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Dual-stage sugar substitution in strawberries with a *Stevia*-based sweetener

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Abstract

The present study introduces and analyzes a new process denominated dual-stage sugar substitution (D3S). This process aims to induce sugar substitution in strawberries. In a first stage, high-calorie sugars (sucrose, fructose and glucose) are partially removed from the fruit samples and in a second stage, low-calorie sugar (stevioside and rebaudioside) is incorporated to the fruit to maintain its sweetness. The process was evaluated by studying the use of ultrasound application in one or both stages of the D3S process. Best performance of the process was obtained by subjecting the fruit samples to ultrasound in the sugar removal stage followed by immersion of the samples in *Stevia*-based solution without application of ultrasound in the sweetener incorporation stage. These operating conditions result in the highest sugar removal during the first stage, highest water loss during the process and highest sweetener incorporation during the second stage of the D3S process. The work described in this research is relevant to the production of dried fruits. A process to produce low-calorie dried fruit is presented. The process removes high-calorie sugars from the fruit and replaces it with a natural low-calorie sugar restoring the sweetness of the fruit.

Keywords: *Stevia rebaudiana,* drying, strawberry, ultrasound, sugar substitution

1. Introduction

Recent sociological trends, including reduced levels of physical activity and the increased availability of inexpensive low-nutrient, high-calorie food products, have contributed to concerns regarding the healthiness of current diets in developed countries (Brownell et al., 2009; Dietz et al., 2009; Drewnowski et al., 2008; Silver & Bassett, 2008). As consumers have become more aware of different diets and their impact on health, food companies and marketers have begun to dedicate more time and effort to develop healthier and more nutritive products.

Foods such as yogurts and carbonated beverages available today have been introduced successfully into the market as low-calorie products incorporating artificial sweeteners. Among these, saccharine and aspartame have been reported as the most widely implemented artificial sweeteners in the food industry (FDA, 2007).

Stevia rebaudiana, a perennial herb native of Brazil and Paraguay, is used to produce a natural sweetener that is used in Asia and South America and that have gained recent attention by many food and beverage multinational enterprises (Panpatil & Polasa, 2008). Steviol glycosides, which are up to 300 times sweeter than sucrose, are found in high concentrations in the leaves of *S. rebaudiana* (Brandle & Telmer, 2007). Sweeteners derived from *S. rebaudiana* show great potential as zero-calorie sweeteners in the snack and quick-meal foods including more specifically in food products based on dried fruits. Such applications currently involve the addition of large amounts of sugar during the dehydration stages and coating of the dry fruit. Consequently, considerable caloric loads result in a fruit product that is sometimes viewed negatively by consumers, limiting consumer acceptance.

Based on the results obtained by Fernandes et al. (2008a, 2008b) and Garcia-Nogueira (2009) for ultrasonic pre-treatment of fruits prior to drying, a new pre-treatment technique called dual-stage sugar substitution (D3S) was proposed in this study. The D3S first stage applies an ultrasonic treatment on the fruit, which removes caloric sugars from the fruit, whereas the second stage is intended to introduce a natural sweetener into the fruit.

Fernandes et al. (2008a, 2008b) and Fernandes & Rodrigues (2007) reported soluble solid losses (mostly sugar) for several fruits in ultrasound-assisted osmotic dehydration experiments. Similarly, Garcia-Nogueira (2009) reported that soluble solid losses on strawberries of approximately 10% of the initial weight of the fruit were obtained when applying ultrasonic pre-treatment in distilled water for 45 min.

