Effect of Carbonation on Watermelon Juice and Study of Antioxidant properties

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1. Introduction

Watermelon *(Citrullus lanatus)* fruit belongs to the vine-like herb, which is regarded as a *Cucurbitaceae*. It originated in South Africa and is prominently grown in various parts of the world [1]. The cultivation requires long warm seasons in the region, making it difficult to access at any time [2]. This problem accounts for making them available throughout the year with minimal processing. Watermelon, in nutritional aspects, consists of a significant part of water and a minor component of fiber, proteins, fat, and minerals [3]. Watermelon is widely considered to contain different vitamins such as niacin, riboflavin, thiamine, and ascorbic acid. Its attractive color, taste, and pleasant odor make it feasible for utilization in various foods [4]. In recent years, watermelon juice has been thermally processed to inhibit the growth of microorganisms and increase their shelf-life. On the contrary, it has created undesirable changes in color, appearance, and flavor [5]. Watermelons generate considerable interest due to their higher Antioxidant activity than any other fruit [6].

Watermelon juice has positively responded to decreasing the risks of chronic diseases, cancers, diabetes, and atherosclerosis [5]. A considerable demand for carbonated soft drinks is seen among people. Consumers are interested in them due to their thirst-quenching and sparkling effects, but they often lack nutritional value [7, 8]. Studies by Tahmassebi and BaniHani [9] showcased that excessive carbonation intake results in dental erosion and an increased risk of type II diabetes and dental caries. Besides dental decay, most carbonated beverages are acidic, causing alternations in intra-esophageal pH [10]. Carbonation is a preservative technique that results in the addition of CO_2 to a liquid. Carbonation prevents the growth of microorganisms in juice. They contribute to the extension of shelf life [11]. The carbonation process is entirely performed under high pressure, due to which smaller bubbles are formed in the liquid when opened, giving rise to a tingling effect [8]. The majority of carbonated beverages contain synthetic chemicals along with flavors. A significant issue can be minimized by using juice extracted from fruit samples and performing carbonation on them without compromising the nutritional value [12]. Carbonating watermelon juice involves juicing, pasteurization, blending, and carbonation, which gives rise to a value-added product. Preservation techniques utilize chemical preservatives at refrigeration temperature [2], thermal treatments [4], pulsed electric field, ultraviolet radiation, sonication, ohmic heating [13], and ultrasound [14]. Despite numerous methodologies for preservation, people are often interested in carbonated beverages. On a shorter note, no recent study has put forward the aspects of carbonated watermelon juice. This study is focusing on the effect of carbonation on watermelon juice in terms of sensory, TSS, titrable Acidity, Shelf-life, Color, and pH.

2. Materials & Methods

2.1. Raw Materials

A fresh Kiran watermelon variety of *Citrullus lanatus* is selected and purchased from the markets of Guntur. The fruit was thoroughly washed using distilled water to remove dirt and pesticides, dried using a dry cloth to remove the surface contaminants, and stored at 4ºC until further steps [4, 15].

2.2. Juice Preparation

The watermelon is cut longitudinally using a stainless-steel knife. The red portion of the fruit is utilized for further processing. Hence white rinds are separated from the red part. The resulting red part is cut into smaller pieces. The juice was extracted using a Preethi Zodiac Centrifugal Juicer. Most of the juice is squeezed out, leaving behind the pulp. A double-layered muslin cloth filters the extracted juice [14].

2.3. Standardization

The Total Soluble Solids are calculated for the prepared juice, and an appropriate adjustment is made with sugar and water. Three samples of different fruit juice content (10%, 12%, 15%) were prepared with a 15º Brix, which were immediately packed in pre-sterilized glass bottles to undergo pasteurization [16]. Sensory evaluation is performed to identify an appropriate sample for carbonation. The beverage is prepared with 10% fruit content and various levels of TSS (10º, 12º, 14º). The juice containing 10% fruit content and 10º Brix is chosen for further evaluation [17].

2.4. TSS Adjustment Calculation

The Total soluble solids are adjusted according to the product being developed. Initially, Brix is corrected with the known quantities of Fruit juice and water.

