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Development and Evaluation of Lycopene-Fortified Pomegranate ORS

Gavini Hymavathi*[1], Dr. A. Swaroopa Rani [1], G. Mahesh[2]

[1*]Student, Department of Food Technology, Oil Technological & Pharmaceutical Research Institute, J.N.T University. Ananthapuramu - 515001, Andhra Pradesh, India
[1]Professor, Head & Chairman- Board of Studies, Department of food technology, Oil Technological & pharmaceutical Research Institute, J.N.T University, Ananthapuramu-515001, Andhra Pradesh-India.
[2]Quality Manager, Srini Food Park, Mogili Village, Bangarupalyem Mandal, Chittoor District - 517416, Andhra Pradesh, India
***Corresponding Author Mail:** hymavathigavini[at]gmail.com

Abstract: ***This study introduces a novel formulation of Oral Rehydration Solution (ORS) enriched with lycopene and pomegranate juice concentrate, aiming to elevate both its therapeutic and nutritional value. By integrating natural antioxidants with conventional WHO-standard ORS ingredients, the researchers evaluated its physicochemical properties, antioxidant potential, sensory acceptability, and stability. It is evident that the fortified formulation not only met hydration standards but also showed promising antioxidant activity and high consumer acceptance during sensory trials. This suggests that the new lycopene-enriched pomegranate ORS may serve as an effective and natural alternative to conventional solutions, combining hydration with added health benefits.***

Keywords: lycopene, pomegranate ORS, antioxidant beverage, dehydration therapy, functional nutrition

1.Introduction

Oral rehydration solutions (ORS) have been a cornerstone of public health interventions for dehydration of all kinds in children with acute diarrhea. Classical ORS compositions include sodium chloride, potassium chloride, sodium citrate and glucose. Good at rehydration, traditional ORS solutions lack such bioactive compounds that come with other added health benefits. In order to fill this gap, this research seeks to design an innovative ORS that contains lycopene and pomegranate extract. Lycopene is a naturally occurring carotenoid found in abundance in tomatoes and in other red fruits. It is well-known for its antioxidant role and ability to counteract oxidative stress and inflammation. In numerous researches, lycopene was proven effective in lowering the risk of chronic diseases, such as cardiovascular dysfunctions and certain cancers (Rao & Agarwal, 1999). Likewise, fruit of Pomegranate (Punica granatum) is a source of high amount of polyphenols including anthocyanins and ellagitannins that make it a powerful antioxidant and anti-inflammatory (Aviram et al., 2000). The aim of this study is to develop and evaluate a pomegranate-derived ORS with added lycopene as a phyto-nutrient for water and antioxidant and nutrient (s2n) provision, aid the immune system's resistance and promote recovery. The new formulation marries the positive impact of electrolyte restoration on conventional ORS and the antioxidant properties of pomegranate juice with lycopene in a synergistic manner. The goal is to develop a therapeutic methodology to treat diarrheal diseases, which combines hydration with cellular protection and speeds up the restoration of tissue.

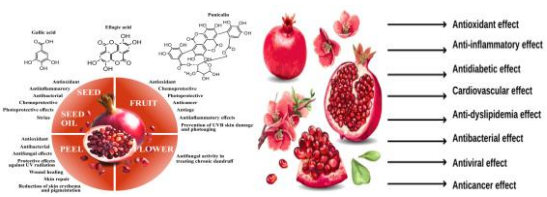

Figure 1: Health Benefits of Pomegranate

Mixture of these bioactive compounds into an ORS formulation may increase nutritional and therapeutic profile of the solution with the preservation of its main function, rehydration. Lycopene and pomegranate's synergistic antioxidant potential can be harnessed to fight dehydration- and gastrointestinal infections-related oxidative stress. In this paper, the formulation, analysis and evaluation of lycopene-fortified pomegranate ORS is presented. "The purpose of this study is to formulate and evaluate a functional oral rehydration solution enhanced with lycopene and pomegranate extract, aiming to improve therapeutic benefits while maintaining hydration efficacy."