The present study is focused on the feasibility of the substitution of sugar in strawberry by *Stevia*-derived natural sweetener using ultrasonic pre-treatments in osmotic solutions. The effects of ultrasound application during both stages of the D3S process for strawberry halves were determined. First-stage comparisons were made for strawberry halves receiving either

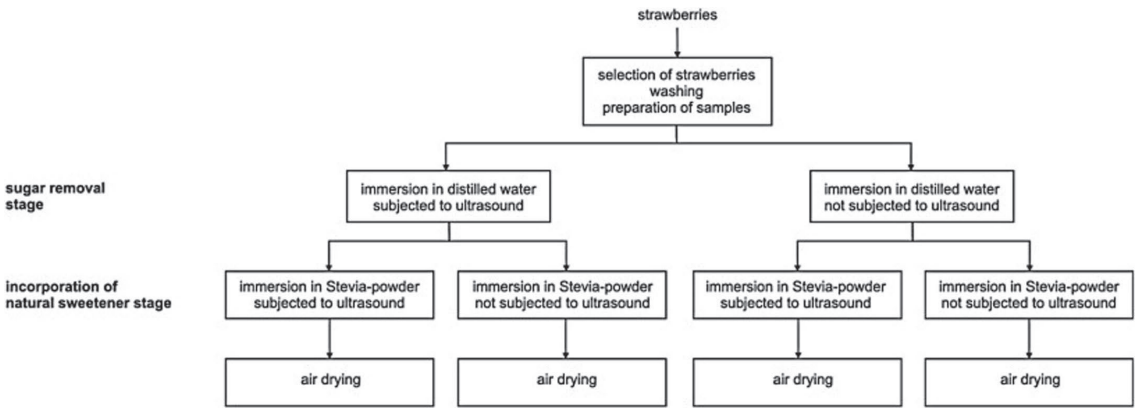

Figure 1. Scheme of the experimental design for the dual-stage sugar substitution process.

an ultrasound treatment or no ultrasonic energy. Second-stage comparisons were made for strawberry halves immersed in solutions of three different concentrations of *Stevia* extracts for three different processing times receiving either an ultrasound treatment or no ultrasonic energy.

2. Materials and methods

The D3S process was designed to induce sugar substitution in strawberries. A scheme of the experimental design is showed in Figure 1 and will be explained in detail in the next sub-sections. In the first stage, high-calorie sugars (sucrose, fructose and glucose) are partially removed from the fruit samples and in the second stage, low-calorie sugar (stevioside and rebaudioside) is incorporated to the fruit to maintain its sweetness.

2.1. Preparation of samples

Strawberries, *Camarosa* cultivar (mostly conic and long conic shaped) were purchased from retail markets in Lincoln, NE, USA. Strawberries were cut in half along their long axes. Each half was weighed to select sample halves within a range between 4 and 9 g.

Strawberries were classified based on a relative standard of maturity, shape and color. Such classification stage was intended to select similar berries to be used in every experiment, as well as to discard ripe, damaged or moldy samples. Upon selection, leaves were removed manually and strawberries were cut in halves. The initial moisture content of berries was determined by heating strawberry halves in a drying oven (Marconi model MA-085) at 60 °C for 48 h following AOAC method 934.06 (AOAC, 1990). The initial concentration of soluble solids (°Brix) in the berries was calculated using a refractometer (Atago 35Brix, Japan).

2.2. Sugar removal stage

The first D3S pre-treatment stage (sugar removal stage) was based on the results of Garcia-Nogueira (2009) that showed high soluble solid loss from strawberry halves immersed for 45 min in distilled water at an ultrasound frequency of 25 kHz.

Experimental units (comprised by two strawberry halves randomly selected) were assigned to a pre-treatment combination and run in triplicate. The pre-treatments carried out at 0 kHz (i.e. no use of ultrasound) were considered as the control runs. The first-stage pre-treatments, incorporating ultrasound, were performed using an ultrasonic bath (Aquasonic US Cleaner Model 150HT; internal dimensions: 35 × 30 × 15 cm). The operating frequency of the ultrasonic bath was 25 kHz. Flasks containing the experimental units assigned to ultrasound pre-treatments were placed inside the bath reservoir, containing a liquid medium of distilled water. Temperature of the liquid medium was maintained at 30 °C. Flasks containing experimental units assigned to control pre-treatments were placed on an isolated counter approximately 3 m away from the ultrasonic bath.

