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Development of a process for sugar fortification with vitamin-A

Desarrollo de un proceso para fortificación de azúcar con vitamina-A

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Abstract

A seeded cooling crystallization batch process was developed for sugar fortification with retinyl palmitate seeds (RP, vitamin-A). Sugar solubility in water was measured in presence of RP. Linear and natural cooling profiles were induced with stirring speeds of 90 and 180 rpm. RP encapsulated in the product concentration was measured and the percentage of degradation was determined. Fortified crystals were stored under ambient conditions and the RP percentage was measured during 60 days. In addition, organoleptic tests were performed to determine differences in taste of fortified sugar against commercial sugar. The results showed that the natural cooling profile and agitation rate of 180 rpm increased the RP encapsulation and decreased the degradation percentage. An increment in RP seeds produced a larger RP encapsulated concentration. The presence of RP did not cause significant changes in either the solubility or taste of fortified sugar. Finally, the percentages of RP in the product obtained in this study were greater, at 30 and 60 days of storage, than those reported for the traditional product.

Keywords: Degradability, encapsulation, fortification, retinyl palmitate, sugar.

Resumen

Se desarrolló un proceso discontinuo de cristalización por enfriamiento con sembrado para la fortificación de azúcar con palmitato de retinilo (PR, vitamina-A). Se midió la solubilidad del azúcar en agua en presencia de PR. Se probaron perfiles de enfriamiento lineal y natural con agitación de 90 y 180 rpm. Se midió la concentración del PR encapsulado en el producto y se determinó el porcentaje de degradación. Los cristales fortificados fueron almacenados a condiciones ambientales y se les midió el porcentaje de PR durante 60 días. Además, se realizaron pruebas organolépticas para determinar diferencias en sabor del azúcar fortificado contra azúcar comercial. Los resultados mostraron que el perfil de enfriamiento natural y una agitación de 180 rpm aumentaron el encapsulamiento de PR y disminuyeron su porcentaje de degradación. El aumento de semillas de PR produjo un incremento en la concentración de PR encapsulado. La presencia de PR no provocó cambios significativos ni en la solubilidad ni en el sabor del azúcar fortificado. Finalmente, los porcentajes de PR en el producto obtenido en este estudio, fueron mayores, a los 30 y 60 días de almacenaje, que los reportados para el producto tradicional.

Palabras clave: Azúcar, degradabilidad, encapsulación, fortificación, palmitato de retinilo.

1 Introduction

Vitamin-A is responsible for strengthening the vision, the proper functioning of the immune system against infections and growth. Over the past years, vitamin-A deficiency has become a serious health problem in several countries (World Health Organization - WHO, 2009; Social Security Mexican Institute - IMSS, 2014). Epidemiological studies have shown that severe deficiency of vitamin-A has caused blindness in children and strongly contributes to their mortality. Dary and Arroyave (1996a) presented the following three strategies already suggested in different countries for increasing vitamin-A level: modification of population's diet through changes in food production, distribution and consumption patterns; periodic distribution among children of high doses of vitamin-A; and fortification of foods with vitamin-A.

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The third strategy, using sugar as vehicle, has proved to be the most promising strategy to reduce vitamin-A deficiency (Dary and Arroyave 1996a). The selection of sugar, as an agent to be fortified, was done based on the assumption that sugar was widely consumed by the population in amounts that showed minimal daily variation. This fact made easy to determine the amount of vitamin-A needed to fulfill the WHO requirements.

Vitamin-A occurs in many forms, such as retinol, retinal, retinyl acetate or retinyl palmitate (RP), and provitamin-A carotenoids (β-carotene, α-carotene, etc.). The physicochemical characteristic of vitamin-A, as retinol, prevents it from being used as fortifier because it is an oily, hydrophobic and sensitive to some physical and chemical factors such as heat, moisture, exposure to air and light, and acid or alkaline environments that reduced its stability during processing, distribution and storage. Allwood and Martin (2000) found that retinol was very sensitive to light and degraded by photolysis. Dary and Arroyave (1996c) informed that environments with a pH lower than five tended to oxidize retinol, but they described that vitamin-A produced as retinyl palmitate has proved to be a suitable fortifier product less sensitive than retinol.

Dary and Arroyave (1996b) described the traditional sugar fortification process with RP. This process mechanically mixed bulk sugar with a RP premix in a ratio of 1000:1. Vegetable oils and antioxidants were added to the mixture to increase the adhesion of RP to the crystals and prevented the oils to become rancid over time. Diguet *et al*. (2016) described other approaches to produce a premix of sugar formed by a sugar core surrounded by a special coating that might contain polysaccharides, oligosaccharides or other nutrients. A disadvantage of the traditional fortification process is that RP, oils, and antioxidants remains attached to the surface of sugar crystals leaving them exposed to light, moisture and heat that promote their chemical deterioration. De Gracia and Murillo (1993) reported for retail fortified sugar a decreases of RP concentration from 10 to 30% during preparation of the premix and 18 to 35% after one-year storage depending on the storage and weather conditions. Another disadvantage of the traditional preparation process is that the mechanical premix dilution does not guarantee a homogeneous distribution of RP in the mixture.

