

## Fermentation Studies and Nutritional Analysis of Drinks Made from Water Extract of *Hibiscus sabdariffa* Calyx (Sobo), Juices of *Citrus sinensis* (Orange) and *Ananas comosus* (Pineapple)

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**Abstract:** Pure orange juice, pineapple juice and sobo (the water extract of the calyx *Hibiscus sabdariffa*) as well as sobo-orange and sobo-pineapple mixtures with or without added preservatives have been analyzed for titratable acidity, ascorbic acid and protein contents. The study shows that freshly prepared sobo is the most acidic, followed by orange juice, while pineapple juice is the least acidic. The ascorbic acid content (vitamin C) in percent decrease in the order orange juice>sobo>pineapple juice, while the protein content varies in the order sobo>pineapple juice>orange juice. The ascorbic acid content of the mixture increases as the sobo content decreases for both sobo-orange and sobo-pineapple mixtures. The nitrogen and protein contents of sobo-pineapple mixtures were higher than those of sobo-orange mixtures. The fermentation of the three drinks and their mixtures has also been studied by measuring the pH as a function of time. The pH of the sobo-orange and sobo-pineapple mixtures increase with time and increase in the orange juice or pineapple juice content of each mixture, although the change in pH with time of the sobo-orange mixtures were higher than those of the sobo-pineapple mixtures. The variation of pH with time is a measure of the stability or shelf-life of each mixture. Thus the combination of the results of titratable acidity, nutritive value and shelf-life showed that the combination of sobo with orange juice or pineapple juice is superior to the sobo, orange juice or pineapple juice alone.

**Key words:** *Hibiscus sabdariffa*, *Citrus sinensis*, *Ananas comosus*, antioxidant, nutritional analysis

### INTRODUCTION

Citrus juices are popular because of their desirable flavour and perceived health benefits. These health benefits are due to the phenolic compounds, the flavonoids and vitamin C present in the fruit. These compounds are natural anti-oxidants (Ames *et al.*, 1993; Cutler, 1984, 1992; Osawa *et al.*, 1990) and some of their functions include anticancer, antiviral, anti-inflammatory activities and inhibition of aggregation of human platelets (Benavente-Garcia *et al.*, 1997). Dietary intake of flavonoids has been shown to be inversely related to coronary heart disease mortality (Hertog *et al.*, 1993, 1995; Knekt *et al.*, 1996). The action of these phenolic compounds as antioxidants is by virtue of the free-radical scavenging properties of their constituent hydroxyl groups. The extended conjugation across the flavonoid structure and the increased number of the hydroxyl groups enhance the antioxidant properties by allowing them to act as reducing agents, hydrogen- or electron-donating agents or single oxygen scavengers (Kanner *et al.*, 1994; Salah *et al.*, 1995; Vinson and Hontz,

1995). Considerable efforts have been made towards isolation, identification and quantitative analysis of the compounds that are responsible for these characteristics and qualities of fruits (Attaway, 1977; Rouseff *et al.*, 1980; Fellers *et al.*, 1986; Marini and Balestrieri, 1995; Mouly *et al.*, 1994; Ooghe and Detavernier, 1997).

The colour, taste and aroma quality of citrus juices have a pronounced influence on consumer preferences and purchase decision. Anthocyanins, the red pigments and other phenolic compounds in fruits degrade and polymerize easily with the influences of temperature and time (Pilando *et al.*, 1985; Spanos and Wrolstad, 1992; Shahidi and Naczk, 1995). The effect of this degradation and polymerization leads to colour loss, deterioration and haze and sediment formation. Efforts have been made by several researchers to minimize this problem. One of the methods employed involves the application of chemical additives e.g., sulphites and its derivatives (Martinez and Whitaker, 1995) but the shortcoming of this method is that the chemical additives constitute health hazards to the consumers. Various

sulphite substitutes such as a combination of ascorbic acid or erythorbic acid with citric acid and cysteine have been employed but they also are oxidized irreversibly and therefore, do not meet the shelf life requirements (Sapers and Miller, 1993). Reports have also shown that enzymes like pectinases have been successfully employed to inhibit polymerization (Flores and Heatherbell, 1984; Maurer, 1973) and the unwanted enzymes and micro organisms have been eliminated by heating the juice to 80-85°C, followed by cooling to 50°C and depectinizing (Schobinger, 1986).