In each first-stage pre-treatment trial, three flasks (one for each time interval in the subsequent second D3S pre-treatment for sugar substitution) were placed in the ultrasonic bath and three flasks were left outside on the counter as mentioned previously (controls). Hence, each D3S pre-treatment trial consisted of 6 experimental units. The first pre-treatment stage was repeated 18 times (triplicate for each of the time:solution concentration:ultrasonic frequency combinations of the second D3S stage). Overall, 108 pre-treatment combinations were studied.

After completion of each pre-treatment trial, strawberry halves were removed from flasks, strained, and blotted with absorbent paper to remove excess solution. Weights for each experimental unit after the first stage of D3S pre-treatment were recorded.

2.3. Incorporation of natural sweetener

Pre-treatments continuing in the second D3S stage of sugar substitution by a *Stevia*-based natural sweetener were structured in three time intervals: 10, 20 and 30 min; three solutions: distilled water and two osmotic concentrations (25% and 50% w/w); and two frequency levels (0 kHz and 25 kHz). Osmotic solutions were prepared by mixing *Stevia* powder with distilled water. The weight ratio between the fruit and the liquid medium was 1:4 to avoid dilution effects (Teles et al., 2006).

Stevia powder was obtained from Puritan's Pride (Oakdale, USA). The powder consisted of 10% steviol glycosides and 90% maltodextrin (w/w). The steviol glycosides in *Stevia* powders contain usually from 64 to 98% of stevioside and from 36 to 2% of rebaudioside A and C (Kovylyaeva et al., 2007; Hearn & Subedi, 2009). The *Stevia*-based powder used in this study contained 88% of stevioside and 12% of rebaudioside A and C. Determination of the steviol glycosides content was carried out by HPLC following the method described by Kolb et al. (2001).

The second-stage ultrasound treatments were performed in the same ultrasonic bath used in the first stage. Treatment trials at 0 kHz were carried out on an isolated counter approximately 3 m away from the ultrasonic bath. The second-stage pre-treatment was carried out in separate 250 mL Erlenmeyer flasks filled with 100 mL of the corresponding solution. Temperature

of the liquid medium in the ultrasonic bath was kept constant at 30 °C. After each second-stage sugar substitution trial, strawberry halves were removed from the solution, drained and blotted with absorbent paper to remove excess solution. Weights for each experimental unit after each pre-treatment were recorded.

Additionally, the osmotic potential (OP) of the commercial *Stevia*-compound solutions utilized in the D3S experiments was calculated to determine differences in the rate of mass transfer versus sucrose-based solution values from ultrasound-assisted osmotic dehydration experiments in strawberry halves by Garcia-Nogueira (2009). Equation (1) was used to calculate the osmotic potential of the system.

$$\Pi = -RT\sum_{i=1}^{n} C_i \quad (1)$$

where C_i is the concentration of the components of the soluble solids in the osmotic solution (mol/L), R is the ideal gas constant (8.314 J/K mol), T is the temperature (K) and P is the osmotic potential (MPa).

2.4. Air-drying

After the two D3S stages, the strawberry halves were placed in Petri dishes (flat surface up) in a single-layer arrangement and dried in a forced circulation air-drying oven (Marconi model MA-085, Brazil). Air temperature in the oven was set to be at 60 °C. Cross-flow air moved from side to side of the dryer at 0.5 m/s, flowing parallel to the width of the dryer shelves. Air relative humidity (16%) was determined by psychrometry. Moisture loss from the strawberry halves during the drying period was monitored by weighing samples and recording weights every 30 min for the first 5 h and every hour thereafter. After 24 h of drying, the final weight of the strawberry samples was recorded.

2.5. Water loss and soluble solids gain determinations

The response variables of water loss (WL) and soluble solids gain (SG) were determined using the weight of strawberry halves before and after the pre-treatment trials, as well as the moisture content (wet basis) of strawberries, before and after the pre-treatment. Water loss and soluble solids gain (soluble gain assumed as simple carbohydrates and *Stevia* compound gain only) were calculated according to Equations (2) and (3).

$$WL(\%) = \frac{(w_i \cdot X_i - w_f \cdot X_f)}{w_i} \cdot 100 \quad (2)$$

$$SG(\%) = \frac{(w_f \cdot X_{sf} - w_i \cdot X_{si})}{w_i} \cdot 100 \quad (3)$$

where, w_i is the initial fruit mass (g) before pre-treatment; w_f is the final fruit mass (g) after pre-treatment; X_i is the initial fruit moisture content on wet basis (g water/g total fruit mass) before pre-treatment; X_f is the final fruit moisture content on wet basis (g water/g total fruit mass) after pre-treatment; X_{si} is the initial fruit dry solid matter content (g dry matter/g total fruit mass) before pre-treatment; and X_{sf} is the final fruit dry matter content (g dry matter/g total fruit mass) after pre-treatment.

