

Osmotic Dehydration and Freezing as Combined Processes on Apple Preservation

A. Bunger^{1,*}, P.C. Moyano², R.E. Vega², P. Guerrero¹ and F. Osorio³

¹*Department of Food Science and Chemical Technology, Universidad de Chile, Vicuña Mackenna 20, Santiago, Chile*

²*Department of Chemical Engineering, Universidad de Santiago de Chile*

³*Department of Food Science and Technology, Universidad de Santiago de Chile*

Combined processes effects of osmotic dehydration in sucrose solutions and freezing on apple cubes preservation were analysed. Two multifactorial experimental designs, in two levels, were conducted consecutively to quantify the effects of the following factors: temperature, osmotic dehydration time, concentration of the osmotic medium and freezing rate. The response variables considered were: sensory evaluation, colour, texture, water activity (a_w) and reducing and total sugars. The first experimental design selected fast freezing as the best process to preserve texture and colour of the fruit. From the second experimental design, under fast freezing, were obtained the following optimal levels: 55°Bx for the concentration of the osmotic medium, 35°C for the syrup temperature and 60 min for the osmotic dehydration time. A test of acceptability was performed under these conditions with 80 potential consumers on a 7-point hedonic scale, which gave 93% acceptance. Glass transition temperature (T_g') of the maximally cryoconcentrated liquid was -41.89°C for the product processed under optimum conditions. Significant correlations ($P \leq 0.05$) were found between sensory and instrumental responses.

Keywords: apples, sensory evaluation, osmotic dehydration, freezing, cryostabilisation

INTRODUCTION

The increase in consumer preferences for minimally processed fruits and vegetables have prompted researchers to study "combined methods" as a preservation technique. The synergistic or additive effect of combined inhibiting factors may permit the production of food with improved quality over that preserved by only one technique, e.g. heat treatment, dehydration or freezing (Ferrando and Spiess, 2003).

Freezing is a suitable way to preserve some quality parameters of foods, such as colour, flavour and nutrients. However, freezing and frozen storage almost always cause physical and chemical changes that lead to a loss of quality (Haard, 1997; Martínez-Monzó et al., 1998).

Cryostabilisation is currently under active research as a means of protecting products stored for long periods at typical freezer temperatures (-18°C) from deleterious changes in texture, structure and chemical composition (Slade and Levine, 1991). The procedure

involves the incorporation of cryoprotectors (low molecular weight solutes) or cryostabilisers (high molecular weight solutes) with the aim of increasing the glass transition temperature (T_g') of the maximally cryoconcentrated liquid phase in the food (Martínez-Monzó et al., 1998; Forni et al., 1997). According to Slade and Levine (1991), the lower the difference between T_g' and the storage temperature, the higher the product stability.

The incorporation of these cryomaterials can be done through osmotic dehydration, i.e. by immersing the food in a hypertonic solution that contains the appropriate cryomaterial. During the osmotic dehydration, the product is in contact with a low water activity solution and a two-way mass transfer occurs: water is transferred from the product to the solution, and in the opposite direction, solute is transferred from the solution to the product (Spiazzi and Mascheroni, 1997; Salvatori and Alzamora, 2000; Moyano and Zúñiga, 2003). If the dehydrated food is frozen afterwards, it will be characterised by higher T_g' values when sugars of suitable molecular weight and structure are used as cryomaterials.

Dehydrofreezing is a variant of freezing in which a food is dehydrated to a desirable moisture and then frozen (Robbers et al., 1997; Li and Sun, 2002). A reduction in moisture content reduces the amount of water, thus lowering refrigeration load during freezing, with additional lower cost of packaging, distribution and storage (Biswal et al., 1991; Li and Sun, 2002).

*To whom correspondence should be sent

(e-mail: abunge@uchile.cl).

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Successful applications of dehydrofreezing have been reported on green beans (Biswal et al., 1991), strawberries (Sormani et al., 1999), kiwis (Robbers et al., 1997) and papayas (Moyano et al., 2002).

The objective of this work was to study the preservation of apple cubes (var. *Granny Smith*) through the combination of osmotic dehydration and freezing processes.

MATERIALS AND METHODS

Samples

Apples var. *Granny Smith* were provided by a local food purchaser and kept at 2°C until processing. Commercial sucrose (IANS S.A., Chile) was used to prepare the osmotic syrup.

