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EXTRACTION OF CAFFEINE FROM TEA AND DEVELOPMENT OF CAFFEINATED FRUIT JUICE

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A B S T R A C T

Developing a new product was the objectives of this work. For this purpose isolated caffeine from tea was added to fruit juices to make a drink that would fulfill the need of nutrition as well as stimulation. Three fruits (Pineapple, Orange, Malta) were chosen for the experiment and caffeine was extracted from tea by liquid – liquid extraction method. Extracted caffeine from made tea was purified before addition to the beverage. Preservatives, added sugar or any other additives were not used in the juice. Fresh & Caffeinated juices were stored at three different temperatures 25°C, 4°C and -15°C to observe their shelf life as well as physicochemical and microbial changes during six days of storage. Fresh and caffeinated juices stored at room temperature (25°C) became unacceptable after 1 day of storage, in case of 4°C it was 5 days. The juices stored at deep freezing temperature (-15°C) remained acceptable up to 21 days. During storage pH of fresh and caffeinated juices were increased while acidity and vitamin C content decreased gradually. Microbial analysis showed presence of fungus in fresh and caffeinated juices and microbial loads were counted by SPC (Standard Plate Count) method.

Keywords: Caffeine, fruit juice, physicochemical, microbial, sensory analysis.

INTRODUCTION

The active ingredient that makes tea and coffee valuable to humans is caffeine. Global consumption of caffeine has been estimated at 120,000 tons per year, making it the world's most popular psychoactive substance (Barone and Roberts, 1996). The principal dietary sources of caffeine are overwhelmingly coffee and tea. Coffee accounts for some 54 per cent of ingested caffeine, while tea accounts for some 43 per cent. The remaining 3% consists mostly of caffeine ingested in the form of cocoa and chocolate products, various fabricated soft drinks (Golding, 1995). Taken in moderation, caffeine is harmless and may even be beneficial. Daily intake of 250-300 mg of caffeine a day is a *moderate* amount. In humans, the lethal dose of caffeine is between 150 and 200 mg per kilogram (Frary *et al.,* 2005). There have been more than 19,000 studies on caffeine and coffee in the past 30 years in an attempt to determine its exact effects on the human body. One of the most thorough and exhaustive studies was done by Harvard University, in which they examined 126,000 people over an 18-year period. The findings indicate that people who drink one to three cups of coffee per day are up to 9 % less likely to contract diabetes (Rogers and Dernoncourt, 1998). Other studies have shown similar results in many facets of human health. Caffeine has also shown to be beneficial in asthma, stopping headaches, boosting mood, lower risk of colon cancer and even preventing cavities (Lieberm, 1991). Fruit juices are nutritious and rich in vitamins and minerals. Caffeinated fruit juices could provide stimulation to the central nervous system, allowing the consumer to feel more alert while providing a variety of vitamins, minerals and nutrients not obtained through consumption of other carbonated beverages, soda, coffee and tea. It could be a healthful and enjoyable alternative to the use of over-the-counter caffeine pills, which some consumers prefer over other caffeinated beverages that include ingredients that are not healthful. It could also permit consumers who may be too ill to tolerate harsh liquids like coffee, tea, soft drinks or energy drinks to enjoy the benefits of caffeine

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without additional digestiveinterruption, while providing those consumers nutrients that are lost and needed during illness, as well as hydration. It could be attractive to athletically inclined individuals and health-conscious individuals that wish to avoid unnecessarily potent carbohydrates and refined sugars that are common in other caffeinated beverages such as soft drinks. The juice could also be attractive to parents, who would certainly be expected to prefer their older children drinking caffeinated fruit juices as an alternative to soft drinks, coffee or tea (Flook and Williams, 2001).

MATERIALS AND METHODS

Raw material: Fruits (ripened pineapple, Orange, Malta) & tea bags were collected from local market Sylhet, Bangladesh.

Extraction of caffeine from tea: Caffeine was extracted manually from tea (10g) by liquid – liquid extraction method. The procedure used to make tea was simply "steeping" the tea with very hot water (200 ml) for a few minutes which extracted most of the caffeine. Since caffeine is white, slightly bitter, odorless, crystalline solid, it is obvious that water extracts more than just caffeine. Tea leaves contain tannins, which are acidic, as well as a number of colored compounds and a small amount of intact chlorophyll (soluble in dichloromethane). To ensure that the acidic substances remain water soluble and that the caffeine will be present as the free base, sodium carbonate (5g) was added to the extraction medium. When the brown aqueous solution was subsequently extracted with dichloromethane (60ml), primarily caffeine dissolved in the organic solvent. Evaporation of the solvent made off crude caffeine, which on sublimation yields a relatively pure product.

