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INVESTIGATION TO EVALUATE ASCORBIC ACID, CAROTENOID AND SUGAR CONTENT IN DIFFERENT BRANDS OF PROCESSED AND PACKED APPLE JUICES

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ABSTRACT

The aim of this investigation was to evaluate ascorbic acid, carotenoid and sugar content in different types of processed and packed apple juices for experimentation. Four types of packed apple juices namely Appy fizz, Ditto, Real and Onjus were purchased from local market of Dehradun. All the four brands of apple juice showed acidic pH and Real brand is most acidic having pH of 3. Differences were observed in the Brix, viscosity and density of all these apple varieties. Thus Ditto juice possessed maximum nutritional components compared to Appy fizz, Real and Onjus packed apple juices. Ascorbic acid content was maximum in Ditto and least in the Onjus packed apple juice. Ascorbic acid was mainly responsible for the radical-scavenging capacity of the AQ (aqueous fraction) apple juice fractions. β carotene was not found in any of the brands of packed apple juices. It could be concluded that packed apple juice is a good nutritional source. Ditto comes out to be the best suited apple type for obtaining packed apple juice.

***Key words* :** ascorbic acid, carotenoid, brands, processed, apple juice.

INTRODUCTION

What are juices?

Juice is the liquid naturally contained in fruit or vegetable tissue. It is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents. For example, orange juice is the liquid extract of the fruit of the orange tree. Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or electric juicers. Many commercial juices are filtered to remove fiber or pulp, but high-pulp fresh orange juice is a popular beverage. Juice may be marketed in concentrate form, sometimes frozen, requiring the user to add water to reconstitute the liquid back to its "original state". However, concentrates generally have a noticeably different taste from that of their "fresh-squeezed" counterparts (Kurowska et al, 2000). Other juices are reconstituted before packaging for retail sale. Common methods for preservation and processing of fruit juices include canning, pasteurization, freezing, evaporation and spray drying.

Juices available in market

Popular juices include apple, orange, grapefruit, pineapple, tomato, passion fruit, mango, carrot, grape, cherry, cranberry, guava, and pomegranate. It has become increasingly popular to combine a variety of fruits into single juice drinks. Popular blends include cran-apple (cranberry and apple) and apple and blackcurrant (Franke et al, 2005). A demonstration of this trend is that prepackaged single fruit juices have lost market share to prepackaged fruit juice combinations. A number of new companies have had considerable success supplying prepackaged fruit juice permutations on the basis of this transition.

Juice bars have also become common place across most of the western world and offer similar juice blends. Juice is also commonly found in many cooking recipes from various cultures. The most popular are lime and lemon juice which help to add a slightly more sour or acidic taste to dishes.

Health benefits

Juices are often consumed for their health benefits. For example, orange juice rich in vitamin C, folic acid and potassium, is an excellent source of bio-available antioxidant phytochemicals (Franke et al, 2005) and it significantly improves blood lipid profiles in people affected with hypercholesterolemia (Kurowska et al, 2000). Prune juice is associated with a digestive health benefit. Cranberry juice has long been known to help prevent or even treat bladder infections, and it is now known that a substance in cranberries prevents bacteria from binding to the bladder (Drug Watch: Cranberry juice reduces bacteriuria and pyuria).

Fruit juice consumption overall in Europe, Australia, New Zealand and the USA has increased in recent years. West Europe Fruit Juice Market Research, Trends, Analysis TOC, are probably due to public perception of juices as a healthy natural source of nutrients and increased public interest in health issues. Indeed, fruit juice intake has been consistently associated with reduced risk of many cancer types (Lewis et al, 2009) and might be protective against stroke (Feldman EB, 2001).

Aim of study

The aim of this investigation was to evaluate ascorbic acid, carotenoid and sugar content in different types of processed and packed apple juices.

MATERIALS AND METHODS

1. Procurement of packed apple juices

The different brands of apples were purchased from the local market of Dehradun. The selected brands include APPY FIZZ, REAL, ONJUS and DITTO.

