

Optimization of osmotic dehydration of pineapple (*Ananas comosus* L.) using the response surface methodology

Optimización de la deshidratación osmótica de piña (*Ananas comosus* L.) aplicando superficies de respuesta

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ABSTRACT

The response surface methodology was used to optimize the effects of temperature (25 - 45°C) and citric acid concentration (0.5 - 2.5% w/w) in osmotic dehydration of pineapple in a sucrose solution. A 3² factorial design was used with weight loss (WL, %), moisture loss (ML, %) and solid gain (SG, %) as responses. The models obtained for all the responses were significant ($P \leq 0.05$) without a significant lack of fit. The results suggest that WL, ML and SG can reach 42.62%, 36.54% and 292.16% respectively, after 4 to 6 h of the process, with 100% sensory acceptance and reductions in microbial counts of more than two log cycles, using the conditions defined by the optimization (44.99°C and 2.48% citric acid).

Key words: citric acid, osmotic drying, post harvest, fruit.

RESUMEN

Se aplica la metodología de superficies de respuesta para optimizar el efecto de la temperatura (25 - 45°C) y la concentración de ácido cítrico (0,5 - 2,5% p/p) en la deshidratación osmótica de piña en soluciones de sacarosa. Se utiliza un diseño factorial 3² con pérdida de peso (PP, %), pérdida de humedad (PW, %) y ganancia de sólidos (GS, %) como respuestas. Los modelos obtenidos para todas las respuestas son significativos ($P \leq 0,05$) sin carencia de ajuste significativa. Los resultados sugieren que se puede alcanzar 42,62%; 36,54% y 292,16%, para PP, PW y GS, respectivamente, después de 4 - 6 h de proceso, con aceptación sensorial del 100% y reducciones en los recuentos microbianos de más de dos ciclos logarítmicos, utilizando las condiciones definidas en la optimización (44,99°C y 2,48% de ácido cítrico).

Palabras clave: ácido cítrico, secado osmótico, poscosecha, frutales.

Introduction

The pineapple (*Ananas comosus* L.) is consumed as fresh fruit, sliced or dried (Saxena *et al.*, 2009), worldwide. Due to its short shelf life (4 - 6 d) (Hajare *et al.*, 2006), multiple strategies have been evaluated to prolong its durability (Hajare *et al.*, 2006; Peiró-Mena *et al.*, 2007; Fernandes *et al.*, 2008; Chauhan *et al.*, 2009; Saxena *et al.*, 2009; Torri *et al.*, 2010; Ramallo and Mascheroni, 2010). For cut products, durability is even lower (Soliva-Fortuny and Martín-Belloso, 2003), mainly due to some changes in the physicochemical and physiological characteristics (Toivonen and Brummell, 2008) resulting from increased enzymatic action due to cell damage caused by cutting (Torres *et al.*, 2007). Post-harvest losses of fruits in general account for 20 - 25% of total fruit production in developed countries (El-Ghaouth *et al.*, 2004; Zhu, 2006; Sharma *et al.*, 2009) and even more in developing countries (Sharma *et al.*, 2009).

Osmotic dehydration (DO) is a food preservation technique that relies on the reduction of water activity and humidity of the product, which has advantages over other dehydration techniques because it preserves the sensory and nutritional characteristics of foods (Ozdemir *et al.*, 2008; Fernández *et al.*, 2005), since it does not require high temperatures, in addition it has low investment costs which facilitates its implementation in rural areas, and is an excellent alternative for conservation of cut products. Its processes involve simultaneous water loss and solute gain (Saxena *et al.*, 2009), in which the mass transfer depends on variables such as pressure, temperature (Corzo and Gómez, 2004), concentration of solutes (Corzo and Gómez, 2004), fruit/syrup ratio and degree of agitation, among others (Fernandes *et al.*, 2008).

