ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effect of the Citric and Malic Acid Additives on the Storage Stability and Sensory Parameters in Lemonade.

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ABSTRACT

On account of better sensorial contribution as well as superior fortification against spoilage, acid additives in commercial fruit drinks hold great importance in the field of food preservation and additives. The principal objective of this study was to find an optimal ratio between citric and malic acid additive in lemon juice for better sensory perception which was assessed by conducting a survey involving 20 volunteers using nine point hedonic scale and the ideal ratio was found to be 80:20% of citric and malic acid respectively. Further the comparative effects of various citric and malic acid additive concentration ratios on the shelf life of refrigerated lemon juice were investigated by analysing the total microbial count, pH and total soluble solids on a per week basis.
Keywords: Lemon juice, Acidic additive, Citric acid, Malic acid, Sensory analysis, Hedonic scale.

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ISSN: 0975-8585

INTRODUCTION

With the rising health conscious of consumer, the fruit juice market has seen an unprecedented rise in sales [1] and side by side there has been a phenomenal increase in fruit juice based disease outbreaks [2]. Likewise several methods exist to prevent spoilage however the preservation of organoleptic characteristics is also an important criterion that has to be kept in mind [3] and in this regard most of the preservation methods may not succeed [1].

In this scenario food acidifiers find great use, the global market for preservatives is flourishing [4] and expected to reach 37.7 billion by 2018 [5], they have been finding an increased utility in fruit juice processing as years go by their use is predominated by the need to extend shelf life, reduce oxidative deterioration, reduce food borne disease outbreaks and in some instances flavour enhancement [6].

Organic acid additives oppress the growth of microbes by intensifying the hydrogen ion concentration, and have emerged as a more viable option not just due to its preservative capabilities [7] but also because of their sensory enhancement property in citrus fruits[8].

Commercially citric acid[9] and malic acid [10] are most widely produced acid additives, their usage is considered as Generally Regarded As Safe (GRAS) status and their usage is regulated by the Codex alimentarius guidelines.[11]

Citric acid, a weak tri-carboxylic acid found in its most concentrated form naturally in lemon fruit [12] and possesses significant food preservative action and metal chelating property, it is currently the most widely used acid additive today, it imparts a tart flavour to food and beverages, it also finds application in detergents, pharmaceuticals and medicine [9].

Malic acid an another important organic acid though possesses lesser antimicrobial effect as compared to citric acid, still holds far greater potential in a health conscious market fuelled by the calorie counting, it can mask the lingering aftertaste of artificial sweeteners. Further its pleasant lingering taste also makes it an ideal candidate for its synergises with citric acid as their binary mixtures gives rise to a more authentic and preferable taste profile [13].

Lemon juice presently occupies 11.3% of the fruit beverage market in USA alone and is one of the most commercially produced beverage [14], in the 18thcentury it was recognized to have anti prussic activity[15]and later the antiprussic compounds were recognized to be the vitamin C[16]. It is now widely recognised inhibitor of urinary crystallisation and of extreme importance for patients with hypocitraturia [17] and attributing to its beneficial health effects, consumer market is ever expanding.

In the present study we present the synergistic activity of citric and malic acid in lemon juice as a more viable combination in fruit juice acid additives for a far superior sensory perception without significant loss of shelf life.

MATERIALS AND METHODS

Preparation of lemon juice

Ripe lemons (*Citrus limonum*) were purchased from a local grocery market in Vellore (Tamil Nadu, India) and refrigerated till usage. The Juice from freshly squeezed lemons was filtered using plastic sieve net and Whatman no 1 filter paper and diluted by mixing 10 mL filtered juice with 90 mL distilled water, 11 g of sugar was added as per taste and the amount of total acid additive content to be supplemented was determined to be 3g/L using 'just about right' method employing 9 point hedonic scale and 20 trained volunteers. The additive quantity and juice formulation were performed keeping under consideration the various sensory characteristics while keeping the industrial products as reference for control.

Preparation of Seven acid additive variants

Six variants of citric and malic acid in the ratio 100, 95:5,90:10,80:10, 66:33 , 33:66 and control without acid addition were made by dissolving citric acid (Sigma Aldrich) and malic acid (Thirumalai Chemicals Pvt. Ltd India). For weekly analysis all the variants were stored in 15 mL Falcon tubes and kept refrigerated at 7˚C, for analysis juice samples were de-refrigerated at room temperature for thirty minutes followed by vortex and shaken to mix the suspended components uniformly.

