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NUTRITIONAL AND MICROBIAL ANALYSIS OF DIFFERENT BRANDS OF APPLE JUICES SOLD IN GILGIT CITY

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ABSTRACT

In the present study microbiological and physico-chemical characteristics of four samples of commercially available apple juices were examined. These samples were Haleeb Just Fruit (Apple Nectar), Nestle Fruita Vitals (Apple Nectar), Olfruite (Apple Power Up) and Tops Pulpy (Apple Drink). All juices were found To have a divers degree of chemical compositions. Acidity was more than the standard acidity. Among the products, TOPS pulpy Juice was found deficient in certain chemical compositions. Besides, all the juices were found free of microbiological contamination as indicated by total viable count (TVC). Similarly, all samples were Found to be free of contamination by coliform bacteria (both total coliforms and fecal coliforms). Yeast and mold were also absent in all the samples. These results show that apple juice brands analyzed in the present report are free of contamination and microbiologically fit for human consumption.

KEY WORDS: Apple drinks, nutritional and microbial conditions.

INTRODUCTION

Malus domestica_belongs to family rosaceae are popularly known as apple tree. The apple is pomaceous of the tree species (Potter, 2007). It is the most widely Similarly, all other samples were tested accordingly. and most broadly known of the various members of Genus malus that are used for humans (Alpha, 2010). In Pakistan, apples have been grown as commercial fruit plants in Balochistan and Khyber-Pakhtunkhwa provinces since long. Because of its attractive income, apple plantation was taken up by the growers in swat, Dir, Mansehra, Parachinar, Chitral, Hunza, North and South Waziristan Agencies. The climate

(cold and high altitude) of Gilgit Baltistan of Pakistan is very favorable for apple tree thus high qualities of apple are grown in this region. As far as health is concerned, apples possess phenolic compounds which may be cancer-protective and demonstrate antioxidant activity. The predominant phenolic phytochemicals in apples are quercetin, epicatechin, and procyanidin B2 (Lee *et al.* 2003). Apples are a widely consumed, rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes. In the laboratory, apples have been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. The phytochemical composition of apples varies greatly between different varieties of apples, and there are also small changes in phytochemicals during the maturation and ripening of the fruit. Storage has little to no effect on apple phytochemicals, but processing can greatly affect apple phytochemicals. The purpose of this study was to determine the nutritional status of apple juices available in different brands and to review the most recent literature regarding the health benefits of apples and their phytochemicals. Since the phytochemical, phytochemical bioavailability and antioxidant behavior and the effects of variety, ripening, storage and processing have been thoroughly discussed (Jeanelle and Rui, 2004).

Apple juice has a significant concentration of natural phenols of low molecular weight (including chlorogenic acid, flavan-3-ols, and flavonoids) and procyanidins. That may protect from diseases associated with aging due to the antioxidant effects which help reduce the likelihood of developing cancer and Alzheimer's diseases (Lawson, 2006). Research suggests that apple juice increases acetylcholine in the brain, possibly resulting in improved memory. Despite having some health benefits, apple juice is high in sugar. It has 28 g carbohydrates (24 g sugars) per 8 ounces. This results in 130 calories per 8 ounces (protein and fat are not significant), which is comparable to non-diet soft drinks such as Pepsi and 7 Up. Also like most fruit juice, apple juice contains a similar amount of sugar as the raw fruit, but lacks the fiber content (Henriette *at el*., 2008). Apple juice is also rich in iron, but you shouldn’t take too much of it as the antioxidants in it will bind the iron and it won’t be absorbed into your blood stream. This of course will lead to iron deficiencies and eventually diseases like anemia .The juice are also an antioxidant, and people who smoke or suffer breathing problems should take it to help detoxify and therefore clean the lungs for easier breathing. Although apple juice contains a lot of the nutrients that are needed for daily function, it has no calories that can make you gain weight, even when you add artificial sweeteners to it (Cappuccino and

Sherman, 2002). Furthermore, Apple juice contains fructose and sorbitol, substances that have been shown to be incompletely absorbed by most people. As this might have clinical consequences, especially in young children, we investigated the absorption of the carbohydrate content of apple juice in apple juice consuming toddlers with chronic nonspecific diarrhea as compared to controls, using the breath hydrogen (H2) test. Incomplete absorption of the carbohydrates from 250 ml of apple juice, as indicated by a maximum breath H2 increase of greater than or equal to 20 parts per million (ppm), was found in all nine patients (mean SEM 57 8 ppm), and in five out of eight controls (22 7 ppm) (P less than 0.01). Six patients were retested with apple juice "enriched" with glucose, which is known to improve fructose absorption. The maximum breath H2 increase as well as the area under the breath H2 curve decreased significantly. It was thus estimated that fructose accounted for 80% of the incomplete absorption and sorbitol for 20%. Elimination of apple juice from the diets of the nine patients resulted in normalization of both the frequency and the consistency of the stools. Incomplete absorption of the carbohydrates, particularly fructose, from apple juice seems to be quite common, and may contribute to chronic diarrhea in young children (Knee kens *et al*., 1989).

