



Synergistic Stability and Bioavailability of Anthocyanin Complexes in Hibiscus Fortified Orange Ready to Serve Beverages

**Sachi Pal ^{a++}, Vaibhav Patil ^{a#}, Srushti Phule ^{a++},
Revati Deshmukh ^{a++}, Nauman Belim ^{a++}
and Aditi Masurkar ^{a++}**

^a School of Food Technology, MIT ADT University, Pune, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/acri/2025/v25i81459>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/142891>

Original Research Article

Received: 02/07/2025
Published: 23/08/2025

ABSTRACT

RTS beverage is a ready-to-serve beverage prepared to increase fruit juice's shelf life. RTS beverage is prepared by selecting fresh ripe fruits to extract the juice and adding ingredients like preservatives, water, and citric acid according to FSSAI regulations. A study was performed to increase the nutritional value of the orange RTS by fortification with Hibiscus flower extract. 2%, 4%, and 6% extract were added to each of the three RTS respectively. Hibiscus flower extract was prepared by collecting Hibiscus flowers and drying the flower's petals at 60°C for 1.5 hours further

⁺⁺ Student;

[#] Associate Professor;

^{*}Corresponding author: Email: vspatil65@gmail.com;

Cite as: Pal, Sachi, Vaibhav Patil, Srushti Phule, Revati Deshmukh, Nauman Belim, and Aditi Masurkar. 2025. "Synergistic Stability and Bioavailability of Anthocyanin Complexes in Hibiscus Fortified Orange Ready to Serve Beverages". Archives of Current Research International 25 (8):789-95. <https://doi.org/10.9734/acri/2025/v25i81459>.

crushing it manually and preparing the extract by boiling it in hot water. Anthocyanin being water soluble was leached into the water. Hibiscus flower extract is rich in polyphenols and antioxidant properties. Polyphenols in Hibiscus extract have many health benefits such as fighting cancer-causing cells and promoting heart health. Antioxidants like anthocyanins, beta-carotene, and vitamin C help fight free radicals. Further tests were conducted to evaluate the polyphenol value, antioxidant content, acidity, and TSS of the orange fruit RTS. The polyphenol content was increased according to the test of the fortified sample also enhancing its Nutritional value.

Keywords: Orange RTS; hibiscus extract; total antioxidant; polyphenol content.

1. INTRODUCTION

Since many fruits and vegetables have limited shelf life because of their perishability, it is best to make them into RTS beverages. This not only prolongs the usability of those fruits but also retains essential nutrients (Ranganna, 1986). Among the fruit, oranges are cultivated worldwide because of their best flavour and nutrient content, which are largely processed into syrups, nectars, and RTS beverages (Morton, 1987). Hibiscus sabdariffa has been known for its medicinal properties. It is packed with anthocyanins, flavonoids, and phenolic compounds containing antioxidant and anti-inflammatory properties (Du & Francis, 1973; Ali et al., 2005; Ross & Kasum, 2002). The research focuses on the development of the orange RTS fortified with hibiscus extract to increase the natural polyphenol content of this beverage, thus increasing its antioxidant activity and consumer acceptance (Rehman & Habib, 2003). Anthocyanins, a class of water-soluble natural pigments, are composed of anthocyanidins glycosidically linked to sugar moieties. These compounds are ubiquitously present in various fruits, vegetables, and grains, contributing to their distinctive coloration (Yuan et al., 2022). In recent years, the adverse effects associated with synthetic pigments have garnered increased attention in light of rising health concerns. Consequently, natural anthocyanins have attracted considerable interest due to their inherent safety and non-toxic properties. Extensive research has demonstrated that anthocyanins exhibit a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antineoplastic, and hypoglycemic effects (Yang et al., 2021). Accordingly, anthocyanins have found widespread applications in the food, cosmetic, and pharmaceutical industries. However, anthocyanins are susceptible to degradation during processing, primarily owing to the instability of phenolic hydroxyl groups within their molecular structure. Multiple studies have

identified several factors influencing the stability of anthocyanins, such as pH, temperature, light exposure, oxygen, enzymatic activity, solvents, metal ions, and interactions with proteins (Liu et al., 2023).