2.6. Experimental design and statistical analysis

A split–split–split plot design was used to evaluate the interaction among the process variables and their effects on water loss and soluble solids gain.

The D3S process variables were: ultrasound frequency in the sugar removal stage (two levels: no ultrasound application (0 kHz) and with ultrasound application (25 kHz)); ultrasound frequency in the sweetener incorporation stage (two levels: no ultrasound application (0 kHz) and with ultrasound application (25 kHz)); *Stevia* solution concentration (three levels: 0, 25 and 50% w/w); and time of immersion in the *Stevia* solution (three levels: 10, 20 and 30 min). One-way analysis of variance (ANOVA) was calculated using the SAS Software v9.0. The significant differences within the variables were determined at $p < 0.05$. Tukey's HSD test was employed for comparison of means where significant differences occurred within the process variable combinations in terms of water loss and soluble solids gain responses.

3. Results and discussion

The initial moisture content for strawberries was 0.904 ± 0.005 g-water/g-fruit. Final moisture content of pre-treated berries varied and was used to calculate the water loss and sugar gain values shown in Table 1.

Pre-treatments resulting in the greatest water loss (5.38%) were observed in strawberry halves subjected to ultrasonic energy in both stages of the process (sugar removal stage and sweetener incorporation stage) and immersed in the 50% w/w *Stevia*-based solution for 30 min. Water loss trends in terms of processing time and ultrasonic application were similar to those reported by Garcia-Nogueira (2009) in strawberries and by Fernandes et al. (2008a, 2008b) and Rodrigues and Fernandes (2007a, 2007b) in other tropical fruits. The water loss values observed in this study (~ 1 to 5%) for both osmotic solutions were lower than those observed by Garcia-Nogueira (2009) (~ 3 to 7%). The difference in water loss is attributed to the difference in osmotic pressure between sucrose solutions used by Garcia-Nogueira (2009) and *Stevia* solutions used in this study. The osmotic potentials of the 25% and 50% sucrose solutions are, respectively, 1.84 kPa and 3.68 kPa, whereas the osmotic potentials of the 25% and 50% *Stevia*-based solutions are, respectively, 0.64 kPa and 1.29 kPa. As such, strawberry halves will exhibit lower water loss values when immersed in *Stevia*-based solution.

A significant three-way interaction (ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * concentration of the *Stevia*-based solution) was found by statistical analysis ($p < 0.05$) of water loss values obtained from air-dried strawberry samples subjected to the D3S process. Water loss mean values for ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * concentration of the *Stevia*-based solution are presented in Table 2.

Water loss values observed for strawberries pre-treated in distilled water were not significantly different among the frequencies in either stage of the D3S process. The use of the *Stevia*-based solution, either at 25% or 50% with or without ultrasound, significantly ($p < 0.05$) increased water loss when compared to water loss for strawberries pre-treated in distilled water. Water loss in *Stevia*-based solutions was significantly higher (~ 3%) when ultrasonic energy was applied in both stages of the D3S process.

Even though the osmotic pressure of *Stevia*-based osmotic solutions is much lower than that of sucrose osmotic solutions used by Garcia-Nogueira (2009), maximum water loss values obtained for both osmotic concentrations (25% and 50%) in the ultrasound-assisted osmotic solution using sucrose and

Table 1. Water loss, soluble solids gain and incorporation of *Stevia* during the sweetener incorporation stage of the D3S process.