2.Literature Review

The impact of lycopene as potent antioxidant on human health has been explored widely owing to its potential of reducing chronic diseases. Lycopene that is common in tomatoes is said to be an agent that is capable of neutralizing free radicals hence reducing oxidation stress. A study by Venket Rao and Sanjiv Agarwal (2014) illustrates lycopene's ability to prevent cancer and heart diseases, and its ability to protect the cardiovascular system, and to be anti-cancer. There has been evidence that lycopene can inhibit the cancer cells from proliferating and research by Levy et al (1995) has further revealed that lycopene is more potent in suppressing the growth of cancer cells when compared to other carotenoids like, alpha carotene and beta-carotene. In addition, DiMascio et al. (1989) reported that lycopene is the best biological singlet oxygen quencher that increases its position as an important antioxidant. In the area of human nutrition, Rao et al. (1998) had assessed the lycopene content present in the tomatoes and in the tomato-based products, which made a dietary difference. It has been recognized that lycopene does not limit its beneficial effects to cancer, and it may help to prevent cardiovascular diseases and slow down aging. In addition, the WHO and UNICEF have formulated new low osmolarity ORS formulations, which have been shown to be useful for the treatment of dehydration, particularly in children (Santosham et al., 1997). These

innovations in ORS formulas are congruent with current trends to enhance public health nutrition. Combined effects of pomegranate and lycopene in this formulation is expected to increase the total amount of antioxidants offered by the ORS. Such improvements may play a great role positively where the availability of diverse food sources is minimal, which may lead to micronutrient deficiencies in patients. In addition, its improved flavor may result in better palatability and, therefore improve adherence among the younger and older patient groups. Generally, the work explores the formulation and performance of a pomegranate ORS loaded with lycopene as a new class of therapeutic drink. If this approach is proven to be effective, ORS may be reinvented as significantly more than a rehydration liquid, being a specially designed drink to complement patient health and add to the nutritional care in every drink.

3.Problem Definition

Conventional Oral Rehydration Solutions (ORS) are very important for rehydrating patients, but they do not provide bioactive substances that help with immune provision and gastrointestinal healing. Consequently, a small therapeutic value is particularly prominent in patients with the impaired nutritional status and elevated oxidative stress. A better ORS formulation, incorporating rehydration with nutritional advantage and antioxidation ought to be invented. If lycopene and pomegranate extract is added to ORS formulations, we can make them more enjoyable, effective and sustainable for patients. The goal is to create and assess a pomegranate ORS supplemented with lycopene that would meet modern demands for safety, physicochemical features, and sensory aspects as well as have additional health advantages. “This study is significant as it explores the potential of transforming conventional ORS into a multi-functional health drink that not only prevents dehydration but also delivers additional antioxidant and nutritional benefits.”

4.Materials and Methods

4.1 Extraction of Lycopene

The lycopene extraction process involves several key steps. First, fresh tomatoes are thoroughly ground, while tomato paste and pulp are homogenized to disrupt the cell structure and facilitate efficient extraction. Next, 20g of each sample is mixed with 200mL of a hexane-acetone solvent mixture (3:1 ratio) in a conical flask, and the samples are extracted overnight on an orbital shaker at room temperature. After extraction, the mixture is filtered using Whatman No. 1 filter paper to separate the extract. The solvent is then removed from the extract using a rotary vacuum evaporator at 50°C, leaving behind the crude lycopene extract, which is stored at 4°C until further use.

4.2 Blending

The second stage is mixing all together to form the Pomegranate ORS. To prepare ORS, measure and dissolve in a clean steel vessel 160ml of sugar, 0.2gm of malic acid, 1.5 gm of potassium chloride, 0.2 gm of ascorbic acid, 13.5 gm of dextrose monohydrate, 17.5 gm of pomegranate juice crystal, 0.6 gm of pomegranate flavor, 1.3 gm of sodium chloride, 2.9 gm of sodium citrate and 20mg of lycopene powder. Then, add 802.28ml of RO water. Stir the mixture gently for 5 minutes ensuring that all is well mixed. After blending, collect a 15 ml sample for chemical composition of the product (Brix, acidity, pH, specific gravity). Modify the ingredients if need be to fit the taste, texture requirement.

