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Influence Of Ultrasound On Physical Stability Of Custard Apple Pulp.

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ABSTRACT

Ultrasound (Sonication), is a non-thermal technique that has been widely used in food industries as a preservation tool for number of food processing areas. The purpose of using ultrasonicator as a preservation tool is to enhance the stability of the custard apple pulp. The current work evaluates the effect of sonication (0, 2, 4, 6, 8, 10 mins, at 35°C, pulse rate 2sec (on), 3sec (off), at 40% amp) on the physical stability of custard apple pulp. Sonication altered the antioxidant activity determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, pulp sedimentation, serum cloudiness (determining turbidity), and surface morphology studied using scanning electron microscopy(SEM). It was found to increase the surface area of suspended particles while decreasing the particle size and the pulp cloudiness.Sonication treatment concluded that stability can be maintained for the pulp, thus increasing the shelf life eventually. The observations indicated that with sonication treatment, improvement can be brought to the quality as well as the stability of the custard apple pulp, thus making it commercially available in the future.

Keywords: Ultrasonication; Custard apple pulp; Morphology; Sedimentation index; Stability

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INTRODUCTION

Custard apple (Annona reticulate) is a small fruit from deciduous Annonaceae family, originating from carribean and Central America. It has been largely produced for its pulp to make pulp and cultivated in many tropical countries and also in many parts of the world like India, Australia and Taiwan. Custard apple mainly consists of vitamin C, an anti-oxidant and it helps to neutralize free radicals and furthermore it is helpful for treating various digestion problems. Several of the researches have also explored the advantage of custard apple in treating many intestinal problems like ulcers, abscesses and boils. The custard apple fruit can be used for tending infections causing diarrhoea and dysentery when the fruit is in unripe form.

With the advancement of scientific researches and high availability of knowledge, the consumers are expecting the food in the market to be safe, fortified with necessary nutrition while at the same time retaining of natural taste and freshness. Many techniques in the food industry can extend the shelf life of a product, mainly thermal processes, but they mostly result in loss of nutritional qualities and physicochemical parameters[1]. Thus, a new technique is to be introduced which can overcome this problem, successfully ensuring the extension of the shelf life, improving the quality of the food product without undergoing any drastic change in its nutritional quality.

Sonication is thus an innovative technique as well as a non-thermal process with which the desired purpose can be fulfilled[2]. It not only avoids degradation of nutrition but it minimises the flavour loss, improves the overall quality of the product and inactivates the microbial growth[3]. Moreover, it has reduced processing time with low energy usage, which makes it more advantageous over other machines[4].Thus, the intention of this work is to assess the impact of ultrasound on physical stability of custard apple pulp.

MATERIALS AND METHODS

Preparation of custard apple pulp

From a local market, fresh custard apples were purchased in the area of VIT University, Vellore. Custard apples were peeled off and seeds present in it were removed. A household table top pulp extractor was used to extract the custard apple pulp. The resulting pulp was then subjected to ultrasonication.

Ultrasound treatment

Optimization of the samples was done at various time intervals and sonication was performed on control sample (0 min) and working samples for 2, 4, 6, 8 and 10 mins at a constant temperature of 32°C, pulse rate at 2 sec on - 3 sec off and amplitude 40% by using an ultrasonicator (Sonics and Materials, INC. 53, Church Hill Road, Newtown, CT, USA, Model: VCX500). All the pulp samples were sterilized and transferred to air tight tubes immediately after the sonication treatment for further analysis.

Pulp characterization

Structural morphology

To assess the effect of sonication on the custard apple pulp, the sonicated samples (2-10 mins) and the control sample (0min) was observed under SEM (ZEISS-EV018, USA).To obtain the results, the samples were kept onto a glass slide covering the part with a cover slip[5].

Pulp sedimentation

Pulp sedimentation was assessed by using 15ml graduated tubes (sterilized) having conical bottom. The samples were stored at room temperature for a total of 15 days. As illustrated by Augusto et al.[5], Silva et al.[6], Yu et al.[7], the sedimentation index (IS) was calculated by the following formula:

$$IS = \text{Sedimentation } \frac{\text{volume}}{\text{Total}} \text{sample volume}$$

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Serum cloudiness

The serum cloudiness was assessed after centrifuging the custard apple pulp at 3,000rpm for 10 min at 20°C (C24BL Model, Remi Electrotechnik Ltd., India). The optical density (absorbance) of the supernatant was measured at 660 nm using a spectrophotometer (UV Mini-1240, SHIMADZU Corp., Japan) and thus related to the turbidity of the pulp directly[7] [6].