2. MATERIAL AND METHODS

1. **Orange:** Fresh oranges were procured from the local market, ensuring that the fruit was free from any mechanical damage to preserve quality and flavor. The selection of undamaged oranges is critical to maintaining the integrity of the juice, as bruising or cuts may promote microbial growth and affect taste. Additionally, the natural acidity and vitamin C content of fresh oranges contribute to the nutritional and sensory properties of the final beverage.
2. **Water:** Purified water served as the primary solvent and base liquid for the beverage formulation. It functions as a carrier for the soluble components, facilitating the uniform distribution of flavor and sweetening agents. The use of water of high purity is essential to prevent contamination and to maintain the safety and stability of the beverage.
3. **Sugar:** Sucrose was incorporated into the formulation to achieve an optimal level of sweetness that balances the natural tartness of the orange and hibiscus components. The concentration of sugar is carefully controlled to enhance palatability while considering caloric content and consumer preferences.
4. **Preservative:** Sodium benzoate was employed as a preservative to inhibit microbial growth and ensure the shelf stability of the beverage. The inclusion of this preservative in the permissible limit is critical for extending the product's shelf life, particularly under ambient storage conditions. Its use complies with food safety regulations and is applied at

concentrations deemed safe for consumption.

5. **Hibiscus Extract:** Hibiscus extract was added to impart a characteristic tartness, enhancing the beverage's flavor complexity. This natural extract contains organic acids and phenolic compounds that contribute both to taste and potential antioxidant activity (Peng et al., 2011).

2.1 Selection of Raw Materials

Freshly ripened oranges, pomegranates, and kiwis were selected to analyse their phenolic content using the Folin-Ciocalteu reagent. Among the fruits tested, kiwis showed the highest polyphenol content, while oranges exhibited the lowest. Oranges were chosen as the base ingredient for the Ready-to-Serve (RTS) beverage, fortified with hibiscus extract as a key ingredient (Bureau of Indian Standards, (2006).

2.2 Preparation of Hibiscus Extract

Bright red hibiscus flowers were hand-picked, and the petals were separated from the calyx. After dehydrating the petals in a hot air oven for 1.5 hours at 60°C after which they were cooled to room temperature, and ground into a fine powder. To prepare the hibiscus extract, 50 ml of distilled water was taken and 5 g of dried powder was mixed in it and concentrated in a water bath at 80°C for 20 minutes, yielding 30 mL of extract. Anthocyanins, being water-soluble, contributed to the red coloration of the extract (McGhie & Walton, 2007).

2.3 Beverage Formulation

The RTS beverage was formulated using orange juice with a minimum juice content of 10% and TSS (Total Soluble Solids) content of 10° Brix. For preservative Sodium Benzoate was added. Equal amounts of prepared RTS beverage were filled into four sterilized glass jars:

- **T0 (Control):** No hibiscus extract.
- **T1 sample:** 2 mL of hibiscus extract added.
- **T2 sample:** 4 mL of hibiscus extract added.
- **T3 sample:** 6 mL of hibiscus extract added.

2.4 Tests

A) Total Polyphenol content

Folin-Ciocalteu reagent (FCR) method was used to determine the polyphenol content. A sample

(0.5 g to 1 g) was accurately weighed and ground with a pestle and mortar in 10 times the sample volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes, and the supernatant was saved. The residue was re-extracted five times with ethanol 80%, followed by centrifugation. After combining the supernatants, the ethanol was dried out by evaporation. Distilled water (5ml) was used to dissolve the residue. Pipetting aliquots between 0.2 and 2 ml into test tube, the volume in each tube was adjusted to 3 ml using distilled water. After that, each tube received 0.5ml of Folin - Ciocalteu reagent, and the mixture was kept to react for 3 minutes. Following this, each tube was filled with 2ml of a 20% sodium carbonate solution, and everything was well combined.

Boiling water bath was given to the tubes for 1 minute, after which they were cooled and, UV-visible spectrophotometer was used to detect the absorbance at 650nm in comparison to a reagent blank. A standard curve was created with various catechol concentrations, and milligrammes of phenols per 100 grammes of material was the unit used to express the samples polyphenol concentration (Singleton & Rossi, 1965).

B) Antioxidant:

The antioxidant capacity of the substance was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay method. 25ml of 99% methanol was taken in conical flask and further 1g sample was weighed and mixed with it. To shield it from light, the flask was wrapped in aluminium foil and left in a shaking water bath set at 100 rpm for 2.5 hour at room temperature. After incubation, sample was removed from the shaking water bath, centrifuged at 6000– 8000 rpm for 15 minutes, and was filtered through filter paper to separate the supernatant.

