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Effect of thermal and thermosonication on anthocyanin stability in jamun (*Eugenia jambolana*) fruit juice

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Abstract

The purpose of this investigation is to study the influence of high intensity ultrasound, sterilization and pasteurization on the stability of anthocyanin in jamun fruit juice. Different ultrasound process parameters for the treatments are compared to the classical thermal treatment like pasteurization and sterilization. For ultrasound treatments, three parameters were varied: temperature (80°C and 90°C), amplitude (80% and 100%) and time (5 and 10 minutes). Sterilization was carried out at F_0-3. It was found that anthocyanin content after sterilization was reduced by 51.35% compared to untreated juice. Pasteurization (80°C for 5 min) reduced anthocyanin by 34% when compared to untreated jamun fruit juice. It was found that anthocyanin degradation increases with increase in temperature and time of pasteurization. After ultrasonic treatment (80% amplitude for 5 min), the degradation of anthocyanin was 21.9% when compared to the untreated juices. After treatment with thermosonication (80°C-5 min and 80% amplitude-5 min) it was found that anthocyanin degatradation slightly higher than that of pasteurization (80°C-5 min) and degradation was 35.4%. From the results it can be concluded that anthocyanin retention was greater in sonicated fruit juice compared to pasteurized, sterilized or thermosonicated fruit juices. Thermosonication can replace conventional heat processing methods for maximum retention of total anthocyanin content.

Introduction

Conventional thermal processing of fruit juices remains the most widely adopted technology for shelf-life extension and preservation of fruit juice. However, consumer demand for nutritious foods, which are minimally and naturally processed, has led to interest in non-thermal technologies. Recent regulations by the FDA have required processors to achieve a 5 log reduction in the numbers of the most resistant pathogens in their finished products. This rule comes after a rise in the number of food borne illness outbreaks and consumer illnesses associated with consumption of untreated juice products. The ruling has accelerated the search for novel non-thermal processes that can ensure product safety, yet maintain the desired nutritional and sensory characteristics. Non-thermal technologies are preservation treatments that are effective at ambient or sub-lethal temperatures, thereby minimizing negative thermal effects on food nutrition and quality parameters.

In recent years, a lot of effort has been put into the investigation and development of new methods of food processing that have reduced impact on the nutritional content and quality of food, but significantly reduce microbial activity. The amount of required heat could thereby be partially reduced or considerably eliminated (Ugarte-Romero *et al*., 2007). The color of foods is one of the most important quality factors for vegetables and plays a considerable role in the overall acceptability of foods. Color is a component of total appearance and incorporates visual recognition and assessment of the surface and subsurface properties (Nisha *et al*., 2011).

Ultrasound is defined as sound waves having a frequency that exceeds the hearing limit of the human ear (~20 kHz). Some animals utilize ultrasound for navigation (dolphins) or hunting (bats) using the information carried by back scattering sound waves. Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products. Ultrasound is applied to impart positive effects in food processing such as improvement in mass transfer, food preservation, assistance of thermal treatments and manipulation of texture and food analysis (Knorr *et al*., 2011). Based on frequency range, the applications of ultrasound in food processing, analysis and quality control can be divided into low and high energy. Low

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energy (low power, low intensity) ultrasound has frequencies higher than 100 kHz at intensities below 1 W•cm^{-2}, which can be utilized for non-invasive analysis and monitoring of various food materials during processing and storage to ensure high quality and safety.

High energy (high power, high-intensity) ultrasound uses intensities higher than 1W•cm^{-2} at frequencies between 20 and 500 kHz, which are disruptive and induce effects on the physical, mechanical or chemical/biochemical properties of foods. These effects are promising in food processing, preservation and safety. This emerging technology as been used as alternative to conventional food processing operations for controlling microstructure and modifying textural characteristics of fat products (sonocrystallization), emulsification, defoaming, modifying the functional properties of different food proteins, inactivation or acceleration of enzymatic activity to enhance shelf life and quality of food products, microbial inactivation, freezing, thawing, freeze drying and concentration, drying and facilitating the extraction of various food and bioactive components.

