Dual-stage sugar substitution in *Tommy Atkins* mango

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ABSTRACT: The dual-stage sugar substitution technique (D3S) was used to induce sugar replacement in mango. It involved two stages, in which high-calorie sugars were partially removed from the fruit samples in the first stage and, in the second one, low-calorie sugar was incorporated into the mango. Ultrasonic waves can be applied in one or both stages and their use was also evaluated in this study. Results showed that submitting samples to ultrasonic waves (25 kHz) in both stages and their immersion in Stevia-based solution (250 or 500 g kg^{-1}) in the second stage for 10, 20 and 30 min of processing gave higher water loss during the process, while greater solids gain could be achieved by applying ultrasound only in the first stage. Samples were also evaluated in terms of some quality parameters. The use of this technique resulted in samples with higher values of total phenolic content and changes in color parameters (L*, a* and b*). When samples were subjected to ultrasonic waves in both stages, a higher carotenoid retention was observed.

Key words: carotenoids, color, *Mangifera indica*, phenolics, preservation

Substituição de açúcar em dois estágios na manga *Tommy Atkins*

RESUMO: A técnica de substituição de açúcar em dois estágios (D3S) foi utilizada para induzir a troca de açúcares na manga. Esta técnica envolve dois estágios, nos quais os açúcares de alto teor calórico são parcialmente removidos das amostras de frutas no primeiro estágio e, no segundo, açúcar de baixa caloria é incorporado à manga. Ondas ultrassônicas podem ser aplicadas em um ou ambos estágios e seu uso também foi avaliado neste trabalho. Os resultados indicaram que submeter as amostras às ondas ultrassônicas (25 kHz) nos dois estágios e sua imersão em solução a base Stevia (250 ou 500 g kg^{-1}) no segundo estágio por 10, 20 e 30 min de processamento resultou em maiores perdas de água durante o processo, enquanto maiores valores de ganho de sólidos foram obtidos pela aplicação do ultrassom apenas no primeiro estágio. As amostras também foram avaliadas em relação a alguns parâmetros de qualidade. O uso desta técnica resultou em amostras com maiores valores de fenólicos totais e em mudanças nos parâmetros de cor (L*, a* e b*). Quando as amostras foram submetidas às ondas ultrassônicas em ambos estágios, uma maior retenção de carotenoides foi observada.

Palavras-chave: carotenoides, cor, *Mangifera indica*, fenólicos, preservação

Introduction

Mango has limited storage life and is susceptible to chilling injury (Sogi et al., 2015). Therefore, some techniques are necessary to increase its preservation and storage period and to reduce the wastes from the harvest season (Pu & Sun, 2016).

Food demand has increased in the past years not only with respect to its quantity but also its quality, requiring changes in techniques for food processing (Pingret et al., 2013). Consumers are demanding for products based on natural and healthy appeal, with low content of sugar (Oliveira et al., 2012). Thus, low-calorie sweeteners with a potential to replace sugar have been investigated (Gasmalla et al., 2014).

Steviol glycoside is an attractive non-caloric sugar substitute for the food industry (Crammer & Ikan, 1986). It has some advantages when compared to other sugars: its source is natural, stability at temperatures up to 200 °C, acid-stable, not fermentable (Kroyer, 2010), and acceptable sensory characteristics (Prakash et al., 2008). It finds extensive use in several countries to sweeten food products (Elkins, 1997). Garcia-Noguera et al. (2010) reported that it has a great potential to be used in dried fruit products and proposed a new technique called dual-stage sugar substitution (D3S). In the D3S first stage, caloric sugars are removed from the fruit, while in the second one a Stevia-derived sweetener is incorporated by the material. This technique was applied to Malay apples and strawberry (Garcia-Noguera et al., 2010; Oliveira et al., 2012), where the use of ultrasonic waves during the D3S process was reported to enhance mass transfer (sugar removal in the first stage and/or stevia incorporation in the second one).

This work studied the use of the D3S technique for the sugar substitution in mango by Stevia natural sweetener using ultrasonic pretreatments in osmotic solutions. The effect of ultrasound application during the D3S stages was determined. The samples were also evaluated for total phenolic and total carotenoids contents, and total color difference.

Material and Methods

The experiments were carried out at the Laboratory of Chemical Processes, Department of Chemical Engineering (DEQ), Federal University of Pernambuco (UFPE), in Recife, Pernambuco state, Brazil.