Some attempts to protect high value particles from adverse environmental conditions have been reported on the literature. Pulido and Beristain (2010) described a spray drying encapsulation of ascorbic acid using chitosan as wall material. Viveros-Contreras *et al*. (2013) encapsulated ascorbic acid atomizing the alginate solution into CaCl2, followed by freeze-drying. Fuentes-Ortega *et al*. (2017) analyzed the microencapsulation of sesame oil by spray drying. Pérez-Alonso *et al*. (2015) determined the stability of phenolic compounds coated with biopolymers and mead. Reus *et al*. (2014) described a particle formation and encapsulation of isonicotinamide and caffeine within a single process. Ling and Chadwick (2017) described crystallization of acetaminophen and sulfathiazole inside microporous particles of alginate and carboxymethyl cellulose.

On the other hand, cooling crystallization under different cooling profiles have been frequently used to control particle size distribution. Quintana-Hernandez *et al*. (2004) studied natural, lineal and cubic cooling profiles in batch crystallization of sugar in the range of 40-70 °C. Natural and lineal cooling profiles presented faster responses and high yield. Cubic profiles had low yields due to the low supersaturation in the total analyzed batch time. Bohlin and Rasmunson (1992) suggested that a linear or weakly non-lineal cooling curve usually produced larger crystals than natural cooling curve as long as the batch time was larger. Sanchez-Sanchez *et al*. (2017, 2020) analyzed different operation conditions for sugar cane batch crystallization and included changes in vacuum pressure and total evaporation time. Quintana-Hernandez *et al*. (2005, 2016) used the metastable zone width as a strategy to limit solute concentration that favor growth over nucleation.

In the present work, it was developed a cooling crystallization process for sugar fortification with vitamin-A. The effects of cooling profiles, agitation rates and type of seeds on the encapsulation and degradation of RP were investigated. Furthermore, the effects of RP on the solubility of sugar in water and flavor of fortified sugar were analyzed, and the percentage of RP on stored fortified sugar was measured over a 60-day period.

2 Materials and methods

2.1 Materials and reagents

Sugar cane was purchased from Distribuidora de azúcar (Celaya, Mexico). Vitamin-A, as retinyl palmitate powdery with 500,000 IU/g was provided by

F. Hoffmann-La Roche (Basel, Switzerland), it had a mean of 3.94 μm and a standard deviation of 2.76 μm. Ethanol (99.9%), hexane (99.99%), and distilled water were purchased from SUQUIBA (Celaya, Mexico).

2.2 Experimental apparatus

Solubility experiments were carried out in a jacked glass vessel with a volume of 400 mL. Temperatures were controlled with a programmable temperature thermostat (Lauda RP1800, Germany) with accuracy (0.01 K). Constant agitation (50 rpm) was provided with a mechanical agitator Armfield (England). Sugar concentration (°Brix, g sugar/100 g solution) was measured with an Abbe refract meter Atago DR-A1 (USA). Sugar and RP samples were weighted on an analytical balance Mettler Toledo AG245 (USA) with accuracy (0.01 mg).

Cooling crystallization experiments were performed in a three-liter stainless steel crystallizer provided with four fixed vertical baffles equally spaced, and a heat exchange jacket. An agitator Janke & Kunkel RW20DZM (Germany) with a double blade connected to an electrical motor provided the necessary agitation in the range of 50-1200 rpm. Temperatures were measured with J-type thermocouples and recorded with a data acquisition equipment cDAQ 9401 (National Instruments, USA). A Huber HS40 (Germany) system was programmed with eqs. (1)-(2) to generate natural and lineal cooling profiles. T represented the programed temperature in °C and t corresponded to the time in minutes.

$$T_{natural} = 70 - 30[1 - exp(-t/12)] \quad (1)$$

$$T_{lineal} = 70 - 7.5t/60 \quad (2)$$

RP concentrations were measured with a spectrophotometer Perkin Elmer Lambda 25 (USA). Sugar seeds crystal size distribution was measured with a Master Sizer S (Malvern Instruments, England) and crystal habit was observed with an optical microscope Iroscope MG-18 (Mexico).