Osmodehydrated products are part of the so-called intermediate moisture products and should be consumed in a relatively short time or be subjected to conservation steps

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(Fernandes *et al.*, 2008; Ramallo and Mascheroni, 2010). One area for improvement for these products is their microbial load, since it may affect the shelf life of packaged products, especially cut ones (Zapata *et al.*, 2004; Castelló *et al.*, 2009). For these reasons, the search for strategies to reduce the microbial load of osmodehydrated products, not including additional processing steps, could reduce postharvest losses, while maintaining costs, thus providing added value to fruits and vegetables, improving the income level of farmers, enabling them to better manage prices even at harvest and allowing the possibility of using lower quality fruit.

DO kinetics of the pineapple using sucrose syrup as a function of the solution concentration and temperature has been studied (Parjoko *et al.*, 1996; Silveira *et al.*, 1996; Lombard *et al.* 2008) and the diffusion coefficients have been determined (Ruiz-López *et al.*, 2010). Losses of up to 30% have been reported for citric acid (CA) (Peiró-Mena *et al.*, 2007) and ascorbic acid (Ramallo and Mascheroni, 2010) in pineapple and other fruits (Moraga *et al.*, 2010) during DO, which can affect quality in osmodehydrated products.

It is known that suitable combinations of pH and water activity (A_w) can be very effective in controlling microorganism in food. For example, it has been found that pretreatment with acid solutions improves the inactivation of *Escherichia coli* in dehydrated apples (Derrickson-Tharringtona *et al.*, 2005). Citric acid has been widely accepted as effective in reducing the surface pH of different types of cut fruit (Soliva-Fortuny and Martín-Belloso, 2003). Combinations of low pH and low A_w have been reported to promote the development of microorganisms with greater resistance (Tiganitas *et al.*, 2009), that is, some of these combinations do not effectively inhibit problem microorganisms (Derrickson-Tharringtona *et al.*, 2005). These results make it necessary to carefully evaluate the combination of preservation methods before suggesting an application for a process.

The response surfaces are very effective tools for optimization, which have been used in different food processes among which DO is include (Azoubel and Murr, 2003; Corzo and Gómez, 2004; Valdez-Fragoso *et al.*, 2007; Ozdemir *et al.*, 2008; Chauhan *et al.*, 2009). The main advantage is that they reduce the number of experiments needed to obtain statistically valid results (Ozdemir *et al.*, 2008) and are faster and more informative than traditional assessments which evaluate one variable at a time (Ozdemir *et al.*, 2008).

The aim of this study was to optimize the osmotic dehydration of pineapple cubes, through the combined effect of temperature (T) and citric acid concentration (CA), using the response surface methodology to maximize weight loss (WL), moisture loss (ML) and solid gain (SG), seeking to increase solid gain (sucrose and citric acid) to neutralize acidity losses that may occur in the process and reduce the microbiological risk. Additionally, we evaluated the microbiological quality and sensory acceptance of the osmodehydrated pineapple cubes in the established optimal conditions to ensure the microbiological and sensory quality of the product.

Materials and methods

Sample preparation. Pineapples of the Manzana variety with °Brix between 9 and 11 were selected, and obtained commercially in Medellín; washed and disinfected with sodium hypochlorite at 50 mg L⁻¹ for 10 min; peeled, cored and cut into 1 cm cubes.

Osmotic treatments. The treatments were carried out under atmospheric pressure conditions with containers at rest, using sucrose solutions of 45°Brix and citric acid concentrations between 0.5 to 2.5% (w/w). The pineapple cubes were placed in a fruit/syrup ratio of 5/1 (g/g). The samples were then subjected to a water bath with a temperature control (D-77960 Jubalo®) using temperatures between 25 - 45°C for 4 h. These conditions were defined based on literature reports (Ruiz-López *et al.*, 2010; Lombard *et al.*, 2008).

Analytical determinations. The moisture analysis was carried out in a forced air oven (Binder, model termolab 53®), using the AOAC oven drying method 934.06 (AOAC, 1995). The °Brix was measured with a refractometer (Milton Roy Company® LR 45227) and weight determinations with an analytical balance (Sartorius CP 2245®). Each analysis was done in triplicate.

For the calculation of weight loss (WL) and moisture loss (ML), equation 1 was used.

$$WL/ML\% = \frac{(w_i - w_f)}{w_i} \times 100 \quad (1)$$

where: w_i is weight/moisture initial and w_f is weight/moisture final.