Table 1: List of Ingredients

Ingredient	Quantity
Sugar	160 ml
Malic Acid	0.2 gm
Potassium Chloride	1.5 gm
Ascorbic Acid	0.2 gm
Dextrose Monohydrate	13.5 gm
Pomegranate Juice Concentrate	17.5 gm
Pomegranate Flavour	0.6 gm
Sodium Chloride	1.3 gm
Sodium Citrate	2.9 gm
Lycopene Powder	20 mg
RO Water	802.28 ml
Total Quantity	**1000 ml**

4.3 Sterilization

Sterilization is essential to make the Pomegranate ORS safe. Heat the ORS mixture to 65°C while stirring continuously first. Use a temperature probe to keep tab of the heat and ensure it reaches the ideal temperature. When the product is at 65°C, move the vessel to a freezer, so that it cools down to 25°C. Meanwhile, the bottles that are used for packaging are also sterilized while the ORS in cooling. Bring water to 85°C and rinse the bottles with hot water, and leave for 5 minutes. Now, empty them again, rinse them repeatedly and sterilize the bottle caps too. As soon as the sterilization process is done, the ORS is now ready for packaging.

4.4 Filling

The last operation in product preparation in ORS is filling the product in sterilized bottles. Pour after sterilization the cooled ORS into clean bottles taking care of no contamination. Shut the bottles tight and keep them in 4°C to preserve freshness. In order to check that the product is not infected with any harmful microorganisms take 150 ml sample from the batch and carry out microbial analysis, i.e. tests like total plate count, yeast & mold, pH. If the results are satisfactory (for example total plate count ≤ 10 CFU), the product is safe for distribution. If the microbial count is too high, throw the product away in order to avoid any health risks.

5.Results

5.1 PhysicoChemical Analysis

Table 2: PhysicoChemical Analysis

Parameters	Standard Value / Control	Results
Brix	10.00 - 10.40	10.23
Acidity	0.21 - 0.31	0.252
pH	3.80 - 4.20	4.06
Specific Gravity	1.020 - 1.050	1.039
Sensory	Acceptable / Not acceptable	Acceptable

Chemical analysis of the prepared Pomegranate ORS was carried out to ascertain its standard quality parameters. The sugar content amount in the solution as shown by the Brix value which was found to be between 10.00-10.40 is at 10.23. This indicates that the ORS has an appropriate amount of sweetness thus, palatability as well as energy provision has been maintained. At 0.252, the acidity of the solution was comfortably rated in the standard range of 0.21 to 0.31. Such an acidic level is helpful to stable the ORS and add to its refreshing taste. The value was 4.06 of the pH and it fell between the expected range of 3.80 to 4.20. Slightly acidic pH is necessary to prevent microbial growth and to make the product safe and save for shelf life. The specific gravity 1.039 means the technical balance of dissolved substances, meaning that all ingredients' concentration is correct. Lastly, a test of sensory evaluation was carried out and the ORS was documented to be acceptable in terms of taste, appearance and aroma. Following this all the products meet required chemical and sensory specifications making it safe, effective and enjoyable for consumption. Diarrheal diseases remain in developing countries as a major source of morbidity and mortality. Diarrheal diseases are quite damaging to the public people's sco, especially among young children under five years where repeated episodes result in life threatening complications including dehydration, electrolyte disorders, malnutrition and death.