Pre-treatment conditions						
Ultrasonic frequency applied to the sugar removal stage (kHz)	Ultrasonic frequency applied to the sweetener incorporation stage (kHz)	Concentration of the *Stevia* solution used in the sweetener incorporation stage (% w/w)	Processing time of the sweetener incorporation stage (min)	Water loss (%)	Soluble solids gain (%)	Incorporation of steviol glycosides (%)
0	0	0	10	− 0.64 ± 0.48	− 1.25 ± 0.58	0.00 ± 0.00
			20	− 0.69 ± 0.39	− 1.46 ± 0.38	0.00 ± 0.00
			30	− 1.19 ± 0.24	− 1.99 ± 0.84	0.00 ± 0.00
		25	10	1.12 ± 0.31	1.01 ± 0.82	0.10 ± 0.08
			20	2.68 ± 0.35	1.83 ± 1.43	0.18 ± 0.14
			30	3.82 ± 0.77	2.27 ± 1.15	0.23 ± 0.11
		50	10	0.89 ± 0.18	1.36 ± 0.52	0.14 ± 0.05
			20	0.88 ± 0.15	5.54 ± 1.37	0.55 ± 0.14
			30	1.82 ± 0.27	5.95 ± 1.02	0.59 ± 0.10
0	25	0	10	− 1.88 ± 0.95	− 0.99 ± 0.36	0.00 ± 0.00
			20	− 3.58 ± 1.11	− 2.07 ± 0.88	0.00 ± 0.00
			30	− 3.88 ± 0.67	− 1.87 ± 0.49	0.00 ± 0.00
		25	10	3.39 ± 1.11	0.75 ± 0.70	0.08 ± 0.07
			20	2.91 ± 1.88	1.96 ± 1.35	0.20 ± 0.13
			30	1.98 ± 0.86	2.36 ± 1.02	0.24 ± 0.10
		50	10	0.74 ± 0.41	1.62 ± 1.23	0.16 ± 0.12
			20	1.93 ± 0.09	6.07 ± 1.20	0.60 ± 0.12
			30	5.08 ± 0.24	6.49 ± 1.06	0.65 ± 0.10
25	0	0	10	− 5.27 ± 0.47	− 3.14 ± 0.47	0.00 ± 0.00
			20	− 4.33 ± 0.76	− 3.87 ± 0.76	0.00 ± 0.00
			30	− 5.03 ± 1.12	− 6.03 ± 1.12	0.00 ± 0.00
		25	10	1.17 ± 0.61	1.02 ± 0.72	0.10 ± 0.07
			20	3.01 ± 0.75	2.94 ± 1.32	0.29 ± 0.13
			30	3.47 ± 1.18	4.51 ± 0.60	0.45 ± 0.06
		50	10	1.17 ± 0.61	2.94 ± 1.52	0.29 ± 0.15
			20	3.01 ± 0.75	9.78 ± 0.54	0.98 ± 0.05
			30	3.47 ± 1.18	15.20 ± 1.75	1.52 ± 0.18
25	25	0	10	− 6.27 ± 1.58	− 3.57 ± 1.06	0.00 ± 0.00
			20	− 6.33 ± 0.88	− 4.27 ± 0.93	0.00 ± 0.00
			30	− 5.97 ± 1.32	− 5.70 ± 1.15	0.00 ± 0.00
		25	10	4.06 ± 1.50	4.06 ± 0.75	0.41 ± 0.07
			20	2.91 ± 1.88	2.91 ± 1.18	0.29 ± 0.12
			30	2.36 ± 1.31	2.33 ± 0.52	0.23 ± 0.05
		50	10	1.54 ± 0.41	3.08 ± 1.68	0.31 ± 0.17
			20	2.03 ± 0.09	9.72 ± 2.35	0.97 ± 0.23
			30	5.38 ± 0.60	7.52 ± 2.91	0.75 ± 0.29

Table 2. Water loss (%) for three-way (ultrasound frequency in the 1st stage * ultrasound frequency in the 2nd stage * concentration of the *Stevia* solution) interaction in air-dried strawberry halves after D3S process with stevia compounds.