The soluble solid gain (SG) was calculated as shown in equation 2.

$$SG\% = \frac{(^{\circ}Brix_f - ^{\circ}Brix_i)}{^{\circ}Brix_i} \times 100 \quad (2)$$

where: $^{\circ}Brix_f$ represents final Brix degrees and $^{\circ}Brix_i$ represents initial Brix degrees.

Statistical analysis. eighteen experiments were carried out according to a 3^2 factorial design with 2 replicates, using the factors temperature (T) (25, 35 and 45°C) and citric acid concentration (CA) (0.5, 1.5 and 2.5% w/w) and the response variables WL, ML, and SG.

The response surface methodology assumes that there is a polynomial function that relates the responses to the independent variables in the process (factors) (Montgomery, 1991). Therefore, the experimental data obtained from the factorial design (Tab. 1) were fitted to a polynomial of the form found in equation 3 (Montgomery, 1991).

$$\text{Response} = a_0 + a_1X_1 + a_2X_2 + a_{12}X_1X_2 + a_{11}X_1^2 + a_{22}X_2^2 \quad (3)$$

where the response is WL, ML or SG, the a_i are adjustable constants and X_1 , X_2 , are T and CA, respectively.

We developed the analysis of variance (ANOVA) with a confidence level of 95%, which includes the statistical significance of each of the terms of the adjustable model (Value - P), the estimated effects in each term (a_i), the coefficient of determination of the model (R^2) and the lack of fit in order to establish the accuracy of the model to represent the data. The resulting models were optimized to determine the levels of the variables that give the maximum values of the responses.

Microbiological analysis. Analysis was performed in triplicate on samples of fresh pineapple and osmodehydrated pineapple with and without citric acid. Mold - yeasts and aerobic mesophilic were counted. For comparison of means, this study applied the Least Significant Difference (LSD) method with a confidence level of 95%, using the software Statgraphics Plus 5.1®.

Sensory analysis. Sensory evaluation was performed using a panel of 50 untrained judges, made up of students and staff of the Food Department of the Universidad de Antioquia. The sensory method was an acceptance test: I like - I do not like. The minimum number of responses was established for a 5% probability according to a binomial test.

Results and discussion

To define the conditions of CA and T that maximize the values of the parameters WL, SG and ML, a 3^2 factorial

design was developed, which is presented in Tab. 1 with the runs of the experiment in a random form and the values of the response variables obtained in each run.

TABLE 1. 3^2 Factorial design for the osmotic dehydration of pineapple.

Treatment	T (°C)	CA (%)	WL (%)	ML (%)	SG (%)
1	45	2.5	42.37	34.16	253.66
2	35	0.5	26.12	20.70	161.96
3	25	0.5	28.60	20.24	207.48
4	35	2.5	32.65	25.98	200.61
5	45	0.5	39.51	31.27	250.92
6	35	1.5	30.72	23.48	193.25
7	25	1.5	29.04	21.76	206.80
8	45	1.5	42.57	34.14	247.56
9	25	2.5	33.76	19.84	198.64
10	45	2.5	43.93	34.22	257.93
11	25	1.5	29.68	21.74	203.40
12	35	2.5	31.98	23.75	195.70
13	45	0.5	38.57	29.48	237.20
14	25	2.5	35.33	21.04	203.40
15	25	0.5	26.28	18.53	172.11
16	35	1.5	28.40	23.94	185.28
17	35	0.5	27.79	19.38	180.98
18	45	1.5	44.10	31.32	252.14

T, temperature; CA, citric acid; WL, weight loss; ML, moisture loss; SG, solid gain.

Results of the ANOVA of the central composite design are shown in Tab. 2, indicating the statistical significance of each factor for each response variable (Value - P).

TABLE 2. Analysis of variance (Value- P) of the central composite design.

Factor	PP (%)	PW (%)	GS (%)
T	0.0000	0.0000	0.0000
CA	0.0000	0.0007	0.0227
TxT	0.0000	0.0001	0.0000
TxCA	0.0909	0.1145	0.9732
CAXCA	0.7583	0.0636	0.3924
No adjustment	0.1243	0.1284	0.7280
R^2	96.18	95.19	91.83

T, temperature; CA, citric acid; WL, weight loss; ML, moisture loss; SG, solid gain.

