Optimisation of Common Acidulant (Fruitaric Acids) to Enhance Organoleptic Quality and Shelf Life of Fruit Juices

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ABSTRACT
Citric, Malic, and Tartaric acids are widely used additives in the food Industries. These additives have been identified for their individual role in enhancing the organoleptic properties and shelf life of different fruit products but there is limited information pertaining to combined effect of these additives. On optimization an appropriate combination of these acids (fruitaric acid) would thus become as one single dative that can be used in several of the mixed fruit or common fruit flavours particularly in the confectionery, fruit juices, and other related products. These additives would contribute to a better flavour profile and shelf life of the fruit product than when used singly. Thus the current research was carried out to optimize the ratio of citric, malic and tartaric acid to enhance shelf life and organoleptic properties of fruit juices. In this research following ratios of citric to malic to tartaric acid were used for formulation of different test samples, viz. S1=25:37.5:37.5, S2=50:25:25, S3=75:12.5:12.5, S4= 100:0:0 and S5 as reference sample without any additive. Fruit juices selected in the current study were Apple and Mango juices. To evaluate the shelf life of product plate count, yeast count, pH, and total soluble solids were analysed for five weeks. Sensory analysis was performed for all the formulated test sample of the fruit juices to find out the best test sample and obtained result were analysed by SPSS software version 12 for windows. It is evident from this study that the organoleptic property and shelf life of product will be enhanced if acidulant is added as per sample S3.

Keywords: Acidulant, Citric acid, Malic acid, Mango and Apple, Tartaric acid.

INTRODUCTION
Citric acid, Malic acid, and Tartaric acid, along with various other acids are the natural ingredients of various fruits and these acidulant are used by the food industries to develop different types of fruit, and fruit based product specifically, viz. citric in citrus fruits, malic in apple and tartaric in tamarind. These acids are responsible for protecting the fruit from spoilage by pathogenic and food spoilage microorganism and hence provide a natural shelf life to fruit and fruit based product particularly in the confectionery, fruit juices, and other related products. Nowadays in food industry these acids are added in various product to enhance shelf life and organoleptic property of food product instead of using another preservative such as sorbic acid, benzoic acid, Erythorbic and other artificial preservative, because regular consumption of these preservative with food product causes various side effects to consumer. Citric acid, malic acid, tartaric acid and other naturally occurring acids in fruits is Generally Regarded As Safe (GRAS) compound and has minimum side effect when used as additive. More over these acids are involved in multitude of applications related to Food Industry. It works as preservative, flavouring agent as well as acidulant. The most important property of these acids which attracts the food industry is antimicrobial activity, because of this property it inhibits the growth of various pathogenic and food spoilage microorganisms and hence preventing the food product from spoilage and enhance shelf life. These antimicrobial properties of citric acid, malic acid, and tartaric acid are mainly due to their acidity, and relative effectiveness of these acids has been measured in terms of their minimum inhibitory concentration. Different acids have different acidic strength and thus they have varying minimum inhibitory effects, however, previous studies have shown that both the ionized and the non ionized form, concertedly promotes inhibitory effect. To be more precise, several factors, which include, number of carboxyl group, number of hydroxyl group and the nature of the bond, present in the acid, describe characteristic acidity. Organoleptic property of a food product includes a range of different attributes which is modified by additional addition of various flavouring agent. Similarly extra addition of these acids enhances different sensory attributes such as flavour, texture, smell, taste, sourness, consistency etc. Based on the sensation of different sensory characters, consumer decides toward the uptake of a particular food article. As earlier mentioned that, the different acids have different potential to inhibit the growth of microorganism, similarly different acids impart different taste and flavour in food product based on their concentration. Hence to attain the best potential of these acids, proper combinational ratio of citric acid, malic acid, and tartaric acid is required, that can overcome present artificial preservative readily available in the market.

In this study, we have scientifically explored the possible ratios of each acid and tried to optimise the ratio which can work as single dative for the best quality of product, which can enhance flavour as well as shelf life of fruit and fruit based product. The products selected for this work...
were Apple (*Malus domestica*) and Mango (*Mangifera indica*) juice. To study the effect of these additives on the organoleptic property, sensory analysis was done, while to analyze shelf life, various analytical methods were used ranging from plate count, yeast count, acidity, and total soluble solid, and all of it were computed for a window of 5 weeks.

**MATERIALS AND METHODS**

**Sample Preparation**

Citric acid (C), malic acid (M) and tartaric acid (T) were collected from the Thirumalai Chemicals India Pvt. Ltd. Fruit samples of apple and mango were purchased from the supermarket complex of VIT University, Vellore.

Total five test sample for each fruit juice was prepared, by adding different amounts of citric to malic to tartaric acid in fruit juices in the following ratio, S1=[C: M: T = 25: 37.5: 37.5], S2=[C: M: T = 50: 25: 25], S3=[C: M: T = 75: 12.5: 12.5], S4=[C: M: T = 100: 00: 00] and S5 as a reference sample without any additive. Net amount of additive added in the fruit sample were 0.3% (w/v).

