

Development of Orange RTS by fortification with Hibiscus extract.

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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 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). 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).

2. Materials and Methods

- 1] Orange - From the local market which was fresh with no mechanical damage
- 2] Water- Base liquid for Beverage
- 3] Sugar- To Balance the sweetness
- 4] Preservative- To ensure shelf stability (i.e. -sodium benzoate)
- 5] Hibiscus Extract- It will give a slight tart or tangy taste.

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.

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).

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.

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 and 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.

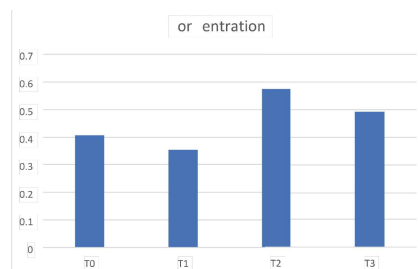
C) Acidity and pH Measurement Acidity and pH levels were determined using standard AOAC methods (AOAC, 2016).

3. Results and Discussion

A) Polyphenol content:

There was a great difference in polyphenol content among the samples. During concentration trials, the highest polyphenol content was observed at T2 concentration of 0.575 while the lowest was at T1 with 0.353. This shows that T2 can be potentially enriched with polyphenols and has the potential to become a health-oriented beverage. T3 also recorded a higher content of polyphenols at 0.493 when compared with T1 and T0 (Scalbert & Williamson, 2000).

Concentration	SAMPLES Av Average Absorbance (nm)			
	T0	T1	T2	T3
0.5	0.408	0.353	0.575	0.493
1.0	0.496	0.517	0.647	0.737



B) ANTIOXIDANT CONTENT:

The DPPH assay revealed notable antioxidant activity in fortified samples, with T2 showing the most balanced performance. This supports hibiscus's role as a functional ingredient in RTS beverages (Vasanth Rupasinghe & Clegg, 2007).

Table no 2:- Antioxidant Activity of Orange RTS Infused with Hibiscus Extract (DPPH Assay) Sample DPPH % Inhibition (Mean ± SD)

Sample	Inhibition %
Control	54.16 ± 0.26
T1	58.92 ± 0.19
T2	63.14 ± 0.28
T3	69.14 ± 0.30

C) PH Levels

PH data across the samples were relatively uniform, ranging from acidic to between 3.0 and 3.5. The highest pH was exhibited by T0 at 3.5, whereas the pH of T1, T2, and T3 were relatively similar, at 3.0. This uniformity illustrates that samples were stable in terms of acidity, an essential consideration for product consistency and shelf life (Upadhyay & Mishra, 2015).

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

D) Acidity

Samples presented reduced acidity at a slight rate; however, the lowest acidity was recorded at the. T3 level with 0.57%, followed closely by T2 with 0.64% and both T0/T1 at 0.682%. Lower acidity in the T3 level may contribute to a balanced flavour profile, thus improving consumer acceptance. This may result from a lower rate of compositional change in the samples due to possibly influenced processing conditions or ingredients (Upadhyay & Mishra, 2015).

Table 4 : Acidity Level

Sr. No.	SAMPLES	ACIDITY % (w/v)
1	T0	0.68 ± 0.03
2	T1	0.68 ± 0.04
3	T2	0.64 ± 0.05
4	T3	0.57 ± 0.06

E) Sensory Test

Sensory test was conducted to evaluate the acceptance of the prepared RTS beverage based on color , Flavor , Taste, aroma and overall acceptance. The results are that T2 was most acceptable due to sweet and tart taste and pleasant aroma compared to T1 and T3. T3 has most sour taste and slimy texture due to high concentration of hibiscus extract even though it had a attractive color. T1 was not acceptable because of its low nutrient content.

Table 5 :- Sensory Evaluation Table

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	7.3 ± 0.4	7.9 ± 0.3	8.4 ± 0.5	7.5 ± 0.4
Acceptance				

4. Conclusions

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.

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