Bioactive compound analysis

Determination of DPPH free radical scavenging activity

According to the technique stated by Yi et al.[8]DPPH free radical scavenging activity was assessed with slight modification. In a tube, 2.0 mL of sample was mixed with 2.0 mL of 0.2 mMethanolic DPPH solution. The reaction mixture was then allowed to stand for 30 min in dark at room temperature. Using spectrophotometer (UV Mini-1240, SHIMADZU Corp., Japan) the absorbance was measured at 517 nm. % Inhibition of DPPH free radical scavenging activity was determined by the following equation:

$$\% \text{ Inhibition} = \left(\text{Absorbance of control} - \text{Absorbance of} \frac{\text{sample}}{\text{Absorbance}} \text{ of control}\right) \times 100$$

Determination of total phenolic content

Using spectrophotometer (UV Mini-1240, SHIMADZU Corp., Japan) the total phenolic content was determined by Folin–Ciocalteu colorimetric method[9].

Statistical analysis

Data obtained in the study (bioactive compound analysis) were represented as mean value ± standard deviation (SD). Completely Randomized Design (CRD) was used with One-way ANOVA at a significance level of $P < 0.05$, significant differences between mean values were performed by Tukey's range test. Statistical analyses were determined by using GraphPad Prism 5.01 software (AnalyticalSoftware, La Jolla, CA, USA).

RESULTS AND DISCUSSION

Structural morphology

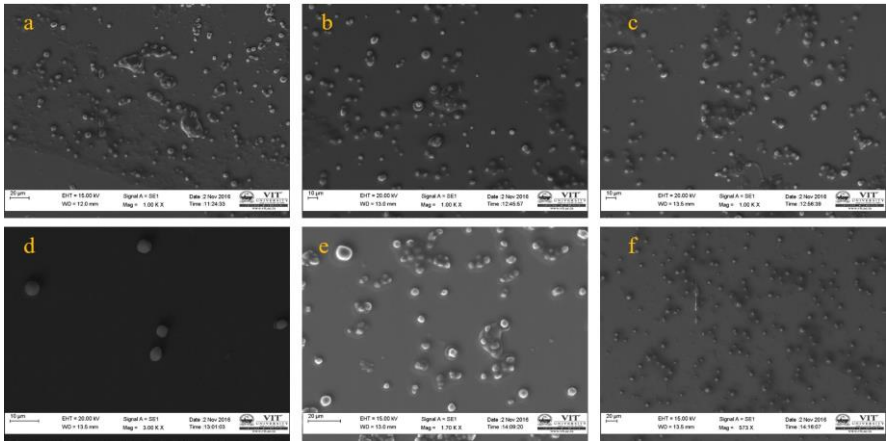

Fig 1: Effect of ultrasonication on the microstructure of the custard apple pulpsonicated for (a) 0 min (Non Sonicated)(b) 2mins (c) 4 mins (d) 6 mins (e) 8 mins(f) 10 mins.

Fig. 1. depicts the microstructures of custard apple pulp. Initially, the unsonicated sample (0min) and the sonicated sample (2mins) showed deformable and irregularly structured particles with the presence of both large and small particles. The large particles are part of whole cells or aggregates while the smaller

particles are referred to internal components suspended or other cell parts present in the pulp sample. In the process of sonication (4, 6, 8 and 10 mins) the observed particles which are suspended undergone breakage resulting in the formation of more number of smaller particles comprised of cell wall fragments, cells, fibrous particles and polymers. Thus, with the increasing sonication time disruption of custard apple cells and cluster was observed followed by increase in the surface area of the suspended particles in the sample permitting for a total cell disintegration when the pulp undergone sonication till 10 mins. Hence, a clear difference was assessed between the control sample (0min) and the sonicated samples (2, 4, 6, 8 and 10 mins) [10][7] [6].

Pulp sedimentation

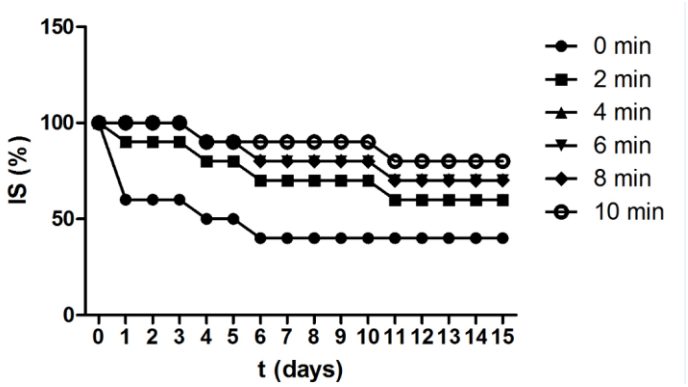

Fig 2: Effect of Sonication on the sedimentation index (IS)of the custard apple pulp samples during 15 days of storage.