For 300ml of Juice preparation consisting of 10% Fruit content and 10% TSS

Required Juice: $\frac{10}{100} \times 300\ mL = 30\ mL$

TSS of Fresh Watermelon juice: 8%

TSS content present in the juice: $\frac{8}{100} \times 30\ mL = 2.4\ mL$

The final TSS in the juice is 10% as per FSSAI standards. Then the amount of sugar to be added will be $0.1 \times 300 - 2.4 = 27.6g$

2.5. Mixing

All the ingredients will be added to the watermelon juice and the required amounts of sugar and water. These ingredients undergo mixing in the Preethi Zodiac Blender, ensuring a proper mix of all the constituents and proper distribution of particles. Therefore, the prepared juice is checked for TSS, Titrable Acidity, Color, Microbial Analysis, pH, and Specific Gravity [18, 19].

2.6. Pasteurization

The homogenized juice samples are held in pre-sterilized glass bottles. Pasteurization is performed using a water bath equipped with a temperature setting. The pasteurization temperature is adjusted to 74ºC for 45secs. The internal temperature of glass bottles is monitored using a thermometer. Time and temperature combinations are observed and are cooled to inhibit the cooking process. Further, they are stored at 7ºC to complete the pasteurization process [20].

2.6. Carbonation

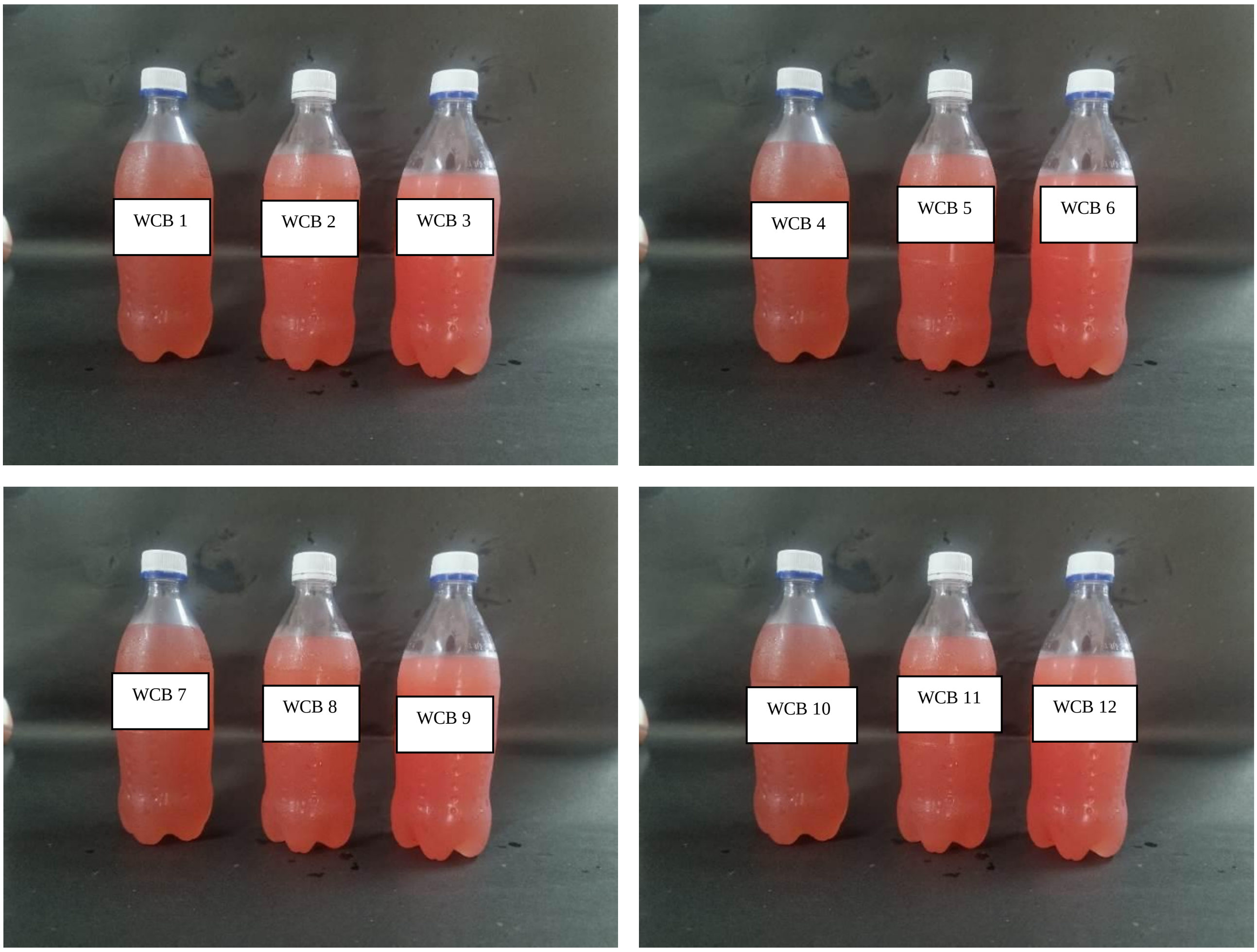
The carbonation is performed at various levels of pressure, viz. 80, 90, and 100 [21] were accomplished on a manually operated carbonation machine. The prepared beverage is filled in specially designed containers that hold 300ml and are placed in the unit. Carbon dioxide is plunged into the juice and set at pressure, and it is sealed immediately with airtight caps [17, 22, 23].

