

Physico-Chemical Studies on the Effect of Preservatives in Storage of ABC Juice

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Abstract:- Consuming the fruit and vegetable juices are considered as a simple and easy method to gain maximum health benefits. Preserving the nutritive value of these juices is important during their storage. So, preservatives and additives are used to increase their shelf life, along with ensuring the product safety. In this study, three kinds of natural preservatives were used to preserve a juice blend obtained from apple, carrot and beetroot under ambient room temperature and refrigerated conditions. The samples were examined every day for any changes in physical parameters and after 7 days, an aliquot of juice was studied for its chemical properties. The sample with lemon juice as preservative and stored under ambient room temperature had no contamination with least amount of reducing sugars and total proteins. Hence, the results observed in this preliminary study corroborate the usage of lemon juice as an effective natural preservative for preserving juice blend of apple, beetroot and carrot.

Keywords:- ABC Juice, Lemon Juice, Natural Preservative, Storage.

I. INTRODUCTION

Fruits and vegetables are considered as a rich source of nutrients and possess huge health benefits. Generally, nutritionists recommend an intake of the fruits and vegetable juices as an effective method to acquire a good health. Also, many studies relating to the consumption of fruit and vegetable juice blends have proven to offer protection against many diseases, such as cancers, neurodegenerative diseases, and cardiovascular diseases [1]. Preserving such blends must be aimed towards improving shelf life, ensuring the product safety, and retain the product's nutritive value. This can be achieved by using natural or artificial preservatives.

Apples (*Malus domestica*) are among the most popularly and frequently consumed fruits. These fruits are rich in bioactive components such as polyphenols, tannins, antioxidants, vitamins B6, K and C. Apples are also a rich source of fiber [2, 3, 4]. Beetroot (*Beta vulgaris*) is one of the

richest dietary sources of antioxidants, naturally occurring nitrates, vitamins and minerals such as phosphorus, calcium, magnesium, sulfur, and an excellent source of foliate, manganese, iron and many antioxidants [5, 6, 7]. The presence of these components in beetroot is helpful to humans in lowering blood pressure levels, reducing the risk of cardiovascular attacks and also to reduce the formation of tumors in stomach and colon [8, 9, 10]. Similarly, Carrots (*Daucus carota*) are high in fibers, carotenoids, vitamins C and E, and phenolics such as *p*-coumaric, chlorogenic, and caffeic acids. Oral intake of carrot juice also displays other beneficial physiological effects including reduced oxidative DNA damage, increased levels of plasma antioxidants and reduced inflammation [11, 12].

A blend prepared from apple, carrot and beetroot juices (ABC juice) is considered beneficial for many health ailments [13]. Hence, a preliminary study on increasing the shelf life of such blended juice is reported here. Three kinds of additives were used to preserve the juice at refrigerated and ambient room temperature for seven days and the observations are detailed in this study.

II. MATERIALS AND METHODS

Apple, beetroot, carrot, salt, sugar, lemon juice, electric blender, strainer, glass bottles, measuring scale, sterile gloves and refrigerator.

➤ Preparation of ABC Juice:

Healthy and fresh ABC (apple, beetroot and carrot) were washed thoroughly in clean water and rinsed in distilled water. The items were then wiped dry and peeled for further process. ABC was then chopped into medium sized cubes (*Fig. 1*) and weighed in the electronic balance (Shimadzu BL-220H). 100g of each chopped ABC was weighed and blended in the mixer separately to obtain a fine paste. This paste was passed through the strainer individually to obtain the juice without any residues.



Fig 1 Apple, Beetroot and Carrot Considered for Preparation of Juice

➤ *Sterilization of the Glass Bottles:*

The borosilicate glass bottles (pickle jars) were washed and placed in the hot air oven to dry. Later, it was autoclaved at 121°C for 15 minutes.

➤ *Storage and Observation of the Samples:*

Individual juices from ABC were mixed together and 100 ml of this juice was added into the sterilized (autoclaved) bottles. 1.5% of salt or sugar was added to two different bottles with ABC juice and labeled respectively. 1.5 ml of juice extracted from one whole fresh and clean deseeded lemon was added for the third bottle containing the ABC juice. These three bottles were considered for storage under room temperature (normal conditions). Similar set of three more bottles with same amount of preservatives were prepared for storage under refrigeration conditions (Fig 2).

One bottle without any added preservatives was considered as control in each set. All the bottles with ABC juice and preservatives were labeled as S1 to S6 (S1: ABC juice + Salt, S2: ABC juice + Sugar, S3: ABC juice + lemon juice, S4: ABC juice + Salt (refrigerated condition), S5: ABC juice + Sugar (refrigerated condition), S6: ABC juice + lemon juice (refrigerated condition)) and those without any preservative were labeled as C1: control 1 (room temperature), and C2: Control 2 (refrigerated condition). S1 to S3 along with C1 was stored under room temperature and conditions. S4 to S6 along with C2 was stored in the refrigerator. The bottles were observed regularly and the results were recorded.