During the processing of fruit into fruit juices, not all the nutrients get into the fruit juice, a good percentage still remain in the pulp (Maria *et al.*, 1999). Man needs mixed flavonoids in his daily diet, as it has been shown that the average daily western diet contains approximately 1 g of mixed flavonoids (Maurer, 1976). No single fruit or fruit juice from a single botanical plant contains the right composition of all the nutrients/flavonoids needed by man. To get the required mixed flavonoids needed by man, fruit juices made from different fruits must be mixed together. Packaged fruit juices and wines have been made from mixed fruit juices (Belitz and Grosch, 1987).

Juices with low pH usually have longer shelf life though it is not good for people with stomach problem, so it needs to be blended with juices with high pH or juices that can moderate the pH. Fermentation of fruit juices reduces the acid content hence pasteurization and depectinizing and low temperature are employed to inactivate the microorganism responsible for fermentation (Flores and Heatherbell, 1984).

Sobo extract which is prepared by heating the calyx of *Hibiscus sabdariffa* in water, has a cooling and refreshing taste. It has a brilliant and attractive red colour with sour and agreeable acidic taste which aid digestion. It has diuretic and choloretic properties, its acts as intestinal antiseptic and it's a mild laxative. It is used to treat heart and nerve diseases, high blood pressure and calcified arteries (Asolkar *et al.*, 1992; Chopra *et al.*, 1956, 1969; CSIR, 1948-1992). The red colour is due the presence of anthocyanins (Pilando *et al.*, 1985). In our earlier studies (Odebunmi *et al.*, 2002, 2003) we found sobo extract to have high protein and acid contents, as also observed by Al-Kahtani and Hassan (1990) and Ibrahim *et al.* (1971). The high acid content of sobo extract makes it hazardous for consumption by people with stomach problems like ulcer, but orange juice and pineapple juice which are high in ascorbic acid but low in acidity can be blended with it (Odebunmi *et al.*, 2002,

2003; Ibrahim *et al.*, 1971). The mixing of fruit juices is desirable if one needs a fruit drink that contains the mixed flavonoids, which are high in nutrient quality.

This study involved the mixing of the water extract of the calyx of *Hibiscus sabdariffa* (sobo extract) with orange and pineapple juices and the determination of the protein and vitamin C contents of the mixture. The fermentation study of the sobo -orange and sobo-pineapple mixtures were also conducted. The choice of orange or pineapple juices was due to the abundance of these fruits during their seasons and the high rate of waste resulting from under usage of these fruits. The use of these fruits will be economically reasonable and sensible. Also the calyx of the *Hibiscus sabdariffa* which is used in making the sobo extract is easily available and cheap.

## MATERIALS AND METHODS

The calyx of *Hibiscus sabdariffa* along with extracts of orange and pineapple fruits were used in the course of this study. The calyx of *Hibiscus sabdariffa* was used in preparing sobo drink while the extracts of orange and pineapple fruits were used in preparing the fruit juices. The samples for ascorbic acid (vitamin c) analysis were first decolourized using commercial activated carbon supplied by May and Baker. Standard solutions of sodium thiosulphate and potassium trioxiodate in 0.05M H<sub>2</sub> SO<sub>4</sub> (acid medium) were used in the titration.

During fermentation studies, citric acid and sodium metabisulphite were used as preservatives, while 0.02M NaOH and phenolphthalein as indicator were used for the measurable titratable acidity.

**Preparation of the drink samples:** The sobo drink was prepared by continuous extraction of the calyx of *Hibiscus sabdariffa* through heating in water at about 100°C for 1 h. Thereafter, the used calyx was separated from the desired sobo extract by filtration (Odebunmi *et al.*, 2002, 2003). Orange fruit was washed thoroughly, peeled, cut into small pieces, squeezed and the extract obtained was sieved to separate the juice from the orange tissue and seeds. The pineapple juice was prepared by first washing the fruit, followed by peeling of the fruit, then the edible succulent tissue was sliced and blended. Sieving was then done to separate the juice from the coarse, fibrous tissue. Samples of the sobo extract, orange and pineapple juices were added to measured quantities of sugar, flavour essence and preservatives prior to analysis.