2.6.1 Sample Composition

Fig,1 Samples Prepared and their composition

Table.1 Sample Composition for 200 mL Carbonated Watermelon Juice (WCB)

	Week 1	**Week 2**	**Week 3**	**Week 4**
60 PSI	WCB 1	WCB 4	WCB 7	WCB 10
80 PSI	WCB 2	WCB 5	WCB 8	WCB 11
100 PSI	WCB 3	WCB 6	WCB 9	WCB 12



3. Physicochemical Analysis

3.1. TSS Measurement

A handheld refractometer is utilized to calculate the Total Soluble Solids present in the juice. Before utilization, the instrument is calibrated to zero with the help of distilled water [16, 24].

3.2. Titrable Acidity

The total Titratable Acidity of this juice is estimated by titrating the juice sample against a Standard Alkali of 0.1N of NaOH in the presence of a phenolphthalein indicator [2]. The sample of 20 mL juice is made up to 50 mL by adding water. The 10 mL of sample aliquot is taken in a conical flask to which a few drops of phenolphthalein indicator are added and titrated against 0.1N of NaOH [14, 15].

$$\text{Total Acidity (\%)} = \frac{Titre\ Value \times Normality\ of\ NaOH \times Volume\ made\ up \times Equivalent\ weight\ of\ acid}{Volume\ taken\ for\ estimation \times Weight\ of\ Sample \times 100}$$

3.3. pH

The pH values of the prepared juice sample were analyzed using a pH meter at room temperature [22, 25]. Before testing, the pH meter is calibrated using a standard buffer solution [15].

3.4. Specific Gravity

An empty specific gravity bottle is used to calculate the specific gravity of the sample. Initially, the weight of the empty bottle is recorded, regarded as (A_{sg}), along with the water being filled and measured again, which is observed as (B_{sg}). The sample weight is determined by filling the bottle with carbonated watermelon juice (C_{sg}). Specific gravity is finally calculated as [16, 21]

$$\text{Specific Gravity} = \frac{C_{sg} - A_{sg}}{B_{sg} - A_{sg}}$$

3.5. Sensory Evaluation

Prepared Watermelon Carbonated beverages undergo sensory evaluation for freshly prepared and stored samples. They were evaluated for color, texture, aroma, taste, mouthfeel, and overall acceptability. A 9-point hedonic scale is suggested by [22], ranging from (9) immensely liking to (1) extremely dislike. A total of 10 semi-trained panelists were selected to perform the sensory evaluation. The evaluation was performed for three different samples for seven days, based on the pressure exerted by the CO_2 on the Carbonated beverage prepared [14, 23].

3.6. Free Radical Scavenging Activity using DPPH Method

Initially, prepare the DPPH solution by mixing 2 mg in 50 mL of 99 % Methanol and storing it in a dark and cool place. Preparation of samples of various concentrations involves the addition of 0.1 µl Carbonated Watermelon Juice in 0.4 µl of Methonal to which 950 µl of DPPH is added. Similarly, such concentrations are prepared until 0.3 µl of Carbonated Watermelon Beverage. After adding DPPH, the samples are incubated in a dark room for 30 min. After the incubation, the samples are analyzed for the % DPPH Activity with the help of absorbance collected in UV-Vis Spectroscopy at 517 nm. The absorbance values increase with the increase in concentration. Concerning the absorbance, the % DPPH Activity is calculated. Before checking the sample's absorbance, the UV-Vis Spectroscopy is calibrated with the help of a Blank and Control Sample. The blank is the methanol sample, and the Control sample is a Methanol and DPPH solution composition.

$$\%\ DDPH\ Activity = \frac{Ab_{(Control)} - Ab_{(Sample)}}{Ab_{(Control)}} \times 100$$

3.7. Viscometer Analysis

The pulp's viscosity was determined using a Brookfield viscometer and spindle no. 3 (Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 30 revolutions per minute. The result is stated in centipoise (cp).