Ultrasound frequency used in the sugar removal stage (kHz)	Ultrasound frequency used in the sweetener incorporation stage (kHz)	Concentration of the *Stevia* solution in the sweetener incorporation stage (% w/w)		
		0	25	50
0	0	− 4.39 ± 0.31 a	0.04 ± 0.01 b	0.04 ± 0.01 b
0	25	− 3.52 ± 0.26 a	0.28 ± 0.03 b	0.28 ± 0.02 b
25	0	− 4.04 ± 0.17 a	0.03 ± 0.01 b	0.03 ± 0.01 b
25	25	− 4.28 ± 0.18 a	2.78 ± 0.19 c	2.91 ± 0.12 c

* Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

Table 3. Soluble solids gain (%) for three-way (ultrasound frequency in the 1st stage * ultrasound frequency in the 2nd stage * concentration of the *Stevia* solution) interaction in air-dried strawberry halves after D3S process with *Stevia* compounds.

Ultrasound frequency used in the sugar removal stage (kHz)	Ultrasound frequency used in the sweetener incorporation stage (kHz)	Concentration of the *Stevia* solution in the sweetener incorporation stage (% w/w)		
		0	25	50
0	0	− 5.86 ± 0.86 b	1.93 ± 0.67 c	4.46 ± 0.63 d
0	25	− 11.60 ± 0.75 a	1.68 ± 0.25 c	5.73 ± 0.32 d
25	0	− 10.70 ± 0.87 a	3.38 ± 0.18 d	9.97 ± 0.70 e
25	25	− 11.50 ± 0.93 a	2.53 ± 0.39 c	6.66 ± 0.81 d

* Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

Table 4. Soluble solids gain (%) for three-way (ultrasound frequency in the 1st stage * ultrasound frequency in the 2nd stage * time immersed in *Stevia* solution) interaction in air-dried strawberry halves after D3S process with stevia compounds.*

Ultrasound frequency used in the sugar removal stage (kHz)	Ultrasound frequency used in the sweetener incorporation stage (kHz)	Time immersed in *Stevia* solution (min)		
		10	20	30
0	0	− 0.68 ± 0.40 d	0.19 ± 0.42 e	1.02 ± 0.41 f
0	25	− 2.68 ± 0.49 a	0.31 ± 0.33 d	− 1.19 ± 0.45 c
25	0	− 2.36 ± 0.22 b	1.55 ± 0.34 g	3.34 ± 0.24 h
25	25	− 2.23 ± 0.66 b	1.00 ± 0.17 f	− 1.10 ± 0.35 c

* Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

the D3S process were very similar. This observation can be explained by considering that subjecting strawberry halves to ultrasound for 45 min in the first stage of D3S process likely resulted in the formation of microscopic channels, which ultimately allowed for increased water loss in the *Stevia*-compound solutions during the second stage of the D3S process.

The D3S process resulting in the greatest sweetener gain (15.20%) was strawberries subjected to ultrasound in the sugar removal stage followed by immersion in the 50% w/w *Stevia*-based osmotic solution for 30 min without ultrasonic application. The trends observed were similar to those observed by Garcia-Nogueira (2009) in ultrasound-assisted osmotic dehydration of strawberries in sucrose solutions and in other tropical fruits as reported by Rodrigues and Fernandes (2007a, 2007b).

Garcia-Nogueira (2009) observed that in strawberry halves, sugar gain in sucrose osmotic solutions increased with longer periods of osmotic immersion. However, soluble solids gain in *Stevia*-based solutions was lower than in sucrose-based osmotic solutions. This result may be also attributed to the difference in osmotic pressure between the sucrose solutions used by Garcia-Nogueira (2009) and *Stevia*-based solutions applied in this D3S study.

Sweetener gain values in the D3S process consisting of two stages of ultrasonic exposure (in both sugar removal stage and sweetener incorporation stage) were higher than those obtained when the samples are not subjected to ultrasound (0 kHz) in the sugar removal stage. But the sweetener gain values in the D3S process consisting of two stages of ultrasonic exposure (in both sugar removal stage and sweetener incorporation stage) were lower than those obtained when the samples were subjected to ultrasound in the sugar removal stage and not subjected to ultrasound during the sweetener incorporation stage. As such, the D3S process should be carried out subjecting the samples to ultrasonic waves during the sugar removal stage followed by immersion of the samples in sweetener solution (without applying ultrasound).