### Nutritional analysis of the drinks

**Nitrogen and crude protein determination (Macro Kjeldahl Method):** A sample of the drink was carefully weighed and transferred into a Kjeldahl flask. About 8 g of catalyst mixture made up of 96% anhydrous sodium sulphate, 3.5% copper sulphate, 0.5% selenium oxide and 20 mL of concentrated sulphuric acid were added. The content was heated until digestion was complete. The content was allowed to cool to room temperature and then washed with ammonia-free water into a 250 mL volumetric flask. The flask was filled to the mark with distilled water using a graduated pipette. Then, 5 mL of the digested sample was transferred into a micro Kjeldahl distillation apparatus and excess concentrated sodium hydroxide solution was added to make the solution strongly alkaline. This is to cause all ammoniacal nitrogen to be reduced to ammonium sulphate. This was then followed by the addition of 50 mL of boric acid and screened methyl red indicator to the distillate as it collects into a conical flask. This was subsequently titrated against 0.01M hydrochloric acid solution. The titration continued until about 50 mL of the distillate has been collected.

**Ascorbic acid content determination:** The determination of the vitamin C content of each sample was carried out using the technique of Iodometric titration (AOAC, 1980). This involved adding 20 mL of 0.5M  $H_2SO_4$  solution to 50 mL of each decolourized sample and followed by adding 2 g of potassium iodide. Thereafter, 25 mL of the standard potassium trioxiodate solution was pipetted into each sample solution, the pale-yellow colour of the solution was observed. The ascorbic acid in the sample was analysed by generating excess water-iodine solution. The iodine was obtained from a standard solution of potassium trioxiodate and potassium iodide. After the ascorbic acid has reacted the remainder was titrated with a standard thiosulphate solution.

The procedure for nitrogen, crude protein and ascorbic acid determination as described in above was carried out on the different sobo-orange and sobo-pineapple mixtures in order to determine the nitrogen content, crude protein content and ascorbic acid content in the different mixtures.

**Fermentation studies:** The fermentation of sobo extract, orange juice and pineapple juice was monitored at 25°C by measuring the pH of the solutions for 72 at 12 h intervals. The effects of addition of citric acid and sodium metabisulphite on the rate of fermentation of the three different drinks were also

determined by measuring the pH of each solution at 25°C for 10 days. The fermentation studies of the different sobo-orange and sobo-pineapple mixtures were equally carried out by measuring the pH of each mixture for a period of 7 days.

## RESULTS AND DISCUSSION

The data in Table 1 are the acidity of sobo extract, orange juice and pineapple juice as a function of time. The data show that freshly prepared sobo extract is the most acidic of the three samples with a concentration of 0.83M, followed by orange juice (0.31M), while pineapple juice is the least acidic (0.13M). The presence of hibiscus, malic, oxalic, citric and 3-indolyl acetic acids are responsible for this high acid concentration sobo extract (Ibrahim *et al.*, 1971). We observed further that the acid concentration of the three samples remained constant for the first 12 h, after which it started to drop. The sobo extract recorded the highest drop of 0.83 to 0.26 after 72 h and this was followed by orange juice. This suggests that the rate of fermentation is highest in sobo extract. The pineapple juice has the least acid content and it also recorded the least drop from 0.18 to 0.07M after 72 h. This might suggest high concentration of anti oxidants in the pineapple juice than the other two samples (Kanner *et al.*, 1994; Salah *et al.*, 1995; Vinson and Hontz, 1995). Even though sobo extract recorded the highest acid drop of the 3 samples, the terminal acid concentration of 0.26M is still high particularly for people with stomach problems like ulcer. This high acid content would impact a desirable preservative effect on the extract which will make it to have a longer shelf life than the other two samples (Shihata *et al.*, 1983).

Table 2 compares the pH of sobo extract, orange and pineapple juices with citric acid and sodium metabisulphite added as preservatives and without preservatives. The data show that sobo extract with or without preservatives show the lowest pH values for the 10 days the fermentation study lasted. This implies that sobo extract is preserved for the ten days (Al-Kahtani and Hassan, 1990). Pineapple juice shows the highest pH

Table 1: Acidity of sobo extract, orange juice and pineapple juice

Time (hours)	Sobo extract (M)	Orange juice (M)	Pineapple juice (M)
0	0.83	0.31	0.13
12	0.83	0.31	0.13
24	0.63	0.25	0.10
36	0.60	0.21	0.09
48	0.43	0.14	0.08
60	0.31	0.14	0.08
72	0.26	0.13	0.07

Table 2: pH of sobo extract, orange juice and pineapple juice with and without preservatives at 25°C