Power ultrasound is an emerging and promising alternative technology for food processing applications (Mason *et al*., 2005). Power ultrasound has been reported to enhance certain quality parameters of orange juice (Tiwari *et al*., 2008) and be effective against food borne pathogens in orange juice (Valero *et al.*, 2007), apple cider and milk (D'Amico *et al*., 2006). Ultrasound has been identified as a potential technology to meet the FDA requirement of a 5 log reduction in pertinent microorganisms found in fruit juices (Salleh-Mack and Roberts, 2007). Advantages of sonication include reduced processing time, higher throughput, lower energy consumption (Zenker *et al.*, 2003) while reducing thermal degradation effects.

Eugenia jambolana Lam (jamun fruit) belonging to the family Myrtaceae is a large evergreen tree indigenous to the Indian subcontinent. The trees are famous for their fruits and their colloquial names, which include Java plum, Portuguese plum, Malabar plum, black plum, Indian blackberry (Swami *et al.*, 2012), Jamun fruit contains an array of health promoting compounds. Juices prepared from jamun fruit contain a relatively high content of anthocyanins (Sagrawat *et al*., 2006). The red color of jamun fruit is due to the presence anthocyanins. The most abundant anthocyanin in food is the glucoside forms of cyaniding, malvidin, delphinidin, peonidin, petunidin and pelargonidin. Anthocyanins of jamun fruit are unstable and may degrade due to various processing conditions including; pH, light, oxygen, enzymes, ascorbic acid and thermal treatment. Non-thermal techniques have been reported to have minimal effect on anthocyanin content of berries such as high pressure processing of strawberry juice (Baliga *et al.*, 2011). The objective of this study is to investigate the effect of high intensity ultrasound, pasteurization, sterilization and thermosonication in the stability of anthocyanin in jamun fruit juice.

Materials and Methods

Juice preparation

Fresh Jamun (*Eugenia jambolana*) fruit were purchased from Mysore Local Fruit Market, India. The seed was removed manually. Seed removed jamun fruit was subjected to blending. The pulp was filtered by using muslin cloth for juice extraction. This juice was used for processing.

Thermal processing

Thermal processing of juice was done by sterilization (F_0-3) and pasteurization. Sterilization was carried out by Retort (IN-PACK STERILIZATION RETORT Alpha Steritech, Bangalore) at F_0-3. For this 200 ml of genuine fruit juice were filled into retort pouches, sealed and kept in a retort for thermal processing. For pasteurization different time-temperature was taken. i.e.; 80°C for 5 minutes and 10 minutes, 90°C for 5 and 10 minutes. Pasteurization was done by using a water bath (CONTINENTAL-NEW DELHI). 100 ml of juice was taken in a beaker and kept in a water bath for pasteurization.

Non-thermal processing (Ultra sound treatment or Sonication)

Jamun fruit juice (100 ml) was placed in a beaker, which served as treatment chamber. An ultrasonic processor was introduced into the vessel. Ultrasonication was carried out with 80% and 100% amplitude. Jamun fruit juice samples were treated ultrasonically for 5 and 10 minutes; i.e., 80% amplitude 5 minutes and 10 minute and 100% amplitude for 5 and 10 minutes. For this study 12 samples were ultrasonically treated. The laboratory use of this sonication system includes discontinuous use of ultrasound probe that is immersed in the sample, and which is intended for small scale testing.

Combination processing (Thermo sonication)

Jamun fruit juice samples (100 ml) were taken in a beaker and pasteurized by Water bath (CONTINENTAL, NEW DELHI) at 80°C and 90°C for 5 minutes and 10 minutes; i.e., 80°C for 5 minutes and 10 minutes, 90°C for 5 minutes and 10 minutes. The pasteurized jamun fruit juice was treated with ultrasound at 80% and 100% amplitude for 5 and 10 minutes; i.e., 80% amplitude for 5 minute and 10 minutes, 100% amplitude for 5 minutes and 10 minutes.