Tommy Atkins mangoes were purchased in a local market (Recife, Brazil), transported to the laboratory and stored at 4 °C until processing. Throughout the research, firm and healthy mangoes, without injuries of pathological, mechanical or physiological nature were used in the experiments. The fruits were washed in tap water, the peel was removed manually and the pulp was sliced (3.0 x 5.0 x 0.5 cm) using cutters designed for this purpose.

The average initial moisture content of mango was 0.864 ± 0.002 kg water kg^{-1} fresh fruit, the soluble solids content was 14 ± 0.3° Brix, the reducing sugars content was 8.70 ± 0.28%, pH was 3.97 ± 0.03, acidity was 0.39 ± 0.07% citric acid, and water activity was 0.97 ± 0.12.

The D3S process parameters and conditions (e.g. solution concentration, immersion time, ultrasound frequency) was based on Garcia-Noguera et al. (2010) and in preliminary tests. The experiments were performed in triplicate.

For the first stage, conical flasks with distilled water and two mango samples (near 10 g each batch) were placed in an ultrasonic bath (frequency of 25 kHz) (Unique, model USC-2850A, Brazil). The weight ratio between the fruit and the liquid medium was 1:4 (Azoubel et al., 2010). Experiments were performed at 30 °C with and without submitting fruit samples to ultrasonic waves.

After 30 min, samples were removed from the solution, drained, blotted with absorbent paper and weighed. For the moisture content determination, the samples were placed in a drying oven at 105 °C for 24 h (Tecnal, TE-395, Brazil). The water loss (WL) and solid gain (SG) were calculated according to Azoubel et al. (2010).

For the second stage, it was used three immersion times (10, 20 and 30 min), two Stevia solution concentrations (250 and 500 g kg^{-1} solution) and the samples were submitted (25 kHz) or not (0 kHz) to ultrasonic waves. Experiments were conducted as explained in the first stage. Stevia powder donated by Steviafarma (Brazil) was used as the natural sweetener.

Total carotenoids and total phenolic contents were determined according to Rodriguez-Amaya (1999) and Singleton et al. (1999), respectively.

The color parameters (L*, a* and b*) of mango were determined using a colorimeter (Minolta, CR400, Japan). The color change after processing (TCD) was determined according to Medeiros et al. (2016).

The quality analysis was carried out in triplicate.

Analysis of variance (ANOVA) of the results was performed to determine significant differences among samples. Means were compared by Tukey test at 0.05 probability level.

Results and Discussion

The initial moisture content for mango was 0.864 ± 0.002 kg water kg^{-1} fresh fruit. The final moisture content of processed samples varied and it was used to calculate the water loss and solid gain values.

During the first stage, water was incorporated into the fruit, while it lost solids to the water medium (Table 1). This was expected as the concentration gradient favored this behavior. Thus, the moisture content of the fruit became higher (increased by 2.67 and 4.69% for 0 and 25 kHz, respectively). A similar trend was reported by Azoubel et al. (2010), which obtained bananas with higher moisture content after the fruit pretreatment.

The first D3S stage had as objective the removal of high-calorie sugars, like glucose and fructose, and this sugar loss was considered equivalent to the loss of soluble solids (Oliveira

Table 1. Water loss (WL) and solid gain (SG) of mango after the first D3S stage

Ultrasonic frequency applied (kHz)	WL	SG	Moisture content
	(%)		
0	-3.05 ± 0.17	-2.22 ± 0.02	88.75 ± 0.25
25	-4.31 ± 0.27	-4.03 ± 0.03	90.49 ± 0.71

et al., 2012). As negative values were obtained for SG, it was confirmed the loss of solids from the samples during this stage. The higher soluble solids loss obtained for mango was observed when it was submitted to ultrasonic waves.

The results for the mass transfer parameters evaluated in the second D3S stage are presented in Table 2. For water loss, it can be observed that when the solution concentration was higher, there was a significant increase ($p < 0.05$) in water loss. It was not statistically significant only when mangoes were not submitted to ultrasonic waves in the first and second stages and the immersion time was 10 min in the second stage. Mendes et al. (2013) reported that increasing solution concentration promoted higher water loss due to an increase in the osmotic pressure. There is also a general trend when the samples were submitted to ultrasonic waves of a significant (95% confidence level) continuous increment of water loss when immersion time in Stevia solutions increased from 10 to 30 min. The greatest water loss (11.61%) was obtained when the fruit was submitted to ultrasound in the first and second stages and immersed in the higher Stevia solution concentration for 30 min. Similar results were observed when this process was applied to strawberries (Garcia-Noguera et al., 2010) and in Malay apples (Oliveira et al., 2012). However, in the case of Malay apples, samples were immersed for a longer time (45 min).