**Sensory analysis**

Organoletic property of formulated test sample prepared with varying concentration of citric acid, malic acid and tartaric acid were evaluated by 50 volunteer (35 male, 15 female) selected from VIT University Vellore, India. Volunteer were asked to taste the products upon their consent and to indicate how much they like or dislike the product on a six point hedonic scale (1-Excellent, 2-good, 3-fair, 4-moderate, 5-neutral, 6-awfull) for different organoleptic property such as Consistency, Sweetness, Soursnss, Overall taste and Overall mouth feel after taste. Formulated test sample were randomized and served to each volunteer. Volunteer were asked to read through the questionnaires and meaning of each attribute was explained to volunteer to avoid any misinterpretation prior to evaluation.

**Shelf life analysis**

For shelf life evaluation of fruit juice microbial load in the formulated variants were evaluated by plate count method. Spread plate method was used to analyse the bacterial load and yeast count in fruit juice at various concentrations of the acidulant. Nutrient agar was used to analyse the bacterial load whereas SDA (Sabouraud dextrose agar) has been used to analyse the yeast count. The plating was done at regular intervals of 7 days for five weeks after the date of additive being added to the fruit juices. Test sample to be inoculated on the media was of $10^3$ dilution factor. Entire set of experiment was done in triplicate and based on this, average number of colony were counted by colony counter.

Sugar content of the juice sample was analysed by refractometer and result were measured in terms of Brix. One degree of Brix is one gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight (%w/w). Acidity of the sample was analysed by pH pen.

**Statistical Analysis**

Obtained data from result of sensory analysis were analysed by SPSS software version 12 for windows. The data represented as mean score (average score of acceptance) for each variant. To determine the difference between mean score one way analysis of variance (ANOVA) was used for all sensory attribute at ps0.005.

**RESULTS AND DISCUSSION**

**Sensory Analysis**

Sensory analysis based on six point hedonic scale (1-Excellent, 2-good, 3-fair, 4-moderate, 5-neutral, 6-awfull), for various Organoleptic property has been schematically represented in Figure 1 for the mango juice and Figure 2 for the apple juice. The lower mean value represents the highest acceptability of the formulated product whereas higher mean value represents least acceptability by consumers. From the figure 1 and 2 it is clear that S5 (Control, without any additive) has highest mean value which represents its least acceptability by the consumer as compared to other sample under investigation. Based on the consumer acceptability for all the five sensory attributes under investigation, S4 can be considered as the best preferred sample, followed by S3. Similarly, for the apple juice S1 sample has shown the maximum acceptability by the consumers, followed by S3.

**Figure 1:** Sensory Analysis of Mango Juice

**Figure 2:** Sensory Analysis of Apple Juice

Note: X-axis- Formulated test sample, Y-axis- Average score of acceptance.
Shelf life Analysis

Bacteriological analysis

Bacterial load of all test samples has been summarized in Table 1 and graphically depicted in figure 3. The number represented in the table is the CFU/ml at $10^3$ dilution (colony forming unit). Microbial load is an important factor in the quality of minimally processed fruit and fruit based product for food industry. Thus in this research citric, malic and tartaric acids were added with varying concentration in different formulated test sample in different ratio to prolong the shelf life of product. During this study it has been observed that bacterial load in formulated variants were very less till third week while rapid increase in bacterial load was observed in fourth week, and in fifth week the test sample were heavily contaminated and were difficult to count all formulated test sample. During storage period of five weeks it was observed that formulated sample S3 has better control over spoilage as compared to other sample, till second week total 10 CFU was observed in both the fruit juice sample, and at the end of 4th week 260 and 350 CFU was found in mango and apple juice respectively while S2 variant was second best in terms of controlling of spoilage with 460 and 530 CFU at the end of 4th week for mango and apple juice respectively.

Table 1: Plate count result of mango and apple juice

<table>
<thead>
<tr>
<th>Test sample</th>
<th>1st Week M</th>
<th>A</th>
<th>2nd Week M</th>
<th>A</th>
<th>3rd Week M</th>
<th>A</th>
<th>4th Week M</th>
<th>A</th>
<th>5th Week M</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>130</td>
<td>120</td>
<td>550</td>
<td>630</td>
<td>U.C</td>
<td>U.C</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>90</td>
<td>60</td>
<td>460</td>
<td>530</td>
<td>U.C</td>
<td>U.C</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>30</td>
<td>260</td>
<td>350</td>
<td>U.C</td>
<td>U.C</td>
</tr>
<tr>
<td>S4</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>50</td>
<td>80</td>
<td>240</td>
<td>430</td>
<td>490</td>
<td>U.C</td>
<td>U.C</td>
</tr>
<tr>
<td>S5</td>
<td>70</td>
<td>80</td>
<td>14</td>
<td>150</td>
<td>260</td>
<td>260</td>
<td>870</td>
<td>940</td>
<td>U.C</td>
<td>U.C</td>
</tr>
</tbody>
</table>