Purification of caffeine by Sublimation: At first the apparatus was set up - a small filter flask was connected to a rubber hose, which was in turn connected to a vacuum. A glass cold finger was fitted into the top opening of the flask with a rubber adapter and positioned according to the amount of sample to be sublimated. The dry caffeine powder was placed at the bottom of the flask and the test tube was filled with crushed ice. The bottom of the test tube was rest about 1 cm from the bottom of the filter flask. The test tube and flask was clean and dry. The entire apparatus was positioned directly on top of a hot plate. Caffeine melts at 238°C and sublimes at 178°C.

Preparation of caffeinated fruit juice: Juice extractor was used to extract fruit juice. Pulps were removed at the time of juice preparation. Extracted juices were pasteurized at 75˚C for 30 minutes. No additives were used for juice preparation. Purified Caffeine was added to the pasteurized juice (152 mg per liter).

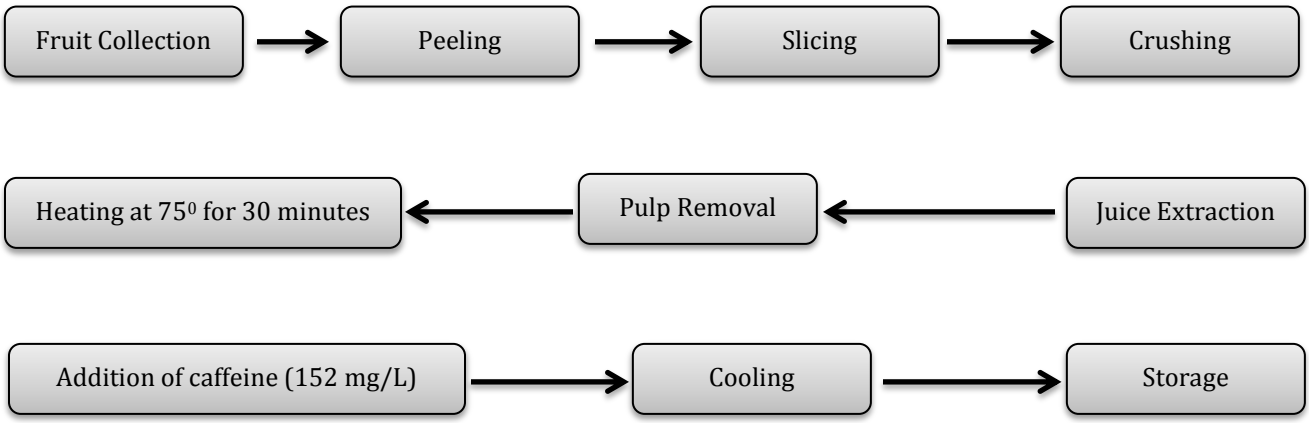

Figure 1: Flow chart of preparation of caffeinated fruit juice.

Physicochemical analysis of fresh and caffeinated fruit juices: The pH of juice was determined with a pH meter taking 50 ml of juice in a beaker. Refractometer was used to determine soluble solids content (% sugar or °Brix). The acid content was measured using a titration with sodium hydroxide. Amount of vitamin C was determined by using iodometric method.

Microbiological analysis: Total yeast and molds count was obtained by the spread plate technique after a three days incubation period at 37 ^{o} $_{C}$ on Sabouraud Dextrose Agar (SDA). Data was expressed in CFU. The standard plate count was done by the pour-plate spread plate

technique after three days incubated at 37°C on Plate Count Agar (PCA) plate.

Sensory evaluation of caffeinated fruit juices: The Sensory evaluation of the caffeinated fruit juices were carried out for consumer acceptance and preference using 10 panelists. Sensory properties were evaluated based on color, flavor, texture and overall acceptability using a nine point Hedonic scale where 1 represents extremely dislike" and 9 "extremely like" respectively.