2.3 Analytical method for RP determination

RP concentration was measured using Dary and Arroyave (1996c) technique. Briefly, weight 10 g of fortified sugar or liquor and dissolve in 30-40 mL 0.1 N NaOH. To ensure complete dispersion of RP beadlets, heat the solution in a water bath at 50 °C for 15 minutes. Cool at room temperature. Transfer to a 50 mL volumetric flask and make up to 50 mL with 0.1 NaOH and mix. Prepare three 20 mL test tubes with 4 mL of the solution. Add 4 mL of absolute ethanol to each tube and mix for 5 seconds. Add 5 mL of hexane to each tube and mix vigorously for 30 seconds to ensure complete extraction of RP. Allow separation of the top organic solvent phase. Transfer the organic phase to a 1 cm light path spectrophotometer cuvette and read the absorbance at a wavelength of 325 nm. RP concentration, expressed as retinol concentration is evaluated using Eq. (3).

$$C_R = 0.546\left(\frac{Abs}{a}\right)\left(\frac{V_h}{V_{az}}\right)\left(\frac{V_i}{p}\right) \quad (3)$$

where Abs is the registered absorbance. a is the absorbance coefficient for retinol (0.092 mL/μg cm). V_h is the hexane volume added (5 mL). V_{az} is the volume of the aliquot analyzed (4 mL). V_i is the total volume prepared from the initial solution (50 mL). p is the weight of sugar or liquor sample (10 g) and 0.546 is the molar relationship between retinol and retinyl palmitate.

2.4 Experimental procedure

2.4.1 Influence of retinyl palmitate on the solubility of sugar

A factorial design was used to analyze the solubility of mixtures of sugar with RP. The response variable was the sugar concentration expressed in ° Brix (g sugar/100 g solution). The analyzed factors were retinol concentration (C_R) at three levels (0, 15 and 30 μg retinol/g sugar) and saturation temperature at two levels (40 °C and 70 °C). The concentration of 15 μg retinol/g sugar represents the average concentration recommended by WHO (2009) in fortified sugar with vitamin-A (10 μg retinol/g sugar minimum and 20 μg retinol/g sugar maximum per day). The concentrations of 30 μg retinol/g sugar was included taking into account the possible degradation of the RP during the crystallization process. Temperatures of 40 °C and 70 °C were chosen from previous crystallization operating limit conditions (Quintana-Hernandez *et al*., 2004).

Solubility measurements were determined following the procedure reported by Maldonado (2005). Briefly, sugar crystal are dried at 105 °C during two hours. After, they are cooled to ambient temperature in a desiccator. The required mass of sugar for saturating a volume of 100 mL of water

is determined using eqs, (4)-(5) reported by Quintana-Hernandez *et al*. (2005).

$$C_s = -0.000701T^2 + 0.264T + 60.912 \quad (4)$$

$$m_s = \left(\frac{C_S}{100 - C_S}\right)100 \quad (5)$$

where C_s is the sugar saturation concentration in the solution (°Brix), T is the saturation temperature (°C), and m_s is the required mass of sugar.

Aqueous unsaturated solutions are prepared at ambient temperature, and heated up to saturation temperature. The solution is stirred at 50 rpm and sugar is completed dissolved. In a cyclic manner, 0.1 g of sugar is added to the solution up to the point where it is no longer possible to dissolve more sugar (concentration of sugar in the solution remained constant). Experiments at different conditions were done three times to verify repeatability and the results were analyzed with MINITAB, using an analysis of variance (ANOVA) with a 95% confidence level.

2.4.2 *Experimental designs*

The influence of cooling profiles, agitation rate and type of seeds on the degradation of RP was analyzed. Two experimental designs were proposed. In the first one, an experimental design 2^2 (E1.1-E4.1) analyzed the effects of cooling profiles (lineal and natural) and agitation speed (90 and 180 rpm). Solutions with 2844 g of sugar and 0.0426 g of retinyl palmitate were dissolved in 900 mL of water at 70 °C during 30 minutes. Then, the solutions were cooled during 240 minutes following natural or lineal cooling profiles. Every 10 minutes, a sample of 10 mL was removed and filtered (Whatman 40). Crystals were dried under vacuum for 30 minutes at room temperature in a desiccator. The mass of crystals was weighted. The sugar solution concentration was evaluated by difference between the initial sugar loaded and the mass of crystals weighted. Relative supersaturation, S, was calculated using Eq. (6). Where C_i was the sugar concentration at temperature T_i, and C_s was the saturation concentration at the same temperature.

$$S = \frac{C_i - C_s}{C_s} \quad (6)$$

The amounts of encapsulated and non-encapsulated retinyl palmitate were measured, and the RP degradation percentage (PRD) was calculated with Eq. (7).

$$P_{RD} = \frac{M_I C_I - M_L C_L - M_C C_C}{M_I C_I} \times 100 \quad (7)$$

where M_I represented the initial sugar mass, M_L the mass of the final filtrate (liquor), M_C the final mass of crystals, C_I the initial concentration of retinol (15 μg/g of sugar), C_L was the final retinol concentration in the liquor (μg/g of solution) and C_C the final retinol concentration in crystals (μg/g of sugar).