Experimental Design and Statistical Analysis

Two consecutive multifactorial experimental designs, in two levels, were performed to simultaneously study the effect of the factors that affect the combination of osmotic dehydration and subsequent freezing processes, through the following responses: sensory evaluation, colour, texture, water activity (a_w) and reducing and total sugars.

The first design was a diagnostic design, a fractional factorial of the type 2^{4-1} (Box et al., 1989) to study four factors: osmotic dehydration temperature (25 and 40°C) and time (45 and 90 min), concentration of the osmotic medium (40 and 60°Bx) and freezing rate (slow and fast). This design required 8 experimental runs (Table 1).

Based on results of the first design, a full factorial 2^3 design with two central points was performed (Box et al., 1989) with the following factors (Table 2): concentration of the osmotic medium (55 and 65°Bx) temperature (35 and 45°C) and osmotic dehydration time (30 and 60 min).

Statgraphics Plus software for Windows version 4.0 (Manugistics Inc., Statistical Graphics Corporation,

Rockville, USA) was used for statistical analysis of the experimental data. The statistical significance of the effects was analysed simultaneously by means of normal probability charts, Pareto charts and ANOVA ($P \leq 0.05$). Correlation was measured by Pearson's coefficient ($P \leq 0.05$).

Processing

Pre-treatment

Trials were performed on apples (var. *Granny Smith*) of the same batch. Apples were hand-washed, peeled, cored and cut into uniform cubes of $1 \times 1 \times 1 \text{ cm}^3$.

Osmotic Dehydration

Apple cubes were fixed in a stainless steel basket between grids and immersed in the osmotic medium in a thermostatically controlled water bath (Blue M). Sucrose solutions were used as osmotic agent, prepared with tap water and containing 1% ascorbic acid and 0.2% citric acid (based on the water content) to avoid enzymatic browning. The basket was mechanically rotated at 30 rpm to ensure a good mass transfer. The ratio of sucrose solution to apple pieces was 6:1 (w/w). The concentration levels, osmotic dehydration time and temperature of the osmotic solution were determined through 2 experimental designs. The osmodehydrated apple cubes were stored at 2°C for 24 h before freezing to homogenise the internal moisture and sugar concentration.

Freezing

Two freezing rates were used: a slow freezing rate in an air blast freezer (own manufacture) operating with air at -28°C and a velocity of 0.55 m/s for 1.5 h, and a fast freezing rate by means of a cryogenic freezer (CES model Box Freezer, Belgium) operating with liquid nitrogen at -63°C for 10 min. Frozen apple cubes were stored at -18°C for 15 days and thawed at room tem-

Table 1. Standard matrix of the first experimental design (2^{4-1}).

Run No.	Concentration (C, °Bx)	Temperature (T, °C)	Time (t, min)	Freezing rate* (F)
1	40	25	45	Fast
2	60	25	90	Fast
3	40	40	90	Fast
4	60	40	45	Fast
5	40	25	90	Slow
6	60	25	45	Slow
7	40	40	45	Slow
8	60	40	90	Slow

*Fast = cryogenic freezing; Slow = Air blast freezing.

Table 2. Standard matrix of the second experimental design (2³).

Run No.	Concentration (°Bx)	Temperature (°C)	Time (min)
9	55	35	30
10	55	45	30
11	65	35	30
12	65	45	30
13	55	35	60
14	55	45	60
15	65	35	60
16	65	45	60
17	60	40	45
18	60	40	45

perature ($20 \pm 1^\circ\text{C}$) until equilibrating temperature previous to the analyses.

Design Responses

Sensory Evaluation

Twelve assessors (4 male and 8 female) aged between 22 and 35 were recruited and selected using International Standards (ISO, 1993). This panel received additional training during six 2-h sessions for quantitative descriptive analysis (QDA) and quality of apple cubes. The response variables were obtained by means of a quantitative descriptive analysis (QDA) on a 10cm non-structured linear scale (Meilgaard et al., 1991) and a quality rating test (Muñoz et al., 1992) on a 7-point numerical scale (1 – very bad, 7 – very good). The following QDA descriptors and their respective anchor words were selected by panel consensus during the training period: browning (none, intense), hardness (soft, hard), manual firmness (soft, hard) and sweetness (none, intense). The measured attributes of the quality-rating test were colour, flavour, texture and overall quality. Samples were randomly evaluated in 9 sessions (2 samples per session). Each assessor was provided with water to cleanse the palate between tastings.