RESULTS AND DISCUSSION

Status of caffeine content in caffeinated fruit juice during storage: This analysis was done to find out any changes in the amount of caffeine in caffeinated fruit juice during storage. For this purpose, caffeine was extracted from caffeinated pineapple juice. Fifty ml juice was extracted three times with 20 ml portion of Dichloromethane (CH_2Cl_2) which gave 41 ml of Dichloromethane (CH_2Cl_2) extract. The extract was taken into an empty beaker (75.678 g), after few hours Dichloromethane (CH_2Cl_2) evaporate and left crude caffeine in the beaker. Weight of the beaker with crude caffeine was taken and found 75.683g. Amount of crude caffeine (0.005g) was calculated by subtracting weight of the empty beaker (75.678g) from weight of the beaker with crude caffeine (75.683g).This analysis was done at the date of caffeinated pineapple juice preparation (15-11-2011). After 6 days storage caffeine was extracted from pineapple juice using the same procedure and

Table 1: Physicochemical analysis of fresh and caffeinated fruit juices.

Sample	Days	Parameter											
		PH			TSS (0 Brix)			Acidity (g/100ml)			Vitamin C (mg/L)		
		25°C	4°C	-15°C	25°C	4°C	-15°C	25°C	4°C	-15°C	25°C	4°C	-15°C
Fresh Pineapple Juice	0	4.2	4.2	4.2	10.2	10.2	10.2	0.74	0.748	0.748	334	334	334
	2	-	4.7	4.7	-	10.2	10.2	-	0.730	0.731	-	295	295
	4	-	4.8	4.8	-	12.1	12.2	-	0.68	0.68	-	283	283
	6	-	-	4.8	-	-	14.2	-	-	0.676	-	-	272
Caffeinated Pineapple Juice	0	4.1	4.1	4.1	10.2	10.2	10.2	0.74	0.74	0.74	334	334	334
	2	-	4.7	4.7	-	14	14.2	-	0.72	0.72	-	295	295
	4	-	4.8	4.8	-	14	14	-	0.66	0.67	-	283	284
	6	-	-	4.8	-	-	15	-	-	0.66	-	-	272
Fresh Orange Juice	0	4.2	4.2	4.2	8.0	8.0	8.0	1.28	1.286	1.286	380	380	380
	2	-	4.5	4.6	-	8.4	8.4	-	1.179	1.178	-	265	265
	4	-	4.7	4.7	-	8.4	8.4	-	1.170	1.170	-	341	343
	6	-	-	4.8	-	-	8.8	-	-	0.723	-	-	323
Caffeinated Orange Juice	0	4.2	4.2	4.2	8.2	8.2	8.2	1.27	1.275	1.275	380	380	380
	2	-	4.6	4.6	-	8.4	8.4	-	1.171	1.170	-	365	365
	4	-	4.7	4.7	-	8.4	8.4	-	1.13	1.13	-	341	344
	6	-	-	4.8	-	-	8.9	-	-	0.857	-	-	323
Fresh Malta Juice	0	4.5	4.5	4.5	8.8	8.8	8.8	1.06	1.06	1.06	469	469	469
	2	-	4.7	4.7	-	8.8	8.8	-	1.03	1.04	-	456	456
	4	-	5.0	5.1	-	8.8	8.8	-	1.02	1.02	-	442	441
	6	-	-	5.2	-	-	8.9	-	-	0.081	-	-	423
Caffeinated Malta Juice	0	4.5	4.5	4.5	8.4	8.4	8.4	1.06	1.06	1.06	469	469	469
	2	-	4.7	4.6	-	8.2	8.2	-	1.05	1.05	-	456	456
	4	-	5.0	5.1	-	8.2	8.2	-	1.02	1.02	-	442	442
	6	-	-	5.2	-	-	8.8	-	-	0.940	-	-	425

and same amount of juice and Dichloromethane (CH_2Cl_2). Calculation gave the same juice using the same procedure and same amount of juice and Dichloromethane (CH_2Cl_2). Calculation gave the same amount of crude caffeine as found earlier (0.005g). It means that the status of caffeine content in caffeinated fruit juice does not change during storage. Same analysis was also done for caffeinated Malta and caffeinated orange juice and the findings were same as caffeinated pineapple juice.

