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Biochemical and HPLC analysis of apple juice

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***Abstract*---**Fruit juices are important source of food and diet. Fruit juices have become important in recent years due to the overall increase in natural fruit. Juice consumption as an alternative to the traditional caffeine containing beverages such as coffee, tea, or carbonated soft drinks. HPLC of organic acids of fruit juices indicates the presence of different organic acids having different concentration in different samples of apple juice. Different peaks of organic acids were then compared with standard table of organic acids.

***Keywords*---**fruit juices, HPLC analysis, organic acids.

Introduction

Juice is the liquid that is naturally contained in fruit and vegetables. It can also refer to liquids that are flavoured with these or other biological food sources such as meat and seafood. It is commonly consumed as a beverage or used as an ingredient or flavouring in foods. Juice is prepared by mechanically squeezing or macerating fruit or vegetable flesh without the application of heat or solvents. Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic diseases, such as cardiovascular disease and cancer, and phytochemicals including phenolics,flavonoids and carotenoids from fruits and vegetables may play a key role in reducing chronic disease risk. 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 (Boyer and Liu , 2004). Organic acids give fruit products their characteristic tartness and vary in combination and in concentrations among different juices. The organic acid profile can be used to identify a juice or verify its purity. Typically, organic acids in fruit juices are identified and quantified by using methods such as HPLC. In this procedure , reversed phase column is used to separate and identificate six organic acids. Because several of the analytes are extremely difficult to resolve, a aqueous mobile phase is needed to enhance interaction between the acids and the C18 stationary phase (Leopold *et al*., 2006). Besides analytical methods involving colorimetric reaction and enzyme assay,chromatographic techniques allow

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simultaneous analysis of most of the organic acids . In this field, high performance liquid chromatography (HPLC) is one of most promising and more used techniques, either by direct determination or by the analysis of derivative products. Most of procedures developed until now for food and beverage analysis utilize either reverse phase partition chromatography (Czajkowska and Jaroniec, 1997; Fransson and Ragnarsson,1998) or ion exchange chromatography (Casella and Gatta, 2001; Guillén et al., 1998; Linget et al., 1998) with a refractive index (RI), UV spectrophotometric, conductimetric orelectrochemical detection (Buchberger, 2000) .The separation of organic acids with liquid chromatography and their quantitative determinations are extremely difficult because there is no difference between their structural similarities and spectral characteristics. Besides,

pKa values of most of the organic acids are rather similar and this situation limits the usage of pH for chromatographic separation (Aktaset al., 2005) .The present study was designed, on one side, to determine the organic acid distribution of citrus juices prepared from different species of citrus fruits: sweet orange,and apples of different fruit juice companies to separate, identify and quantify major organic acids in natural citrus juices using HPLC using standards of particular concentrations with photodiode array detection (DAD), which will identify compounds not only with their retention times but also with their individual spectra . A reversed phase HPLC method for separation and quantification of organic acids (oxalic,citric, tartaric, malic, ascorbic and lactic acids) in fruit juices was developed. The chromatographic separation was performed with a Surveyor Thermo Electron system at 10°C by using a potassium di hydrogen orthophosphate buffer (pH 2.8) as mobile phase, an 6 Hypersil Gold aQ Analytical Column and diode array detection at λ=254 nm for ascorbic acid and λ=214 nm for the other organic acids. Organic acid profiles of ten species of Citrus: sweet orange, minneola, clementine, mandarin orange, pomelo, lemon, lime, sweetie, white and pink grapefruit were established. Species significantly affect the organic acid distribution of citrus fruit juices. In all citrus juices, the most abundant organic acid was citric acid, ranging from 6.88 to 73.93 g/l. Citrus juices are good sources of ascorbic acid (0.215-0.718g/l). The average ascorbic acid was the highest in lemon juice followed by sweet orange juice, sweetie and white grapefruit (Nour et al., 2010).