A second 2^2 experimental design was proposed (E1.2-E4.2) to verify the influence of the cooling profiles and the type of seeds (pure RP and a mixture of RP + sugar) on the degradation of retinol. To compensate the degradation effects of retinol during the crystallization process found in the previous stage, the initial retinol concentration (C_{IR}) was calculated with Eq. (8).

$$C_{IR} = \left(\frac{1500}{100 - P_{RD}}\right) \quad (8)$$

For these experiments, agitation rate was constant at 180 rpm and the batch time increased to 300 minutes to produce more fortified sugar. Saturated solution at 70 °C were prepared with 0.05342, 0.5342, 0.05839 and 0.5839 g of RP for experiments E1.2-E4.2 respectively. In addition, experiments E1.2 and E3.2 were supplemented with 8.0 g of sugar seeds (mean of 89.99 μm and a standard deviation of 38.48 μm). In order to have a seed homogeneous crystal size distribution, sugar seeds were recrystallized according to the method described by Maldonado (2005) and RP particles were used as they were purchased. Again, every 10 minutes, a sample of 10 mL was taken and analyzed. All experiments were done by triplicate and an analysis of variance with MINITAB was carried out with a significance level of 5%.

2.4.3 *Retinyl palmitate degradation over time*

Fortified sugar produced at experiments E1.2 to E4.2 was stored in dark paper bags at ambient temperatures (15 to 30 °C) and moistures from 40 to 60%. All bags were kept at the same conditions and away from daylight. Every 10 days, three samples of 10 g from each bag were removed and the retinol degradation percentage was evaluated. An analysis of variance was used to compare degradation percentages among the four different operation conditions using a significant level of 5%.

2.4.4 *Influence of retinyl palmitate on the taste of sugar*

Twenty participants (10 male and 10 female), ages between 20 and 22 years old were asked to taste two 10 mL samples prepared with commercial sugar (solution A) and fortified sugar (solution B). Both solution had a concentration of 0.025 g of sugar/mL of water. Each participant received two numbered samples containing one of the possible combinations AA, AB, BA or BB. The samples were randomized to the participants and only the applicator knew the type of samples delivered. Sufficient water was provides to participants to rinse their mouth after testing each sample. After the test, each participant wrote down on a card the numbers of their samples and answered the yes or no question. "Is there any difference in flavor between the sample pairs?" Participants responses were compared against the actual values (AA-no, BB-no, AB-yes and BA-yes). Correct answers were coded as one, and incorrect answers as cero. The established null hypothesis was "there were no statistical differences among the four experimental groups." An analysis of variance was performed to check differences among the four groups at a significant level of 5%.

3 Results and discussion

3.1 *Influence of vitamin A on the solubility of sugar*

Table 1 shows the solubility values calculated with Eq. (4) for pure sugar and the experimental measurements at different RP concentrations. Experimental results showed a slight decrease in sugar solubility in water when the RP concentration increased at 40 °C, and it remained almost constant at 70 °C. On the other hand, sugar solubility in water increased considerably with temperature. Table 2 shows the results of the analysis of variance for the experimental design. The ANOVA showed that there was no statistical difference due to RP concentration ($p > 0.05$) but it was due to temperature factor ($p \ll 0.05$).

Table 1. Calculated and experimental concentration of sugar in water with the addition of retinyl palmitate at 40 °C and 70 °C.

Concentration	**Temperature**	
	40 °C	**70 °C**
mg retinol/g sugar	**Sugar concentration °Brix**	**Sugar concentration °Brix**
0[a]	70.35	75.96
0[b]	70.3 70.33 70.3	75.9 76 75.89
15[b]	70.2 70.15 70.19	75.85 75.9 75.11
40[b]	70.15 70.1 70.1	75.9 75.9 75.87

[a] calculated with eq. (2), [b] experimental

Table 2. ANOVA of effects of retinol concentration (0, 15 and 30 μg/g sugar) and temperature (40 °C and 70 °C) on the saturation solubility of the sugar-water system.

Source	**DF**	**SS**	**MS**	**F-value**	**p-value**
Concentration	2	0.145	0.073	2.09	0.16
Temperature	1	141.681	141.681	4076.49	0
Error	14	0.487	0.035		
Total	17	142.313			

Table 3. Retinol degradation percentage as function of cooling profiles and agitation rate for operation batch times of 240 minutes.