Colour

Colour was measured using a Minolta Chroma Meter CR-200b (New Jersey, USA) attached to a data processor DP-100, using the CIE Lab L^* , a^* and b^* colour space, calibrated with a white tile. Fifteen measurements of surface on different apple cubes were made for each sample under CIE D_{65} illuminant conditions, 0° viewing angle and a measuring area with a diameter of 8mm. Hue angle, chroma and colour difference were obtained through the following equations (Calvo and Durán, 1997):

$$\text{Hue angle} = \tan^{-1}(b/a)$$

$$\text{Chroma} = (a^2 + b^2)^{1/2}$$

$$\text{Colour difference } (\Delta E) = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$$

Texture

Texture was measured using a texture analyser TA.XT2i interfaced with a data processor Texture Expert version 1.0 (Stable Microsystems, UK). A compression test was carried out with a P/25 probe, which is a flat-ended aluminium probe of 25mm diameter, at a crosshead speed of 0.5mm/s and compressing the apple cubes at 40%. Ten measurements were made for each sample. A puncture test was also performed with an HDP/MPT probe at a crosshead speed of 0.1 mm/s. This probe is also known as a multiple pea test rig, and is designed to penetrate 18 samples simultaneously, using a multiple probe head with 18 needle probes of 2mm diameter. The apple cubes were crossed completely and made three measurements. The response variables considered for both tests were: initial slope, maximum rupture force, distance to produce the maximum force and area under the curve (work).

Reducing and Total Sugars

Determination was done by the spectrophotometric 3,5 dinitrosalicylic acid method DNS (Miller, 1959). Absorbance of coloured complex was measured at 600nm in an 8453 UV-visible spectrophotometer (Agilent Technologies, California, USA) provided with UV-visible Chem Station software. The reducing sugars were determined on a filtered and diluted sample. For total sugars determination, first a diluted and filtered sample was hydrolysed with concentrated HCl solution until pH 1, and then followed the method given above. Measurements were made in triplicate.

Water Activity (a_w)

The a_w was measured at 25°C with an electric hygrometer Humidat-IC (Novasina, Switzerland).

Other Analyses

Glass Transition Temperature

Differential scanning calorimetry (DSC) was performed in a TA Model 910 (TA Instruments, New Castle, USA) on selected samples, choosing two experimental runs, the product processed under the optimum conditions found, and a fast frozen control sample without osmotic dehydration. The procedure

described by Anglea et al. (1993) was employed. Analyses were performed in duplicate.

Moisture

Moisture was determined in a vacuum oven until constant weight according to AOAC method 920.151 (AOAC, 1990). Analyses were performed in duplicate.

Product Acceptability

Acceptability of apple cubes obtained under the optimised conditions was evaluated with 80 frequent apple consumers on a 7-point hedonic scale (1 – dislike it very much, 7 – like it very much) (Meilgaard et al., 1991).

RESULTS AND DISCUSSION

First Experimental Design

Concentration of the osmotic medium was the most statistically significant factor that affected the major number of responses in the 2^{4-1} design (Table 3). Next

in importance were temperature and freezing rate, which affected nearly half the number of responses, compared to the concentration effect. High concentration of the osmotic medium increased firmness of apple cubes measured by compression, puncture and sensory descriptive test (Table 4). It also helped to preserve the colour, as shown by a sharp decrease in a^* and colour difference (ΔE). Sugar absorption increased, as shown by the increase of sensory sweetness, total sugars and sucrose content (Table 4).

The increase in temperature (factor T) prevented enzymatic browning, as shown by the significant decrease ($P \leq 0.05$) of a^* and an increase in sensory colour quality and overall quality (Table 3).

Freezing rate (factor F) affected mainly texture and colour (Table 3), showing that a change from fast to slow freezing negatively affected colour quality and product firmness, as it produced higher cellular damage due to formation of relatively larger ice crystals.

Time (factor t) showed contradictory effects on apple quality, since an increase in time increased firmness and sucrose content but diminished sensory sweetness (Table 3). This decrease in sweetness can be attributed to a higher uptake of acids from the osmotic

Table 3. Effects of the first fractional factorial experimental design (2^{4-1}).