Review of Literature

Apples contain a variety of phytochemicals, including quercetin, catechin, phloridzin and chlorogenic acid, all of which are strong antioxidants. The phytochemical composition of apples varies greatly between different varieties of apples, and there are also small changes in phyto chemicals during the maturation and ripening of the fruit. Storage has little to no effecton apple phytochemicals, but processing can greatly affect apple phytochemicals. While extensive research exists, a literature review of the health benefits of apples and their phytochemicals has not been compiled to summarize this work. The purpose of this paper is to review the most recent literature regarding the health benefits of apples and their phytochemicals, phytochemical bioavailability and antioxidant behavior, and the effects of variety, ripening, storage and processing on apple phytochemicals (Boyer and Liu , 2000). Historically, fruit juice was recommended by paediatricians as a source of vitamin C and an extra source of water for healthy infants and young children as their diets expanded to include solid foods with higher renal solute. Fruit juice is marketed as a healthy, natural source of vitamins and, in some instances, calcium. Because juice tastes good, children readily accept it. Although juice consumption has some benefits, it also has potential detrimental effects Pediatricians need to be knowledgeable about juice to inform parents and patients on its appropriate uses. The organic acids are extensively used in different food products for various purposes. The organic acids and their sodium salts are commonly used in foods as preservatives. Phillips (1999) studied effect of lactic acid and citric acid on the growth of Acetobacter butzleri in culture, alone and in combination. He found that at 0.5%, 1.0% and 2.0% lactic and citric acids inhibited A. butzleri growth; 2% sodium lactate showed effectiveness in inhibiting growth over 8 h incubation but not over longer periods. Sodium citrate was found to be more effective than sodium lactate. Niacin alone inhibited A. butzleri growth at 500 IU/mL over 5H. It did not enhance the effect of sodium citrate inhibition but it did augment the effect of sodium lactate alone over 8 h. They concluded that lactic and citric acids and their salts can effectively be used to check growth of Microorganisms .The organic acids have not only antimicrobial effects but the organic acid producing bacteria are also considered as antimicrobial agents due to acid and antibodies production which lowers the pH of food/substrate to suppress the growth of other microorganisms The reviewed material indicated that as organic acids are the recommended food additives by FAO and are used for 11 taste, aroma and texture at the same time these are also food preservatives. With the application of these organic acids the growth of pathogenic bacteria like E. coli, Yersinia, Salmonella and Leuconostic, is not only reduced instead the organic acids are helpful in destruction of these pathogenic bacteria. Similarly the organic acids producing culture also have antimicrobial powers either by acids or antibiotics production. Organic acids occur throughout nature and are used extensively in food systems. With a characteristically sour taste, organic acids have an important role in the flavor of fruits and their juices by balancing the sugar/acid ratio (Arthey and Ashurst, 1996). The inhibitory effect of organic acids depends on the undissociated form, as well as its ability to donate hydrogen ions in an aqueous system (Uljas and Ingham, 1998).Thus, organic acids can act on a cell by affecting both the external and internal pH. In both culture media and food system, the varying bacteriostatic and bactericidal effects of organic acids have been demonstrated. In one study, survival of E. coli O157:H7 was greater in acidified apple juice compared to acidified TSB, suggesting a protective effect of juice constituents. However, contrary to other studies, acidified apple juice enhanced survival compared to untreated apple juice at 40°C, suggesting a protective effect from the acid under refrigeration (Uljas and Ingham, 1998).

Citric Acid

Citric acid [HOOC-CH2-COH(COOH)-CH2-COOH] is one of the more widely used food acidulants. It is a common constituent of fruits, namely citrus fruits, and imparts a pleasant sour taste.

Malic Acid

Malic acid (HOOC-CHOH-CH2-COOH), along with citric acid, comprise the main organicacids in fruits (Arthey and Ashurst, 1996). In apples, malic acid is the predominant organicacid (Nagy et al., 1993).

Tartaric Acid

Unlike most other fruits, the main organic acid in grapes is tartaric acid (HOOCCHOH-CHOH-COOH) (Nagy et al., 1993).

Material used in Experiments

- Apples
- Balance for weighing out apple pieces
- Pectinase
- Filter Paper
- Plastic wrap,
- Disposable plastic spoons for stirring,
- Two 1 ml syringes or pipets,
- Two small funnels
- Two 100 ml graduated cylinders
- Two 100 ml beaker
- Water bath
- Distilled water,
- Timer or clock.
- Chemicals Used:
- pH indicator
- Iodine solution
- Fehling solution A and Fehling solution B
- Ammonium chloride solution
- Ammonium hvdroxide
- Ammonium oxalate
- Indole Tryptophanase
- Methyl Red
- Voges-Proskauer
- Citrate
- Apparatus / Instruments used
- Magnetic stirrer
- shaker
- water bath
- Auto pipettes
- Weighing balance
- Centrifuge machine
- Whtmann filter paper
- Centrifuge tubes
- HPLC Apparatus