Keeping in view the aforementioned extraordinary benefits of apple and its byproducts, this study was conducted with the following aims and objectives.

1. To check the physicochemical and nutritional values of apple juice sold in Gilgit Baltistan Market.
2. To inspect the microbial analysis of different brands of apple juice.
3. To build awareness among the people about the properties and health benefits of Apple juice.

MATERIALS AND METHODS

1. Determination of pH

For determination of pH (hydrogen ion concentration) in the products, a method of AOAC (2000) was adopted and digital pH meter was used. Sample solution was taken in the beaker and directly inserted the electrode into the solution. When the first reading was completed, the electrode was wiped with distilled water and dried-up with tissue paper. Similarly, as a continue series, all other samples were determined accordingly.

2. Determination of Moisture Content

The moisture content was determined according to AOAC (2000). In this regard, the sample materials were taken in a flat-bottom dish (pre-weighed) and kept overnight in an oven at 100–110 ^{0}C and weighed. The loss in weight was regarded as a measure of moisture content which was calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

3. Determination of Total Solids

Total solids were determined by deducting percent moisture from hundred as described by James (1995)

Total solids (%) = 100 - moisture content (%)

4. Determination of Ash Content

For determination of ash content, method of AOAC (2000) was followed. According to the method, 10 g of each sample was weighed in a silica crucible. The crucible was heated in a muffle furnace for about 3–5 hours at 500 ^{0}C. It was cooled in desiccators and weighed. To ensure completion of ashing, it was reheated again in the furnace for half an hour more, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or grayish white). Weight of ash gave the ash content and was calculated by the following formula.

$$\text{Ash (\%)} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

5. Determination of Titratable Acidity

Titratable acidity as tartaric acid was determined according to the method of AOAC (2000). Each sample of the products was treated with 0.1N NaOH solution using titration kit. Whereas, 3-5 drops of phenolphthalein indicator were used. The volume of alkali used was noted and calculated using following formula.

$$\text{Titratable acidity (\%)} = \frac{1 \times \text{Eq. Wt. of acid} \times \text{Normality of NaOH} \times \text{titer}}{10 \times \text{Weight of sample (g)}} \times 100$$

6. Microbiological Analysis

Total Viable Count (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mold in a sample. To be specific, the count actually represents the number of colony forming units (CFU) per g (or per ml) of the sample. This is an empirical

measurement because organisms occur singly, in pairs, clusters, or packets, and no single growth medium or set of physical and chemical conditions can satisfy the physiological requirements of all organisms in a sample. A TVS is achieved by plating dilutions of the culture until 30-300 colonies appear on a single plate after incubation.

Apple juice samples of various brands [Haleeb Just Fruit (Apple Nectar), Nestle Fruita Vitals (Apple Nectar), Olfruite Apple Power Up, Tops Pulpy Apple Drink] were collected from local markets of Gilgit city. Diluted the apple juice sample 10 times (1/10 x) and 100 times (1/100 x) using 1ml pipette, transferred 1 ml aliquot of diluted sample to sterilized Petri plate. Pour 25 ml sterilized medium into Petri plate. Mixed thoroughly so that sample is completely blended with medium and medium was left to solidify in plates. Incubated plates were left in inverted position for 24 hours at 37°C.

For detection of coliform bacteria, measured aliquots of the sample to be tested are added to a lactose fermentation broth containing an inverted gas vial. Because these bacteria are capable of using lactose as a carbon source (the other enteric organisms are not), their detection is facilitated by use of this medium. In this experiment the lactose fermentation broth also contains a surface tension depressant, bile salt, which is used to suppress the growth of organisms other than coliform bacteria. In the present report, MacConkey broth is used instead of lactose broth for improved results. Tubes of lactose medium are inoculated with 10 ml, 1 ml, and 0.1 ml aliquots of the fruit juices sample. The series consists of at least three groups, each composed of five tubes of the MacConkey broth. The tubes in each group are then inoculated with the designated volume of the juices sample as described under procedure. The greater the number of tubes per group, the greater the sensitivity of the tubes is presumptive evidence of the presence of coliform bacteria in the juice sample. The presumptive test also enables to obtain some idea of the number of coliform organisms present by means of the most probable number test (MPN)**.** The MPN is estimated by determining the number of tubes in each group. For fecal coliforms, all the materials and methods are same as for total coliforms except the temperature of incubation, i.e. 45°C. Observation and results are also same as for total coliform.