To adjust the final model, factors without significant effects ($P>0.05$) were eliminated which appear in the ANOVA (Tab. 2), and the R^2 and the lack of fit of the resulting model were determined. Both data are presented in Tab. 2 for adjusted models of WL, ML and SG, which appear in equations 4, 5 and 6 respectively. The a_i coefficients in these equations correspond to the effects of the factors in terms of their actual values. The R^2 values ($> 91\%$) and lack of fit ($P\leq 0.05$) in Tab. 2 suggest that these polynomials adequately represent the relationship between responses and factors.

$$PP\% = 85,58 - 4,00T + 2,76CA + 0,07T^2 \quad (4)$$

$$PW\% = 43,79 - 1,93T + 1,62CA + 0,04T^2 \quad (5)$$

$$GS\% = 549,35 - 24,02 T + 8,27CA + 0,38T^2 \quad (6)$$

In Figs. 1, 2 and 3 one sees that all responses have an upward trend with increasing values of the factors (T and CA).

Effect on the percentage of weight loss

The weight reduction (WL) is an indirect measure of water loss in osmodehydrated products, which is important in transportation and storage for high volumes. Tab. 2 demonstrates that WL was significantly affected by T ($P = 0.0001$) in its linear and quadratic terms. The positive and negative indications of T for the linear and quadratic terms respectively, in equation 4, indicate that increases in T can positively or negatively affect the WL, depending on the range of values of T found in the system and that exist regionally for this variable, for which WL reaches extreme values (Fig. 1) (Azoubel and Oliveira da Silva, 2008). Increasing T accelerates all kinetic processes; thereby increasing the solid gain and water loss (Silveira *et al.*, 1996; Lombard *et al.*, 2008) due to the effect it has on the coefficients of mass transfer and effective diffusion in the fruit (Alakali *et al.*, 2006). For some values of T, solids gain can be preponderant, generating an increase in weight, which in turn causes a decrease in WL, however in other ranges of T, water loss may be more significant, with which an increases in WL could be observed, as shown in Fig. 1, for values of T above 32°C, results that correspond to those found by Azoubel and Oliveira da Silva (2008), working with the Tommy Atkins mango.

The CA significantly ($P = 0.0000$), positively affects WL, meaning the higher the acid concentration, the higher the WL. Noting that the increased concentration of solutes increases the flow of water (Silveira *et al.*, 1996; Lombard *et al.*, 2008), because its increase favors the coefficients of mass transfer and effective diffusion in the fruit (Alakali *et al.*, 2006).

Effect on the percentage of moisture loss

It is evident that the ML is one of the most important parameters in dehydration. According to Tab. 2, the ML has significant dependence on T in the linear ($P = 0.0000$) and quadratic ($P = 0.0001$), such as that of CA ($P = 0.0007$), which is due to the effect that both variables have on the coefficients of mass transfer and effective diffusion of water (Silveira *et al.*, 1996; Alakali *et al.*, 2006, Lombard *et al.*, 2008). The contradictive indications of T for the linear and

quadratic terms in equation 5 are explained in the same way as WL in Equation 4, insomuch as by the presence of an extreme point for ML (Fig. 2). But in this case, the negative indication of T in the linear term represents regions where the largest entry of solute can promote the formation of surface layers on the fruit, which are barriers to the diffusion of water (Zapata *et al.*, 2002; Giraldo -Bedoya *et al.*, 2004; Fernandes *et al.*, 2009).

The positive effect of CA on ML (equation 5) rules out the formation of a layer of solute on the fruit surface as caused by the CA in the working range, demonstrating that this phenomenon may be due to sucrose, as has already been reported (Zapata *et al.*, 2002; Giraldo-Bedoya *et al.*, 2004; Fernandes *et al.*, 2009). This significant effect of CA is not consistent with the findings of Moy *et al.* (1978), which states that acidifying sucrose solutions did not increase water loss in the mango.