Determination of organic acids in fruit juices using HPLC

Material used: Juice samples: Real apple, Tropicana apple, Mix fruit juice. Standard table of organic acid for comparing with the organic acids of samples. Method : HPLC was performed by using H 3 PO 4 as a mobile phase. All samples were loaded with a microsyringe. The absorbence was set as 210 nm. The time was set for 10 minutes. Different peaks of juice sample were observed and matched with the peaks of standards of different organic acids.

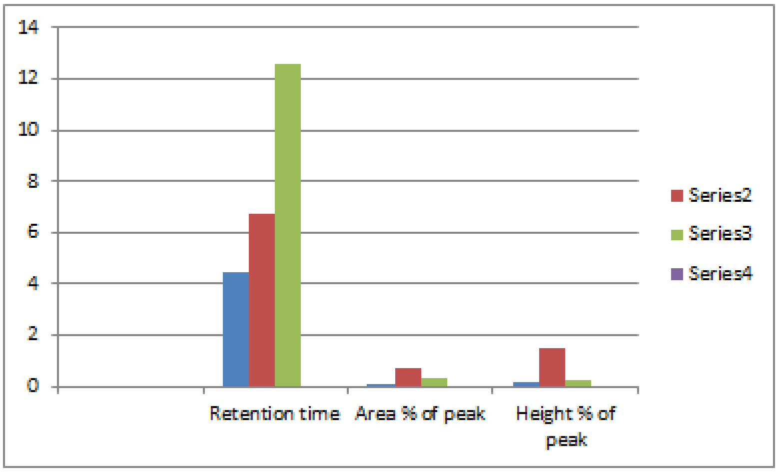
Results of HPLC

Following Results were obtained from HPLC.

Results of HPLC for Appy Fizz

Organic acid	Retention time	Area % of peak	Height % of peak
Tartaric acid	4.490	0.081	0.177
Lactic acid	6.736	0.709	1.503
Citric acid	12.557	0.304	0.231

Table shows that Appy fizz contain Lactic Acid more as compared to Tartaric acid and citric acid.

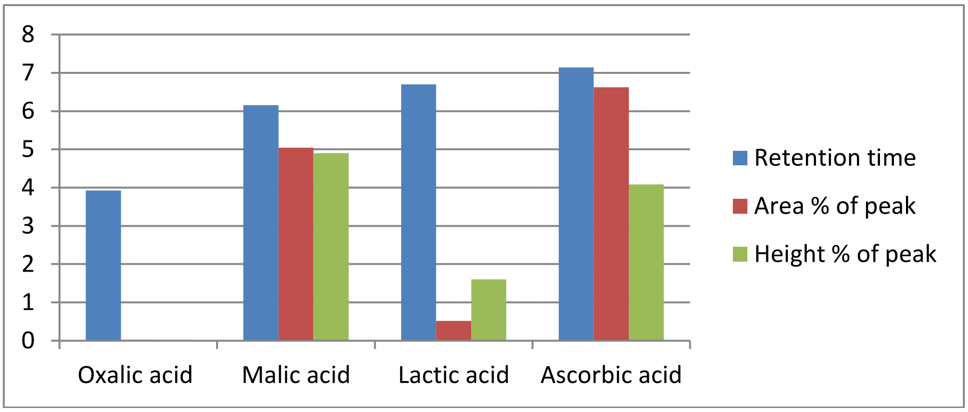


Mix Juice

HPLC results of mix juice

Organic acid	Retention time	Area % of peak	Height % of peak
Oxalic acid	3.924	0.026	0.024
Malic acid	6.158	5.043	4.902
Lactic acid	6.700	0.521	1.607
Ascorbic acid	7.140	6.619	4.083

Table shows that mix juice contain Ascorbic acid more as compared to Oxalic acid, Malic acid, Lactic acid.