RESULT AND DISCUSSIONS

The purpose of this research was to evaluate the physicochemical and microbial analysis of different brands of apple juices sold in Gilgit market. The results after analysis are as under

Table-1: Determination of pH of various brands of apple juices.

S.No.	Brand name	R1	R2	R3	Mean
1	Haleeb Just Fruit (apple nectar)	3.56	3.20	3.41	3.39*
2	Nastel Fruita Vitals (apple nectar)	3.26	3.61	3.20	3.35*
3	Olfruite Apple Power UP	3.28	3.21	3.35	3.28*
4	TOPS Pulpy (apple drink)	3.20	3.50	3.41	3.37*

**Values represent mean of three replications.*
**Means followed by same letter in a column are not significantly different at P> 0.05.*

Table-2:Determination of Percent moisture in various brands of apple juices

S.No.	Brand name	R1	R2	R3	Mean
1	Haleeb Just Fruit (apple nectar)	89.082	89.063	89.074	89.073*
2	Nastel Fruita Vitals (apple nectar)	89.742	89.732	89.721	89.731*
3	Olfruite Apple Power UP	88.808	88.605	88.703	88.705*
4	TOPS Pulpy (apple drink)	97.216	97.214	97.215	97.215*

**Values represent mean of three replications.*
**Means followed by same letter in a column are not significantly different at P> 0.05.*

Table-3: Determination of percent solids in various brands of apple juices

S.No.	Brand name	R1	R2	R3	Mean
1	Haleeb Just Fruit (apple nectar)	10.17	10.15	10.16	10.16*
2	Nastel Fruita Vitals (apple nectar)	9.377	9.354	9.364	9.365*
3	Olfruite Apple Power UP	11.191	11.171	11.180	11.180*
4	TOPS Pulpy (apple drink)	8.351	8.362	8.362	8.361*

**Values represent mean of three replications.*
**Means followed by same letter in a column are not significantly different at P>0.05.*

Table-4: Determination of percent ash content in various brands of apple juices

S.No.	Brand name	R1	R2	R3	Mean
1	Haleeb Just Fruit (apple nectar)	0.219	0.217	0.218	0.654*
2	Nastel Fruita Vitals (apple nectar)	0.027	0.025	0.026	0.026*
3	Olfruite Apple Power UP	0.001	0.001	0.002	0.001*
4	TOPS Pulpy (apple drink)	0.084	0.065	0.076	0.075*

**Values represent mean of three replications.*
**Means followed by same letter in a column are not significantly different at P>0.05.*

Table-5: Determination of percent Titratable acidity in various brands of apple juices

S.No.	Brand name	R1	R2	R3	Mean
1	Haleeb Just Fruit (apple nectar)	4.2	4.1	3.5	3.93*
2	Nastel Fruita Vitals (apple nectar)	3.3	3.1	3.0	3.13*
3	Olfruite Apple Power UP	3.7	3.5	3.4	3.53*
4	TOPS Pulpy (apple drink)	3.5	3.4	3.3	3.4*

*Values represent mean of three replications.
*Means followed by same letter in a column are not significantly different at *P*>0.05.

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Table-6: Total Viable Count

Sr. No.	Brand	Total Viable Count (CFU/ml)
1	Haleeb Just Fruit (Apple Nectar)	Nil
2	Nestle Fruita Vitals (Apple Nectar)	Nil
3	Olfruite Apple Power Up	Nil
4	Tops Pulpy Apple Drink	Nil

Table-7: Total Coliforms

Sr. No.	Brand	Total Coliforms (MPN/100ml)
1	Haleeb Just Fruit (Apple Nectar)	Nil
2	Nestle Fruita Vitals (Apple Nectar)	Nil
3	Olfruite Apple Power Up	Nil
4	Tops Pulpy Apple Drink	Nil

Table-8: Fecal Coliforms

Sr. No.	Brand	Fecal Coliforms (MPN/100ml)
1	Haleeb Just Fruit (Apple Nectar)	Nil
2	Nestle Fruita Vitals (Apple Nectar)	Nil
3	Olfruite Apple Power Up	Nil
4	Tops Pulpy Apple Drink	Nil

