

## Microbiological Status of Soymilk- Fruit Juice Drink as Affected by Orange and Pineapple Juice Replacement

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### ABSTRACT

The effect of orange and pineapple fruit juice replacement on microbiological status of soymilk-fruit juice drink was studied. Soybean seeds, fresh ripe orange and pineapple fruits were processed into soymilk, and fruit juices respectively. The orange and pineapple juices were blended at equal proportion to get mixed fruit juice (MFJ). MFJ was used to replace soymilk (SM) at 10%, 20%, 30%, 40% and 50% levels without addition of any chemical preservatives. The microbiological status of the soy-mixed fruit juice (SMFJ) was evaluated. Microbial examination showed that bacteria count was higher in SMFJ<sub>5</sub> (50:50) ( $40 \times 10^2$  Cfu/ml) and lower in MFJ (100% mixed fruit juice) ( $11 \times 10^2$  Cfu/ml). There was no coliform growth, suggesting absence of faecal contamination. Fungal growth was higher ( $24 \times 10^2$  Cfu/ml) at 40 % juice dilution. Microbes such as *Bacillus sp.*, *Staphylococcus sp* and *Penicillin* were isolated in the samples. The morphology of the microbes was ascertained thereby providing information for the preservation of the beverage and encouraging its processing.

**Key words:** Fruit juice, Microbiological, Orange, Pineapple, Soymilk.

### INTRODUCTION

Fruits are good sources of micronutrients especially minerals and vitamin C. Oranges and pineapples are available in almost all parts of rural Nigeria at affordable prices, and can be processed into fruit juices with longer shelf-life; this can be useful in bridging the nutrient gap experienced in Nigeria and many other developing countries. The high rate of micronutrient deficiencies in Nigeria has been attributed partly to poor dietary habits (Obizoba *et al.*, 2004). Researchers have shown that for optimal antioxidants protection of the body, carotenoids available in fruits like orange, pawpaw, mango and flavonoids found in citrus fruits such as oranges, lime and grapes should be taken on daily basis. Fruits can check constipation and help

to reduce weight. They are rich in vitamin C, which prevent scurvy, body malfunctioning and general malnutrition (Baje, 2004).

Pineapples are rich in vitamin C although there is variation of vitamin contents from fruit to fruit and the quality of pineapple is dependent on a number of factors which include; variety, nutrition, exposure, weather conditions such as light intensity, rainfall and seasonal changes, and ripeness (Marvin, 1978). He further reported that the flavour and composition of pineapple juice are dependent on the composition of the fresh fruit from which it was processed. The edible portion of the pineapple fruit as reported by Okaka (1997) constitute about 60% of the fresh fruit and contains approximately 85% water, 0.4% protein, 14% sugar, 0.4% fat, 0.5% fibre, 1101U

vitamin C, 301U vitamin A. The main carbohydrates in pineapple are sucrose, fructose. The main amino acid is asparagine. It also contains some important enzymes which includes, peroxidase, indo-zyl-acetic acid, phosphatase and bromelin (Ihekoronye and Ngoddy 1985),

Sweet orange is native to north eastern India, Southern China, but has spread to other tropical and subtropical region of the world (Pantastico, 1975). The proximate composition of edible portion is water 86%, protein 0.6%, fat 0.1%, calcium 25mg, iron 0.3mg, vitamin A 120IU, vitamin C 50IU, thiamine 0.6mg (Ihekoronye and Ngoddy 1985). Orange are eaten fresh or processed by canning the segment, bottling or freezing the juice or comminute fruit and by drying the juice to give powdered product (Ihekoronye and Ngoddy 1985). Orange juice is probably the most widely recognized and accepted worldwide. Nutritionally, orange fruit is a source of quick energy in the form of sugar. It contains significant amount of vitamin C and folic acid (Brickln, 1993)

Soybean (*Glycine max*) value in human nutrition is derived from its superiority to other edible legumes in terms of dietary quantities. These include total digestible nutrients and therefore metabolise energy, percentage protein, protein biological, protein efficiency ratio, percentage lysine, vitamin and mineral (Adeyeye and Ajemole, 1992). According to Salunkhe *et al.* (1992). Soybean contains proteins and oil ranging from 32.4 to 50.2 % and 13.9 to 23% and Iwe(2003) justify the claim by opining that variation in protein and oil contents in soybeans is due to locality where the beans are grown and cultivars of the beans. Soybean also contains about 32% carbohydrate which includes starch, sugar, lignin and cellulose (crude fibre) and other minor carbohydrates such as pectin substances, arabinogalactans.

