Quality parameters of freeze-dried peach snack

1. Introduction

Peaches are a seasonal fruit, usually harvested when they reach maturity. Fruits picked before physiological maturity will not ripe satisfactorily and will be smaller, very firm in texture, with low sugars, reduced flavour and colour, while those harvested at a more mature stage will be softer, higher in sugar and water content (Gupta and Jawandha, 2010; Fernandez-Trujillo *et al*., 2000); This will lead to a higher perishability and a rapid softening during shelf life affecting quality parameters and consumers acceptance (Crisosto *et al*. 2006; Ferrer *et al*., 2005; Crisosto *et al*., 2004).

To increase shelf life in fruits, drying is the oldest process (Cakmak and Yıldız, 2011). Dried fruits have been considered as an alternative fat-free snack and have recently gained much more attention (Sette *et al*., 2017; Zhang *et al*., 2014). Most dehydrated fruits are produced by air drying. A disadvantage of this method is a substantial quality degradation in appearance (shrinkage, drying-up, darkening), nutrients, flavor, and the low rate of rehydration (Devahastin and Niamnuy, 2010). Higher quality products can be obtained using freeze drying methods. Freeze drying involves crystallization of water in ice crystals, which subsequently sublimate, thus leaving a porous dried product with high-quality (Mujumdar and Law, 2010). Very limited studies regarding the potential use of peach snacks applying freeze drying have been reported.

On the other hand, quality in peaches has always been measured in terms of the traits of the fruit, mainly through evaluation of the physical and chemical properties that best describe the progress of maturation and ripening (Karimi *et al*., 2012) because they provide a common language among researchers, producers and handlers (Echeverría et al., 2015). Among many quality parameters, colour is considered the most important visual attribute in the perception of the product quality; it is critically appraised by consumers and often is the basis for their selection or rejection. Consumers tend to associate color with texture, flavor, safety, storage time and nutrition because it correlates well with physical, chemical, and sensorial properties. Along with colour, texture also plays an important role in the overall acceptance of food quality by consumers (Pieniazek and Messina, 2016a). Texture in food has been defined as "all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, visual and auditory receptors" (Lawless and Heymann, 1998)

An interesting alternative for analyzing the surface of food products and quantifying appearance characteristics is to use computerized Image Analysis Techniques (Saini *et al*., 2014; Mendoza *et al*., 2012). Image analysis can be a useful tool for characterizing food morphology because the highly irregular structures of many food materials elude precise quantification by conventional means. This technique allows obtaining measurements from digitalized images providing objective evaluations of the morphocolorimetric features of samples, a method that is more quantitative and less biased than the common method of visual perception, which is prone to variation due to the personal opinions of inspectors or trained panels (Kono *et al*., 2014). When microscopic techniques such as Scanning Electron Microscopy (SEM) and Images Analysis are used together, they become a powerful tool to evaluate microstructure changes of a product; cell size and number of cells can them be measured and quantified from the projected image. Employing image processing with SEM, some important sensory attributes such as texture, could be predicted by processing the surface and cross section images of a product.

The aim of the present research was to evaluate the effect of freeze drying on quality parameters of stored peaches applying conventional and non-conventional techniques. This process will benefit increases in consumption of fat-free snack peach and will decrease the effect of wasting unnecessary fruit.

2. Material and Methods

2.1 Sampling and freeze drying process

Peaches (Prunus Persica *cv Snow Giant*) (P) were obtained at a local farmer. Preliminary evaluation to set storage conditions, temperatures, etc. was performed in order to improve analysis. Optimized parameters were applied as follows: One fruit sample (n=20) was used for fresh fruit determinations and the other (n=100) was stored at (21 ± 1) °C at 90% relative humidity (RH). Peaches were sampled out, unpeeled and sliced as chips with a porcelaine knife into 10 mm thick sections and freezed dried every 4 day interval (T_1, T_2, T_3, T_4) during 16 days of storage.

Freeze drying was carried out using a non continuo's equipment (Rificor, Buenos Aires, Argentina) (Messina et al., 2016). Parameters applied were the following: freezing temperature: (-50 ± 1) °C until 12 h; Drying process: (40 ± 1) °C at maximum vacuum (pressure: 0.346 Pa) during 12 h. Samples were vacuum packaged with polyethylene bags (Lumenpol®, Argentina) of 350 × 180 × 150 mm dimension and 90 microns, individually identified and stored in a dark place at room temperature until analysis.