Table_9: Yeast and Mold

Sr. No.	Brand	Yeast and Mold (CFU/ml)
1	Haleeb Just Fruit (Apple Nectar)	Nil
2	Nestle Fruita Vitals (Apple Nectar)	Nil
3	Olfruite Apple Power Up	Nil
4	Tops Pulpy Apple Drink	Nil

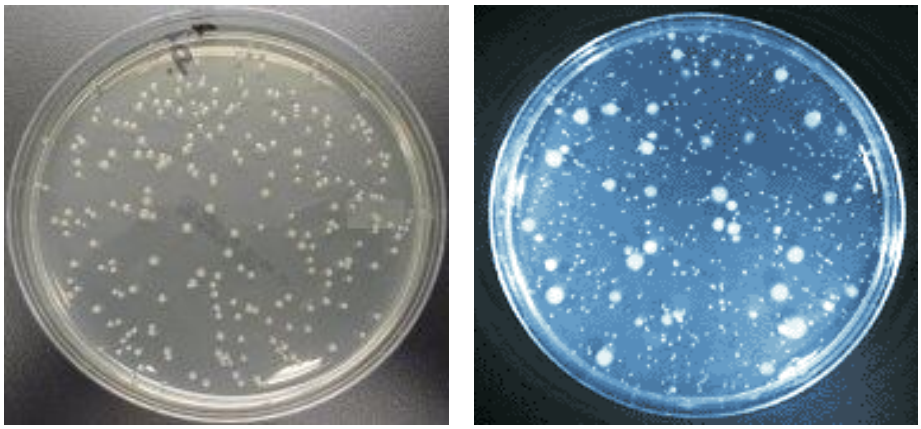

Figure: Nutrient agar plates after 24 hour incubation

OBSERVATION

All plates were examined after 24 and 48 hours of incubation. The microbial colonies were counted on each plate using colony counter. Total number of colonies were multiplies by dilution factor. Results are reported as CFU/ml.

Figure-1: MacConkey broth tubes for total coliforms after 24 hour incubation. Note color change and CO_2 bubble formation in tube No.1

Fecal Coliforms: Color change and CO_2 bubble formation observed in tube No. 3 and 4 Below.

Figure-2: *MacConkey broth tubes for fecal coliforms after 24 hour incubation.*Yeast and Mold

Total Coliforms

Coliform bacteria produce acid as a result of lactose fermentation. *E. coli* produces greater quantities of acid from lactose than other coliform species. When this occurs, the medium surrounding the growth also becomes red because of the action of acid that precipitates the bile salts, followed by absorption of the neutral red present in Mac-Conkey medium. All tubes were examined after 24 and 48 hours of incubation.

1. Positive: 10% or more of gas appears in the tubes in 24 hours.
2. Doubtful: Gas develops in a tube after 48 hours.
3. Negative: There is no gas in a tube after 48 hours.

The most probable number (MPN) was determined and recorded for each sample using MPN table. Results were reported as MPN/100 ml.

All plates were examined after 24 and 48 hours of incubation. The microbial colonies were counted on each plate using colony counter. Results are reported as CFU/ml.

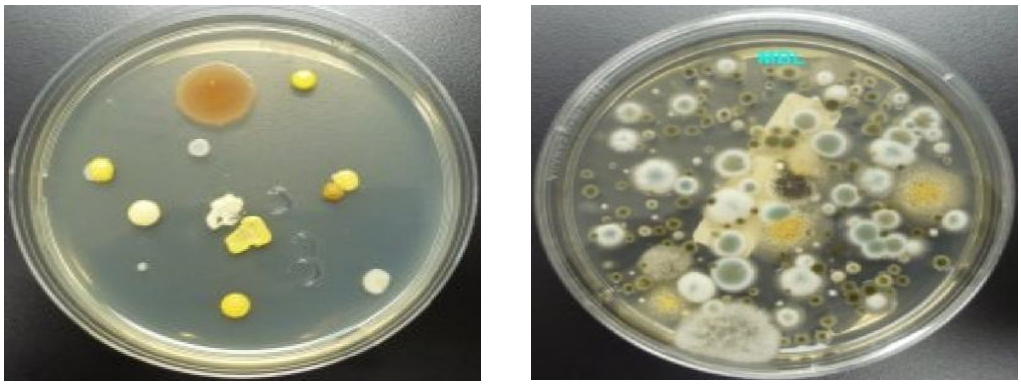

Figure-3: Potato dextrose agar plates after 24 hour incubation

1. DISCUSSION

Apple juices and drinks are made from different varieties of apples depending on the season. Therefore, the physical and chemical properties of apple juices can be varied. Results in Table-1 revealed that all brands of apple juices have appropriate pH value meeting the standards whereas Apple Nectar (Haleeb just fruit) and Nastel Fruita Vitals have the pH value 3.39 and 3.35 respectively are very close to the standard pH. Akpan and Kovo (2005) reported that an apple drink should have 3.5-4.0 pH as this is the standard pH for direct fruit drinks. Thus, our findings are in agreement with other studies and we can claim that our analysis process is authenticated. The standard pH is maintained for different reasons because pH has significant impact on the shelf life (it is reported that lower pH of sample is favorable for higher shelf life) and taste of the product. Our results have established the real range of pH variations in different brands of apple juices for the authenticate use of these products. These results indicate that there were no significant ($P>0.05$) differences between different brands of juice as analyzed.