Soymilk when properly formulated, closely resemble cow milk and mix and attractive alternative to conventional milk (Iwe, 2003; Ahmed, 1984). Cow and soymilk have approximately the same protein contents (3.5 to 4%) and their amino acid profile show a very close relationship. The difference is that, soymilk does not contain lactose,

which constitutes a problem (lactose-intolerance) in infants consuming cow milk. Soymilk products were adopted as weaning food for babies and for treating malnourished children, whereby hundreds of Kwashiorkor and Oedema affected children were saved by feeding them with soymilk and other soy preparations (Iwe, 2003). Since soymilk is relatively inexpensive source of protein with least incidences of cardiovascular diseases and lactose intolerance, the use of soymilk in various diets is on the increase (Osundahunsi, 2003). Soy-protein has been found to promote health by lowering blood cholesterol and so have been used in prevention or treatment of heart diseases (Messina, 1995). According to Messina *et al.* (1994), a cup of soy milk and half cup of tofu per day lowers the risks for a wide range against various cancers. Therefore, the objective of this work is to determine the microbial status of soymilk - fruit juice drink.

## MATERIALS AND METHODS

Fresh ripe pineapple, sweet oranges and soybean seeds were purchased from a local market in Imo state, Nigeria. All analyses were done at National Root Crop Research Institute Umudike, Umuahia, Abia State, Nigeria.

### Sample preparation

#### Soymilk preparation

Soybean seeds were thoroughly sorted to remove immature seeds, stones and foreign materials. 1kg of the cleaned soybean was washed and soaked in 3,000ml of water for about 14h at room temperature (27°C). The soaked beans were blanched for 5min, drained, dehulled, milled and water added to it (ratio of 1:3 beans to water). The resultant slurry was filtered in a filter press (Muslin cloth) and pasteurized at 93°C for 15min.

#### Fruit juice preparation

The fruits (orange and pineapple) were washed in a clean tap water, peeled manually with a sharp knife and sliced into cubes. 1kg of the sliced cubes of the mixture of orange and pineapple were pulped in a monanex blender. The resultant pulp was expressed through a cheese cloth to obtain a clear mixed fruit juice (MFJ).

**Soymilk – Mixed Fruit Juice Drink Preparation**

The mixed fruit juice (MFJ) was blended with soymilk (SM) at varying proportions on percentage basis (100:0, 90:10; 80:20, 70:30, 60:40 and 50:50). The resultant blends were homogenized and pasteurized at 80°C for 10s in a water bath, hot-filled into sterile bottle, cooled to room temperature (27°C) and stored in a refrigerator at 4°C until analysed.

**Microbiological examination**

The method used for media preparation, microbial load count and biochemical characterization were as described by Ogbulie *et al.* (2001).

**Method of microbial Load count**

Serial dilution, pour plate count and streaking methods were used for microbial load count of the beverages. Ten milligram serial dilution of the different samples were carried out by pipetting 9ml of distilled water into each test tube and plugged with cotton wool and sterilized by autoclaving for 15min at 121°C, they were put in the test tube rack and allowed to cool to 37°C before introducing the samples of the beverages.

Then to each of the tube containing the 9ml of sterile distilled water, each of the different samples of beverage were introduced with 1ml sterile pipette into the test tube and plugged with cotton wool, shaken gently for proper mixing. With another sterile 1ml pipette, the homogenate was collected.

Molten agar at 45°C was poured into culture plate and swirled clockwise and anticlockwise

gently for thorough mixing and allowed to solidify. The homogenates of the different samples were collected and streaked with a sterile inoculating loop aseptically. The plates were properly labelled before incubating at a temperature of 35°C for 48h after which the number of Organisms were determined by direct plate colony count.

**Wet Mount Microscopy**

One drop of lactophenol cotton blue was placed on a microscope slide and with a sterile inoculating wire; some growth from each of the different colonies from the culture plates was carefully placed over the preparation which was then viewed under x10 and x40 objective of the light microscope. Observation were made and recorded accordingly.

**Identification of Fungi**

The genera were distinguished according to the morphology of their vegetation and reproductive structures as seen under the microscope.

**Gram Staining for Bacterial Isolates**

With a sterile wire loop, suspected bacterial colonies were in turn used to prepare smears on clean microscope slide, dried and heat fixed. It was then stained with 0.5% aqueous crystal violet and allowed to stand for 60sec, washed off with distilled water, stained with lugols iodine for 60sec, rinsed out with distilled water, decolorized with alcohol for 30sec, rinsed with distilled water, flooded with 1% aqueous safranin for 60sec as a count strainer, rinsed with distilled water, blotted dry with filter paper. The slides were then examined under the