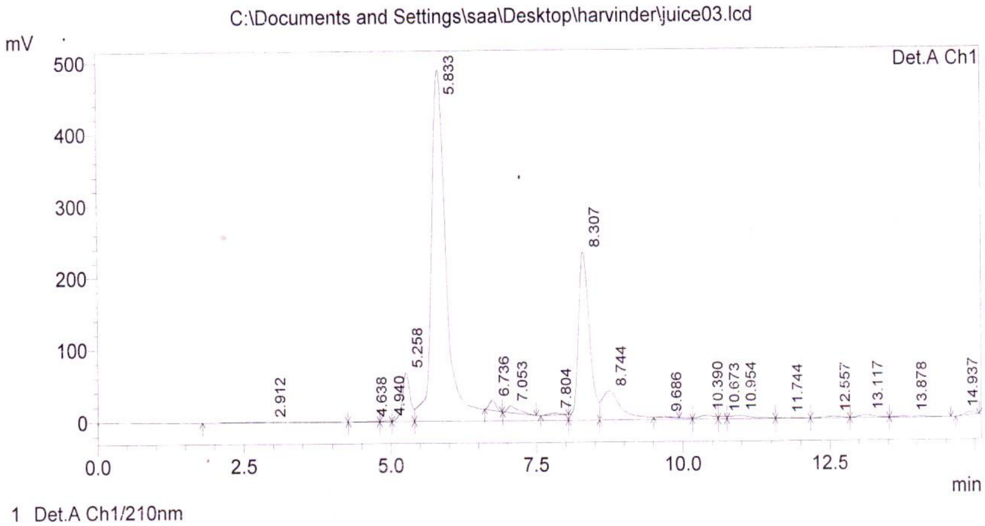


HPLC Results of Apple Juice

Organic acid	Retention time	Area % of peak	Height % of peak
Malic acid	6.102	3.544	3.643
Lactic acid	6.700	5.187	4.894
Citric acid	12.754	0.104	0.094

Table shows that the Apple juice contains more Lactic acid as compared to Malic acid and Citric acid.

Chromatogram of Appy fizz

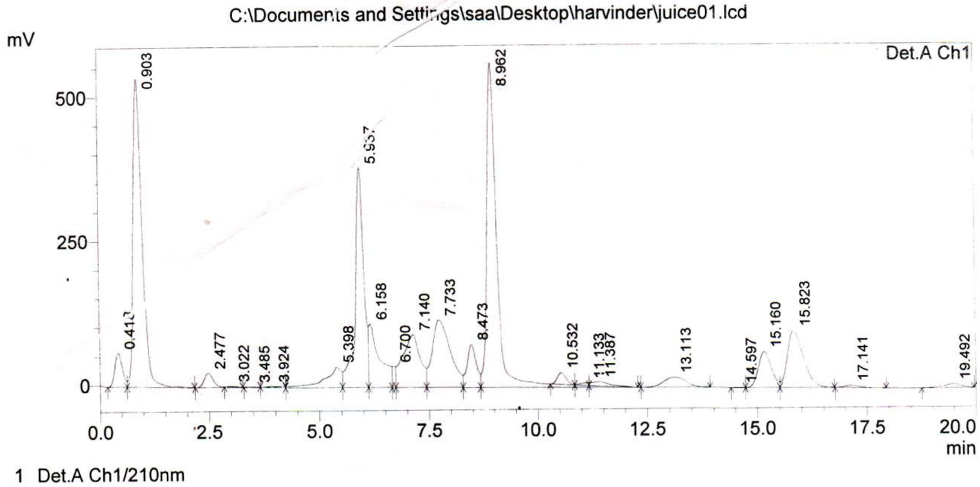


PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.912	55437	812	0.374	0.092
2	4.638	8292	425	0.056	0.048
3	4.940	11958	1565	0.081	0.177
4	5.258	629782	67558	4.251	7.650
5	5.833	9282475	487497	62.658	55.205
6	6.736	105012	13277	0.709	1.503
7	7.053	126598	9477	0.855	1.073
8	7.804	37865	2627	0.256	0.298
9	8.307	3064773	233488	20.687	26.441
10	8.744	993289	39939	6.705	4.523
11	9.686	13416	1010	0.091	0.114
12	10.390	89977	5128	0.607	0.581
13	10.673	25805	2901	0.174	0.329
14	10.954	112442	4392	0.759	0.497
15	11.744	17393	845	0.117	0.096
16	12.557	45095	2042	0.304	0.231
17	13.117	80179	4085	0.541	0.463
18	13.878	61126	1842	0.413	0.209
19	14.937	53713	4159	0.363	0.471
Total		14814626	883067	100.000	100.000