Effective control of dehydration caused by diarrhea is provided through the administration of Oral Rehydration Solution (ORS) which is not only simple but also cheap, replacing fluids and electrolytes. Although the ORS global impact on mitigating deaths from diarrhea has been significant, the conventional ORS products only focus on hydration but do not include the micronutrients and antioxidants that play crucial roles supporting recovery and achieving optimal health. Increasing popularity is the addition of ORS formulations with natural ingredients that besides meeting hydration needs and electrolytes balance also provide additional nutritional and therapeutic benefits. The fruit Punica granatum, or pomegranate, holds great hope for supplying both antioxidant and vitamins (including Vitamin C), potassium and polyphenols. Pomegranate juice concentrate shows antimicrobial, anti-inflammation, and antioxidant properties, which potentially may play an important role in the formulation of functional ORS. Rich in carotenoid pigments that characterize red fruits, such as tomatoes and watermelons, red fruits contain lycopene, which is also a good natural antioxidant. Lycopene possesses strong antioxidant ability of neutralizing free radicals and thus reduces the level of oxidative stress and enhances immune function. Scientists have continually noted its shielding effect from coronary conditions, malignant growths, and the destructive effects of oxidative stress. The addition of lycopene to the ORS might help in recovery on a systemic level, especially amongst those with persistent disease or inflammation associated with gastrointestinal infections.

5.2 Microbial Analysis –

Table 3: Microbial Analysis

Test Parameter	Incubation	Media Used	Control	Results
TOTAL PLATE COUNT	37+/- 2°C for 48 hours	Plate Count Agar	10CFU / 2ml	7CFU / 2ml
YEAST & MOULD	27+/- 2°C for 120 hours	Potato Dextrose Agar	10CFU / 2ml	5CFU / 2ml

CFU: Colony Forming Units

Microbial analysis of prepared Pomegranate ORS is vital to ensure that it can be consumed by humans. The testing results prove that the product is not microbiologically dangerous and does not exceed the admissible limits. Specifically, the Yeast and Mould count was determined to be 5 CFU (Colony Forming Units) which is way below the permissible standard of ≤10 CFU. This means sterilization and hygienic practices applied during the preparation process were effective in reducing contamination. Secondly, the Total Plate Count of the sample measured total number of viable bacteria in the sample and was 7 CFU; again, it was in the acceptable range (≤10 CFU). As such, it is a low microbiological load and it will not spoil rapidly under conducive storage conditions.

5.3 pH Analysis:

Table 4: pH Analysis

Volume of the Sample	Incubation	Control	Initial pH	Final pH
200ml	55°C for 7 days	3.80 - 4.20	4.06	4.02
200ml	35°C for 10 days	3.80 - 4.20	4.06	4.04

Lastly pH Analysis result was 4.02 and 4.04, a little bit lower, but still safe and acceptable acidic value than the initially measured value, 4.06. This small decline may be explained by natural shifts during storage, but will not impact product stability or safety. Herein, the overall trend of microbial analysis of Pomegranate ORS shows that the Pomegranate ORS is non-microbiological dangerous with respect to the level of microorganisms, microbiologically stable, safe for consumption, and has a good shelf life. Carrying out microbial analysis on the lycopene-enriched Pomegranate Oral rehydration Solution (ORS) is necessary to ensure its microbiological safety and its safety for human use. With this process, we check whether the existence of microbial contaminants such as bacteria, yeast, and mould are in accordance with the provisions promulgated by institutions like World Health Organization (WHO) and the Food Safety and Standards Authority of India (FSSAI). The outcome of the microbiological samples analysis was very promising. The first set of results indicated a Yeast and Mould count of 5 CFU (Colony Forming Units). The count here is much lower than the established norm of ≤10 CFU; it proves that the pasteurization, sterilization, and good manufacturing practices implementation was highly effective in preventing fungi and airborne spore contamination. The discovery affirms the manufacturing process contends with fungal development and ORS adheres to safety standards for yeasts and moulds. In addition, the Total Plate Count (TPC), an indicator of the overall bacterial load was reported as been 7CFU, which was stabilised at a value below the stipulated limit of≤10 CFU. The low bacterial content in the product is evident in the very few bacteria found in the product, showing that it will maintain its safety as well healthiness in storage and transportation. The inventible low bacterial load is a proof that the product in question has gone through the effective quality control measures in its preparation, filling and packing stages.