In a general way, solid gain values tended to significantly increase ($p < 0.05$) as immersion time and solution concentration increased. As observed by Garcia-Noguera et al. (2010), when ultrasound was applied in both stages, the solids gains were slightly higher than those obtained when no ultrasonic waves were applied (0 kHz) in the first stage. However, the D3S process resulting in higher sweetener gain (3.56%) was obtained for mangoes submitted to ultrasonic waves in the first stage (sugar removal stage) followed by immersion in the 50 g kg^{-1} Stevia-based solution for 30 min without ultrasonic

application. The same behavior was observed by Garcia-Noguera et al. (2010) for strawberries. The authors reported that during the first stage the formation of micro-channels in the fruit tissue structure caused by the application of ultrasonic waves favored the Stevia incorporation in the second stage. The higher sweetener (Stevia) gain without the application of ultrasound in the second stage was attributed to the lower osmotic pressure of the Stevia-based solution and to the fact that ultrasound could cause soluble solids extraction from the fruit, reducing the entry of solids into the strawberry. Thus, the process could be performed without using ultrasound in the second stage in order to recuperate the mango initial sweetness.

The food industry is interested in technologies that can promote microorganism control in foods without changing its initial quality (nutritional and sensory characteristics). Studies about the use of ultrasound and its interference on the quality of fruit juices are common. However, for a potential use by the food industry, an evaluation of products submitted to new technologies is necessary (São José et al., 2014).

Table 3 shows the obtained values for the evaluated quality parameters before and after two processing conditions of the osmotic process. Those conditions were chosen based on higher values of mass transfer parameters (WL and SG) and were named D3S 1 (ultrasound in the first stage for 30 min followed by immersion of samples in a 500 g kg^{-1} Stevia-based solution for 30 min at 0 Hz) and D3S 2 (ultrasound in the first stage for 30 min followed by the sample immersion in a 500 g kg^{-1} Stevia-based solution for 30 min at 25 Hz), respectively.

Total phenolic content (TPC) for fresh and treated samples are significantly different ($p < 0.05$) (Table 3). TPC was higher after the D3S process, but no significant difference between the two D3S treated mangoes was observed. Both treated samples were submitted to ultrasonic waves in the first stage. Bhat et al. (2011) reported that ultrasonic waves increased the total

Table 2. Water loss (WL) and solids gain (SG) after the osmotic process

Osmotic dehydration				WL	SG
Ultrasound frequency at first stage (kHz)	Ultrasound frequency at second stage (kHz)	Stevia-based solution concentration at the second stage (g kg^{-1})	Sample immersion time in the second stage (min)	(%)	
0	0	25	10	1.94 ± 0.11	1.20 ± 0.04
			20	1.02 ± 0.00	0.03 ± 0.00
			30	0.61 ± 0.16	1.33 ± 0.02
		50	10	2.70 ± 0.19	- 0.43 ± 0.02
			20	3.87 ± 0.68	- 0.09 ± 0.09
			30	3.66 ± 0.08	2.00 ± 0.01
0	25	25	10	0.47 ± 0.00	- 1.9 ± 0.00
			20	2.05 ± 0.31	- 2.05 ± 0.03
			30	3.84 ± 0.00	1.00 ± 0.18
		50	10	6.62 ± 0.11	1.73 ± 0.02
			20	6.16 ± 0.07	1.73 ± 0.01
			30	8.82 ± 0.48	2.10 ± 0.08
25	0	25	10	2.68 ± 0.30	0.45 ± 0.03
			20	5.15 ± 0.20	1.44 ± 0.03
			30	4.78 ± 0.30	1.88 ± 0.04
		50	10	6.72 ± 0.43	0.73 ± 0.05
			20	7.88 ± 0.65	3.18 ± 0.10
			30	9.74 ± 0.37	3.56 ± 0.06
25	25	25	10	4.83 ± 0.31	0.91 ± 0.04
			20	4.96 ± 0.21	1.51 ± 0.03
			30	7.10 ± 0.07	2.24 ± 0.01
		50	10	9.76 ± 0.20	1.97 ± 0.03
			20	8.16 ± 0.02	2.33 ± 0.00
			30	11.61 ± 0.47	2.99 ± 0.08