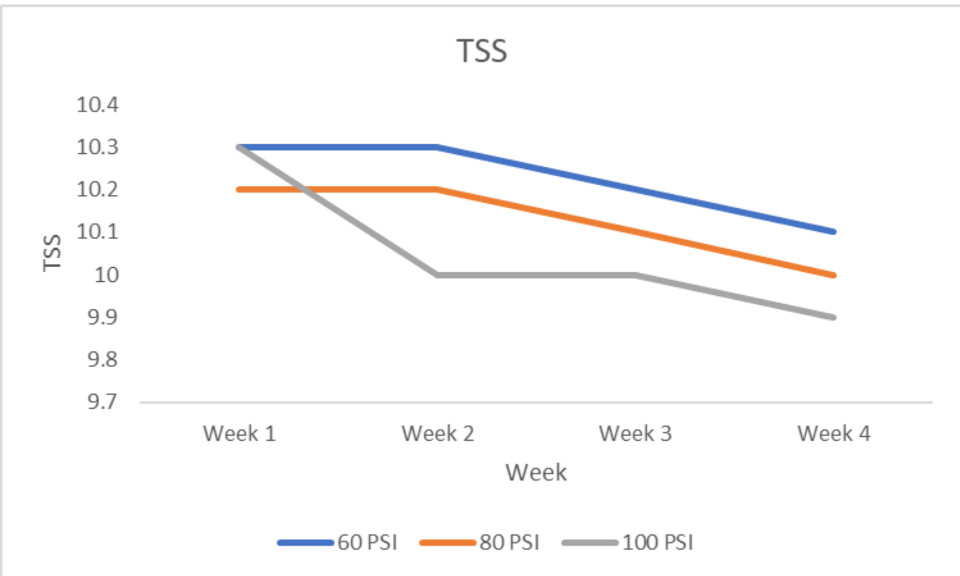
4. Results & Discussions

4.1. TSS

The Total Soluble Solids of the Watermelon Juice remained the same in most cases, with no significant change throughout 4 weeks. The prepared sample has 10º Brix at the initial stages, dropping to over 9.9 º Brix. This represents the minimal change in the medium's sugar composition and fruit content. After the required time, the product displayed various changes in the medium, such as coagulation and the product's aroma.

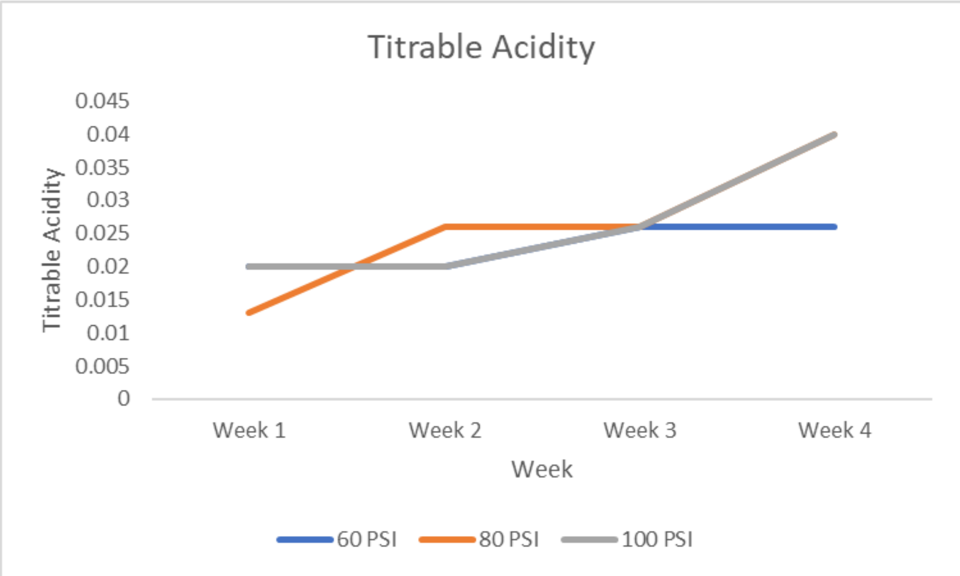
Table.2 TSS Results (º Brix) Fig.2 TSS Analysis