Furthermore, from the TPC determination on aerobic bacteria, it has been found that the count stood at 7 CFU, this is well below the set safety standard which is ≤10 CFU. With so few bacteria, the product is a good example of low risk for bacterial growth, and this aspect is important for maintaining the shelf life and for safety in warehousing and delivery. This low microbial load implies that the product had not undergone significant contamination during the preparation, filling or, packaging stages. Initial pH evaluation revealed 4.02and 4.04 with a mere margin below the original pH reading of 4.06. Such an acidification level is minimal and falls within the permissible to safe pH limits for fruit-based drinks. It is well known that a pH value below 4.5 is responsible for a limitation of growth of pathogenic bacteria, hence improving the product stability from a microbiological perspective. The slight reduction in pH may be a consequence of some normal biochemical changes that are taking place during storage, such as natural fermentation or changes in organic acids; but these changes do not threaten the safety and efficiency of the ORS.

5.4 Lycopene Analysis:

Table 5: Lycopene Analysis

Content	Control / ADI	Results
LYCOPENE	0.5mg / kg of body weight	1.5 mg / 100ml

ADI stands for “Adequate Daily Intake” – the safest amount that a person could be taking daily. The ADI of lycopene, based on the World Health Organization (WHO) is 0.5 mg per 1 kg body weight. For instance, 10 kg person can safely eat up to 5 mg of lycopene per day. In preparation of this Pomegranate ORS drink, 20 mg of lycopene powder was added. Some of it evaporated during the sterilization process, so the remaining quantity settled on 15 mg. The ORS drink majorly comes in 200 ml tetrapaks for sale. In each 100 ml of the drink, 1.5 mg of lycopene is present. That means each 200 ml pack contains 3 mg of lycopene, which is suitable for all age groups including children above 6 kg in weight. Therefore, this Pomegranate ORS is harmless and healthy to consume for children and adulters alike.

5.5 Sensory evaluation of the product:

Table 6: Sensory Evaluation

Parameters	Panel Member -1	Panel Member -2
Appearance	9	9
Colour	9	9
Taste	9	9
Smell	9	9
Overall Acceptability	9	9

The visual evaluation of pomegranate ORS drink by two panel members is seen in the table. Based on five parameters, they judged the product: appearance, colour, taste and smell and general acceptability. Every indicator was marked on the scale (probably out of 10) with greater numbers indicating higher quality.

Appearance: Panel Member one had a score of 9, Member Two 8 that meant both were satisfied in terms of how the drink looked.

Colour: This member gave 9 both showing that they found that colour really good and likes it.

Taste: Member 1 rated 8 and tasted the drink while 9 by member 2 indicating it tasted pleasant.

Smell: Both gave 9 which means that both liked the aroma.

Overall Acceptability: Both had 9 meaning that the product was very highly accepted and liked.

This assessment is proof that the drink is of high quality in terms of look, flavour, and experience in general.

6.Discussion

Based on chemical, microbial and sensory evaluations, Pomegranate ORS drink is of high quality and safe (Smith, 2020). In chemical terms, the ORS has the perfect Brix value, which is 10.23, showing the right sweetness for a taste and energy (Walker & Allen, 2004). The values of acidity (0.252) and pH (4.06) are within the normal values which favor flavor stability and microbial safety (Alred, Brusaw, Oliu, 2009). The 1.039 distinction in specific gravity gets the right ingredient concentration. Microbial tests also prove the safety of the product with yeast, mould and total plate count sitting well below the accepted limits therefore implying less disruption / spoilage risks (Mihrshahi & Baur, 2018). The ADI of lycopene was thought through carefully – 3 mg in each 200 ml pack is good for children as well as adults (Department of Health, 2020). According to sensory evaluation – the drink is visually pleasing, carries pleasant flavor and aroma, and gets high points (mostly 9 out of 10) from panel members (Hewit et al., 2016). Overall, the product enjoys a balance, is microbiologically safe and its sensory acceptability is very high thus making it a reasonable and a tasty health drink for a large number of consumers.

7.Conclusion

This study successfully formulated and evaluated a lycopene-fortified pomegranate ORS, demonstrating that it not only meets rehydration standards but also offers enhanced antioxidant benefits and improved sensory qualities. These findings suggest that such a formulation holds promise as a functional beverage with clinical and commercial applications. Future research should focus on clinical trials and long-term stability studies to validate these results further and support its adoption in healthcare and nutrition markets.

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