Moisture content of food is of great importance to every food processor as a number of biochemical reactions and physiological changes in food depend very much on the moisture

content (Onwuka, 2005). Besides moisture content in product also indicate as how much has been added in the product for economic benefits by the manufacturer ignoring the standard usage of water. The results in Table-2 show the percent moisture content i.e. 89-97 in apple juices of all brands from 1-4 respectively. All the juices were found in accordance with the standard of Codex except TOPS pulpy apple drink. By describing the standard moisture content in direct drink juices, Pushpkar and Babeley (2001) reported that standard apple nectar should have 85-90% moisture content. Therefore, our findings authenticate all brand juices having standard moisture content except TOPS pulply juice. Our study suggests that TOPS Pulpy juice must be reviewed to maintain the standard moisture content since more water have been used by the manufacturer for his own economic benefit.

This study also analyzed the concentration of total solids in all four brands of apple nectars. Piyasena *et al*. (2002) reports that an apple drink must have 10-12% total solids. When our analysis were compared with the findings of other researchers as mentioned above it become evident that apple nectars from 1-3 (Table-3) stands on international standard having 10-11% total solids except TOPS pulpy juice i.e. 8.36% which obviously falls in lower standard. Both the studies authenticate good quality of apple nectars based on having standard level total solids. Besides, our analysis becomes valid as quality determination of chemical compositions. Total solids are measured of the amount of material dissolved in water. This material can include carbonate, bicarbonate, chloride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, organic ions and other ions (American Public Health Association, 1998). Like other food compositions, ash is also important composition because through Ash content, minerals are estimated. Ash is the inorganic residue remaining after the water and organic matter (Nielsen, 1998). The most widely used methods for determining minerals are ashes which are destroyed by heating and that they have a low volatility compared to other food components (James, 1995). These results are also in agreement with other scientists. Our study analysis revealed that ash content in all apple juices is very low except Haleeb just fruit. Results shows the concentration of ash content i.e. 0.654, 0.026, 0.001 and 0.075 in product 1-4 respectively which are much below the standard level. Shahnawaz *et al.* (2009) reported that drinkable juice of fruits must contain about 0.25-0.30%. From the results, it can be stated that the manufacturers have used less quantity of raw material of apple. Results in Table-5 revealed that percent acidity in the juices was more. The standard acidity in drinkable juices should not be increased from 0.5-0.6% (shanawaz *et al.* 2009). The high acidity content in these analyzed juices may be due to the demand by the end users i.e. Pakistani

people or it may be an analysis error. Whatsoever the case may be, the acidity was too much high.

Juices and nectars are acidic foods which may be contaminated either by yeast, molds and bacteria. Maturin and Peeler (2001) reported that spoilage of acidic foods is most often due to contamination with aerobic acid-tolerant bacteria as well as yeasts, molds and fungus. Thus, enumeration of these microorganisms is an important aspect of evaluating the microbiological quality of acidic foods. Potter and Hotchkiss (1960) described that foods undergo varying degrees of deterioration during storage. Deterioration may include losses in organoletpic desirability, nutritional value, safety and aesthetic appeal. Foods may change in color, texture, flavor or another quality attributes. In this study, shelf life or spoilage of stored products were examined through microbial enumeration. In our study results at Tables (6-9) and Figure1-3 indicates that all the juice samples as were analyzed for microbiological contamination through total viable count (TVC) by coliform bacteria (both total coliforms and fecal coliforms), Yeast and mold were absent in all the samples. Thus, the results suggest that apple juices of different brands as analyzed in the present study were contamination free and microbiologically fit for human consumption.

CONCLUSION

This study concludes that all juices were varied in having chemical compositions. Among the juices samples, TOPS apple juice was more deficient in chemical composition than others. According to the microbial study, all juices were free from microbial contamination and fit for human consumption. However, it may be suggested that further studies of these products may be carried out to testify whether the preservatives have been used in permissible level or not so that health hazard due chemical residues could be overcome.

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