Chromatogram of Mix Fruit Juice

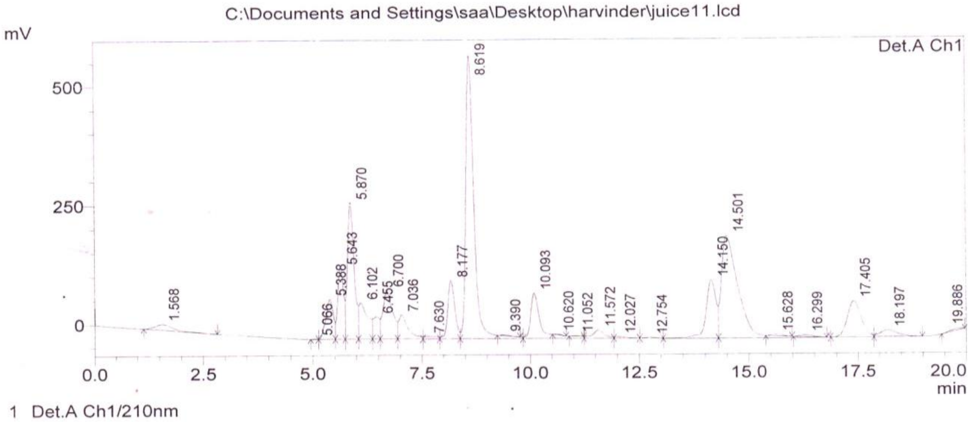


PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.418	722272	59487	1.892	2.637
2	0.903	7555435	535178	19.790	23.722
3	2.477	461627	24832	1.209	1.101
4	3.022	16376	1389	0.043	0.062
5	3.485	5829	446	0.015	0.020
6	3.924	9840	532	0.026	0.024
7	5.398	907277	35698	2.376	1.582
8	5.937	5147819	382899	13.484	16.972
9	6.158	1925118	110600	5.043	4.902
10	6.700	198860	36260	0.521	1.607
11	7.140	2527004	92115	6.619	4.083
12	7.733	3377688	117218	8.847	5.196
13	8.473	1087594	74611	2.849	3.307
14	8.962	8993761	562258	23.558	24.922
15	10.532	280877	20220	0.736	0.896
16	11.133	62282	4743	0.163	0.210
17	11.387	216156	7032	0.566	0.312
18	13.113	602272	16977	1.578	0.753
19	14.597	5626	439	0.015	0.019
20	15.160	1274672	62808	3.339	2.784
21	15.823	2504653	99524	6.561	4.411
22	17.141	95581	4170	0.250	0.185

Chromatogram of Apple Juice



PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.568	371587	12639	1.194	0.600
2	5.066	5138	902	0.017	0.043
3	5.388	991720	83984	3.186	3.988
4	5.643	1430439	150773	4.596	7.159
5	5.870	3045297	288007	9.784	13.675
6	6.102	1102967	76721	3.544	3.643
7	6.455	477133	47635	1.533	2.262
8	6.700	1614548	103079	5.187	4.894
9	7.036	778832	51824	2.502	2.461
10	7.630	111893	5575	0.359	0.265
11	8.177	1402934	123472	4.507	5.863
12	8.619	7686392	595774	24.694	28.288
13	9.390	25724	1767	0.083	0.084
14	10.093	1593169	95858	5.118	4.551
15	10.620	20385	1990	0.065	0.094
16	11.052	13673	1420	0.044	0.067
17	11.572	346031	18154	1.112	0.862
18	12.027	57455	3008	0.185	0.143
19	12.754	32448	1984	0.104	0.094
20	14.150	1938816	123087	6.229	5.844
21	14.501	5714916	215731	18.360	10.243
22	15.628	63454	3557	0.204	0.169

Conculsion

Fruit juices are important source of food and diet. Fruit juices have become important in recent years due to the overall increase in natural fruit. Juice consumption as an alternative to the traditional caffeine containing beverages such as coffee, tea, or carbonated soft drinks. HPLC of organic acids of fruit juices indicates the presence of different organic acids having different concentration in different samples of apple juice. Different peaks of organic acids were then compared with standard table of organic acids.

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