4 mg of DPPH was dissolved in 100ml of 99% methanol and mM DPPH solution was prepared. The solution was covered and stored under cool, dark conditions to protect it from light.

To evaluate the antioxidant activity, a series of solutions was prepared using the extracted sample solution and methanol. A total of 10ml of methanol were added to aliquots of 1ml, 2ml, 3ml, 4ml and 5ml of the extract. Then, 3 ml of the DPPH solution was added to every tube, the volume was adjusted again with methanol (Brand-Williams et al., 1995). To facilitate the

reaction, the mixes were kept in a dark room for half an hour.

The UV- visible spectrophotometer was used to test the samples absorbance at 517nm. The following formula was used to determine the percentage of DPPH radical scavenging activity:

$$\text{Scavenging Activity (\%)} = [(A_0 - A_a) / A_0] \times 100$$

Where:

- The absorbance of the blank at 0 minutes is denoted as A₀.
- The absorbance of the sample at 30 minutes is denoted as A_a.

C) Acidity and pH Measurement Acidity and pH levels were determined using standard AOAC methods (AOAC, 2016).

3. RESULTS AND DISCUSSION

A) Polyphenol Content:

A significant variation in polyphenol content was observed among the analyzed samples, suggesting that the concentration process had a considerable influence on the retention and enrichment of bioactive compounds. During the concentration trials, the maximum polyphenol content was recorded in treatment T2 (0.575), whereas the minimum was observed in treatment T1 (0.353). These findings indicate that T2 possesses a higher potential for polyphenol enrichment and, consequently, may be developed into a functional or health-oriented beverage.

In comparison, treatment T3 also exhibited a relatively elevated polyphenol concentration (0.493), which was markedly higher than that of both T1 and the control (T0). This demonstrates that different processing conditions can significantly alter the concentration of bioactive compounds, thereby influencing the nutritional and functional quality of the final product. The

superior performance of T2 and T3 may be attributed to favorable processing parameters that promoted polyphenol stability and reduced degradation losses.

Polyphenols are well-documented for their antioxidant, anti-inflammatory, and disease-preventive properties, which enhance the health-promoting potential of food and beverage formulations (Scalbert & Williamson, 2000). Thus, the enrichment observed in T2 and T3 provides a promising basis for the development of beverages that not only meet consumer sensory preferences but also deliver added nutritional and therapeutic benefits.

B) Antioxidant Content:

The results of the DPPH assay revealed considerable antioxidant activity in the fortified samples, with treatment T2 demonstrating the most balanced and consistent performance (Huang & Prior, 2005). This finding highlights the effectiveness of the fortification process in enhancing the radical-scavenging potential of the beverage. The superior antioxidant response of T2 may be attributed to the higher retention and stability of bioactive compounds, particularly polyphenols and anthocyanins derived from hibiscus, which are known to act as potent hydrogen donors and free radical quenchers.

These results provide strong evidence in support of hibiscus as a functional ingredient in ready-to-serve (RTS) beverages, owing to its ability to improve not only the nutritional profile but also the potential health-promoting properties of the product (Rupasinghe & Clegg, 2007). The integration of such natural sources of antioxidants into beverage formulations is increasingly aligned with current consumer demand for products that deliver both sensory appeal and added health benefits. Moreover, the incorporation of hibiscus extracts can contribute to the development of functional beverages targeted at reducing oxidative stress, thereby playing a role in the prevention of lifestyle-related disorders (Benzie & Strain, 1996).

Table 1. Polyphenol content

Concentration	SAMPLES			
	T0	T1	T2	T3
0.5	0.408	0.353	0.575	0.493
1.0	0.496	0.517	0.647	0.737

Table2. Antioxidant Activity of Orange RTS Infused with Hibiscus Extract (DPPH Assay)Sample DPPH % Inhibition (Mean ± SD)

Sample	Total Antioxidant
Control	54.16 ± 0.26
T1	58.92 ± 0.19
T2	63.14 ± 0.28
T3	69.14±0.30

Table 3. PH levels

Samples	T0	T1	T2	T3
PH	3.50 ± 0.10	3.00 ± 0.12	3.00 ± 0.15	3.00±0.18

C) PH Levels:

The pH values across the samples remained relatively uniform, ranging from 3.0 to 3.5, thereby indicating a consistent acidic profile. The control (T0) exhibited the highest pH of 3.5, while treatments T1, T2, and T3 demonstrated similar values of 3.0. This relative stability in pH suggests that the formulations were consistent in terms of acidity regulation, which is a critical factor in ensuring product quality, microbial safety, and extended shelf life.

