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Effect of Temperature on Fermentation Rate of Fruit Juices and Their Nutritional Value – A Comparative Approach

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Abstract

Fermentation is an ancient technique of preserving food still in use to produce foods like wine, cheese, sauerkraut, yogurt, and kombucha. Fermented foods are rich in beneficial probiotics with high nutritional values, organoleptic quality and bioavailability of amino acids, vitamins C and A which can transform food into a much healthier food. Fermented fruit juice is a beverage created by the fermentation of fruits under controlled conditions. Pomegranate (*Punica granatum*), orange (*Citrus x sinensis*), lemon (*Citrus limon*), grapes (*Vitis vinifera*), papaya (*Carica papaya*), and pineapple (*Ananas comosus*) are the fruits used in the study. The fruit juices were extracted, pasteurized and inoculated with yeast then maintained between 35 - 40 ^oC followed by adding Fehling reagent to show the red color changes till the fermentation gets completed. The change in carbohydrates, proteins, iron and ascorbic acid content were measured before and after fermentation to show the quality of fermented fruit juice. The highest percentage of carbohydrate (1.25) and iron (2.40) was noted in *Citrus x sinensis*, protein (2.00) in *Punica granatum* and ascorbic content (2.00) in *Citrus lemon*. Estimation and time taken for fermentation indicated that the highest time was taken by lemon (120 minutes) whereas least time by pineapple (40 minutes).

Key Words: Fruit juices, Fermentation rate, Carbohydrate and Biochemical Analysis

Introduction

Fermentation helps break down large organic molecules through the action of microorganisms into simpler ones. Yeast enzymes convert sugars and starches into alcohol, while proteins are converted to peptides and amino acids. The microbial or enzymatic actions on food ingredients tend to ferment food, leading to desirable biochemical changes responsible for the significant modification to the food [1]. It is a process developed to preserve fruits and vegetables for times of scarcity [2]. World Health Organization (WHO) and Food and Agriculture Organization (FAO) recommended intake of a specific dose of vegetable and fruits in daily food to prevent chronic pathologies such as hypertension, coronary heart problems and risk of strokes. The consumers tend to prefer the foods and beverages which is fresh, highly nutritional, health promoting and ready to eat or ready to drink [3].

Fermented fruit juice is a widely consumed beverage around the world. In nations like India, 20-30 % of fruits produced are wasted due to lack of efficient use, post-harvest, and processing technology. This problem can be solved by transforming them into value-added products such as fermented fruit juice [4]. Any fruit with a high sugar content can be used to make fermented fruit juice. In the olden days, fermentation of food was meant for food preservation and flavour improvement [5]. Nowadays, in food and beverages fermentation, various technologies and operations are used. The conversion of perishable and indigestible raw materials into pleasant foods and drinkable beverages add value and high stability [6].

Among the most important factors affecting the quality of fermented fruit juices are the clarification and composition of fruit juice, the sulfur dioxide level added, the interaction with other indigenous microorganisms, the supplementation with nutrients, strain and several inoculated yeasts and fermentation temperature. The most important parameter of fermentation is temperature since it can affect the biochemical reactions and metabolism of the yeasts. Some European wineries still prefer fermentation temperatures between 20 – 25 ^{0}C [7]. A review of literature shows studies on the feasibility of pomegranate [8], quality analysis of wine fruit [9], inoculated fermentation of orange [10], fermented papaya and it's nutritional and health applications [11] are available. However as there is no systematic and comparative study on the effect of temperature on fermentation rate of the selected fruits, the present investigation was carried out focusing their nutritional value.

Experimental Process

Preparation of Fruit Sample Extracts

The six different fruit samples are *Punica granatum, Citrus x sinensis, Citrus lemon, Vitis vinifera, Carica papaya* and *Ananas comosus*. An accurate amount was weighed, washed thoroughly and crushed into a liquid with a help grinding machine. Then the whole extract was filtered and the extract was used for further experiments.