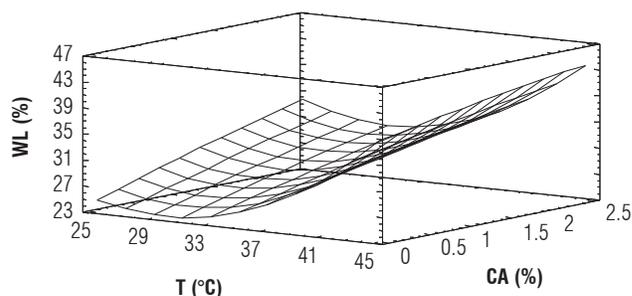


FIGURE 1. Response surface for weight loss (WL) in the osmotic dehydration of pineapple as a function of temperature T and citric acid (CA).

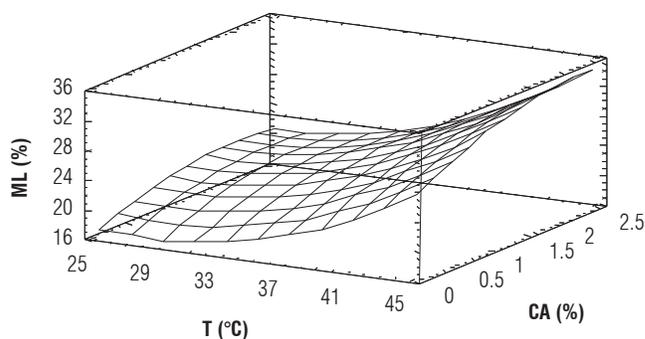


FIGURE 2. Response surface for moisture loss (ML) in the osmotic dehydration of pineapple as a function of temperature T and citric acid (CA).

Effect on the percentage of solid gain

The high SG in some cases is undesirable in DO, because it is associated with changes in the organoleptic properties and because it adversely affects the flow of water to form a shell on the surface of the product (Giraldo-Bedoya *et al.*,

2004). But, if what is sought is the reduction of weight loss in the product while not significantly affecting the cost, a high SG is desirable, as in candied products (Giraldo-Bedoya *et al.*, 2004). According to Tab. 2, the variable T has a significant effect on the linear and quadratic terms ($P = 0.0000$), in equation 6, SG has a behavior similar to the other two evaluated parameters (WL and ML) with a endpoint associated with the reversal of the linear and quadratic terms of T and a positive effect of CA only on the linear term. The turning point could be around 34°C (Fig. 3), a value at which the SG stops off as a function of T and begins an upward course. This is a notable point in the case that you want to have products with a minimum solid gain and are consistent with the results of Azoubel and Oliveira da Silva (2008) with the Tommy Atkins mango. A similar explanation can be given for the two previous parameters, based on the effect that both variables have on mass transfer and effective diffusion in the DO of fruits, as has been reported (Silveira *et al.*, 1996; Alakali *et al.*, 2006; Lombard *et al.*, 2008). It is possible that a solute layer accumulated on the surface of the fruit as a result of increases in T in a working range, can also be a barrier to the passage of solutes into the fruit (Zapata *et al.*, 2002; Giraldo -Bedoya *et al.*, 2004; Fernandes *et al.*, 2009). These results agree with those obtained by Melo *et al.* (2001), who observed decreases in the solid gain in a given temperature range, working with mango in an osmodehydration solution for passion fruit.

In general, the variable with the greatest effect on the three responses (WL, SG and ML) is temperature, indicating that a new goal for the research could be the development of phenomenological models for the process of osmotic dehydration of pineapple. These models could also consider the effect of T on the fluid dynamics of the systems in DO, considering variables such as viscosity, surface tension and chemical potential, among others.

Optimization

The models of equations 4, 5 and 6, were optimized to maximize WL, ML and SG, since all aim to reduce A_w , which in turn is associated with the maximum preservation of the product (Giraldo-Bedoya *et al.*, 2004; Derrickson-Tharringtona *et al.*, 2005; Giraldo *et al.*, 2005; Ngoran *et al.*, 2009; Chauhan *et al.*, 2009) which is the main objective of the present study. Tab. 3 shows the local maximum values of the responses and the values of each of the factors that define them. The results of Tab. 3 are highlighted in Figs. 1, 2 and 3, which show that for the maximum values of the studied CA and T (2.5% and 45°C, respectively) the maximums for WL, ML and SG are provided.