Fig 2 The ABC Juice Prepared on the Day 0 and Ready for Storage at Room Temperature and Refrigerator Conditions.

➤ *Estimation of Sugar and Protein Content in the Samples:*

To estimate the sugar and protein content in the samples preserved, DNS and Bradford’s tests were performed respectively. To obtain the standard calibration curve for estimating sugar content, sucrose at 0, 0.2, 0.4, 0.4, 0.6, 0.8 and 1.0 mg/ml concentration was used. Standard protocol of sugar estimation by DNS method was followed [14]. 0.5 ml of each sample was also considered along with the assay set. Standard calibration curve was plotted for sucrose and the amount of glucose in samples was estimated.

Similarly, the protein content in the samples was estimated using bovine serum albumin (BSA) as standard in varying concentration viz. 0, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml. Standard protocol for estimation of proteins by Bradford’s method was followed [15]. Calibration curve was plotted and the amount of protein in the samples was estimated.

III. RESULTS AND DISCUSSIONS

➤ *Storage and Observation of the Samples:*

The samples stored under room temperature and refrigerator conditions were observed periodically and the changes in their physical appearances were recorded in the sensory evaluation sheet. The observations recorded everyday are depicted in table 1.

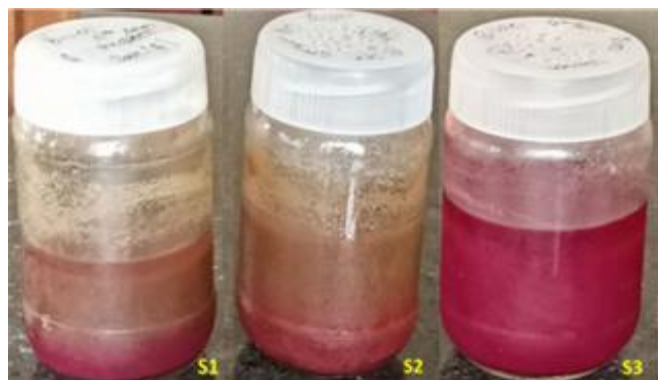


Fig 3 Image of the Samples S1, S2, S3 Stored at Room Temperatures on Day 7

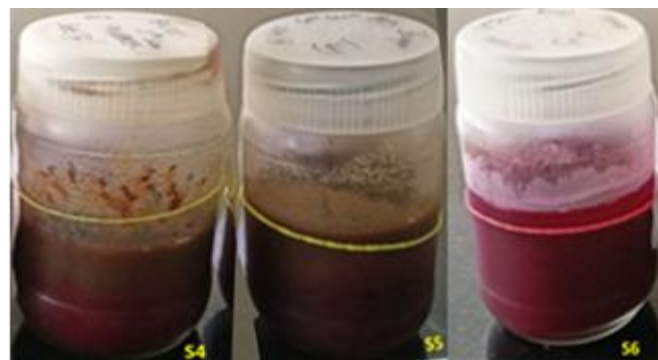


Fig 4 Image of Samples S6, S4, S5 stored at Refrigerated Conditions on day 7.

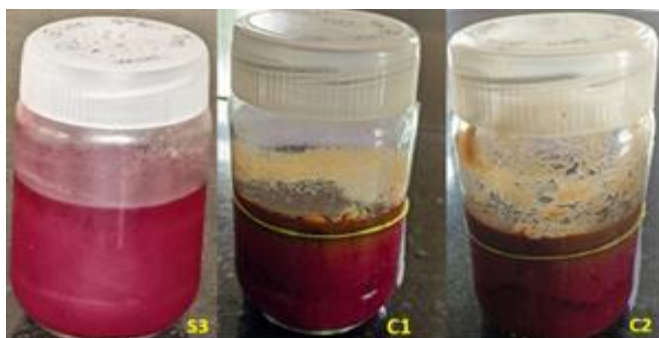


Fig 5 Image of Samples S3, C1 (Room Temperature), C2 (Refrigerator) on Day 7

From Table 1, the S3 bottle containing ABC juice with lemon juice as perseverant showed no contamination until 7 days in room temperature conditions. On Day 0, the ABC juice with and without preservatives (S1 to S6, C1 and C2) were all deep red in color with thick flowy texture and consistency. On day 2, juice in the control bottle under room conditions became reddish brown in color and also cloudy i.e due to absence of preservatives. S2 became cloudy in appearance on day 2 as it had more sugar content in it whereas