Chemicals Required

Fehling's solution, Pasteur's salt solution (Ammonium sulphate -10 g, Potassium phosphate-2.0 g, Calcium phosphate-0.2 g, Magnesium sulphate-0.2 g in 860 mL of water)

Biochemical Analysis

Total carbohydrate was estimated by Anthrone method [12]. Lowry *et al.*, method was followed for quantification of total proteins [13]. Ortho phenanthroline method was adopted to determine the percentage of iron [14]. The percentage of ascorbic acid was analyzed by using 2,6-dichlorophenol indophenols [14]. The absorbance change was monitored with Elico SC-177 Scanning mini spectrophotometer.

Fermentation Process

5.0mL of the juice sample was taken in a clean 250 mL conical flask and it was diluted with 50mL of distilled water.2.0 gram of baker's yeast and 5.0 mL of the solution of Pasteur's salt were added to the above conical flask. The contents of the flask were shaken well and the temperature of the reaction mixture was maintained between 35 - 40 ^{o}C. After 10 minutes, 5 drops of the reaction mixture were taken from the flask and it was added to atest tube containing 2 mL of Fehling reagent. The test tube was placed in the boiling water bath for about 2 minutes and the color of the solution was noted. Appearance of red colour indicates the completion of fermentation process. The procedure was repeated every ten minutes until completion of fermentation.

Results and Discussion

Estimation of Time Taken For Fermentation

The fruit juice gets fermented and gives a red colour with the Fehling's solution, on different time duration. The time taken is noted for every juice. The observed color changes are picturized in figure 1. The results are shown in table 1 and figure 2. It is observed that lemon takes more time to get fermented (120 minutes) whereas pineapple gets fermented in least time (40 minutes).

Figure1. Colour changes during fermentation process of various fruit juices

Table 1. Fermentation process of fruit extracts with different contact time

S.No.	Fruit Extracts	Time Taken for Fermentation (Minutes)
1.	Punica granatum	110
2	Citrus x sinensis	90
3.	Citrus lemon	120
4.	Vitis vinifera	60
5.	Carica papaya	90
6.	Ananas comosus	40

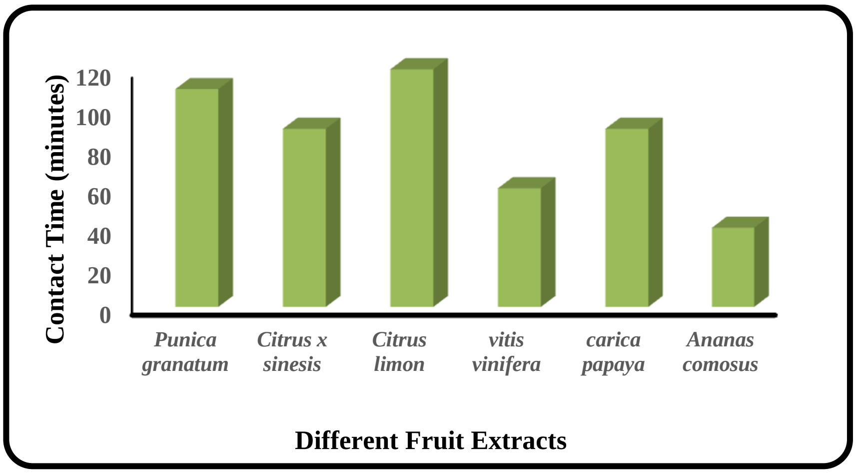

Figure 2. Fermentation of fruit juices with various contact time

Biochemical Analysis

Estimation of Carbohydrates

In the present study, it is noted that the percentage of carbohydrates decreased after fermentation in all the fruit extract of *Punica granatum*, *Ananas comosus*, *Citrus x sinensis*, *Citrus lemon*, *Vitis vinifera* and *Carica papaya*. The glucose released during fermentation is a preferred substrate for microorganisms fermenting the food. A decreasing percentage of total carbohydrate was observed after 24 hour of fermentation. Estimation of carbohydrates before and after fermentation process is noted in the Table 2 and the graphical representation is shown in the figure 3.

