advisable to extract the fruit juices with better methods in an attempt to exploit its antioxidant and antitumor properties (Olah *et al.*, 2002. FAO/WHO, 2001).

Glycosides showed positive results (minute amount) for all the fruit juice extracts with no clear intensity in the extracts. The glycosides have been used for centuries as stimulants in cases of cardiac failure (Olah *et al.*, 2002). This perhaps justifies the already locally established function of the fruits in the treatment and management of hypertension (Kumar et al., 2012).

The physico-chemical properties of the fruit juices were measured before and after probiotication. The acidity of the substrates increased during probiotication. Physico-chemical properties of the freshly prepared fruit juices were of the following values, the juices yielded 400 to 550 mg/kg. The pH were 4.2, 4.0,4.0 and 4.2 for mango, orange, watermelon and grape, respectively, with percentage titrable acidity (as citric acid) range of 0.1±0.01 to 0.4±0.1. The turbidity ranging from 35.0±1.2 to 50±1.0. The reducing sugar (%) and total soluble solid (%) ranged from 9.0±1.0 to 17.2 ±1.3 and 12.2±1.0 to 21.0±0.8, respectively. This result is in accordance the work done by Kumar *et al*. (2009) on physico-chemical analysis of fresh fruit juices.

The Physico-chemical analysis of free cell fermented various probioticated fruit juices at different time intervals (24, 48 and 72 hrs) revealed that the parameters analysed decreases in values as the time increases except for the titrable acidity (%) which increased progressively. However, the reducing sugars were decreased due to utilization of the same for the growth of the bacteria. The pH decreases from 4.2-3.2, 3.8-3.5, 3.8-3.2 and 4.8-3.2, while the titrable acidity increases from 0.49±0.01-0.70±0.01, 0.46±0.06-0.52±0.01, 0.20±0.01-0.40±0.01 and 0.17±0.06-0.28±0.01for mango, orange watermelon and grape, respectively.

A rapid decrease in pH in the beginning of fermentation is of great importance for the quality of the end product (Viander *et al*., 2003). The rapid increase in acidity minimizes the influence of spoilage bacteria. In the slowly acidified medium, lactic acid fermentation can be suppressed by butyric bacteria activity (Karovicová and Kohajdová, 2003).

The changes in pH and acidity during fruit juices fermentation by *L. casei*, although the fruit juices had an initial pH values of 4.0, 3.8,3.8 and 4.2 for mango, orange, watermelon and grape, respectively. The lactic acid culture (*L. casei*) actively fermented the fruit juices and lowered the pH to as low as 3.2, 3.5, 3.2 and 3.8 for mango, orange, watermelon and grape, respectively after 72 h fermentation. Especially, mango fruit juice showed a more rapid drop in pH than the other three fruit juice cultures examined. As shown in the result, mango fruit juice produced significantly more acid during the juice fermentation than the other three juices examined. However, this result agrees with the of Mohan *et al*. (2013), who studied the probiotication of tomato juice by lactic acid bacteria and found out that the lactic acid cultures reduced the pH to 4.1 or below and increased the acidity to 0.65% or higher, and the viable cell counts (CFU) reached nearly 1.0 to 9.0×109/ml after 72 h fermentation.

The lactic acid cultures rapidly fermented the juices and reduced the level of sugar. The sugar present in the mango, orange and grape juices were consumed at a much faster rate than

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watermelon by *L. casei*. For example, *L. casei* reduced the sugar level of the mango juice from an initial value of 17.2±1.3 % to 10.7±0.58, 5.3±0.10, and 3.1±0.11% after 24, 48, and 72 h fermentation, respectively.

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