C1 became thick textured and started developing white clumps of mass on the surface. The first instance of changes in the refrigerated set was observed on day 3 in S5 turning clumpy as it contained sugar as preservative. The contamination was prominent in the form of white cottony growth in C1 on day 4 whereas S2 had turned thick in texture. On day 5, C1, S1, S2 turned reddish brown in color and had a thick texture with white cottony growth and white clump formation, whereas S3 had retained its color, but became thick and slimy in texture. On day 6, white cottony growth or clumps were observed in C1, S1 and S2 samples maintained at room temperatures. S5 and C2 from the refrigerated set started to develop white clumps with a change in texture. S6 started to become mildly effervescent and bubbly due to the presence of lemon juice. Discoloration viz. brownish black to reddish brown was observed in C1, S1, and S2 respectively on Day 7. C2, S4, S5 and S6 were all from refrigerated sets and had thick to clumpy texture with notable white clumps and were more effervescent with a slimy texture (Fig 3, Fig 4, Fig 5). Among all these, only S3 had retained its deep red color and was thick in texture with no visible clumps formation.

TABLE 1: Result table with observations of ABC juice samples with added preservatives stored under room temperature and refrigerated conditions. C1: control 1 (room temperature), S1: ABC juice + Salt, S2: ABC juice + Sugar, S3: ABC juice + lemon juice, C2: Control 2 (refrigerated condition), S4: ABC juice + Salt (refrigerated condition), S5: ABC juice + Sugar (refrigerated condition), S6: ABC juice + lemon juice (refrigerated condition).

Sensory Evaluation Chart								
Day 0								
	C1	S1	S2	S3	C2	S4	S5	S6
Color	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red
Texture	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy
Visible changes	-	-	-	-	-	-	-	-
Day 1								
	C1	S1	S2	S3	C2	S4	S5	S6
Color	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red
Texture	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy
Visible changes	Cloudy	-	-	-	-	-	-	-
Day 2								
	C1	S1	S2	S3	C2	S4	S5	S6
Color	Reddish brown	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red	Deep red
Texture	Thick	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy
Visible changes	White clumps	-	Cloudy	-	-	-	-	-
Day 3								
	C1	S1	S2	S3	C2	S4	S5	S6
Color	Reddish brown	Deep red	Reddish brown	Deep red	Deep red	Deep red	Reddish brown	Deep red
Texture	Thick	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy	Thick flowy
Visible changes	White clumps	-	White clumps	-	-	-	Clumpy	-

Day 4								
	<i>C1</i>	<i>S1</i>	<i>S2</i>	<i>S3</i>	<i>C2</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>
Color	Reddish brown	Reddish brown	Reddish brown	Deep red	Deep red	Deep red	Reddish brown	Deep red
Texture	Thick	Thick	Thick	Thick flowy	Clumpy	Thick flowy	Clumpy	Thick flowy
Visible changes	White cottony growth	-	White clumps	-	-	-	Cloudy	-
Day 5								
	<i>C1</i>	<i>S1</i>	<i>S2</i>	<i>S3</i>	<i>C2</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>
Color	Reddish brown	Reddish brown	Reddish brown	Deep red	Deep red	Reddish brown	Reddish brown	Deep red
Texture	Thick	Thick	Thick	Thick flowy	Clumpy	Thick	Clumpy	Thick slimy
Visible changes	White cottony growth	White clumps	White clumps	-	-	-	White clumps	-
Day 6								
	<i>C1</i>	<i>S1</i>	<i>S2</i>	<i>S3</i>	<i>C2</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>
Color	Brownish black	Reddish brown	Reddish brown	Deep red	Reddish brown	Reddish brown	Reddish brown	Deep red
Texture	Thick	Thick	Thick	Thick flowy	Clumpy	Thick	Clumpy	Thick slimy
Visible changes	White cottony growth	White clumps	White cottony growth	-	-	-	White clumps	Mildly effervescent
Day 7								
	<i>C1</i>	<i>S1</i>	<i>S2</i>	<i>S3</i>	<i>C2</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>
Color	Brownish black	Reddish brown	Reddish brown	Deep red	Reddish brown	Reddish brown	Reddish brown	Deep red
Texture	Thick	Thick	Thick	Thick flowy	Clumpy	Thick	Clumpy	Thick slimy
Visible changes	White cottony growth	White clumps	White cottony growth	-	White clumps	Viscous	White clumps	Mildly effervescent