	Cooling profile	**Agitation rate**	**Crystal mass** M_C	**Liquor mass** M_L	**Retinol concentration in crystals** C_C	**Retinol concentration in liquor** C_L	P_{RD}
Experiment		[rpm]	[g]	[g]	[μg/g$_{sugar}$]	[μg/g$_{solution}$]	[%]
1.1	Natural	90	360.99	3380.85	11.73	8.11	25.84
2.1	Natural	180	375.12	3366.63	13.69	8.6	20.13
3.1	Linear	90	243.81	3498.48	11.24	7.69	30.55
4.1	Linear	180	350.73	3391.74	11.79	7.98	26.84

Table 4. ANOVA of effects of cooling profiles (natural and lineal) and agitation rate (90 and 180 rpm) on degradation percentage of retinol.

Source	**DF**	**SS**	**MS**	**F-value**	**p-value**
Cooling Profile	1	97.8123	97.8123	287.57	0
Agitation	1	66.5523	66.5523	195.67	0
Error	9	3.061	0.03401		
Total	11	167.426			

3.2 Degradation of retinyl palmitate in the crystallization process

Table 3 shows the results of the first 2^2 experimental design. The table include the average results of crystal and liquor masses (MC, ML), retinol concentrations in crystals and liquor (CC, CL) and retinol degradation percentage (PRD) for each run conditions after 240 minutes. The natural cooling profile (E1.1 and E2.1) favored the faster growth of crystals due to higher supersaturation. Similarly, agitation rate of 180 rpm increased mass transfer and RP dispersion (E2.1 and E4.1). Table 4 shows the results of the analysis of variance. At a 5% significance level, both cooling profiles and agitation rate showed to be statistically significant.

The combination of natural cooling profile and higher agitation rate produced the largest mass of crystals, the highest concentration of retinol within crystals and the smallest degradation of retinol. In contrast, stirring rate of 90 rpm and linear cooling profile generated the smallest concentration of retinol within crystals and the largest degradation. Degradation of RP in the production process fluctuated between 20.13% and 30.55% depending on the cooling profile and agitation rate.

Table 5 shows the experimental results of the second experimental design. The increment in the batch time from 240 to 300 minutes caused an increment in both degradation of RP and production of fortified sugar.

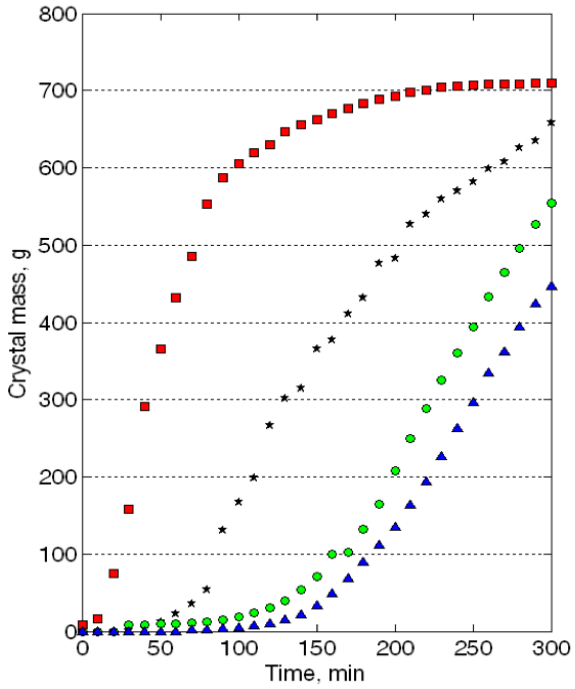

Fig. 1. Fortified crystal masses produces for natural cooling profile (■ E1.2 and * E2.2), lineal cooling profile (● E3.2 and ▲ E4.2), sugar and retinyl palmitate seeds (E1.2 and E3.2), and retinyl palmitate seeds (E2.2 and E4.2).

Table 5. Retinol degradation percentage for second experimental design after 300 minutes operation batch.

				Fortified crystals			Filtrate	
	Cooling Profile	**Type of seed**	$C_{Initial}$	C_C	C_{Cen}	C_{Cen}/C_C	C_L	P_{RD}
Experiment			[$\mu g/g_{sugar}$]	[$\mu g/g_{sugar}$]	[$\mu g/g_{sugar}$]	[%]	[$\mu g/g_{sugar}$]	[%]
1.2	Natural	RP+sugar	18.79	14.72	9.23	62.7	10.03	23.47
2.2	Natural	Pure RP	187.94	148.04	116.32	78.57	100.8	23.53
3.2	Lineal	RP+sugar	20.53	15.3	9.44	61.7	9.82	31.83
4.2	Lineal	Pure RP	205.26	150.15	117.07	77.97	100.2	31.93

$C_{initial}$ retinol initial concentration at each batch.

C_c total retinol concentration in fortified crystals (adhere to surface + encapsulated).