Sensory survey

To find the optimum ratio of citric and malic acid for a suitable sensory perception a survey was conducted employing nine point hedonic scale [18] involving a panel of 20 volunteers, entire set of experiment was conducted twice. Volunteers were selected from among the university students and faculty staff with the selection criteria of consuming fruit juice at least thrice weekly. The age distribution of the volunteers was 70% (20-24) and 30% (25-47) and gender constitution was 60% males and 40% females. Uniform conditions of ambient surroundings were maintained throughout the entire survey, the volunteers were presented with 10 ml of the each seven variants i.e. 100, 95:5, 90:10, 80:10, 66:33, and 33:66 and control juice samples and were asked to rate each variant on the basis of aroma, colour, texture, flavour and overall impression, using a nine-point hedonic scale. The scoring system was as per follows - Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, neither like nor dislike = 5, Dislike slightly = 4, Dislike moderately = 3, Dislike very much = 2, Dislike extremely = 1. A separate entry for comments and suggestions was provided.

Physico-chemical analysis

Total Suspended Solids (TSS) were measured using hand held refractometer meter having measuring range 0-32±0.1-0.15% (Erma, Tokyo) and the measured value were expressed in ˚Brix.

For microbiological analysis total colony count was performed, 0.1 mL juice sample was held at room temperature at 25˚C for 30 min before plating, thoroughly mixed by vortexing, serial diluted in saline water at 10-1 and 10-5 and were plated on solidified nutrient agar media which was analysed for total colony count after a duration of 48 h incubation at 37˚C. The resulting colonies were counted using a digital colony counter and expressed as Log CFU/mL.

Statistical analysis of results

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 the mean scores Turkey's HSD (Honestly Significant Difference) test was used and the entire sensory attribute are reported at $p \leq 0.005$.

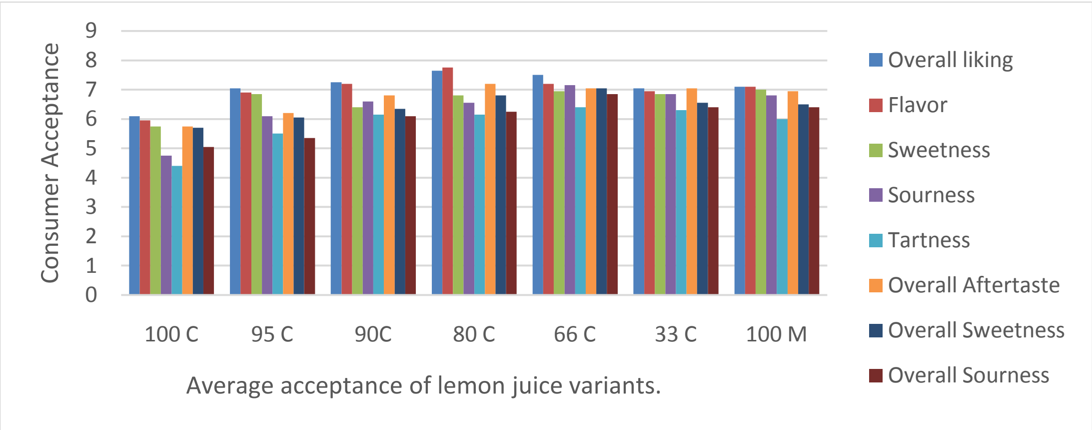
RESULTS AND DISCUSSION

Sensory analysis

Analysis of fruit juice samples using the nine point hedonic scale indicate the average score of acceptance falling between the range for overall taste (6.1 to 7.25), sweetness (5.75 to 6.95), sourness (4.75 to 7.15), flavour (5.95 to 7.75), after taste sweetness (5.7 to 7.05) and aftertaste sourness (5.05 to 6.85).

Figure1: Sensory survey

Samples	Overall liking	Flavor	Sweetness	Sourness	Tartness	Overall Aftertaste	Overall Sweetness	Overall Sourness
100 C	6.1	5.95	5.75	4.75	4.4	5.75	5.7	5.05
95 C	7.05	6.9	6.85	6.1	5.5	6.2	6.05	5.35
90C	7.25	7.2	6.4	6.6	6.15	6.8	6.35	6.1
80 C	7.65	7.75	6.8	6.55	6.15	7.2	6.8	6.25
66 C	7.5	7.2	6.95	7.15	6.4	7.05	7.05	6.85
33 C	7.05	6.95	6.85	6.85	6.3	7.05	6.55	6.4
100 M	7.1	7.1	7	6.8	6	6.95	6.5	6.4



A trend of overall preference towards 80:20 citric: malic acid ratio (average score of acceptance: 7.65) as the optimum concentration of organoleptic choice most preferred, 66:33 citric: malic acid sample (average score of acceptance: 7.5) was a close second on the sensory survey.

For sweetness parameter the variant 66:33 citric: malic acid sample (average score of acceptance: 6.95), for sourness parameter the variant 66:33 citric: malic acid sample, for flavour parameter the variant 80:20 citric: malic acid sample and for after taste sweetness and sourness the variant 66:33 citric: malic acid sample scored highest respectively on the nine point hedonic scale sensory survey.