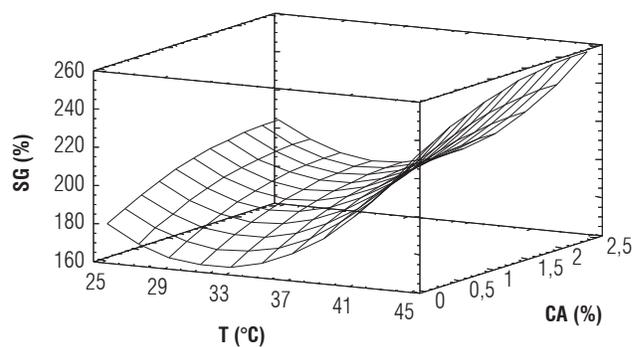


FIGURE 3. Response surface for solid gain SG in the osmotic dehydration of pineapple as a function of temperature T and citric acid (CA).

TABLE 3. Maximum predicted local values in the optimization of osmotic dehydration of pineapple cubes.

Factor	Optimal Value
T	44.99°C
CA	2.48%
Response	Optimal Value
WL	44.54
ML	34.01
SG	257.93

Kinetics of osmotic dehydration of pineapple

To corroborate the validity of the optimization process and evaluate the kinetics of osmotic dehydration of pineapple, a process was developed in duplicate at 45°C and with 2.5% CA, taking data for 7 h (Figs. 4, 5 and 6).

For WL, significant increases occur in the first 6 h (Fig. 4), after which it stabilizes. While in ML and SG, stabilization occurs after about 4 h (Figs. 4 and 6), which is typical behavior in pineapple DO (Parjoko *et al.*, 1996; Lombard *et al.*, 2008).

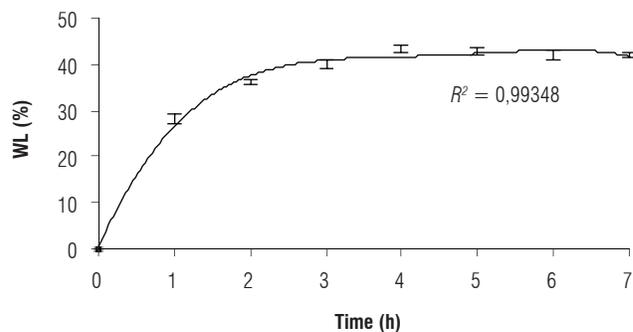


FIGURE 4. Weight loss (WL) in the osmotic dehydration of pineapple with 2.5% citric acid and 45°C. The bars on the columns indicate standard deviation.

The kinetic behavior in Figs. 4, 5 and 6 are explained considering that in the early stages of the process there are a higher chemical potential of water and a lower chemical potential of solutes inside the fruit (sucrose, acid) than in the outside solution. These differences in the chemical potential are the driving force for mass transfer; spurring the movement of solutes into and water out of the fruit (Giraldo-Bedoya *et al.*, 2004; Ozdemir *et al.*, 2008). Over time, the entry and exit of substances reduces the differences in the chemical potential, bringing the system to equilibrium, so that the entrance and exit of material gradually decreases to a stop.

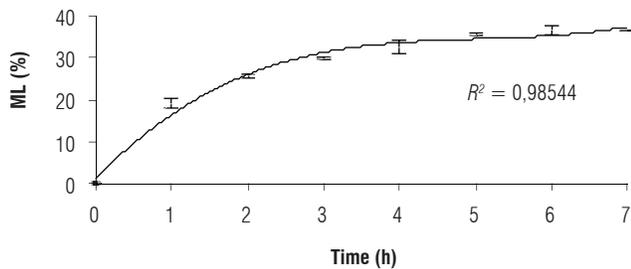


FIGURE 5. Moisture loss (ML) in the osmotic dehydration of pineapple with 2.5% citric acid and 45°C. The bars on the columns indicate standard deviation.