Fig. 2. depicts the sedimentation index (IS) of the control (0 min) and the ultrasonicated samples (2, 4, 6, 8 and 10 mins) after 15days. In the initial 24h, the sample which is unsonicated, exhibited phase separation, which for some fruits is a common phenomenon[11]. Also, it is important to highlight that the unsonicated sample i.e., 0min sample and the sonicated 2mins sample showed rapid sedimentation (IS decreased from 100% to approx. 60%) in 24h. The difference in the sedimentation rate of the initial samples of the pulp could be explained with a possibility of disruption of the larger particle aggregates, since the pulp got ultrasonicated. For large aggregates of particles, the inter particle forces are relatively weak and the shear stress caused is due to sonication, which is sufficient to separate the pulp particles. Therefore, the bigger aggregates of particles which were still existing in the unsonicated sample would sediment rapidly, which shows a scaling down in IS in just 24 hours[5] [6]. But then again, the sonicated samples (4, 6, 8 and 10 mins) exhibited particle sedimentation from the 4th day, thus confirming that stabilization of the custard apple pulp could be attained by sonication treatments. According to the Stokes law, the sedimentation particle velocity is proportional to the magnitude of the particle (diameter) and the difference between the particle's densities and the suspended medium is inversely proportional to the viscosity of the suspended medium[12]. Thus, the depletion in the size of the particle during the process of sonication of the sample can be associated to the increased/greater stability of the samples undergone sonication, and thus ultrasonication can be considered as a significant tool for the prevention of pulp sedimentation[5] [6].

Serum cloudiness

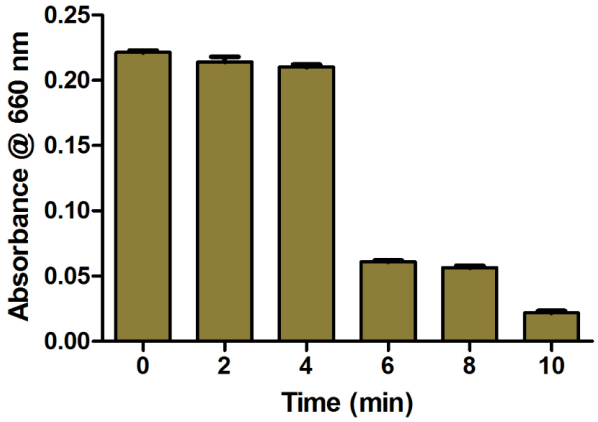

Fig 3: Effect of sonication on serum cloudiness of the custard apple pulp samples.

Fig. 3. shows the result of ultrasonication on serum cloudiness (SC) of custard apple pulp. It is clearly observed that with increase in sonication time from 0 to 10 mins, there is reduction in pulp cloudiness upto the 10min interval. It is because the smaller particles are allowing a greater amount of light to pass through the pulp[7] [6]. Thus decrease in cloudiness with increase in the time of sonication treatment can be imputed as the particle size is getting decreased leading to lowering of the serum cloudiness[5] [6] [7].

Bioactive compound analysis

The total phenolic content is constant for the intervals 0, 2, 4 and 6 mins whereas it significantly ($P<0.05$) reduced in 8 and 10 mins, indicating the reduction in the antioxidant property (Table 1).The percentage inhibition of DPPH free radical scavenging activity significantly ($P<0.05$) decreased in samples sonicated at different time intervals as compared to that of the 0min (Table 1). The percentage inhibition of DPPH free radical scavenging activity and the total phenolic content of the samples are significantly ($P<0.05$) different from each other. The decrease in antioxidant capacity can be imputed to the decrease in total phenolic content in the custard apple pulp[2].

Table 1

Effect of sonication on DPPH (% inhibition) and Total phenol content of the custard apple pulp samples.

%w/w	0 minutes	2 minutes	4 minutes	6 minutes	8 minutes	10 minutes
DPPH (% Inhibition)	80.73± 0.4	78.73± 0.4	72.2 ± 0.1	70.96± 0.6	69.1 ± 0.1	68.13 ± 0.1
Total phenol content (mg)	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.22 ± 0.3	0.2 ± 0.1

CONCLUSION

Our present study manifested the effect of ultrasonication on the physical attributes of the custard apple pulp. The results indicated that the serum cloudiness decreased with increase in the sonication time. Increase in the sonication time enhanced the stability of the custard apple pulp in the sedimentation test. Furthermore, it indicated that sonication of custard apple pulp at lesser time interval is favourable to the consumer from health point of view.

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REFERENCES

[1] Gomez PL,Welti-Chanes J, Alzamora SM. Annu Rev Food SciTechnol 2011; 2: 447–465.
[2] Abid M, et al. UltrasonSonochem2013; 20: 1182–1187.
[3] Piyasena P,Mohareb E,McKellarRC.Int J Food Microbiol2003; 87: 207-216.
[4] Mason TJ, Paniwnyk L, Lorimer JP. UltrasonSonochem 1996; 3: S253-S260.
[5] Augusto PED, et al. Food Res Int2013a; 51: 170–179.
[6] Silva VM, et al. Int J Food Sci Tech2010; 45: 2127–2133.
[7] Yu ZY, et al. J. Food Eng2016; 17: 1-8.
[8] YiZB, et al. LWT-Food SciTechnol2008; 41: 597–603.
[9] Singleton VL, Orthofer R, Lamuela-Raventos RM. Methods Enzymol1999; 299: 152–178.
[10] Augusto PED, et al. Food Res Int2013b; 54: 169–176.
[11] Okoth MW, Kaahwa AR, Imungi JK. Food Control 2000; 11: 305-311.
[12] Kubo MTK, Augusto PED, Cristianini M. Food Res Int2013; 51: 170-179.