C_{cen} encapsulated retinol concentration.

C_{cen}/C_c percentage of encapsulated retinol.

C_L retinol concentration in filtrate (liquor).

P_{RD} degradation retinol percentage.

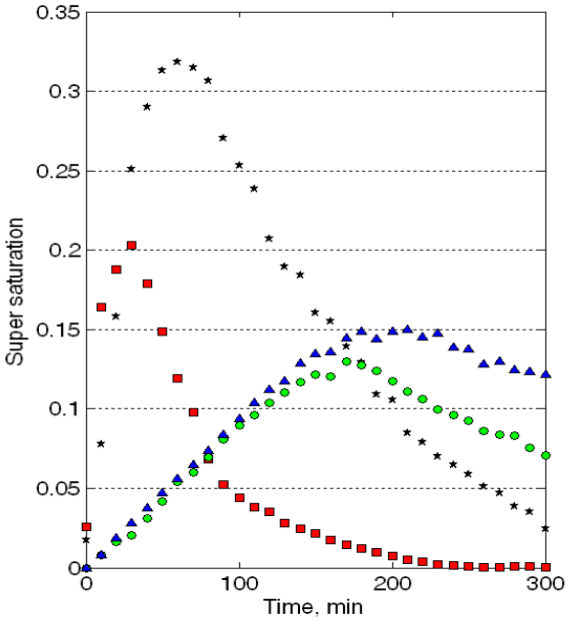

Fig. 2. Supersaturation for natural cooling profile (■ E1.2 and * E2.2), lineal cooling profile (● E3.2 and ▲ E4.2), sugar and retinyl palmitate seeds (E1.2 and E3.2), and retinyl palmitate seeds (E2.2 and E4.2).

A comparison of the percentages of degradation of RP between E2.1 and E2.2 shows an increment of 16.89%, and between E4.1 and E4.2 the increment was 18.86%. Fig. 1 shows the masses obtained at the different experimental conditions. The theoretical mass of crystals that can be obtained by cooling a saturated solution from 70 °C to 40 °C is 709.6 g. This theoretical mass (100% yield) was only achieved at the experimental conditions of E1.2. The yields of fortified sugar at the other experimental conditions (E2.2, E2.3 and E2.4) were 92.7%, 78.2% and 62.9% respectively. It is clear that lineal cooling profiles had lower production rates and lower yields (compared to natural cooling profiles). Higher yields could be achieved by increasing the batch times. However, the degradation of RP would be expected to increase too.

On the other hand, a comparison of fortified sugar yield, for the same cooling profile, showed higher yields when mixtures of seeds were used (E1.2>E2.2 and E3.2>E4.2). This behavior suggested that the adsorption and integration rates of RP to the crystal surfaces were slower than the rates of sugar adsorption and integration. Hsu *et al*. (2018) and Reus *et al*. (2014) reported similar findings on heterogeneous crystallization due to the low affinity between the heterogeneous solutes. In addition, separation of RP between the solid phase and the liquid phase can be analyzed with Eq. (9), where x_{solid} and x_{liquid} are the mass ratio of RP to the solute and the solution, and K, the separation constant, is inversely proportional to the final crystallization temperature (Sangwal and Mielniczek-Brzóska, 2007).

$$K = \frac{x_{solid}}{x_{liquid}} \tag{9}$$

At the beginning of each experiment, operation temperature is high, K is low and x_{liquid} is high (contrary to the values at the end of each experiment). If temperature is kept constant at the end of all experiments, Eq. (9) shows that an increment in the initial concentration of RP in the liquid phase should be compensated with an increment in the concentration of RP in the solid phase. So, from a thermodynamic point of view, the concentration of RP, encapsulated in the solid phase proportionally increases as function of the RP initial concentration.