Total soluble solids (°Brix)

The TSS of Jamun fruit juice was measured by using Hand Refractometer and expressed as °Brix.

Determination of pH

pH of the sample was measured using a pH meter by Eutech Instruments, Singapore.

Titratable acidity (%)

The Acidity of Jamun fruit juice was estimated method suggested by Ranganna (1999) and is expressed in percentage of citric acid.

Ascorbic acid (mg/100 g)

The Ascorbic acid content was determined by 2,6-Dichlorophenolindophenol sodium salt visual titration method as suggested by Ranganna (1999). The dye, which is blue in alkaline solutions and red in acidic solutions, was reduced by ascorbic acid to pink color.

Estimation of reducing, non-reducing and total sugars (%)

Reducing and Total sugars present in the Jamun fruit juice was estimated as per method described by AOAC 1990. Non-reducing sugars present in the sample was calculated as per the formula given below and is expressed in terms of percentage.

Non-reducing sugars (%) = Total sugars (%) – Reducing sugars (%).

Estimation of anthocyanin (mg/100 gm)

The anthocyanin content of genuine fruit juice was estimated by methods suggested by AOAC (1990) and is expressed in mg/100 gm.

Results and Discussion

In this study jamun fruit juice was prepared, immediately after the preparation of juices, the chemical analysis was conducted. Anthocyanin was estimated that 69.45 mg/100 gm. The purpose of this investigation was to examine the influence of high intensity ultrasound and thermal processing on the stability of anthocyanin in jamun fruit juice. Different process parameters of ultrasound (amplitude, time, temperature) have been compared to the classical thermal treatments (pasteurization and sterilization). Anthocyanin content during processing can be influenced by three factors, i.e. ultrasound amplitude level (%), sonication time (min) and temperature (°C). The Chemical analysis of untreated jamun fruit juice was analysed and found to be °Brix 8, pH 3.43, Acidity (% of citric acid) 0.28, Reducing sugar (%) 4.77, Total sugar (%) 7.84, Anthocyanin (mg/100 ml) 69.45 and Ascorbic acid (mg/100 ml) 16.56.

Table 1. Chemical parameters jamun fruit juice after thermal processing

Parameters	Pasteurization				Sterilization
	80°C		90°C		F_0-3
	5 min	10min	5 min	10 min	
pH	3.73	3.81	3.84	3.91	3.63
Acidity (% of citric acid)	0.26	0.27	0.24	0.25	0.28
Reducing sugar (%)	4.83	4.82	4.95	5.11	6.24
Total sugar (%)	7.57	7.28	8.14	7.84	7.61
Anthocyanin (mg/100gm)	45.6	43.9	41.8	40.2	33.8
Ascorbic acid (mg/100gm)	10.84	9.61	9.2	8.51	5.52
°Brix	8	8	8	8	8

Table 2. Chemical parameters of jamun fruit juice after non thermal processing

Parameters	Sonication			
	80% Amplitude		100% Amplitude	
	5 min	10 min	5 min	10 min
pH	3.63	3.74	3.71	3.82
Acidity (% of citric acid)	0.29	0.26	0.26	0.29
Reducing sugar (%)	4.75	4.83	4.79	4.92
Total sugar (%)	7.34	7.46	7.54	7.61
Anthocyanin (mg/100 gm)	54.19	53.87	53.15	51.24
Ascorbic acid (mg/100 gm)	13.89	13.28	13.10	12.75
°Brix	8	8	8	8