	Week 1	Week 2	Week 3	Week 4
60 PSI	10.3	10.3	10.2	10.1
80 PSI	10.2	10.2	10.1	10
100 PSI	10.3	10	10	9.9



4.2. Titrable Acidity

Titrable Acidity discusses the amount of acid percentage in the prepared food component. This particular research focuses on preparing a carbonated fruit beverage, including watermelon. As watermelon is a good source of malic acid, the equivalent weight of this particular acid is 134.09 g/mol. The sample had lower acid content and acidity levels in the initial stages but increased drastically over time. This clearly states that the sample has good sensory properties during the initial weeks, and after week 3, the sample is unsuitable for consumption. The samples prepared with 60 PSI and 100 PSI put forward a good amount of Acidity, whereas the sample prepared with 80 PSI failed to retain the acidity and showed an increase in the levels right from Week 2. Here, the samples prepared from 60 and 100 PSI are



good enough to carry out other physicochemical processes.

Fig.3 Titrable Acidity

Table.3 Titrable Acidity Results (g/L)

	Week 1	Week 2	Week 3	Week 4
60 PSI	0.02	0.02	0.026	0.026
80 PSI	0.013	0.026	0.026	0.04
100 PSI	0.02	0.02	0.026	0.04

4.3. pH

Watermelon Carbonated beverage samples showcased their acidic nature when tested using a pH meter. This confirms the presence of Carbon dioxide, thereby extending the shelf-life of the particular juice material. The pH levels spiked in the initial days when the sample was prepared. Over time, the pH level has dropped, and a constant range is observed. The juice can be termed moderately acidic, which is feasible for consumption. Errors in packaging and storage can cause a decline in the pH. The pH value indicates that the carbonation has been appropriately achieved.

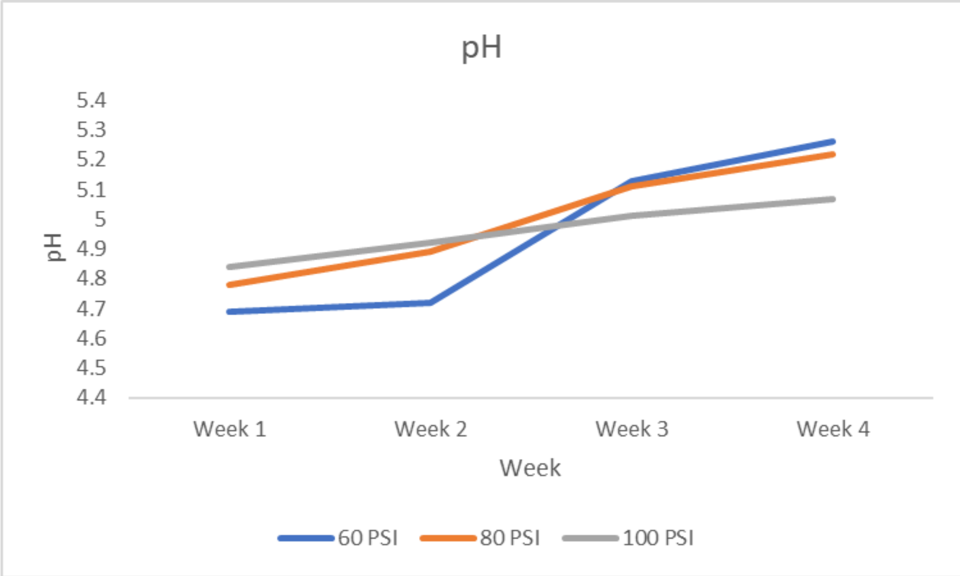


Fig.4 pH Analysis

Table.4 pH Results

	Week 1	**Week 2**	**Week 3**	**Week 4**
60 PSI	4.69	4.72	5.13	5.26
80 PSI	4.78	4.89	5.11	5.22
100 PSI	4.84	4.92	5.01	5.07

4.4. Specific Gravity

The prepared samples showcased nominal differences regarding Specific Gravity in all the cases. The Specific gravity in the case of a watermelon carbonated beverage depends on the fruit content and sugar solids. Samples prepared have similar specific gravities due to the extended storage life due to adding CO_2. The Carbon dioxide that has been added inhibits the growth of various microorganisms and thereby accelerates the product's shelf-life.