Time (Days)	Sobo extract			Orange juice			Pineapple juice		
	I	II	III	I	II	III	I	II	III
1	3.20	3.02	3.40	3.80	3.70	4.15	3.85	3.75	4.20
2	3.25	3.10	4.10	3.85	3.95	4.70	3.90	4.00	4.75
3	3.25	3.25	4.05	3.90	4.00	4.40	4.00	4.05	4.50
4	3.30	3.20	3.70	4.05	3.80	4.20	4.05	4.05	4.35
5	3.35	3.25	3.80	4.20	3.95	4.15	4.30	4.10	4.40
6	3.30	3.30	3.00	4.10	3.70	4.00	3.90	4.15	4.20
7	3.35	3.35	3.10	4.20	3.80	4.00	4.00	4.20	4.00
8	3.37	3.70	3.05	4.25	3.97	3.90	4.20	4.30	4.20
9	3.45	3.80	3.20	4.30	4.10	4.80	4.25	4.35	4.30
10	3.50	3.75	3.25	4.40	4.20	4.90	4.70	4.45	4.50

I = Sample without preservatives, II = Sample with Citric acid, III = Sample with sodium metabisulphite

Table 3: Fermentation studies of different mixtures of sobo-orange and sobo-pineapple mixtures

Days	pH of Mixture									
	Sobo-orange					Sobo-pineapple				
	80:20	60:40	50:50	40:60	20:80	80:20	60:40	50:50	40:60	20:80
1	2.50	2.70	3.00	3.10	3.50	2.70	2.90	3.00	3.30	3.60
2	2.60	2.82	3.08	3.27	3.73	2.77	3.00	3.05	3.39	3.70
3	2.73	2.99	3.33	3.45	3.99	2.95	3.09	3.20	3.76	3.85
4	2.75	3.16	3.46	3.68	4.00	3.00	3.14	3.32	3.82	3.91
5	3.00	3.37	3.49	3.73	4.10	3.09	3.21	3.38	3.88	4.02
6	3.08	3.41	3.62	3.93	4.28	3.13	3.29	3.41	3.94	4.05
7	3.11	3.48	3.73	4.00	4.32	3.20	3.31	3.50	3.99	4.11

values of the 3 samples. With citric acid as preservative, minimal rise of pH for all the samples was observed and the change in pH as the drinks ages is also low. For sodium metabisulphite the effect as a preservative broke down after the second day with a consequent decrease in pH value then an increase in short the change in pH values is does not follow a particular pattern for all the 3 samples. One of them tends to conclude that citric acid is a better preservative than sodium metabisulphite for all the three samples. Generally, the 3 samples with or without preservatives show little difference in pH after 10 days. This is suggestive of inactivation of the enzymes and the presence of anti-oxidants that prevent deterioration of the samples (Fellers *et al.*, 1986; Marini and Balestereri, 1995; Mouly *et al.*, 1994).

The results of fermentation study of the different sobo-orange and sobo-pineapple mixtures are presented in Table 3. The pH of each mixture increases as the content of orange and pineapple increases and also the pH increases as each mixture ages which indicate a decrease in acidity. A comparison of Table 2 and 3 shows that the sobo-orange and sobo-pineapple mixtures in Table 3 have a gradual increase in pH throughout the 7 days which the study lasted but in Table 2, where we have 100% sobo, 100% pineapple and 100% orange juices, the pH never followed the pattern shown in Table 3. The pH was initially increasing but after the 5th day we saw a decrease in value and started increase from seventh day

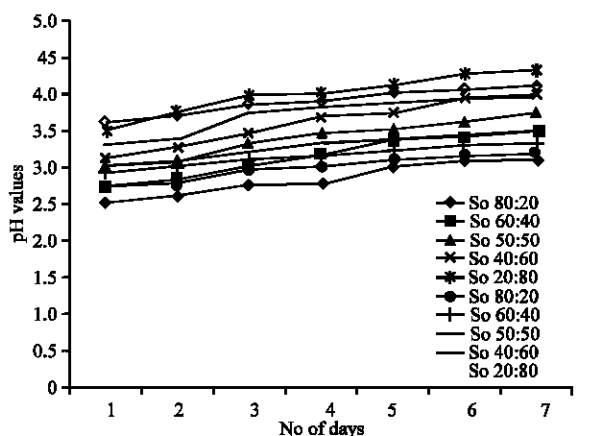


Fig. 1: pH of fruit mixtures for 7 days

again. This tends to show that the mixing of the drinks in whatever proportion gave the mixture a better preservation and longer shelf life.