**Table. 1: Bacterial and fungal count after 48 hours incubation**

| Sample            | Mac-Conkey | Nutrient agar Cful/ml | Above dextrose agar   |
|-------------------|------------|-----------------------|-----------------------|
| MFJ               | -          | 11 x 10 <sup>-2</sup> | 8 x 10 <sup>-2</sup>  |
| SM                | -          | 32 x 10 <sup>-2</sup> | 18 x 10 <sup>-2</sup> |
| SMFJ <sub>1</sub> | -          | 36 x 10 <sup>-2</sup> | 20 x 10 <sup>-2</sup> |
| SMFJ <sub>2</sub> | -          | 34 x 10 <sup>-2</sup> | 22 x 10 <sup>-2</sup> |
| SMFJ <sub>3</sub> | -          | 38 x 10 <sup>-2</sup> | 21 x 10 <sup>-2</sup> |
| SMFJ <sub>4</sub> | -          | 37 x 10 <sup>-2</sup> | 24 x 10 <sup>-2</sup> |
| SMF <sub>5</sub>  | -          | 40 x 10 <sup>-2</sup> | 23 x 10 <sup>-2</sup> |

Key: MFJ = 0:100%, SM = 100:0%, SMFJ<sub>1</sub> = 90:10%, SMFJ<sub>2</sub> = 80:20%, SMFJ<sub>3</sub> = 70:30%, SMFJ<sub>4</sub> = 60:40% and SMF<sub>5</sub> = 50:50%.

oil immersion objective of the microscope. Gram positive bacteria appeared purple while gram negative bacteria appeared pink.

### Spore Staining

The isolate was heat fixed and flooded with 5% aqueous malachite green and steamed intermittently for 2min afterwards, it was placed under running water and then counter stained with 0.5% aqueous safranin for 15sec. This was followed by rinsing with water and drained. Isolates showing presence of spores appeared green while the vegetative cells appeared pink. The position of the spores was recorded.

### Biochemical characteristics

The following biochemical test were carried out for the identification of the bacteria isolates.

### Catalase Test

A platinum wire loop sterilized by flaming was used to pick colonies and placed on a grease free slide where 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) had been dropped and this was mixed thoroughly. Release of white frothy bubbles caused by liberation of free oxygen gas indicated a positive result.

### Oxidase test (wet paper method)

A strip of sterile paper was soaked with the oxidase reagent (tetramethyl-p-phenylene-

**Table. 2: Characterization of bacterial isolates**

| Sample            | Colony characteristics   | Gram stain/cell Characteristics   | Spore stain | Catalase    | Oxidase | Isolates                     |
|-------------------|--|---|-------------|-------------|---------|------------------------------|
| MFJ               | Small golden yellow, Smooth glistering colonies  | Gram +ve  | -           | +ve         | -       | <i>Streptococci</i> sp       |
| SM                | Grayish white colonies with root like spreading out growth   | Gram +ve rod appearing in chains with branches                            | +           | +ve         | -       | <i>Bacillus acreus</i>       |
| SMFJ <sub>1</sub> | (1) Greyish white Colonies with root like growth<br>(2) Small golden yellow smooth glittering colonies | (1) Gram +Ve Cocci clustered cell<br>(2) Gram +Ve rod appearing in chains | -           | +ve         | -       | <i>Lactobacillus</i> sp      |
| SMFJ <sub>2</sub> | (1) Greyish white Colonies with root like growth<br>(2) Small golden yellow smooth glittering colonies | (1) Gram +Ve Cocci Clustered cell<br>(2) Gram +Ve rod appearing in chains | -           | +ve         | -       | <i>Staphylococcus aureus</i> |
| SMFJ3             | As in SFJ2   | As in SMFJ2 SFJ2  | As in SFJ2  | As in SFJ2  | As in   | As in SMFJ2                  |
| SMFJ4             | As in SFJ2   | As in SMFJ2 SFJ2  | As in SFJ2  | As in SFJ2  | As in   | As in SMFJ2                  |
| SMFJ5             | As in SFJ2   | As in SMFJ2 SFJ2  | As in SMFJ2 | As in SMFJ2 | As in   | As in SMFJ2                  |

Key: MFJ = 0:100%, SM = 100:0%, SMFJ<sub>1</sub> = 90:10%, SMFJ<sub>2</sub> = 80:20%, SMFJ<sub>3</sub> = 70:30%, SMFJ<sub>4</sub> = 60:40% and SMFJ<sub>5</sub> = 50:50%, AS = Aseptate, + = Positive, Sp = Sporangiospore, S = Septate, - = Negative, C = Conidia.

diaminedihydrochloride. Then, with a glass rod, a colony of the test organism was rubbed on the test strip. A deep purple blue within 10sec indicated a positive result.