Table.5 Specific Gravity Results

	Week 1
60 PSI	1.04
80 PSI	1.044
100 PSI	1.042

4.5. Sensory Evaluation

The sensory evaluation is done concerning 9 points hedonic scale with 6 different evaluators to bring out the results. The mean of the scores have been taken and put up in Table.6. The color of all the samples remained good till week 3, irrespective of the carbon-dioxide levels. This clearly states that Carbonation does not affect the color aspects of the product. Coming to the appearance, the sample prepared from 100 PSI held well during the storage conditions and time. Regarding taste, 80 PSI and 100 PSI are good enough and have good thirst-quenching properties. The sample of 60 PSI did not give enough feel and savory upon consumption. In terms of aroma, all the samples showcased a fruity flavor and fresh odor, making them uncomfortable for consumption. The sample of 100 PSI had a pleasing aroma and felt due to the high amount of pressure added to the juice.

As mentioned earlier, higher amounts of PSI indicate a good amount of mouthfeel. Finally, the samples did not score well when discussing the overall acceptability. The only sample of 100 PSI put forward a good score for overall acceptability. Over 3 Weeks, the sample deteriorated quickly. 100 PSI samples had good pressure to retard the physical and chemical changes, thereby reducing the damage in the juice prepared, making it convenient to consume even after 3 Weeks.

Table.6 Sensory Evaluation for 60 PSI

	Week 1	Week 2	Week 3	Week 4
Color	8	8	8	7
Appearance	7.6	7.6	7.3	6.3
Taste	6.3	6.3	6.3	6
Aroma	7	7.3	7	4
Mouthfeel	7.6	6.6	6.6	5
Overall Acceptability	7.6	7.3	7	5.3

Table.7 Sensory Evaluation for 80 PSI

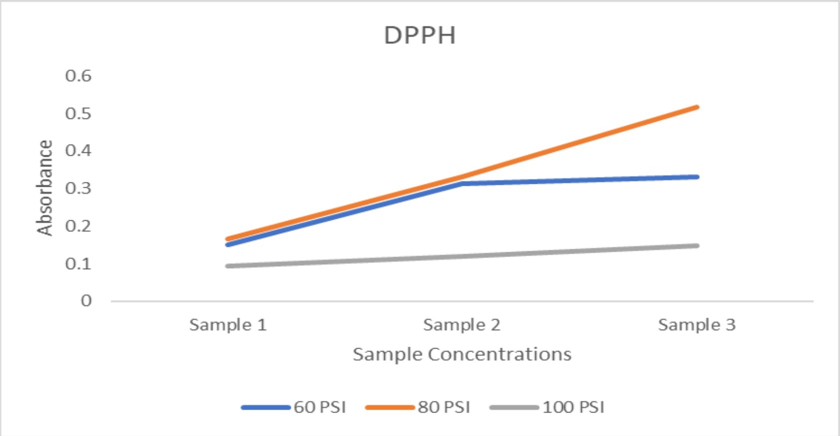
	Week 1	Week 2	Week 3	Week 4
Color	8.3	8.3	8	6.6
Appearance	7.3	7	7	6.3
Taste	8.3	8	7.3	6
Aroma	7.6	7.3	6.6	5.3
Mouthfeel	8	7.3	6.3	6
Overall Acceptability	7.6	7.3	7	5

Table.8 Sensory Evaluation for 100 PSI

	Week 1	Week 2	Week 3	Week 4
Color	8.3	8	8	7.3
Appearance	8	7.3	7.3	7.3
Taste	8.6	8.3	8	7
Aroma	8	8	7.6	7
Mouthfeel	8.3	8.3	8	7.3
Overall Acceptability	8.6	8.3	8.3	6.3

4.6. Free Radical Scavenging using DPPH Method

The Free Radical Scavenging using DPPH Method focuses on the antioxidant property of the lycopene in the watermelon juice. Lycopene destroys the free radicals present in a body or a system. The DPPH, as a free radical, analyses the potentiality of the particular compound. The antioxidant activity is thereby understood concerning absorbance values. The absorbance keeps increasing with the increase in the concentration of the sample. The samples range from 0.1 µl to 0.3 µl. This proves that carbonation is an excellent potential preservation method to increase the shelf-life of prepared watermelon juice. Thereby helping keep up the



good nutritional and health benefits of the sample.