Figure 1 is the graph of change in pH with time. It shows that for all sobo-orange and sobo-pineapple mixtures the change in pH increases with time. It shows further that the change in pH as a function of time is smaller for all the sobo-pineapple mixtures compared with sobo-orange mixtures. This implies that sobo-pineapple mixtures are more stable and should handle longer shelf life without addition of any preservatives. For both the sobo-orange and sobo-pineapple mixtures the pH values for the 40:60 mixtures are higher than those

Table 4: Nutritive value of fortified water extract of *Hibiscus sabdariffa* (Sobo extract)

Sobo: Orange	Ascorbic acid content (%)	Nitrogen content (%)	Protein content (%)
100 : 0	6.47	0.35	2.18
80 : 20	6.48	0.28	1.75
60 : 40	6.49	0.21	1.31
50 : 50	6.50	0.21	1.31
40 : 60	6.51	0.21	1.31
20 : 80	6.52	0.21	1.31
0 : 100	6.51	0.14	0.87
Sobo: Pineapple	Ascorbic acid content (%)	Nitrogen content (%)	Protein content (%)
100 : 0	6.47	0.35	2.18
80 : 20	6.47	0.35	2.18
60 : 40	6.48	0.35	2.18
50 : 50	6.49	0.26	2.15
40 : 60	6.50	0.21	1.31
20 : 80	6.50	0.21	1.31
0 : 100	6.46	0.17	1.06

of other mixtures (Table 3 and Fig. 1). For the sobo-pineapple mixtures, the 40:60 mixture shows a high value of change in pH from day one to day three after which the change becomes more gradual corresponding to a decrease in gradient. By comparison the 40:60 sobo-orange samples show a gradual change in pH from day one to the seventh day that this study lasted. This implies that this mixture has undergone the highest deterioration of all the mixtures. Out of all the sobo-orange mixtures, 80:20 has the least change in pH and this change from day to day is small hence one can assume that the mixture has not shown any breakdown or deterioration. The 50:50 sobo-orange mixtures were also stable for the first four days before rapid deterioration sets in.

The change in pH for 100% sobo, 100% orange and 100% pineapple samples was also shown in Fig. 1. One hundred percent sobo has the minimum change out of the three samples just about 0.1 from day one to day seven. This confirms the high acid content of sobo and the facts that it has not broken down for this 7 days.

It is evident from this study that the blending of this samples better because it high acidity of sobo extract which might be problem to people with ulcea is brought down by orange or pineapple juice without breakdown of the drink mixtures within the days the study lasted. The nutrient of the mixed samples is increase by mixing sobo which is high in protein content with orange juice which is also rich ascorbic acid. For anybody trying to prepare this mixture for home consumption or commercial, one will recommend the sobo-orange mixtures 80:20, 60:40 and 50:50 and sobo-pineapple mixtures 80:20, 60:40, 50:50 and 40:60 for adequate protein and ascorbic acid content. The acid values of these mixtures ratio are also not so high as to cause any health hazard.

The nutritive values of sobo-orange and sobo-pineapple mixtures are shown in Table 4. In the sobo-orange mixture, the ascorbic acid content increases as the sobo extract in the mixture decreases. This is not the case in the sobo-pineapple mixtures, where the initial small

increase was followed by a decrease. The ascorbic acid content was found to be 6.46, 6.47% and 6.51% for 100% pineapple juice, sobo extract and orange juice, respectively. It is therefore, evident that orange juice is higher in ascorbic acid than pineapple juice and sobo extract. The nitrogen and protein contents of sobo-pineapple mixtures were higher than those of sobo-orange mixtures even though the values declined after the 60:40 ratios for the two mixtures. The 100% sobo extract has the highest protein content of 2.18%, followed by pineapple juice 1.06% while orange juice is 0.87%. From this study, the sobo-orange mixtures 80:20, 60:40 and 50:50 and sobo-pineapple mixtures 80:20, 60:40, 50:50 and 40:60 are recommended for consumption for adequate protein and ascorbic acid content. The acid values of these mixtures ratio are also not so high as to cause any health hazard.

## CONCLUSION

In this research showed that; acidity reduction with mixture of pineapple and orange which is good for people who may have stomach problem increase in shelf life and mixing does not affect nutrient quality of the drinks.

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