Water loss reduces weight and moisture, while the solids of the product are increased, ie increased WL, ML and SG. With the entry of solutes, weight and solids are increased, but moisture is reduced, ie reduced WL and increased ML and SG.

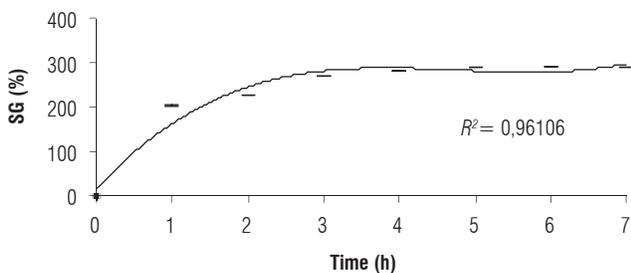


FIGURE 6. Solid gain (SG) in the osmotic dehydration of pineapple with 2.5% citric acid and 45°C. The bars on the columns indicate standard deviation.

Figs. 4, 5 and 6 at 4 h into the process (time of each run in the experimental design) show that WL, ML and SG have values of 43.24, 32.47 and 282.35% respectively, all close to or above those predicted by the optimization process (Tab. 3), corroborating the validity of the models obtained and the submitted optimization. The maximums obtained at 7 h into the process were 43.24, 36.54 and 292.16% for

WL, ML and SG respectively; this maximization is suitable for the preservation of fruit, because it is associated with reduced water availability for microorganisms and enzyme reactions that can damage the product (Zapata *et al.*, 2002; Giraldo *et al.*, 2005; Tiganitas *et al.*, 2009). On the other hand, the increase in WL is desirable for its effect on storage and transportation costs when handling large volumes.

Microbiological analysis

Considering the importance of the microbiological aspect of cut osmodehydrated products (Zapata *et al.*, 2002; Soliva-Fortuny and Martín-Belloso, 2003), it is important to assess the impact that can be obtained from the DO treatment proposed in the present study on said aspect. For this reason, microbiological analysis was performed on fresh produce, osmodehydration with sucrose and osmodehydration with sucrose and citric acid. Tabs. 4 and 5 show that mold-yeasts and aerobic mesophilic microorganisms were significantly reduced in the treatment without citric acid with respect to fresh pineapple ($P \leq 0.05$) and the treatment with citric acid (DOAC) further reduced counts, compared to treatment without citric acid (DO) ($P \leq 0.05$). These differences are statistically significant at 95% confidence and demonstrate the synergistic effect on preserving the product, obtained by using citric acid in the DO process with sucrose.

TABLE 4. Comparisons of the microbiological analyses of fresh pineapple cubes for osmodehydration with sucrose (DO) and osmodehydration with sucrose and citric acid (DOAC).

Treatment	Count (cfu/g)		
	Fresh	DO	DOAC
Aerobic mesophilic	3,64·10 ⁶ a	8,9·10 ³ b	8,0·10 ¹ c
Molds and yeast	1,26·10 ⁶ a	7,03·10 ³ b	5,0·10 ¹ c

Means with different letters in each row are statistically different according to the test of Least Significant Difference ($P \leq 0.05$).

Sensory analysis

To confirm that the product with the conditions obtained for optimization is acceptable from the sensory standpoint, sensory analysis of osmodehydrated samples with 2.5% CA at 45°C was performed and 100% product acceptance was obtained.

Conclusions

The most important variables in DO are WL, ML and SG, and they are significantly affected by temperature and citric acid concentration during the DO of pineapple under the conditions in this study.

Under these conditions, the maximization of the responses was achieved at a temperature of 45°C and with citric acid concentrations of 2.5% w/w, reaching 43.24, 36.54 and 292.16% for WL, ML and SG respectively.

The kinetic changes occur between 4-6 h into the process, after which the system tends toward equilibrium, making it unreasonable to extend the process beyond that time, under the conditions of this study.

Adding citric acid to the osmodehydration solution, besides eliciting a positive response from the variables, has a clearly positive effect by reducing the microbiological counts of pineapple by adapting an acidic pH that is unfavorable for the growth of molds and yeasts, without negatively affecting the sensory acceptance of the osmodehydrated product.

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