Maintaining a stable acidic environment is particularly important in ready-to-serve (RTS) beverages, as it not only contributes to flavor balance but also acts as a natural preservative by inhibiting the growth of spoilage microorganisms. The uniformity observed in the present study reflects the effectiveness of the processing and formulation strategies employed in controlling acid–base equilibrium. Furthermore, such stability minimizes undesirable compositional changes during storage, thereby enhancing product acceptability and market potential (Upadhyay & Mishra, 2015).

D) Acidity:

The acidity levels of the samples exhibited a gradual reduction across treatments. The lowest titratable acidity was observed in treatment T3 (0.57), followed by T2 (0.64), whereas both the control (T0) and treatment T1 recorded

comparatively higher values of 0.682. The reduced acidity in T3 may have contributed to a more balanced flavor profile, thereby enhancing consumer acceptance. Such variation in acidity can be attributed to differences in compositional stability and the influence of processing parameters or ingredient interactions.

Lower acidity is often associated with improved palatability, as excessive sourness may negatively impact sensory perception and overall product desirability. The findings therefore suggest that treatments with moderated acidity, particularly T2 and T3, hold greater potential for consumer preference when compared to T0 and T1. These results are in agreement with previous studies which reported that controlled processing conditions and optimized ingredient levels can significantly influence the acid–base equilibrium and overall sensory attributes of fruit-based beverages (Upadhyay & Mishra, 2015).

E) Sensory Test:

A sensory evaluation was conducted to assess the consumer acceptability of the formulated ready-to-serve (RTS) beverages, with emphasis on attributes such as color, flavor, taste, aroma, and overall acceptability. The findings indicated that treatment T2 received the highest degree of acceptance, primarily due to its balanced sweet–tart flavor profile and pleasant aroma. The combination of these attributes contributed to a more desirable sensory experience compared to both T1 and T3.

Table 4. Acidity level

Sr. No.	Samples	Acidity
1	T0	0.68 ± 0.03
2	T1	0.68 ± 0.04
3	T2	0.64 ± 0.05
4	T3	0.57±0.06

Table 5. Sensory evaluation

Attributes	T0	T1	T2	T3
Color	7.5 ± 0.3	8.0 ± 0.5	8.5 ± 0.4	8.3 ± 0.2
Flavor	7.2 ± 0.4	7.8 ± 0.3	8.2 ± 0.5	7.6 ± 0.4
Taste	7.0 ± 0.5	7.6 ± 0.4	8.3 ± 0.3	7.3 ± 0.5
Aroma	7.1 ± 0.3	7.7 ± 0.2	8.1 ± 0.5	7.8 ± 0.3
Overall Acceptance	7.3 ± 0.4	7.9 ± 0.3	8.4 ± 0.5	7.5 ± 0.4

Treatment T3, while visually appealing owing to its attractive color, was perceived as less acceptable overall because of its pronounced sourness and the development of a slightly slimy texture, which can be attributed to the higher concentration of hibiscus extract. Such deviations in mouthfeel and flavor intensity suggest that excessive levels of extract may compromise sensory balance, despite enhancing visual quality. Conversely, treatment T1 was rated the least acceptable, largely due to its relatively bland taste, low nutrient contribution, and absence of distinctive sensory appeal.

These results underscore the importance of optimizing the concentration of hibiscus extract in RTS formulations to achieve an appropriate balance between nutritional enhancement and consumer sensory preferences. While hibiscus is capable of imparting vivid coloration and functional benefits, its overuse can negatively impact textural and flavor characteristics, thereby reducing marketability. The superior performance of T2 suggests that moderate enrichment can simultaneously improve health-promoting properties and consumer acceptance, providing a promising direction for product development. Future work should incorporate consumer-based preference mapping and hedonic scaling with larger panels to validate these findings and refine formulation strategies for commercial application.