Citric acid most widely used acidifier in foods[19] imparts a rapidly building up tart taste, contrastingly malic acid is responsible for smoother and longer lasting tart taste [20] and a clear organoleptically synergistic effect exists between both the acids[21] as indicted by the results.

Sometimes the hedonic survey result data can be biased indicating a difference between the preferred and actual liking which may be due to the surroundings, sometimes discrepancies occur between the small restriction food samples and the consumption [22].

Physico-chemical analysis

With time spoilage of juice occurred leading to a drastic increase in microbial count figure2, a clear rise in the total colony count can be observed. 100% citric acid proven to be most feasible preservative due to least spoilage over time and a significantly increasing spoilage was observed over decreasing citric acid concentration in the juice samples and maximal in the control, indicative that citric acid is more suitable as a preservative.

Juice products have a limit of microbiological shelf life of 6 log CFU/mL [23], thus all the samples were unfit for consumption after the second week. Citric and malic acid addition were found to reduce the rate of spoilage as compared to the control sample, their antimicrobial effect is well established owing to pH lowering[24],[25] and cell membrane damage [26], several studies have shown citric acid to be a more potent preservative as compared to malic acid.

Figure 2: Microbiological shelf life analyses

week	100c	95c	90c	80c	66c	33c	Control
week1	4	4	4.079181	4.176091	4.176091	4.079181	4.278754
week2	5.875061	5.934498	5.91169	5.991226	5.992995	6.033424	6.079181
week3	5.986772	5.959995	6.041393	6.146128	6.113943	6.079181	6.176091
week4	5.716003	5.880814	5.695482	5.459392	5.50515	5.342423	5.681241

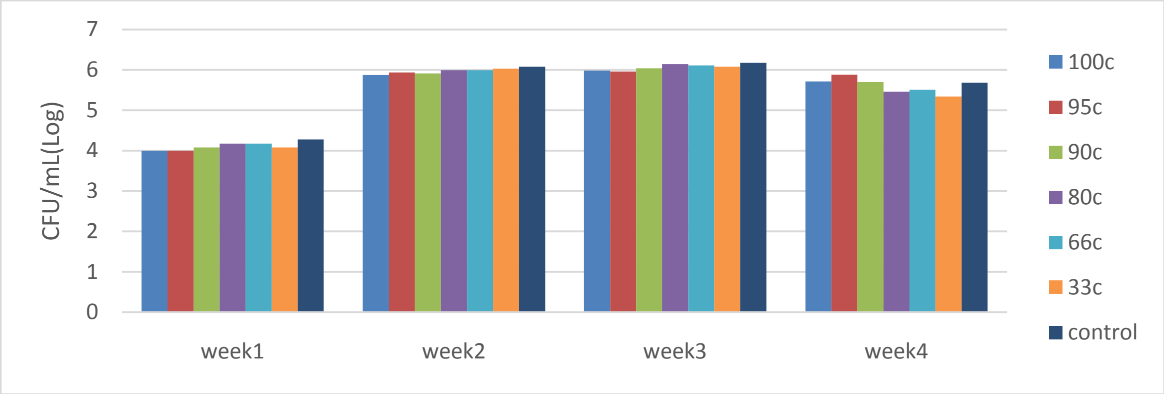


Figure 1: Microbial growth in lemon juice variants during storage.

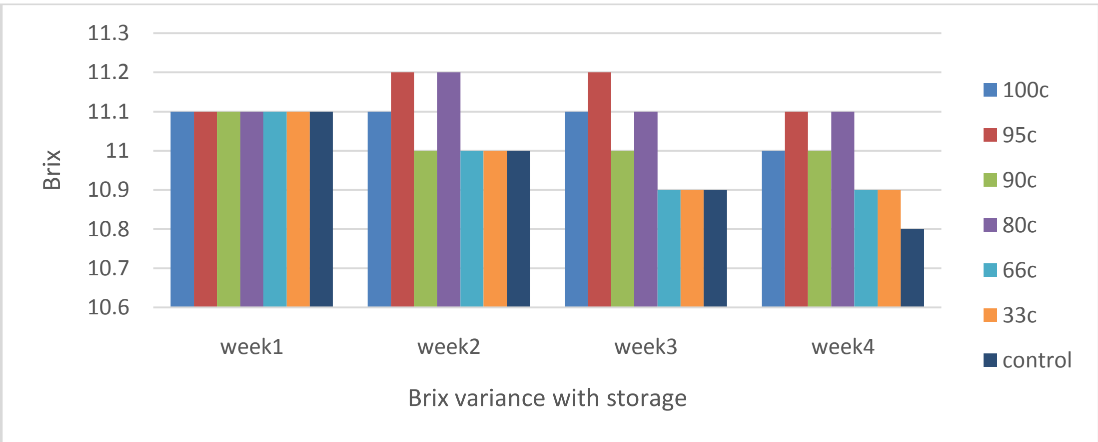
Total soluble solids

A decrease in the total soluble solids (˚Brix) content of the juice was observed with storage, figure3. Over time a drop in Total Soluble Solids was found with increasing concentrations of malic acid and maximal for the control. This can be explained as follows, the microbes in the juice sample with their growth uses sugar [27] leading to a drop in total soluble solids, an accompanying fermentation odour was detected in the samples with prior storage.