➤ *Estimation of Sugar and Protein Content in the Samples:*

At the end of 7 days, the content of reducing sugars in all the samples was more than the control (without any preservatives). The amount of reducing sugar in control 1 (room conditions) and control 2 (refrigerated) was 0.07 mg/ml and 0.06 mg/ml respectively. The sample S3 (ABC juice + 1.5 ml lemon juice stored at room conditions) had the least amount of reducing sugars i.e. 0.028 mg/ml followed by S4 (ABC juice with 1.5% salt stored at refrigerated conditions) had 0.04 mg/ml (Fig 6). The total protein content in control 1 and control 2 was 94 mg/ml and 93 mg/ml. The sample S3 (ABC juice with lemon juice stored under room conditions) had comparatively least amount of protein i.e 88 mg/ml compared to sample S1 (ABC juice with 1.5% salt stored under room conditions) having 92 mg/ml (Fig 7).

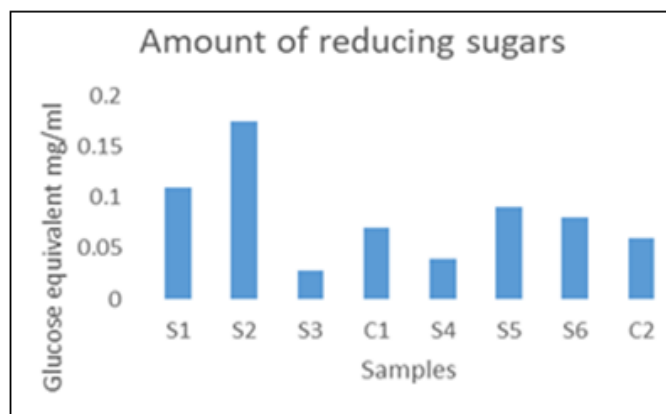


Fig 6 Graph on Total Reducing Sugars in the Samples and Controls

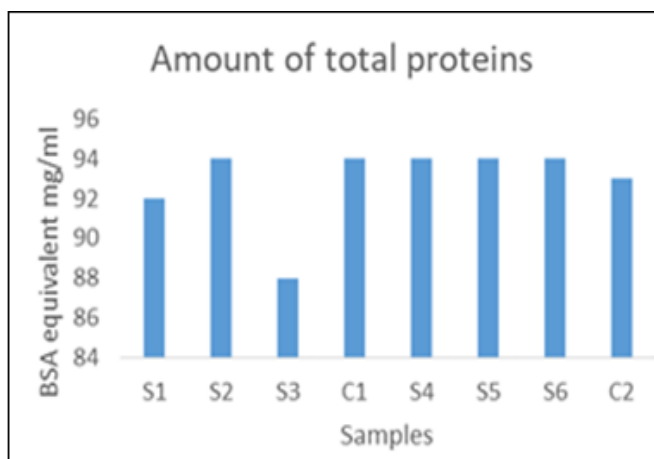


Fig 7: Graph on Total Protein Content in the Samples and Controls.

IV. CONCLUSION AND DISCUSSIONS:

Reference [16] has reported on using 5% of lemon juice as the most acceptable limit, for a preservative whereas [17] describes up to 9% of lemon juice can be used as a preservative in storage of smoothies. Reference [18] have clearly mentioned about lemon juice being a better preservative than other chemical agents for preserving fruit blends. The author has mentioned about bacteriostatic and fungistatic properties of using 4% of lime juice as a preservative in fruit blends. The retention of color in the S3 sample may be accounted to the presence of a potent antioxidant agent preventing the oxidation of ABC blend. This anti-oxidant agent can be the citric acid present in the lemon juice. References [17, 18, 19] have reported on using ascorbic acid and citric acid as an effective preservative to inhibit microbial growth of bacteria and fungi up to 2 weeks. Based on the same grounds, the uncontaminated status of S3 in our study can be due to the increase in acidic nature of the ABC juice, as the addition of lemon juice have decreased the pH and prevented the growth of microorganisms. The decreased amount of reducing sugars in the S3 sample may be due to minimal hydrolysis of polysaccharides into monosaccharide and oligosaccharides. According to [20] 3% of protein content in growth media is sufficient to develop microbes and fungi, whereas in this study, though sample S3 had the least amount of total proteins i.e. 88 mg/ml, due to presence of lemon juice, there was no contamination observed. The only drawback of using lemon juice as a preservative is the change in the overall taste of ABC juice.

Among all the ingredients used in ABC juice blend, apple is generally considered as seasonal fruit and is not easily affordable to everyone. Hence, preparing such blends of apple, beetroot and carrot for consumption is highly recommended in daily diet due to its immense health benefits. Thus, this study is a preliminary effort in illustrating the usage of lemon juice as an effective preservative of ABC juice. Further studies and tests are needed to optimize these results and implement the usage of lemon juice in storing juice blends.

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