Table 2. Estimation of Carbohydrates

S.No.	Fruits	Percentage of Carbohydrate	
		Before fermentation	After Fermentation
1.	Punica granatum	2.45	1.1
2.	Citrus x sinesis	2.81	1.25
3.	Citrus lemon	2.63	1.2
4.	Vitis vinifera	1.90	0.89
5.	Carica papaya	2.27	0.96
6.	Ananas comosus	1.72	0.78

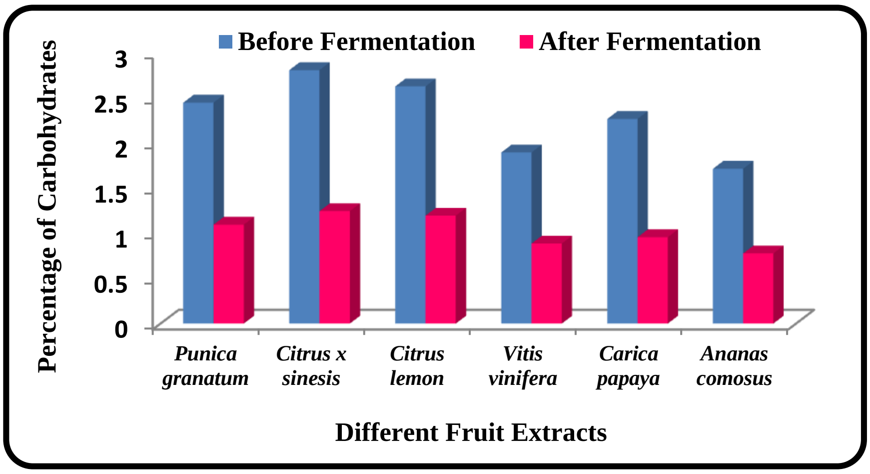

Figure 3. Estimation of Carbohydrates

Estimation of Proteins

The percentage of protein increases after fermentation. Protein is increased either by the accumulation of microbial biomass or by the concentration of protein already in the substrate as carbohydrates are consumed. Estimation of proteins before and after formation in the fruit juice is indicated in Table 3 and shown in the figure 4.

Table 3. Estimation of Proteins

S.No.	Fruits	Percentage of Proteins	
		Before Fermentation	After ermentation
1.	Punica granatum	2	2.92
2.	Citrus x sinesis	1.29	1.6
3.	Citrus lemon	1.05	1.3
4.	Vitis vinifera	0.82	0.8
5.	Carica papaya	0.47	1.1
6.	Ananas cosmosus	0.35	0.5

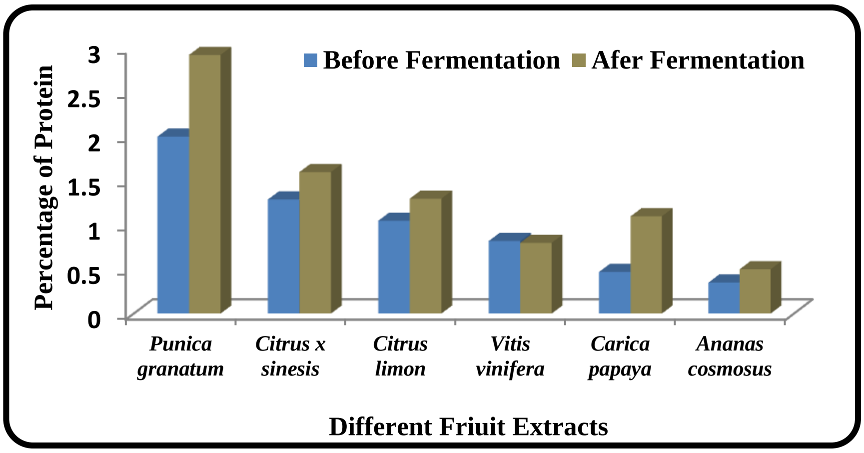

Figure 4. Estimation of Proteins

Estimation of Iron

In this study, the percentage of iron is increased after fermentation. The increased bioavailability of iron from fermented fruits may be due to the effect of the reduction of phytate, increased iron solubility. It is more likely an effect of the increase in hydrated ferric iron (Fe^{3+}) species caused by fermentation. Percentage of iron in fruit juices before and after fermentation is shown in table 4 and figure 5.