Table 3. Chemical parameters of thermosonicated jamun fruit juice

Parameters	Thermosonication							
	80°C-5 min 80% Amplitude		80°C-10 min 80% Amplitude		90°C-5 min 100% Amplitude		90°C-10 min 100% Amplitude	
	5 min	10 min	5 min	10 min	5 min	10 min	5 min	10 min
pH	3.64	3.53	3.73	3.61	3.81	3.67	3.85	3.71
Acidity (% citric acid)	0.28	0.28	0.26	0.24	0.24	0.27	0.24	0.27
Reducing sugar (%)	4.95	4.84	5.10	5.14	5.23	5.17	5.15	5.32
Total sugar (%)	7.14	7.34	8.51	6.91	7.64	8.11	7.28	7.43
Anthocyanin (mg/100 g)	44.83	44.14	43.10	42.60	41.10	40.60	39.40	38.80
Ascorbic acid (mg/100 g)	10.31	10.10	9.22	8.87	8.24	8.10	7.85	7.21
°Brix	8	8	8	8	8	8	8	8

Effect of thermal processing

Samples which were sterilized (F_0-3) show the lowest level anthocyanin i.e. 33.8 mg/100 gm as compared to pasteurized jamun fruit juices at varying temperature and time. The degradation of anthocyanin and ascorbic acid was increased with increasing temperature and time of pasteurization. Titratable acidity, pH, Reducing sugar, Total sugar and °Brix for untreated jamun fruit juice were 0.28% citric acid, 3.43, 4.77%, 7.84% and 8 respectively (Table 1). Treatment of Jamun fruit juice with temperature did not cause significant differences in these parameters. Anthocyanin degradation occurred under heating conditions, likely due to accelerated chalcone formation with prolonged anthocyanin exposure to high temperatures. Anthocyanin degradation rates were directly related to thermal exposure times. Degradation is primarily caused by oxidation, cleavage of covalent bonds or enhanced oxidation reactions due to thermal processing.

Anthocyanins are the bioactive compounds present in different fruits and vegetables, are the basis for the red, blue and purple colors of fruits and vegetables. They are among the most abundant flavonoid constituents of fruits and vegetables. They have a series of conjugated bonds capable of absorbing a light up to 500 nm, which provide the basis for the red, blue and purple colors in different fruits, vegetables. They are readily degraded during thermal processing leading to loss of color and nutritional quality. Anthocyanin degradation results from oxidation during thermal processing and cleavage of covalent bonds with the degree of degradation depending upon the severity of the heat treatment. The degradation rate of anthocyanins increases during processing and storage as temperature rises (Palamidis and Markakis, 1978).

Anthocyanins are rapidly degraded during thermal processing even at pasteurization temperatures (Patras *et al*., 2010). The stability of anthocyanin depends upon many factors besides heat, such as pH, storage temperature, chemical structure of the anthocyanin compound, presence of UV light, oxygen, oxidative and hydrolytic enzymes, proteins and phenolic compounds that could have a protective effect, and the metallic ions that could enhance oxidation (Giusti and Wresland, 2003). The exact mechanism for the stability of anthocyanin is difficult to establish but the phenolic acids such as Ferulic and syringic acids play a role in its stability. Magnitude and time of heating have a strong influence on anthocyanin stability and after 3 h of heating at 95°C only 50% of anthocyanin based pigments in elderberry were retained (Sadilova *et al.*, 2006)

Volden *et al.* (2008) reported that blanching, boiling and steaming resulted in anthocyanin losses of 59%, 41% and 29%, respectively in red cabbage; however the anthocyanins in certain products appear to be more stable. For black carrot, anthocyanins showed reasonable stability during heating at 70 - 80°C (Kirca *et al*., 2006) and 70°C with these differences in stability related to anthocyanin structure and the pH value. The black carrot anthocyanins, show stability to heat and pH as compared to other sources due to the presence of di-acylation of anthocyanin structure. Acylation of the molecule is believed to improve anthocyanin stability by protecting it from hydration Brouillard (1981). The presence of inter and intramolecular co-pigmentation with other moieties, polyglycosylated and polyacylated anthocyanins provide greater stability towards change in temperature, pH and light. Storage temperature had a very strong influence on the stability of black carrot anthocyanins colored juices and nectars