Fig.5 DPPH Analysis

Table.9 DPPH Analysis (Absorbance)

	Sample 1	Sample 2	Sample 3
60 PSI	0.1517	0.3141	0.3314
80 PSI	0.1655	0.3312	0.5178
100 PSI	0.0944	0.1199	0.1473

4.7. Viscometer Analysis

The viscometer readings did not showcase a significant change in the system and were between 3.13 to 4.62 (cp). The sudden change in the medium is due to the physicochemical changes and spoilage in the juice. Over the period, samples have seen coagulation and changes in flavor and aroma, which are indications of spoilage in the juice, causing a sudden rise in the viscosity of the juice.

Table.10 Viscometer Analysis (cp)

	Week 1	Week 2	Week 3	Week 4
60 PSI	3.16	3.41	4.01	4.53
80 PSI	3.19	3.46	4.22	4.62
100 PSI	3.13	3.42	3.85	4.03

5. Conclusion

The prepared samples showcased different levels of correctness over the period. Concerning the analysis, we can conclude that the sample that utilized a higher amount of Carbon dioxide could keep up the juice's shelf-life. The higher PSI, between 80 and 100 levels, showcased many sensory properties, taste, and overall acceptability. Initially, when standardization happened, the samples were analyzed for the sensory and the ability to be carbonated. The conclusion has dropped to lower levels of TSS and Fruit content. This is because carbonation can only be achieved when a particular sample has a lower viscosity and can hold down the gas. During the carbonation process, the sample must be chilled to the lowest level so that the gas will be perfectly balanced and incorporated into the juice. If the sample does not have the proper temperature, it fails to get carbonated, and during the shelf-life, the gas levels decrease. pH levels also have even shot up during the analysis, concluding that the samples have undergone the required carbonation. Over time, the pH changed from lower to moderate levels, showcasing that the gas was escaping from the medium or that the sample was undergoing various changes concerning time. The titrable acidity focused upon the malic acid present in the watermelon.

Along with that, the titrable acidity also includes the presence of CO_2. The 60 PSI samples failed to reach a carbonated beverage's standards during the sensory evaluation. It failed to give a carbonated juice's properties or thirst-quenching qualities. The juice prepared is a clear idea of a sample prepared directly from a fruit, not concentrate. Over the studies and in this experiment, we can conclude that the sample prepared holds down the fruit and fresh odor, which need to be looked into to produce a better-standardized juice. The shelf-life must be focused on for much longer by including various other preservatives if possible.

References

[1] G. Alka, S. Anamika, and P. Ranu, "A review on watermelon (Citrullus lanatus) medicinal seeds," *Journal of Pharmacognosy and Phytochemistry,* vol. 7, no. 3, pp. 2222-2225, 2018.

[2] M. Alam, M. Hoque, S. Morshed, S. Shahriar, and A. Begum, "A study on watermelon (Citrullus Lanatus) juice preserved with chemical preservatives at refrigeration temperature," *Journal of Environmental Science and Natural Resources,* vol. 5, no. 2, pp. 23-28, 2012.

[3] A. Ahmad, R. L. Prasad, D. Kumar, N. Singh, V. Prasad, and A. Paul, "An economic analysis of production of watermelon in Allahabad District, Uttar Pradesh India," *Seed (Kg),* vol. 3, no. 2.8, p. 2.5, 2017.

[4] S. Aghajanzadeh, A. M. Ziaiifar, and M. Kashaninejad, "Influence of thermal treatment, homogenization and xanthan gum on physicochemical properties of watermelon juice: A response surface approach," *LWT-Food Science and Technology,* vol. 85, pp. 66-74, 2017.

[5] B. Ozcelik and M. Yavuz, "Watermelon Juice," in *Handbook of Functional Beverages and Human Health*: CRC Press, 2016, pp. 577-586.

[6] W. S. Choo and W. Y. Sin, "Ascorbic acid, lycopene and antioxidant activities of red-fleshed and yellow-fleshed watermelons," *Advances in applied Science research,* vol. 3, no. 5, pp. 2779-2784, 2012.

[7] F. C. Taylor, A. Satija, S. Khurana, G. Singh, and S. Ebrahim, "Pepsi and Coca Cola in Delhi, India: availability, price and sales," *Public health nutrition,* vol. 14, no. 4, pp. 653-660, 2011.