2. Determination of physical properties of packed apple juice

(a) Soluble Solids

Soluble solids of apple juice were determined by using a Digital Refractometer at room temperature and results were reported as degrees Brix.

(b) pH

pH of the apple juices were determined by using pH meter.

(c)Relative viscosity

Viscosity was measured by using Ostwald's Viscometer. It was cleaned with chromic acid and then thoroughly with distilled water. It is finally washed with alcohol or ether and then dried. Sufficient volume of distilled water is introduced by pipette in lower bulb so that bend portion of tube and half or a little more than half of lower bulb are filled up. Clamp the viscometer in vertical position. Through the rubber bulb attached to upper arm of upper bulb, suck the water until it rises above the upper mark and allow it to flow under its own weight. The time of flow from upper mark to lower mark is measured by starting the stop watch as the meniscus of the liquid just reaches the upper mark and stopping the watch as the meniscus just passes the lower mark. Take at least 3-4 readings with water and then take their mean value and note it as t_2. Now remove the water from the viscometer and dry it. Introduce in lower bulb almost same amount of juice and measure the time of flow of liquid as before. Take at least 3-4 readings and then take their mean value and note it as t_1. Now wash and dry the R.D. bottle and then weigh it empty and then fill it with distilled water and weigh. Remove the water and dry the R.D. bottle and then fill it with juice and weigh.

Calculations

- For Density of juice (d_1) and of water (d_2)

Mass of empty R.D. bottle = a gm

Mass of R.D. bottle + juice = b gm

Mass of the juice = b-a gm

Mass of R.D. bottle + water = c gm

Mass of the water = c-a gm

- $d_1 / d_2 = (b-a) / (c-a)$
- Relative viscosity of the juice = η_1 / η_2

$$\eta_1 / \eta_2 = (d_1 \times t_1) / (d_2 \times t_2)$$

By putting respective values the relative viscosity of the juice can be calculated.

(d) Relative density

The density of any liquid can be measured by using Relative Density bottle commonly called as R.D. bottle. It is slightly round bottomed type of glass vessel, fitted with a glass cork.

R.D. bottle is first washed with chromic acid solution and then with distilled water and finally with alcohol. It is then dried and weighed. The R.D. bottle is then filled with distilled water and stoppered. There should be no bubble inside the bottle. R.D. bottle is again weighed. Water is then poured out and washed with alcohol and dried. R.D. bottle is then filled with juice as before and weighed again.

Let: Mass of empty R.D. bottle = w_1 gm

Mass of R.D. bottle + water = w_2 gm

Mass of R.D. bottle + juice = w_3 gm

Then,

Density of juice (d_1) / density of water (d_2) = $(w_3-w_1) / (w_2-w_1)$

$$d_1 = [(w_3-w_1) / (w_2-w_1)] \times d_2$$

By putting respective values the relative density of the juice can be calculated.

(e) Carbohydrates

Qualitative test of carbohydrate: Anthrone Test

The anthrone test was used for the qualitative estimation of polysaccharides as well as monosaccharides. The test is based on the dehydration of monosaccharides to furfural derivatives, e.g. hydroxymethylfurfural. Furfural derivatives react with anthrone to form a deep green color complex.

1. Place 0.5-1 ml of each sample to separate test tubes.
2. Add 2 ml of anthrone reagent.
3. Mix and place tubes in boiling water bath for 10 min and observe the color for presence or absence of sugar.

3.Determination of Ascorbic Acid in packed apple juice

Ascorbic acid was determined by redox titration. The redox reaction is better than an acid-base titration since there are additional acids in a juice, but few of them interfere with the oxidation of ascorbic acid by iodine.

Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form tri iodide:

$I_2 + I^- \leftrightarrow I_3^-$

Tri iodide oxidizes vitamin C to form dehydroascorbic acid:

$C_6H_8O_6 + I_3^- + H_2O \rightarrow C_6H_6O_6 + 3I^- + 2H^+$

As long as vitamin C is present in the solution, the tri iodide is converted to the iodide ion very quickly. However, when the all the vitamin C is oxidized, iodine and tri iodide will be present, which react with starch to form a blue-black complex. The blue-black color is the endpoint of the titration. This titration procedure is appropriate for testing the amount of vitamin C in vitamin C tablets, juices, and fresh, frozen, or packaged fruits and vegetables

Preparation of reagents

1% Starch Indicator Solution

1. Add 0.50 g soluble starch to 50 ml near-boiling distilled water.
2. Mix well and allow to cool before use (doesn't have to be 1%; 0.5% is fine).

Iodine Solution

1. Dissolve 5.00 g potassium iodide (KI) and 0.268 g potassium iodate (KIO_3) in 200 ml of distilled water.
2. Add 30 ml of sulfuric acid.
3. Pour this solution into a 500 ml graduted cylinder and dilute it to a final volume of 500 ml with distilled water.
4. Mix the solution.
5. Transfer the solution to a 600 ml beaker. Label the beaker as iodine solution.

Vitamin C Standard Solution

1. Dissolve 0.250 g vitamin C (ascorbic acid) in 100 ml distilled water.
2. Dilute to 250 ml with distilled water in a volumetric flask. Label the flask as ascorbic acid standard solution.

Standardizing Solutions

1. Add 25.00 ml of vitamin C standard solution to a 125 ml Erlenmeyer flask.
2. Add 10 drops of 1% starch solution.
3. Rinse buret with a small volume of the iodine solution and then fill it. Record the initial volume.
4. Titrate the solution until the endpoint is reached. This will be when we see the first sign of blue color that persists after 20 seconds of swirling the solution.
5. Record the final volume of iodine solution. The volume that was required is the starting volume minus the final volume as V_1.
6. Repeat the titration at least twice more.

Vitamin C Titration

We titrate samples exactly the same as we did for standard. Record the initial and final volume of iodine solution required to produce the color change at the endpoint.

Titrating Juice Samples

1. Add 25.00 ml of juice sample to a 125 ml Erlenmeyer flask.

2. Titrate until the endpoint is reached. (Add iodine solution until we get a color that persists longer than 20 seconds).
3. Repeat the titration until we have at least three measurements V_2.

Calculations

- Volume × Normality of KIO_3 = Volume × Normality of known Vitamin C solution
  $V_1 \times N_1 = (25 \times 4 \times w) \div$ Equivalent weight Eq. wt.
  Normality of $KIO_3 = (25 \times 4 \times w) \div (V_1 \times \text{Eq. wt.})$
- Volume × Normality of Unknown Vitamin C = Volume × Normality of KIO_3
  $25 \times N_2 = (V_2 \times 25 \times 4 \times w) \div (V_1 \times \text{Eq wt.})$
  $N_2 = (25 \times 4 \times w \times V_2) \div (25 \times \text{Eq wt.} \times V_1)$
  Strength = $[(25 \times 4 \times w \times V_2) \div (25 \times \text{Eq wt.} \times V_1)] \times \text{Eq wt.}$
  $= (4 \times w \times V_2) \div V_1$ gm/lt

NOTE: Eq wt. = Equivalent weight of Vitamin C
w = wt. of vitamin C dissolved in 250 ml distilled water i.e. 0.250 gm
N_1 = Normality of KIO_3
N_2 = Normality of Unknown Vitamin C
V_1 = Volume of KIO_3 used in titrating standard Vit C
V_2 = Volume of KIO_3 used in titrating sample

Thus putting the values in the above formula will give the amount of vitamin C present in grams in per litre of the sample juice.

4. Determination of Carotene in packed apple juice

Principle

β carotene are precursors of vitamin A and hence their estimation in food stuffs is important from nutritional point of view. Carotenes are separated on a column of $Ca(OH)_2$ after their extraction from experimental material by organic solvents. The individual carotene are then determined in the column effluent spectrophotometrically. The values form their respective vitamin A potency is used to derive the total vitamin A content of food stuff.