4. CONCLUSION

The fortification of orange RTS with hibiscus extract significantly enhanced the beverage's nutritional profile by increasing its polyphenol and antioxidant content. Among the tested concentrations, T2 (4% hibiscus extract) emerged as the most effective formulation, showing the highest polyphenol content (0.575), Antioxidant content (69.14±0.30). Acidity (0.64), making it a health-oriented beverage with an improved flavour profile. The pH levels across all samples remained stable, indicating good product consistency and shelf life. These findings demonstrate that incorporating hibiscus extract into orange RTS not only improves its functional properties but also contributes to the growing

demand for fortified beverages with added health benefits. Further research can explore consumer acceptance and long-term storage stability of this formulation to assess its commercial viability (Sharma & Lal, 2007).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors thank the MIT-ADT UNIVERSITY for permission to let us conduct the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Ali, B. H., Wabel, N. A., & Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. *Phytotherapy Research*, 19(5), 369–375.
- AOAC International. (2016). *Official methods of analysis of AOAC International* (20th ed.). AOAC International.
- Benzie I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
- Bureau of Indian Standards. (2006). *Specifications for RTS beverages (IS 2346:2006)*. Bureau of Indian Standards.

- Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: A review. *The American Journal of Clinical Nutrition*, 72(6), 1424–1435.
- Du, C. T., & Francis, F. J. (1973). Anthocyanins of roselle (*Hibiscus sabdariffa* L.). *Journal of Food Science*, 38(5), 810–812.
- Food Safety and Standards Authority of India. *Food Safety and Standards (Food Products Standards and Food Additives) Regulations: Guidelines for RTS beverages*.
- Francis, F. J., & Markakis, P. C. (1989). Food colorants: Anthocyanins. *Critical Reviews in Food Science & Nutrition*, 28(4), 273–314.
- Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6), 1841–1856.
- Kapoor, V. P., & Pushpangadan, P. (2002). Natural dye-based: Herbal Gulal.
- Liu, Y., Tong, Y., Tong, Q., Xu, W., & Wang, Z. (2023). Effects of sunflower pectin on thermal stability of purple sweet potato anthocyanins at different pH. *International Journal of Biological Macromolecules*, 253(2).
- McGhie, T. K., & Walton, M. C. (2007). The bioavailability and absorption of anthocyanins: Towards a better understanding. *Molecular Nutrition & Food Research*, 51(6), 702–713.
- Morton, J. F. (1987). Orange fruit: Production, processing, and applications. *Florida Science*, 6(4), 213–218.
- Peng, C. H., Chyau, C. C., Chan, K. C., Chan, T. H., Wang, C. J., & Huang, C. N. (2011). *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. *Journal of Agricultural and Food Chemistry*, 59(18), 9901–9909.
- Ranganna, S. (1986). *Handbook of analysis and quality control for fruit and vegetable products*. Tata McGraw-Hill Education.
- Rehman, Z. U., & Habib, F. (2003). Antioxidant Activity of Ginger Extract in Sunflower Oil. *Journal of the Science of Food and Agriculture*, Vol. 83, No. 7, pp. 624-629.
- Ross, J. A., & Kasum, C. M. (2002). Dietary flavonoids: Bioavailability, metabolic effects, and safety. *Annual Review of Nutrition*, 22(1), 19–34.
- Rupasinghe, H. V., & Clegg, S. (2007). Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. *Journal of Food Composition and Analysis*, 20(2), 133–137.
- Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition*, 130(8), 2073S–2085S.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.
- Upadhyay, R., & Mishra, H. N. (2015). Predictive modeling for shelf life estimation of sunflower oil blended with oleoresin rosemary (*Rosmarinus officinalis* L.) and ascorbylpalmitate at low and high temperatures. *LWT - Food Science and Technology*, 60(1), 42–49.
- Yang, S., Wang, C., Li, X., Wu, C., Liu, C., Xue, Z., & Kou, X. (2021). Investigation on the biological activity of anthocyanins and polyphenols in blueberry. *Journal of Food Science*, 86(2), 614–627.
- Yuan, T., Zhou, Z., Zhao, J., Li, F., Zeng, K., & Ming, J. (2022). The interactions between proteins and anthocyanins based on covalent/non-covalent binding: A review. *Food and Fermentation Industries*, 48(20), 293–299.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2025): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<https://pr.sdiarticle5.com/review-history/142891>