The ˚Brix drop was observed lowest for 100% citric acid variant and greatest for 100% malic acid variant and the control indicating maximum decrease of sugar content on account of consumption by the microbial fauna.

Figure3: Brix variance with time:

week	100c	95c	90c	80c	66c	33c	control
week1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
week2	11.1	11.2	11	11.2	11	11	11
week3	11.1	11.2	11	11.1	10.9	10.9	10.9
week4	11	11.1	11	11.1	10.9	10.9	10.8

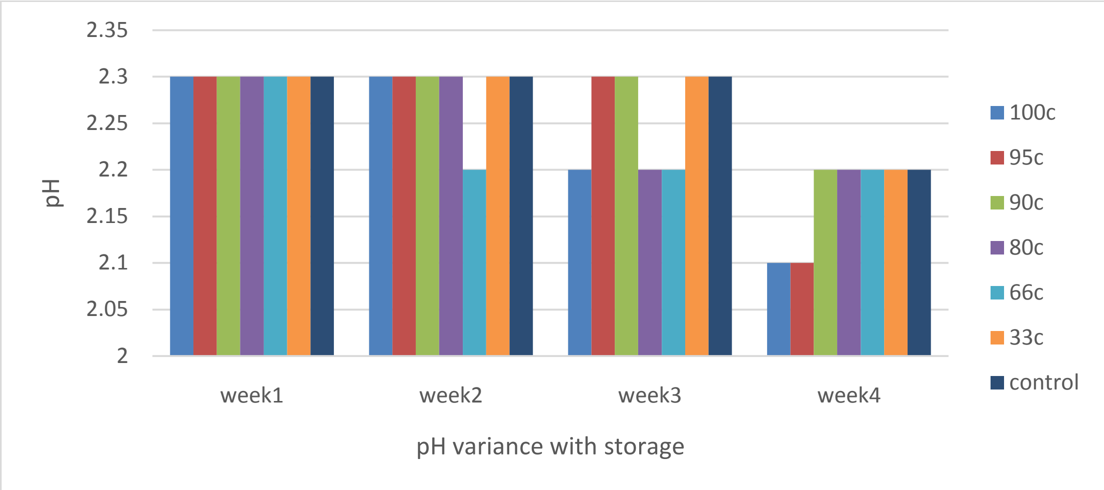


pH

During the storage of lemon juice an overall decrease in the pH was observed, (figure4) which can limit the growth and survival of several bacterial species which results in formation of gas, slime, turbidity and change in acidity [28].

Figure 4: pH variance with storage

day	100c	95c	90c	80c	66c	33c	control
week1	2.3	2.3	2.3	2.3	2.3	2.3	2.3
week2	2.3	2.3	2.3	2.3	2.2	2.3	2.3
week3	2.2	2.3	2.3	2.2	2.2	2.3	2.3
week4	2.1	2.1	2.2	2.2	2.2	2.2	2.2



CONCLUSION

This study aimed at finding an optimum ratio of citric and malic acid additive in fruit juice for better taste and improved shelf life parameters. Results showed that with storage a significant change in the physicochemical properties (pH, titratable acid, total soluble solids and microbiological spoilage) was observed for all the additive acid concentrations (100, 95:5, 90:10, 80:10, 66:33, 33:66 and control) indicating spoilage. An increasing trend towards spoilage was observed with decreasing the concentration of citric acid and malic acid increments. 100% citric acid concentration was found to be most stable towards storage and the stability decreased with decreasing citric acid concentration, 100% malic acid sample was found to be the least stable concentration. Organoleptically, 80:20 (citric: malic acid) sample was found to be the best, indicating that it was the most optimum organic acid ratio preferred as indicated by the survey. More ever 90:10 (citric: malic acid) sample was a close second and it can be suggested that it can be a more viable additive ratio based on its improved shelf life.

We recommend that more studies towards the precise cause of synergistic optimisation of the organoleptic relationship of citric and malic acid additive ratio should be investigated.

ACKNOWLEDGEMENT

The authors wish to express gratitude to Vellore Institute of Technology, Vellore, India for proving facilities to carry out the research, and Thirumalai Chemical India Pvt. Ltd. for the initiative and the opportunity provided.

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