Materials and reagents

- glass column (1x30 cm)
- colorimeter
- petroleum ether – acetone (1:1) mixture
- calcium hydroxide
- sodium sulphate (anhydrous)
- petroleum ether

Procedure

1) Standardization of β carotene

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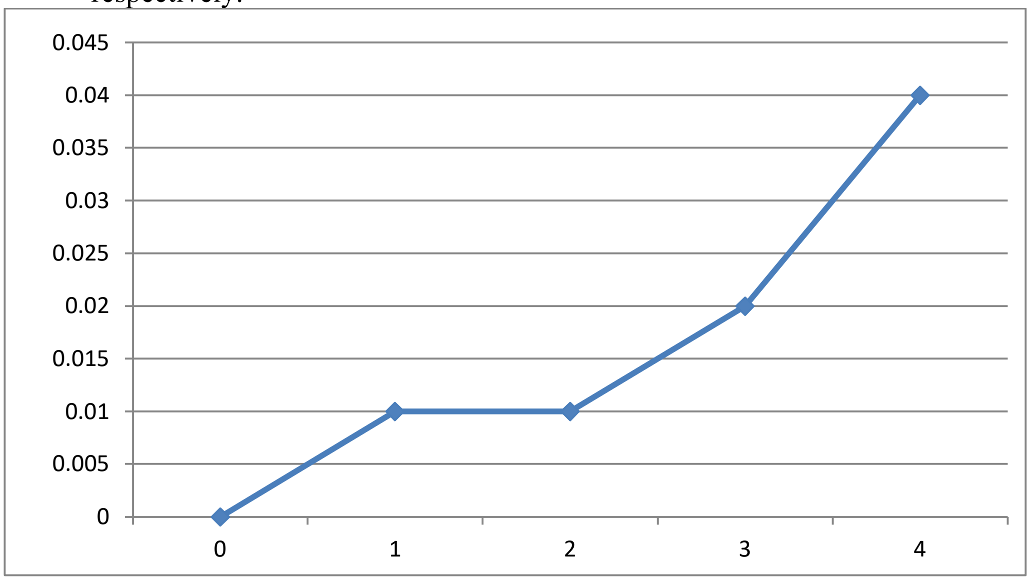
- First of all cover the conical flask with paper or aluminium foil.
- Add 25 mg of β carotene and add 25 ml of petroleum ether – acetone (1:1) mixture.
- The mixture thus formed is called stock solution.
- Take 5 test tubes and add 0ml, 1ml, 2ml, 3ml and 4 ml of stock solution.
- To the test tubes add 0ml, 4ml, 3ml, 2ml and 1ml of petroleum ether – acetone (1:1) mixture respectively.



CONCENTRATION OF STOCK SOLUTION

2) **Preparation of the sample:** Take 25 ml of the sample juice and allow it to stand for some time in 100 ml mixture of petroleum ether – acetone (1:1) mixture. Filter the extract through separating funnel and wash the sample again and again with 100 ml of petroleum ether and acetone (1:1) mixture till all the yellow color is extracted repeat this procedure 2 - 3 times.

3) Take the organic solvent layer and take the optical density of different sample of juice.

4) Record the absorbance of standards and sample fractions at 450 nm and prepare the standard curve. Determine the quantity of β carotene in each of the collected fraction of the sample from the standard curve.

Calculations

Add values of β carotenes of all the fractions of the sample taken into consideration the portion of column effluent of each fraction used for monitoring β carotenes. The true vitamin a value in the sample is expressed in terms of international units. One International Unit of vitamin A is equivalent to 0.6 microgram of β carotene.

RESULTS AND DISCUSSION

Physical and physiochemical characterization

The physical and physiochemical parameters of different brands of apple juices are shown in the following tables:

1. **pH AND BRIX OF DIFFERENT BRANDS OF APPLE JUICES**

S.NO.	***BRAND OF APPLE JUICE***	***pH***	***BRIX***	***ANTHRONE TEST***
1.	APPY FIZZ	3.4	15	+
2.	ONJUS	4.12	12	+
3.	REAL	3.0	16	+
4.	DITTO	3.36	13	+

Table 1: pH and brix of different brands of packed apple juices

+ Sign indicates the presence of sugar in juices.