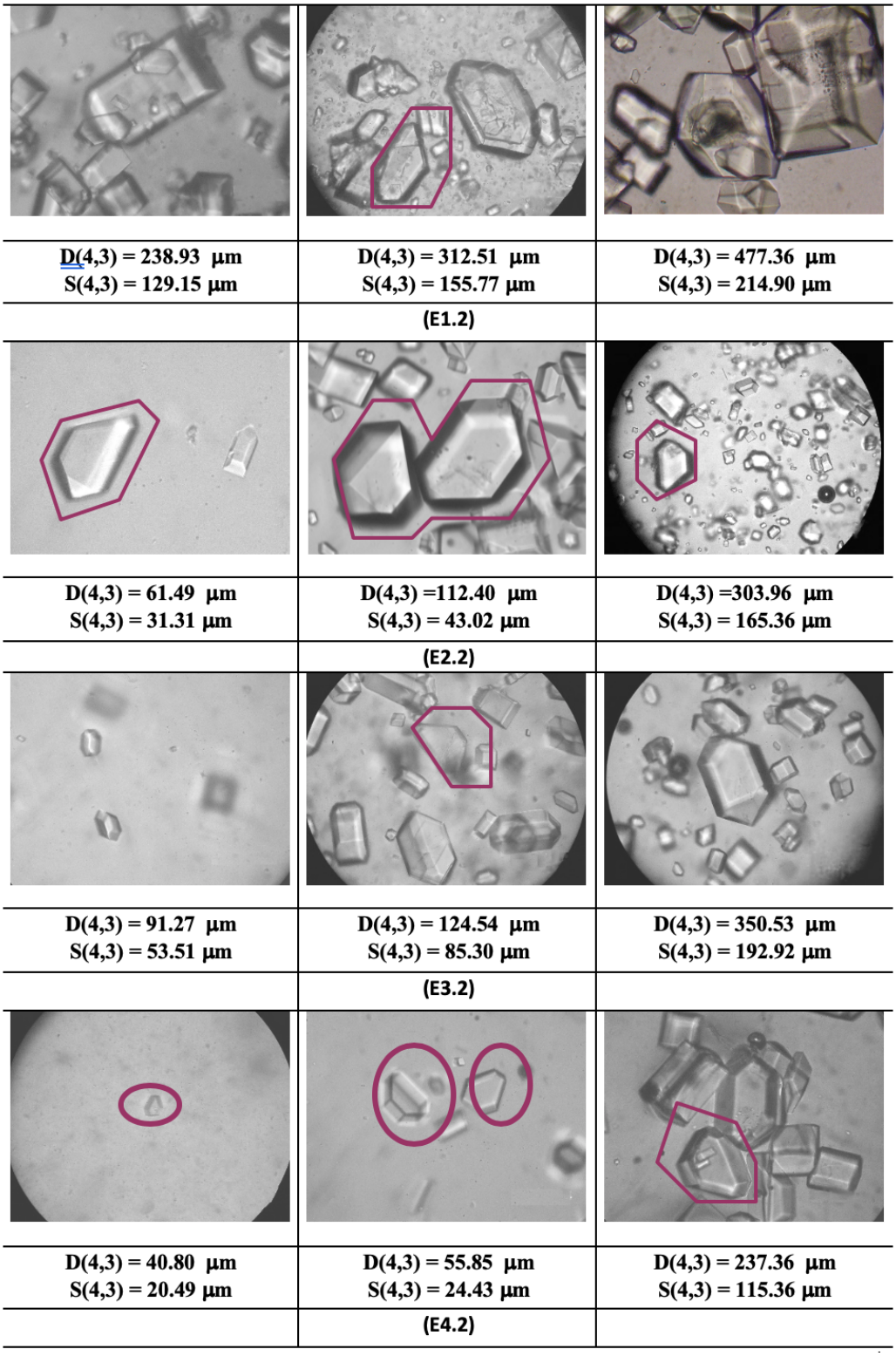

Fig. 3. Images of crystals at times 50,100 and 250 minutes for each experiment.

Table 6. ANOVA of effects of cooling profiles (natural and lineal) and type of seed (RP pure and RP+sugar) on degradation percentage of retinyl palmitate.

Source	DF	SS	MS	F-value	p-value
Cooling Profile	1	210.673	210.673	24061.66	0
Type of seed	1	0.019	0.019	2.19	0.173
Error	9	0.079	0.009		
Total	11	210.704			

Fig. 2 shows the supersaturation profiles as a function of time. Supersaturation increased rapidly with natural cooling profiles, reaching values 0.2029 and 0.3184 for E1.2 and E2.2 respectively. The maximum supersaturations with lineal cooling profiles were 0.1295 and 0.1497 for experiment E3.2 and experiment E4.2. For experiments with pure RP seeds, supersaturation reached higher values due to the lower adsorption and integration rate of RP.

In summary, the maximum production of fortified sugar and the minimum degradation of RP were obtained with natural cooling profiles and a mixture of RP + sugar seeds. Table 6 shows the ANOVA for the second experimental design. The analysis showed that cooling profiles had a strong influence in the RP degradation ($p \ll 0.05$) but the type of seed did not have that influence in the degradation of RP ($p > 0.05$).

Fig. 3 shows the pictures obtained with the optical microscope at times 50, 100 and 250 for the four different operation conditions analyzed. Mixtures of seeds produced bigger crystals (E1.2 and E3.2) because the supplemented sugar seed were bigger than the RP seeds. In all experiments, non-symmetrical crystals appeared. It seemed that adsorption and integration of RP in some crystals facets slowed down their growth. Mantovani (1996) described morphological changes in the crystallization process of sugar in presence of raffinose, glucose and fructose generating asymmetrical crystals. In Fig. 3, some crystals that showed morphological changes in this study were highlighted.