Response	Effects							
	Average	Concentration	Temperature	Freezing rate	Time	CT + Ft	CF + Tt	Ct + TF
Sensory								
Descriptive								
Test ¹ (QDA)								
Sensory Quality ²								
Colour								
Compression								
Puncture test								
Sugars								
a_w								
Browning	4.1	-0.6	-3.5*	-1.4	-0.3	+2.9*	-0.6	+0.3
Hardness	4.4	+1.4*	+0.5	-0.6	+0.5	-0.6	+0.6	-0.1
Manual compression	4.2	+0.8*	+0.4	-0.7	-0.2	-0.8	0.0	0.0
Sweetness	5.6	+0.9*	-0.2	-0.5	-0.9*	-0.2	-0.1	0.0
Colour quality	4.7	+0.3	+2.1*	-1.4*	+0.1	-1.5	+0.2	-0.2
Flavour quality	5.2	+0.7	+0.6	-0.1	-0.1	-0.6	0.0	-0.1
Texture quality	5.0	+0.4	+0.8	+0.1	-0.2	-0.6	+0.1	+0.2
Overall quality	4.9	+0.4	+1.2*	+0.4	+0.1	-1.0*	0.0	-0.1
L^*	61.64	+4.68*	+4.29	-2.89	+2.46	-8.64*	+1.42	-1.60
a^*	-2.62	-3.32*	-4.73*	-1.29	-0.67	+3.03*	20.43	+1.3
b^*	18.30	21.29	23.34*	22.60	+2.24	+3.94*	+1.82	+2.60
ΔE	16.31	25.46*	25.01	+2.78	23.39	+8.69	21.30	+1.87
Hue	1.74	+1.15	+0.25	+0.08	-0.03	-0.18	+0.02	-0.07
Chroma	18.83	-1.21	-2.93	-2.54	+2.08	+3.83	+2.12	+2.53
Initial slope (N/mm)	8.36	+4.32*	+3.23*	-0.15	+3.50*	+1.07	+1.30	+1.71
Maximum force (N)	14.48	+3.09*	+1.97	-4.67*	+2.14	-0.86	+1.88	+1.56
Distance (mm)	3.96	-0.15	-0.20	-0.05	-0.23	+0.11	+0.08	+0.06
Area (Nmm)	25.18	+1.27	+6.11	-11.05*	+3.88	+0.74	+1.87	+4.85
Initial slope (N/mm)	7.78	+3.83*	+1.81	-1.56	+2.52*	+0.33	+1.27	+1.29
Maximum force 1 (N)	20.84	+6.53*	+3.06	-3.33	+3.13	+0.41	+1.35	+1.41
Area 1 (Nmm)	37.83	+5.62*	+2.49	-3.65	+0.01	+1.38	+1.58	-2.10
Maximum force 2 (N)	13.21	+5.81*	+3.31	+0.66	+4.32	+2.92	+1.95	+1.66
Area 2 (Nmm)	93.29	+14.20*	+9.82*	-19.72*	+1.89	+0.84	+3.85	-1.74
Total (%)	40.38	+3.39*	+1.27	+1.20	+1.22	+0.03	+1.02	-0.92
Reducing (%)	18.91	+0.72	-0.06	-0.45	-0.30	+0.35	+0.39	-0.10
Sucrose (%)	21.48	+2.67*	+1.33	+1.64	+2.01*	-0.31	+0.63	-0.82
Water activity	0.966	-0.016	-0.010	-0.008	-0.014	-0.014	-0.007	-0.010

* $P \leq 0.05$.

¹Values based on a descriptive scale of 10cm length.

²Values based on a sensory 7-point quality scale.

Table 4. Change in the response by switching from low to high concentration of the osmotic medium 40 to 60°Bx.

	Response variable	Response change (%)
Sensory descriptive test	Hardness	37.8
	Manual compression	21.1
	Sweetness	17.5
Colour	L^*	7.9
	a^*	-345.0
	ΔE	-40.2
Compression	Initial slope	69.7
	Maximum force	23.9
Puncture test	Initial slope	65.3
	Maximum force 1	37.2
	Area 1	16.0
	Maximum force 2	56.4
Sugars	Area 2	16.5
	Total	8.8
	Sucrose	13.3

medium, together with sucrose uptake, which alters sweetness perception.

Second Experimental Design

Given the previous results, a second design was carried out, in which fast freezing was chosen for all runs. The levels for the factors concentration (C) and temperature (T) were adjusted around the maximum value of the previous design, and the levels for osmotic dehydration time around the minimum value (Table 2).