Table 1 depicts the pH, presence of sugar and soluble solids in different brands of apple juices. All the four brands of juices had acidic nature but maximum acidity was shown by Onjus i.e. 4.12 and Real brand of apple juice had minimum acidic nature i.e. 3.0. In a similar way Onjus, Appy fizz and Real showed maximum amount of carbohydrates as compared to the Ditto brand. Ditto brand had maximum amount of soluble sugar content as compared to other varieties of packed apple juices. The qualitative analysis of sugars by anthrone test had shown presence of sugars in all the four brands of packed apple juices.

2. **DENSITY AND VISCOSITY OF DIFFERENT BRANDS OF APPLE JUICES**

S.NO.	***BRAND OF JUICE***	***RELATIVE DENSITIES (in gm/ml)***	***VISCOSITY (centipoises cP)***

1.	APPY FIZZ	0.903	1.302
2.	ONJUS	0.886	1.195
3.	REAL	0.899	1.278
4.	DITTO	0.935	1.415

Table 2: Viscosity and density of different brands of apple juices

Table 2 states the viscosity and density of different brands of packed apples juices. Density of all the juices was not much different from that of water. Ditto brand of apple juice has maximum viscosity indicating the presence of good amount of soluble solids whereas Onjus has minimum viscosity thus having low content of solids in it. Ditto brand had high value of relative density and Onjus had low value.

3. ASCORBIC ACID AND β- CAROTENE OF DIFFERENT PACKED APPLE JUICES

S.No.	***BRAND OF JUICE***	***ASCORBIC ACID (gm/lt)***	***CAROTENOID (mg/lt)***
1.	APPY FIZZ	0.032	NIL
2.	ONJUS	0.042	NIL
3.	REAL	0.135	NIL
4.	DITTO	0.128	NIL

Table 3: Ascorbic acid content and β carotene content in different brands of apple juices.

Table 3 describes the amount of Ascorbic acid and Carotenoid present in different brands of packed apple juices. Real brand of packed apple juice had maximum amount of ascorbic acid present in it. Appy fizz had minimum amount of Ascorbic acid. Carotenoid content was not found in any of the packed apple juices.

Soluble nutrients are found in packed apple juices but fibres are absent in the juices. These characteristics make the juices easily digestible food supplements. In the present study the physical properties as well as nutritional content was analysed in four brands of packed apple juices namely Appy fizz, Real, Ditto and Onjus. All the apple types showed acidic pH. The high acidity in packed juices could be explained on the basis of technological treatment carried out by the manufacturers, aimed to achieve high acidity- apple juices, which have a better consumer acceptance (Sanchez-Moreno, 2005). Manufacturers add sugars to offset acid taste and get a sweeter product, and this was shown on some of the labels of the commercial apple juices brought for this study.

Real brand of packed apple juice showed maximum Brix (16), density (0.8997) and viscosity (1.302). All these parameters are indicator of the presence of soluble solids including sugars. The present result obtained showed that Real has maximum amount of these compounds. These results are comparable with the earlier studies of apple juices (Handelmann, 2001). The presence of soluble compounds has also been shown in other fruit juices also. In study conducted by Gutierrez et al, 2008 the presence of various nutritional components has been noted in guava juice.

To evaluate the nutritional value of the selected packed apple juices, Ascorbic Acid and β carotene content was also determined. Real juice showed highest amount of ascorbic acid (0.135mg/100gm of juice). When these results were compared with the earlier studies where ascorbic acid content was 2.24mg/250 gm of juice.

The packed juices of all four types did not show the presence of carotene. This may be due to the fact that β carotene is present only in fresh apple but not in packed juices. When the result was compared with the earlier studies it was found that Handelmann 2001 has also said that β carotene is absent in the apple juices.

It could be concluded that packed apple juice is a good nutritional source. Ditto comes out to be the best suited apple type for obtaining packed apple juice.

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