[8] N. D. Solanke, S. Sontakke, and S. Verma, "Study on effect of carbonation on the properties of fruit juices," *International Journal of Current Microbiology Applied Sciences,* vol. 4, pp. 2426-2432, 2017.

[9] J. Tahmassebi and A. BaniHani, "Impact of soft drinks to health and economy: a critical review," *European archives of paediatric dentistry,* vol. 21, no. 1, pp. 109-117, 2020.

[10] T. Johnson, L. Gerson, T. Hershcovici, C. Stave, and R. Fass, "Systematic review: the effects of carbonated beverages on gastro-oesophageal reflux disease," *Alimentary pharmacology & therapeutics,* vol. 31, no. 6, pp. 607-614, 2010.

[11] D. R. Azeredo, V. Alvarenga, A. S. Sant'Ana, and A. U. S. Srur, "An overview of microorganisms and factors contributing for the microbial stability of carbonated soft drinks," *Food Research International,* vol. 82, pp. 136-144, 2016.

[12] N. Pareek, A. Gupta, and R. Sengar, "Preparation of healthy fruit based carbonated whey beverages using whey and orange juice," *Asian Journal of Dairying & Foods Research,* vol. 33, no. 1, pp. 5-8, 2014.
[13] C. Bhattacharjee, V. Saxena, and S. Dutta, "Novel thermal and non-thermal processing of watermelon juice," *Trends in Food Science & Technology,* vol. 93, pp. 234-243, 2019.
[14] S. Yıkmış, "Sensory, physicochemical, microbiological and bioactive properties of red watermelon juice and yellow watermelon juice after ultrasound treatment," *Journal of Food Measurement and Characterization,* vol. 14, no. 3, pp. 1417-1426, 2020.
[15] I. O. ACHAM, M. O. EKE, and J. EDAH, "Physicochemical, microbiological and sensory quality of juice mix produced from watermelon fruit pulp and baobab fruit pulp powder," *Croatian journal of food science and technology,* vol. 12, no. 1, pp. 48-55, 2020.
[16] A. A. Noah and A. Lawal, "EFFECT OF PASTEURIZATION ON THE MICROBIAL, PHYSICOCHEMICAL AND SENSORY PROPERTIES OF WATERMELON JUICE," 2018.
[17] A. Mehtre, S. Thorat, A. Joshi, and P. Chilkawar, "Standardization of carbonated RTS beverage from pomegranate fruits juice," *BIOINFOLET-A Quarterly Journal of Life Sciences,* vol. 9, no. 2, pp. 111-113, 2012.
[18] Y. Wang, W. Li, Y. Ma, X. Zhao, and C. Zhang, "Effect of thermal treatments on quality and aroma of watermelon juice," *Journal of Food Quality,* vol. 2018, 2018.
[19] C. Thongrote, T. Wirjantoro, and A. Phianmongkhol, "Effect of carbonation sources and its addition levels on carbonated mango juice," *International Food Research Journal,* vol. 23, no. 5, 2016.
[20] K. Aganovic *et al.*, "Pilot scale thermal and alternative pasteurization of tomato and watermelon juice: An energy comparison and life cycle assessment," *Journal of cleaner production,* vol. 141, pp. 514-525, 2017.
[21] M. F. B. Shaikh and S. D. Rathi, "Utilisation of buttermilk for the preparation of carbonated fruit-flavoured beverages," *International journal of dairy technology,* vol. 62, no. 4, pp. 564-570, 2009.
[22] B. S. Agarkar, P. Aggarwal, and M. Yaqoob, "Preparation of carbonated sugarcane juice beverages blended with fruit juices," *Agricultural Research Journal,* vol. 56, no. 1, pp. 124-133, 2019.
[23] S. Nale, R. Patil, S. Masalkar, and R. Satbhai, "Standardization of carbonated beverage prepared from pulp and juice of guava fruit," *Journal of Agriculture Research and Technology,* vol. 36, no. 2, pp. 255-258, 2011.
[24] T. Abhinav and S. A. Prakash, "Development of fruit juice blended carbonated beverages," *J Management Research Analysis,* vol. 2, no. 3, pp. 185-189, 2015.
[25] C. Chhaya, P. Rai, G. Majumdar, S. Dasgupta, and S. De, "Clarification of watermelon (Citrullus lanatus) juice by microfiltration," *Journal of food process engineering,* vol. 31, no. 6, pp. 768-782, 2008.