## RESULT AND DISCUSSION

Table 1: shows the bacterial and fungal count of soymilk-fruit juice drink. Bacterial was lowest ( $11 \times 10^2$ Cfu/ml) in fruit juice extract and highest in soymilk sample ( $32 \times 10^2$ Cfu/ml). The bacterial count of the blends (SMFJ) gradually increased with increased soymilk replacement with fruit juice. The highest was recorded at 50% (SMFJ<sub>5</sub>) level ( $40 \times 10^2$ Cfu/ml). The findings of Okafor (1975) revealed that high bacteria load is associated with unpreserved soymilk. Also high protein content of soymilk hampers shelf life prolongation. Micro-organisms flourish most in proteinaceous substrate. Smith and Circle (1992) in their work also wrote that micro-organisms thrive most in a medium that has essential nutrients which enhance microbial synthesis and degradation.

The absence of growth in the Mac-conkey agar suggested the absence of coliform in all the samples indicating that the water used during the production process was potable (not faecally contaminated). Fungi growth after 48h of incubation

showed that, the colony count increased with the blends but highest in sample SMFJ<sub>4</sub> ( $24 \times 10^2$ Cfu/ml). This was expected because as the nutrient level of a food product increases, especially proteins, the level of fungi growth increases (Pelezar *et al.*, 1999). The result of the biochemical analysis as presented in Table 2 showed only two morphological types of bacteria in the sample assayed, namely gram positive cocci and gram negative rod. Bacteria species were found in all the seven samples. Generally, four organisms were isolated from the samples namely; *Streptococcus sp* which was found in the fruit juice, while *Bacillus sp*, *Lactobacillus sp*, and *Staphylococcus* were isolated from the soymilk and the blend samples. Though, the presence of these microbes may not be harmful to consumers, however, some of them, like the *Lactobacillus* assist in the enzymatic breakdown of food and some of them synthesize useful vitamins. *Staphylococcus aureus* is even a normal *microbiota* of human and animals (Tortora *et al.*, 1995). Also, on prolonged storage, these microbes can bring about the microbial spoilage of the blended samples. Pelezar *et al.* (1999) had earlier documented that, *Bacillus aureus* is among the microorganisms responsible for the spoilage of tofu (a soymilk product). The result also agreed with the report of Ogbulie *et al.* (2001) who opined that non-pathogenic genera of

**Table 3: Observed characteristics for fungi identification**

| Samples | Colour on Culture plates      | Types of Hyphae | Columela   | Types of Asexual spore | Probable identity                              |
|---------|-------------------------------|-----------------|------------|------------------------|--|
| MFJ     | White, Brown, Greenish Yellow | As, S<br>S      | +<br>-     | SP<br>C                | Mucor<br><i>Aspergillus</i> Sp                 |
| SM      | Brown<br>Greenish Yellow      | S<br>S          | -<br>-     | C<br>C                 | <i>Penicillium</i> Sp<br><i>Aspergillus</i> Sp |
| SMFJ1   | White, Brown, Greenish Yellow | As, S<br>S      | +<br>-     | SP<br>C                | Mucor<br><i>Aspergillus</i> Sp                 |
| SMFJ2   | White, Brown, Greenish Yellow | As, S<br>S      | +<br>-     | SP<br>C                | Mucor<br><i>Aspergillus</i> Sp                 |
| SMFJ3   | As in SFJ2                    | As in SFJ2      | As in SFJ2 | As in SFJ2             | As in SFJ2                                     |
| SMFJ4   | As in SFJ2                    | As in SFJ2      | As in SFJ2 | As in SFJ2             | As in SFJ2                                     |
| SMFJ5   | As in SFJ2                    | As in SFJ2      | As in SFJ2 | As in SFJ2             | As in SFJ2                                     |

Key: AS = Aseptate, + = Positive, Sp = Sporangiospore, S = Septate, - = Negative, C = Conidia, MFJ = 0:100%, SM = 100:0%, SMFJ<sub>1</sub> = 90:10%, SMFJ<sub>2</sub> = 80:20%, SMFJ<sub>3</sub> = 70:30%, SMFJ<sub>4</sub> = 60:40% and SMFJ<sub>5</sub> = 50:50%.

microorganism such as *Streptococcus*, *Lactobacillus* and *Bacillus aureus* survived pasteurization treatment and eventually spoiled milk product. Fungi species were also isolated as recorded in Table 3. *Mucor*, *Aspergillus*, *Penicillin* and yeast cells were found in the samples.

### CONCLUSION

The result revealed the microbiological status of the samples; showing that the microbial counts of the blends (SMFJ) increased with increased soymilk replacement. The morphology of

the microbes was also ascertained thereby providing information for the preservation of the product. This suggests that the beverage, without preservative be consumed immediately after processing.

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