Dyrby *et al*. (2001) reported greater stability of anthocyanins present in red cabbage at temperature ranges from 20-80°C and at treatment times ranging from 15-360 min as compared to anthocyanins in black currant, grape skin and elderberry in a soft drink model system. This was thought to be due to the protection of the flavylium system through co-pigmentation in cabbage. High temperature together with high pH caused degradation of cherry anthocyanins resulting in three different benzoic acid derivatives (Seeram *et al.*, 2001) also a trihydrobenzaldehyde has been identified as an end product of thermal degradation of anthocyanins (Furtado *et al.*, 1993). Coumarin-3,5-diglycosides are also common thermal degradation products of anthocyanin 3,5-diglycosides (von Elbe and Schwartz, 1996).

Effect of non-thermal processing

Titratable acidity, pH, Reducing sugar, Total sugar and °Brix for untreated jamun fruit juice were 0.28% citric acid, 3.43, 4.77%, 7.84% and 8, respectively. Treatment of Jamun fruit juice with ultrasound irrespective of amplitude level or treatment time did not cause significant differences in these parameters of juice (Table 2). Maximal degradation of anthocyanins and ascorbic acid in the study was observed after treatment at the amplitude of 100% for 10 minutes. It was found that anthocyanin exhibits a high degree of stability to sonication. During sonication a decrease of 18.21 mg/100 gm was observed at the maximum treatment conditions of 100% amplitude for 10 minutes. Anthocyanin degradation increase with increasing amplitude and time of exposure.

Effect of thermosonication

Treatment of Jamun fruit juice with ultrasound irrespective of amplitude level or treatment time and temperature did not cause significant differences in Titratable acidity, pH, Reducing sugar, Total sugar and °Brix (Table 3). The anthocyanin content of fresh jamun fruit juice was 69.45 mg/100 gm. However, at higher amplitude levels and treatment time, anthocyanin content and ascorbic acid was found to decrease. Processing time was shown to have a significant effect on anthocyanin content, as processing time was increased from 5 to 10 min, the level of anthocyanin decreased significantly. Amplitude level was found to have a significant effect on anthocyanin content. Power ultrasound has shown promise as an alternative technology to thermal treatment for food processing (Mason *et al*., 2005). Ultrasound processing of juices is reported to have minimal effect on the degradation of key quality parameters such as color and ascorbic acid (Tiwari *et al*., 2008). They reported a slight increase (2%) in the pelargonidin-3-glucose content of the juice at lower amplitude levels and treatment times, which may be due to the extraction of bound anthocyanins from the suspended pulp. Similarly, weak ultrasonic irradiation is reported to promote an increase in the amount of phenolic compounds found in red wine (Masuzawa *et al*., 2000). Cavities formed by sonication may be filled with water vapour and gases dissolved in the juice, such as O_2 and N_2 (Korn *et al*., 2002). The anthocyanin degradation may also be due to the presence of other organic acid or ascorbic acid and can be related to oxidation reactions, promoted by the interaction of free radicals formed during sonication as shown for ascorbic acid (Portenlanger and Heusinger, 1992).

Conclusion

The results presented in this study, indicates the effect of ultrasound amplitude level and treatment time on color degradation and retention of anthocyanin. Anthocyanins were observed to be significantly influenced by sonication. However, 73% retention of anthocyanin was observed at maximum treatment conditions of amplitude and time. The purpose of the simultaneous use of the combined effect of ultrasound and temperature on the content of anthocyanins in jamun fruit juices was to reduce the temperature and/ or time of thermal processing. The results of this investigation on the effect of combined ultrasound and heat treatment versus thermal processing alone of bioactive compounds in juices also clearly indicate the improved retention levels by the combined treatment. However sonication and thermosonication can be employed for as a preservation technique for processing of jamun fruit juice where high retention of anthocyanin is desired.

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