Osmotic dehydration time was the factor that statistically affected the most number of responses (Table 5). An increase from 30 to 60min produced higher sensory quality (colour, flavour, texture and overall quality) and better colour in both sensory evaluation (decrease in browning) and instrumental measurements (increase of L^* and decrease of a^* and ΔE).

Concentration and temperature affected a low number of responses, and practically no significant interaction effects were found (Table 5). Responses mostly affected were: maximum force, which decreased

Table 5. Effects of the second full factorial experimental design (2^3).

Response	Effects							
	Average	Concentration	Temperature	Time	CT	Ct	Tt	
Sensory Descriptive Test ¹ (QDA)	Browning	4.5	+0.3	-1.8	-3.6*	+1.4	-0.1	+1.8
	Hardness	3.7	-0.6	+0.1	+0.7*	-0.1	+0.5	+0.6
Sensory Quality ²	Manual compression	3.4	-0.1	+0.1	+0.3	-0.5	+0.3	+0.7
	Sweetness	5.9	0.0	+0.1	0.0	-0.1	-0.1	+0.1
	Colour quality	4.3	-0.2	+0.9	+2.1*	-0.3	0.0	-0.8
	Flavour quality	4.9	+0.1	+0.2	+0.6*	-0.2	+0.1	0.0
	Texture quality	4.1	-0.1	+0.3	+0.6*	-0.15	+0.1	+0.1
	Overall quality	4.3	-0.1	+0.6	+1.1*	-0.3	0.0	-0.2
Colour	L^*	66.24	-0.41	+7.11*	+8.25*	-2.57	-0.35	-4.87
	a^*	-0.67	+1.51	-2.76	-5.91*	+1.47	-1.63	+2.89
	b^*	21.06	+3.41	-1.79	-5.08*	+1.33	-1.91	+0.86
	ΔE	12.16	+1.81	-7.75	-10.07*	+3.50	-1.53	+5.97
	Hue	-0.06	+0.07	-0.13	-0.27*	+0.07	-0.06	+0.12
	Chroma	21.39	+3.33	-1.91	-5.08*	+1.3	-1.83	+0.97
	Compression	Initial slope (N/mm)	6.46	-1.75	+1.64	+0.93	-1.60	-0.58
Puncture test	Maximum force (N)	11.77	-3.36*	+1.48	+1.22	-2.21	-0.36	+3.49*
	Distance (mm)	3.70	-0.12	-0.10	-0.21*	-0.03	-0.12	+0.36*
	Area (Nmm)	15.75	-3.37	+1.47	+0.09	-1.04	+1.89	+0.26
	Initial slope (N/mm)	5.12	-0.77	+0.67	+0.86	-0.67	-0.02	+0.62
Sugars	Maximum force 1 (N)	13.43	-1.16	+2.43	+2.21	-0.88	+0.13	+1.44
	Area 1 (Nmm)	23.32	-0.20	+4.55	+3.77	-0.04	-0.88	+2.55
	Maximum force 2 (N)	7.50	-1.04	+1.19	-0.08	+0.38	-1.15	-1.63
	Area 2 (Nmm)	67.09	-9.32	+6.74	+3.11	-0.46	-1.17	+1.88
	Total (%)	36.67	+7.60	+1.35	+1.50	+1.15	-1.80	+1.25
Sugars	Reducing (%)	18.17	+3.00	+0.15	+2.20	+0.25	-1.70	+0.55
	Sucrose (%)	18.51	+4.53	+1.23	-0.68	+0.88	-0.13	+0.68
a_w	Water activity	0.960	-0.011	-0.010	-0.003	+0.007	-0.001	0.000

* $P \leq 0.05$.

¹Values based on a descriptive scale of 10 cm length.

²Values based on a sensory 7-point quality scale.

Table 6. Linear models of the significant effects from the second experimental design.

Response	Equation ³	Optimum value ⁴
Browning ¹	Browning = 10.0–0.12t	2.8
Hardness ¹	Hardness = 2.57 + 0.02t	4.0
Colour quality ²	Colour quality = 1.14 + 0.07t	5.3
Flavour quality ²	Flavour quality = 3.92 + 0.02t	5.2
Texture quality ²	Texture quality = 3.21 + 0.02t	4.4
Overall quality ²	Overall quality = 2.61 + 0.04t	4.9
a*	a* = 8.18–0.20t	–3.62

¹Values based on a descriptive scale of 10 cm length.