Table 2: Yeast count in mango and apple juice

<table>
<thead>
<tr>
<th>Test sample</th>
<th>1st Week M</th>
<th>A</th>
<th>2nd Week M</th>
<th>A</th>
<th>3rd Week M</th>
<th>A</th>
<th>4th Week M</th>
<th>A</th>
<th>5th Week M</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>160</td>
<td>380</td>
<td>130</td>
<td>610</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>270</td>
<td>90</td>
<td>440</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>70</td>
<td>30</td>
<td>240</td>
<td>50</td>
<td>370</td>
</tr>
<tr>
<td>S4</td>
<td>0</td>
<td>10</td>
<td>30</td>
<td>90</td>
<td>20</td>
<td>130</td>
<td>80</td>
<td>450</td>
<td>180</td>
<td>720</td>
</tr>
<tr>
<td>S5</td>
<td>0</td>
<td>40</td>
<td>20</td>
<td>0</td>
<td>60</td>
<td>230</td>
<td>220</td>
<td>560</td>
<td>430</td>
<td>820</td>
</tr>
</tbody>
</table>

Note: - In this table “M” represents Mango and “A” represents Apple.

Yeast count

Yeast (Saccharomyces cerevisiae) is not pathogenic to human being but the food product gets contaminated when the population go beyond limit. During their growth, yeasts metabolize some food component and produces metabolic end product. This causes physical chemical and sensible property of food to change and food product gets spoiled. Due to this reason, to evaluate shelf-life of product, yeast count were analysed for five weeks. Result of this study has been summarized in Table 2 and yeast load in the sample has been graphically presented in Figure 4. Results shows that, formulated sample S2 of mango with additive in the ratio of citric: malic: tartaric acids = [50: 25: 25] has best control over spoilage while S3 sample was second best in terms of controlling of spoilage. Till four weeks there was not even a single colony to be detected in the formulated variant S2 and minimum CFU was found in the final week compare to other test sample. While the apple juice got spoiled faster than mango juice and sample S3 has best control over spoilage by yeast and S2 as second best. In the fifth week minimum number of CFU was found in sample S2 - 440 where as in sample S3 total no of CFU was found 370.
Figure 4: Juice spoilage by yeast count

Analysis of pH and total soluble solid

pH analysis for various formulated test variants of fruit juice has been graphically presented in Figure 5. Interpretation of the following investigation suggests that acidulant with varying concentration can be used as a substitute for the artificial acidity regulator which is added in different food product by food industry. Because the artificial acidulant causes various side effect to consumer on continuous consumption, which can be minimised by the implementation of naturally found acidulant, like citric acid, malic acid, and tartaric acid. During this study it was found that the test sample with acidulant were more stabilised with respect to the reference in terms of pH. Sample S3 and S2 has shown minimum fall in pH for apple fruit juice while for mango juice, sample S1 was found best acidity regulator followed by S2 during the storage period of five weeks.

Figure 5: pH Analysis

Total soluble solid in fruit juice was analyzed by the index of refraction by using Refractometer and the data obtained has been graphically presented in terms of degree brix (Brix) in Figure 6. Here degree brix represent sugar content of the fruit juice. One degree of Brix represents one gram of sucrose (sugar) in 100 ml of fruit juice, it also represents strength of the fruit juice in terms of sweetness. In this study it has been observed that initially in 1st three week total soluble solid remains almost constant but after that a steep decline in the total soluble solid content was observed. At the end of fifth week of analysis, quality of the product was assessed by amount of soluble solid present in fruit juice, and then it was inferred that, maximum the amount of total soluble solid value better would be the quality of the formulated product. During this study it has been observed that for apple juice, formulated test sample (S3) which contains 75% citric acid, 12.5 % malic acid, and 12.5% tartaric acid while for mango, formulated sample (S1) which content 25% citric acid, 37.5% malic acid, and 37.5% tartaric acid has maintained the total soluble solid content in the formulated product better than the other formulated product while in other sample significant fall in total soluble solid were observed.

Note: X-axis- formulated variants, Y-axis-Brix value.

Figure 6: Analysis of Total Soluble Solid

CONCLUSION

In this research it has been observed that by varying the concentration of fruitaric acid, shelf life as well as organoleptic property of food product can be enhanced. Results of sensory analysis have shown that organoleptic property of Mango and apple juices were different for different acidulant concentration. For mango juice sample with 100% citric acid as additive was mostly preferred whereas, for apple, sample with 25% citric, 37.5% malic, and 37.5% tartaric acid as additive was preferred over other formulated samples. While sample containing 75 % citric acid, 12.5% malic acid, and 12.5% tartaric acid was second most sought by the consumer for both the fruit juices. But since sample S3 containing 75% citric acid, 12.5% malic acid and, 12.5% tartaric acid was second preference of consumer for both the fruit juice and also it has prolonged the shelf life of both fruit juices by controlling the microbial growth and maintaining total soluble solid as compared to other formulated variants, hence it can be concluded that, if the acidulates are mixed in the ratio as per test sample S3 then this can act a single dative, and it can be a substitute of artificial preservative to enhance shelf life and organoleptic property of fruit and fruit based product.
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