Table 3. Quality parameters of fresh and processed mango

Sample	Total phenolics (mg GAE g⁻¹ DM)	Total carotenoids (µg g⁻¹ DM)	Color			
			L*	a*	b*	TCD
Fresh	2.74 ± 0.15 a	41.04 ± 0.00 a	58.21 ± 0.54 a	4.21 ± 0.19 a	54.36 ± 0.39 a	-
D3S 1	4.21 ± 0.01 b	20.59 ± 0.29 b	55.56 ± 0.36 b	2.93 ± 0.23 b	43.03 ± 0.38 b	11.71
D3S 2	4.08 ± 0.10 b	36.86 ± 1.80 c	57.33 ± 0.88 a	3.27 ± 0.30 b	48.88 ± 1.22 c	5.63

Samples with the same letter within the same column showed no statistically significant difference for their mean values at 0.05 probability level; D3S 1 - Ultrasound in the first stage for 30 min followed by immersion of samples in a 500 g kg⁻¹ Stevia-based solution for 30 min at 0 Hz; D3S 2 - Ultrasound in the first stage for 30 min followed by the sample immersion in a 500 g kg⁻¹ Stevia-based solution for 30 min at 25 Hz

phenolic content of Kasturi juice. This increase may be related to the pore formation in the vegetal tissue when ultrasonic waves are applied, improving the extraction of polyphenols during sample preparation, as reported by Gamboa-Santos et al. (2013). However, applying ultrasound again in the second stage (D3S 2 sample) did not result in increased TPC when compared to D3S 1 treated mango. It is possible that in this second stage a cell rupture surface caused by cavitation might contribute to the release of phenolics, as observed by Pingret et al. (2013).

Regarding total carotenoids content, the value for control untreated mango was 41.04 µg g⁻¹ DM and it exhibited significant change after the D3S process. Several investigations reported changes in carotenoid content during processing. It is known that carotenoids stability in foods is dependent on some factors, like the presence of light and their physical and chemical characteristics (Provesi et al., 2011).

Ashokkumar et al. (2008) observed that the level of hydroxylation in food could be improved by hydroxyl radicals generated when ultrasonic waves were applied. However, those radicals might affect the quality of some materials because of the antioxidant capacity reduction (São José et al., 2014). Rawson et al. (2011) observed that the hydroxyl radicals generated may result in the decomposition of lycopene, a lipophilic bioactive antioxidant in watermelon juice.

When ultrasonic waves were applied in both osmotic stages, the samples had higher carotenoid contents. This might have happened due to a more prolonged exposure to ultrasound and then the effect of cavitation produced by its application. In addition, the mechanical effect produced by ultrasonic waves permits a higher solvent penetration into the material, increasing the solute transfer rate from the food to the solvent (Zou et al., 2013).

Color parameters of mango are presented in Table 3. Significant differences can be observed between samples that were submitted to the D3S technique and the fresh ones (95% confidence level). D3S treated mangoes have a significant reduction in a* and b* values. In addition, lightness (L*) values also decreased and the fresh fruit presented a lighter color than the processed samples. Similar results were found by previous studies involving ultrasound, as the one reported by Fava et al. (2011), which submitted the grape berry to ultrasonic waves and observed differences in the color parameters.

The total color difference (TCD), calculated based on L*, a* and b* values, is being used to determine the effect of processing on food color (Guiné & Barroca, 2012). The smaller the value of TCD, the smaller is the deviation in color with respect to the reference (untreated sample). The TCD was lower when ultrasound was applied at both D3S stages, thus suggesting that it resulted in mango samples with fewer color changes when compared to the fresh fruit.

Choi et al. (2002) reported that a value for total color difference greater than 2 indicated appreciable differences in the visual impression of many products. Thus, in this study, the change in mangoes color after the process was visually observed.

Cavitation induced by ultrasound may lead to the differences in color, as reported by Sala et al. (1995) for sonicated tomato juice. These authors reported that cavitation is responsible for several reactions, like quicken chemical reaction, accelerate diffusion rates, among others. Mango pulp is rich in yellow-red colored components such as carotenoids (Kaushik et al., 2014) and the processing conditions can affect them. Hence, as the mangoes submitted to ultrasonic waves in both stages had greater total carotenoids content, those samples had the lower TCD value.

Conclusions

1. Submitting mango samples to ultrasonic waves in the D3S process first stage resulted in higher sugar removal.

2. The incorporation of natural sweetener (Stevia) was higher without application of ultrasound in the second stage of the D3S process.

3. Higher total phenolic content was obtained after using the D3S technique in mango samples.

4. Although total carotenoids content and color parameters values decreased with processing, smaller changes could be obtained when using ultrasonic waves in both D3S stages.

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