Table 4. Estimation of Iron

S.No.	Fruits	Percentage of Iron	
		Before Fermentation	Before Fermentation
1.	Punica granatum	1.33	2.2
2.	Citrus x sinesis	1.25	2.4
3.	Citrus lemon	1.16	2
4.	Vitis vinifera	0.91	1.8
5.	Carica papaya	1	1.4
6.	Ananas comosus	1.08	1.6

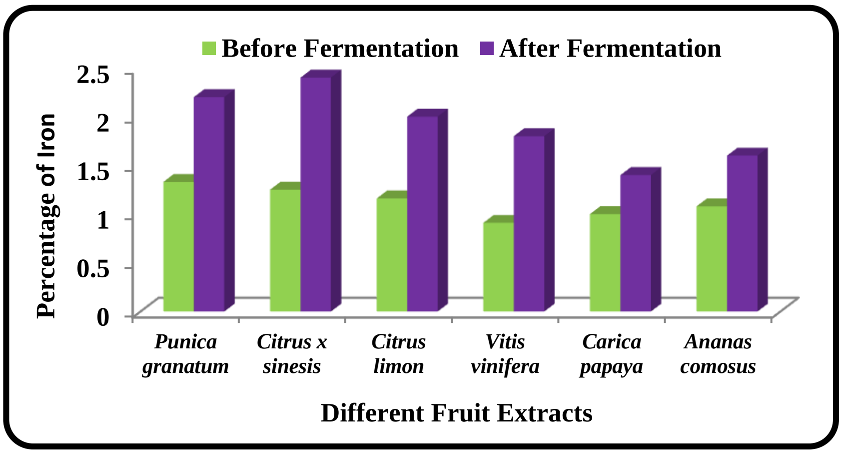

Figure 5. Estimation of iron

Estimation of Ascorbic Acid

Percentage of ascorbic acid before and after fermentation process is noted and shown in Table 5 and Figure 6. Ascorbic acid is an effective quencher of singlet oxygen and other radicals. The fermentation caused a significant increase in ascorbic content of fruit juices. This may be due to the activity of the enzyme ascorbate oxidase that has been produced by the microorganisms associated with fermentation. The highest value for ascorbic acid was observed in *Citrus lemon*.

Table 5. Estimation of Ascorbic acid

S.No	Fruits	Percentage of Ascorbic acid	
		Before Fermentation	After Fermentation
1.	Punica granatum	0.89	1.35
2.	Citrus x sinesis	1.02	1.90
3.	Citrus lemon	1.00	2.00
4.	Vitis vinifera	0.50	0.80
5.	Carica papaya	0.60	1.72
6.	Ananas comosus	0.96	1.60

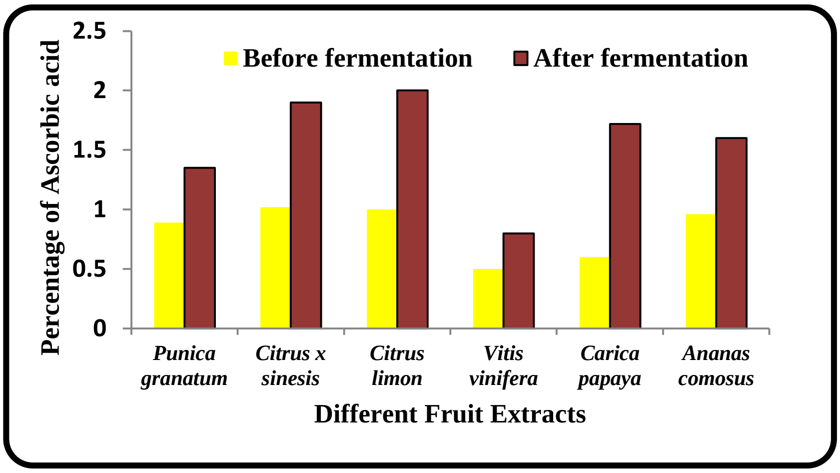

Figure 6. Estimation of Ascorbic acid

Conclusion

The development of an efficient fermentation process that could increase protein, iron and ascorbic acid percentage in fermented fruit juices could improve the nutritional quality and utility. Fermentation helps to reduce non-digestible carbohydrates, enriches the pool of essential amino acids, vitamins and minerals to increase the overall quality and digestibility of food. A significant increase of protein, iron and ascorbic acid was observed in *Citrus x sinesis* and *Citrus lemon* extracts. The use of these two fruits with optimal performance may be considered to be a simple and valuable technology for improving the nutritional, sensory and shelf-life properties. These may be used to develop new products with functional and appearing properties.

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