²Values based on a sensory 7-point quality scale.

³Variables in the equations are coded.

⁴For a time of 60 min.

25% with increasing concentration, and L^* , which increased 12.1% with temperature increase.

Linear models in coded variables were obtained with the above significant effects for a selected set of relevant responses, osmotic dehydration time was the independent variable because this was the only significant effect (Table 6). Hardness and sensory quality (colour, flavour, texture and overall quality) increased with osmotic dehydration time, while browning and instrumental colour parameter a^* decreased. The increase in sensory texture quality and hardness may have been due to both water loss from the fruit and higher solid diffusion into the fruit. This agreed with Tregunno and Goff (1996), who reported that the presence of sugars in apples provided protection during freezing, allowing firmer tissue than for fruit treated in the same manner without sugars. Additionally, SEM has revealed that cells protected by sugars exhibited less damage to the middle lamella and less severe shrinking of the cell content (Torregiani and Bertolo,

2002). Colour quality improvement and browning decrease could be a consequence of higher uptake of citric and ascorbic acid, which are well known browning inhibitors.

From the linear models obtained, high osmotic dehydration time (60 min) was chosen as the best process condition. Since concentration of the osmotic medium and dehydrating temperature were not significant, the low levels of the second design were selected (55°Bx and 35°C), considering a practical and economical point of view.

Correlation Between Sensory and Instrumental Responses

Significant correlations ($P \leq 0.05$) between sensory and instrumental responses were found (Table 7) indicating that both sensory and instrumental methods are suitable for the measurement of colour and texture parameters.

Acceptability Evaluation

The product obtained under the optimum conditions showed a general acceptability of 93% of a total of 80 interviewed consumers (Table 8). Among the measured parameters, texture showed a slightly lower acceptance of 81%, caused by softening of the tissue due to the freezing process.

Effects of T_g' on the Diffusion-Limited Stability and Quality of Frozen Apple Cubes

Diffusion limited changes occurred at very slow rates at a storage temperature at or below T_g' and stability based on diffusion-limited events is excellent

Table 7. Significant correlations ($P \leq 0.05$) between sensory and instrumental responses.

Instrumental response		Sensory response	Correlation coefficient
Colour	a^*	Browning	0.9807
	a^*	Colour quality	–0.9120
	a	Overall quality	–0.9450
	b	Browning	0.8408
	b	Colour quality	–0.7740
Puncture test	b	Overall quality	–0.8056
	Maximum force 1	Hardness	0.9454
	Maximum force 1	Manual compression	0.7464
	Initial slope	Hardness	0.8640
	Maximum force 2	Hardness	0.6912
Compression test	Area 1	Hardness	0.8284
	Area 1	Manual compression	0.8337
	Maximum force	Hardness	0.8417
	Maximum force	Manual compression	0.6802
	Area	Hardness	0.6559
Initial slope	Initial slope	Hardness	0.6685
	Initial slope	Overall quality	0.7025

Table 8. Consumer acceptability of apples processed at optima conditions.

Sensory acceptability	Proportion of consumers (%)			
	Colour	Flavour	Texture	Overall Acceptability
Rejection	3	4	11	3
Indifference	7	0	8	4
Acceptation	90	96	81	93

Based on a 7-point hedonic scale (7 = like it very much, 1 = dislike it very much), grouped as: rejection score (1–3), indifference score (4) and acceptance score (5–7).

Table 9. T_g' values of osmodehydrofrozen apple runs 13, 15, 16 and fast frozen control treatment.

Runs	Description	$T_g' \pm SD$ (°C)
–	Cryogenic control	–44.45 \pm 0.11
13*	55°Bx, 35°C, 60 min	–41.89 \pm 0.23
15	65°Bx, 35°C, 60 min	–38.87 \pm 0.48
16	65°Bx, 45°C, 60 min	–37.12 \pm 0.05

*Optimum treatment.

(Torregiani and Bertolo, 2002). T_g' values for some selected samples were determined. The control treatment (immersed in acid solution and fast frozen, without osmotic dehydration) had a T_g' value of –44.5°C, that was lower than the T_g' value of the osmodehydrofrozen samples (Table 9). The uptake of sucrose during the osmotic dehydration process, explained the increase of T_g' . The optimum treated samples had a T_g' value of –41.9°C, which was lower than the T_g' values obtained for runs 15 and 16, due to a less severe osmotic treatment.

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