3.3 Degradation of the vitamin-A over time

At the analyzed operation conditions, results showed that more than 61% of RP was encapsulated within sugar crystals. In fact, in experiments E2.2 and E4.2, sugar crystals encapsulated up to 80% of RP. These results suggested that RP encapsulation percentage was function of the initial RP concentration. Fig. 4 shows the percentages of retinol as function of time. De Gracia and Murillo (1993) found that the average concentration of retinol were around 81% and 66% after 30 and 60 days of storage respectively. These values were plotted in Fig. 4 as a reference of the fortified sugar obtained with the traditional process. The retinol content in fortified sugar produced with the developed process at any of the analyzed conditions, was higher to the one reported for fortified sugar prepared and stored with the traditional process after 30 and 60 days. In addition, it was observed that crystals produced with an excess of RP in Experiments E2.2 and E4.2 presented higher stability. The global analysis suggested that degradation of RP occurred faster at the crystal surface and the RP encapsulated inside crystals was protected longer at environmental conditions.

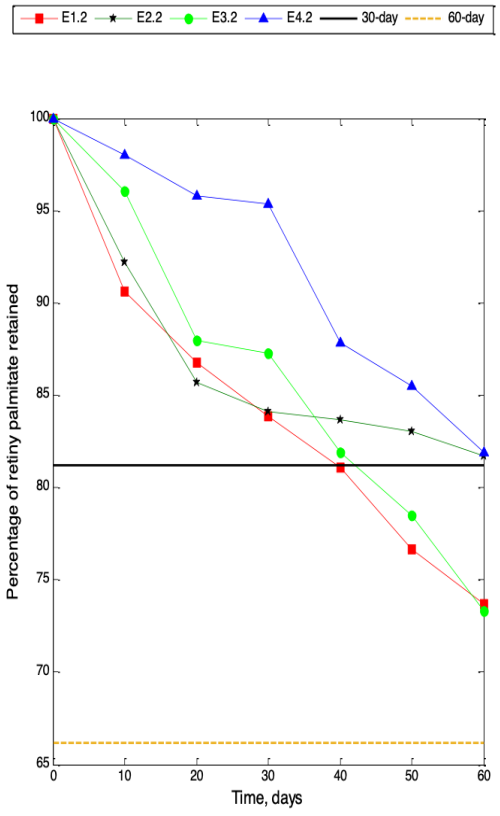

Fig. 4. Percentage of retinol in fortified sugar over time.

Table 7. Results of the organoleptic test.

Participant	Gender	Assigned sample	Actual response	Participant response	Coded comparison*
1	M	AA	no	no	1
2	F	BB	no	yes	0
3	F	BB	no	no	1
4	F	AA	no	yes	0
5	M	AB	yes	no	0
6	M	AB	yes	yes	1
7	F	BA	yes	no	0
8	F	BA	yes	no	0
9	M	BB	no	yes	0
10	M	BB	no	no	1
11	F	AB	yes	no	0
12	F	BB	no	yes	0
13	M	BA	yes	no	0
14	M	AA	no	no	1
15	F	AA	no	no	1
16	M	AB	yes	no	0
17	F	BA	yes	yes	1
18	M	AB	yes	yes	1
19	F	BA	yes	yes	1
20	M	AA	no	no	1

* 1 correct, 0 wrong

Table 8. ANOVA of the effects of RP on the flavor of a sweet solution.

Source	DF	SS	MS	F-value	p-value
Type of delivery pair samples	3	0.6	0.2	0.173	0.551
Error	16	4.4	0.275		
Total	19	5			

3.4 Analysis of the organoleptic experiments

Table 7 shows the participants information and distribution of samples received. Samples AA and BB had an actual response of "no" because they came from the same solution. The column participant response presents the answers they wrote down on the answer sheet. Ten participants (6 males and 4 females) answered accordingly to the actual response and ten did not. Table 8 shows the results of the ANOVA. At a 5% confidence level, there were no differences among the groups. Therefore, no difference in flavor was stablished due to the presence of RP.

Conclusions

The results showed that the proposed fortification process was feasible. Retinyl palmitate particles can be used as seeds and can be encapsulated during the crystallization of sugar, protecting them from the surrounding environment agents. The best production conditions to maximize the mass of fortified crystals and reduce the degradation of RP were natural cooling profiles, agitation rate of 180 rpm and a mixture of RP + sugar seeds. The integration of the RP in the fortified sugar reduced the growth of the crystal facets where the RP is adhered causing changes in the morphology of the fortified crystals. Finally, fortified sugar, produced with the developed process, reduced RP degradation over time and did not produce a perceptible change in flavor.

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