Fresh and caffeinated juices stored at room temperature (25°C) became unacceptable after 1 day of storage and juice stored at cooling temperature (4°C) became unacceptable according to sensory evaluation at the 6th day of storage. During storage pH and TSS of fresh juices increased. This may be due to higher ambient temperature coupled with lower negative humidity which resulted in rapid water loss and caused an increase in TSS. This observation is agreed with those reported by Campos (2001) and Alighourchi and Barzegar (2009). Acidity of fresh juices gradually decreased during storage. Reduction in acidity may be due to the results of a decrease in citric acid. Both citric & malic acid contents declined during cold storage. Above findings are supported by Mukherjee & Singh (1983), Landaniya and Sankar (1996) and Echeverria and Ismail (1987).

Vitamin C content of fresh juices decreased gradually during storage. It may be due to the entry of air and oxygen during handling, packaging, water used for reconstitution. Oxygen can react with other substances and lead to changes in the chemical composition of the juice, resulting in a degradation of vitamin C. These findings are similar to those observed by Kaanane *et al.* (1988), Supraditareporn and Pinthong (2007), Majumder *et al.* (2009), Tiwari (2000) and Cordenunsi *et al.* (2005).

During storage pH and TSS of caffeinated pineapple juice increased and acidity, vitamin C decreased like fresh fruit juices. It seems that caffeine did not bring any significant change in the chemical composition of the juice.

Microbiological Analysis: The results of microbiological analysis of 12 samples of Fresh & Caffeinated fruit juices are shown in table-2. The results showed in the table 2 illustrated lower microbial count at 4°C and higher microbial count at room temperature (25°C). Pasteurization (Heating at 75°C for 30 minute) was done at the time of juice preparation and oxygen barrier packaging/ bottling was not provided. The presence of fungus and other microbes may be due to the absence of oxygen barrier packaging. Supraditareporn and Pinthong (2007), reported that, fungi may develop in a wide array of conditions, but usually require plenty of oxygen and acidic medium, lightly and fully pasteurized juices in oxygen barrier cartons exhibited lower microbial counts, greater ascorbic acid retention.

Table 2: Microbiological Analysis of fresh & caffeinated fruit juice.

Sample Name	SPC (CFU / ml)
Fresh Pineapple Juice (25°C)	1.65×10^{10}
Fresh Pineapple Juice (4°C)	1.15×10^{9}
Fresh Orange Juice (25°C)	1.48×10^{11}
Fresh Orange Juice (4°C)	1.08×10^{7}
Fresh Malta Juice (25°C)	1.50×10^{9}
Fresh Malta Juice(4°C)	1.25×10^{8}
Caffeinated Pineapple Juice (25°C)	1.62×10^{9}
Caffeinated Pineapple Juice (4°C)	1.13×10^{8}
Caffeinated Orange Juice (25°C)	1.40×10^{10}
Caffeinated Orange Juice (4°C)	1.07×10^{7}
Caffeinated Malta Juice (25°C)	1.40×10^{10}
Caffeinated Malta Juice(4°C)	1.20×10^{8}

Sensory analysis: The mean sensory scores of are Caffeinated fruit juices presented in figure two. The panel scores of caffeinated malta juice were highest (7.9, 7.5 and 7.9) with respect to flavor, texture and overall acceptability respectively.

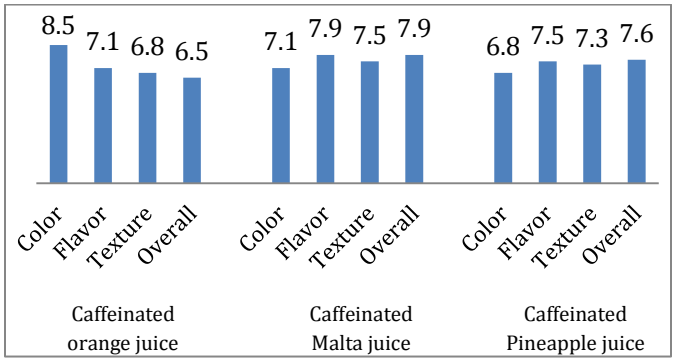

Figure 2: Frequency score of sensory characteristics in caffeinated fruit juices.

The panel scores of caffeinated orange juice were lowest (7.1, 6.8 and 6.5) in case of flavor, texture and overall acceptability respectively but on the basis of color the

panel score of caffeinated orange juice was highest (8.5) in comparison to caffeinated malta and caffeinated pineapple juice.

CONCLUSION

Based on the above results, it could be concluded that caffeine did not bring any significant change in chemical constituent of fruit juices during storage. It did not also bring any significant change in color, flavor, taste and chemical composition of fruit juice.

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