This observation can be explained by the formation of micro-channels in the tissue structure of the strawberry halves during the sugar removal stage (caused by ultrasound application), which will induce higher mass transfer rates between the fruit and the *Stevia*-based solution during the sweetener incorporation stage, which will be translated into higher sweetener gain values. The incorporation of sweetener will be higher without application of ultrasound in the second stage of the D3S process because of the lower osmotic pressure of the *Stevia*-based solution and because ultrasound induces the extraction of soluble solids from the samples, which would reduce the incorporation of *Stevia* by the sample.

A significant three-way interaction (ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * concentration of the *Stevia*-based solution) was found among the different operating conditions applied in the D3S process. Table 3 shows the soluble solids gain values for ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * concentration of the *Stevia*-based solution interactions in air-dried strawberry samples during the DS3 process using *Stevia* in the sweetener incorporation stage.

After the experiments, the concentration of steviol glycosides and maltodextrin in the osmotic solution was determined by HPLC analysis. Steviol glycosides were determined using the method described by Kolb et al. (2001) and maltodextrin content was determined using the method described by Moreno et al. (1999). The results showed that the concentration of steviol glycosides and maltodextrin reduced during the experiments and that the remaining osmotic solution showed to maintain the same initial ratio of steviol glycosides and maltodextrin.

The application of the D3S process consisting of a 45 min ultrasound pre-treatment in distilled water followed by the immersion of the samples for 30 min in a 50% *Stevia*-based osmotic solution yields the maximum sweetener gain and water loss values (15% and 5%, respectively).

The sweetness power of *Stevia*-based sugars is approximately 300× higher than that of sucrose (Brandle & Telmer, 2007), thus the incorporation of 1% of *Stevia*-based powder (steviol glycosides 10% + maltodextrin 90%) into the fruit corresponds to the incorporation of 30% of sucrose. The D3S process resulted in lower soluble solids gain if compared to the osmotic dehydration of strawberries in sucrose solutions, which is interesting because of the higher sweetness power of steviol glycosides, otherwise the final product could be considered too sweet.

A significant three-way interaction (ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * time immersed in *Stevia* solution) was found among the D3S process ($p < 0.05$). Table 4 presents the soluble solids gain values for ultrasound frequency in the sugar removal stage * ultrasound frequency in the sweetener incorporation stage * time immersed in *Stevia*-based solution interactions in air-dried strawberry samples during the DS3 process using *Stevia* extract.

Treatments consisting of application of ultrasound in the first stage only and without application of ultrasound showed significantly different soluble solids gain responses for 10, 20 and 30 min of immersion in both 25% and 50% *Stevia*-based osmotic solutions ($p < 0.05$). Moreover, soluble solids gain in the *Stevia* osmotic solutions was significantly higher ($p < 0.05$) when the samples were subjected to ultrasound in the sugar removal stage. Both water loss and soluble solids gain responses of the samples subjected to the 50% w/w *Stevia*-based

solutions represented the highest values when compared to the results observed for the 25% w/w *Stevia*-based solution. Maximum soluble solids gain was reached after 20 min in the sweetener incorporation stage.

4. Conclusion

Application of the D3S process consisting of a 45 min ultrasonic pre-treatment in distilled water and subsequent osmotic immersion without ultrasonic application in *Stevia*-based solutions proved to be a feasible method to substitute high-calorie sugars from the fruit (sucrose, glucose and fructose) by low-calorie sugars (stevioside and rebaudioside).

The reduced osmotic potential of high molecular weight compounds (*Stevia*-based powder consisting of 10% steviol glycosides and 90% maltodextrin) greatly influenced the rates of water loss and soluble solids gain. Despite lower soluble solids gain in the D3S process, the high sweetness power of *Stevia* extract (approximately 300× higher than that of sucrose) would ultimately result in a sweet flavor in fruit samples produced by the D3S process.

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