In order to analyze microstructure of freeze dried rehydrated samples (FDRP), rehydration was performed with tap water at 98 °C. The duration of rehydration process was fixed in 3 min, as after that time period there was no more absorption of water by the samples.

2.2 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used for the observation of the microstructure of P, FDP and FDRP. Samples were cross sectioned using a scalpel; the cut was always performed in the same direction. P and FDRP samples were gradually dehydrated in an ethanol series (25%, 50%, 75%, and at 100%, 10 min), once in acetone (100%, 10 min) and then dried at room temeparture. All solvents used in these experiments were of high purity and purchased from Sigma–Aldrich®.

Samples were mounted on holders and coated with a gold film. Microscopic evaluation was performed using a Scanning Electron Microscope (SEM 515, Philips, Amsterdam, Netherland). Observations of the samples at magnification of 250, 500 and 1000X were obtained for image analysis (Model Genesis Version 5.21.). Brightness and contrast are the most important variables to be controlled during the acquisition of images; therefore, the values of these parameters were kept constant for each magnification during the process of the especific image acquisition (Pieniazek and Messina, 2015).

2.3 Colour Analysis

Samples were illuminated using a lamp (model TL-D Deluxe, Natural Daylight, 18W/965, Philips, NY, USA) with a colour temperature of 6500 K (D65, standard light source) and a colour-rendering index (Ra) close to 90%. The four fluorescent tubes (60 cm long) were situated 35 cm above the sample and at an angle of 45° with the sample. Additionally, light diffusers covering each lamp and electronic ballast assured a uniform illumination system. A Color Digital Camera (CDC) (Canon Eos Rebel, Japan) was located vertically over the sample at a distance of 12.5 cm. The angle between the camera lens and the lighting source axis was around 45°. Lamps and CDC were inside a wooden box with internal walls that were painted black to avoid the light and reflection from the room (Girolami *et al*., 2013).

Eighteen images from one side of each sample and eight regions of interest of each image were taken on the matte black background using the following camera settings: manual mode with the lens aperture at f of 4.5 and speed 1/125, no zoom, no flash, 3088 × 2056 pixels resolution of the CDC and storage in JPEG format. The algorithms for pre-processing of full images, image segmentation and colour quantification were processed by

Adobe Photoshop cs6 (v13.0 Adobe Systems Incorporated, 2012). *L*, *a* and *b* values were transformed to CIE *L**, *a** and *b**.

2.4 Grey level co-occurrence matrix and image texture analysis

Eighteen images (1024×800 pixels) were captured using an Scanning Electron Microscopy (1000X) and stored as bitmaps in a grey scale with brightness values between 0 and 255 for each pixel constituting the image. The size of each sample (region of interest: 122×122 pixels) was the same for all the evaluated magnifications. Textural property was computed from a set of GLCM probability distribution matrices for a given image. The GLCM shows the probability that a pixel of a particular grey level occurs at a specified direction and distance (d = 1) from its neighboring pixels. Grey level co-occurrence matrix is represented by Pd,θ (i, j) where counts the neighboring pair pixels with grey values i and j at the distance of d and the direction of θ (16) (Pieniazek and Messina.,2016b; Pieniazek *et al.*, 2015).

Five image texture features (Correlation (COR), Energy (ASM), Homogeneity (HOM), Entropy (ENT) and Contrast (CON)) were calculated using MATLAB 8.4 (The MathWorks, Inc., MA, USA) (Eq. 1-5):

$$\mathrm{CON} = \sum_{i=0}^{n-1}\sum_{j=0}^{n-1}(i-j)^2\,\mathrm{Pd},\theta(i,j) \qquad \text{(Eq. 1)}$$

$$\mathrm{ENT} = -\sum_{i=0}^{n-1}\sum_{j=0}^{n-1}\mathrm{Pd},\theta\,(i,j)^2\,\mathrm{LogP}(i,j) \qquad \text{(Eq. 2)}$$

$$\mathrm{HOM} = \sum_{i=0}^{n-1}\sum_{j=0}^{n-1}\frac{\mathrm{Pd},\theta(i,j)}{1+|i-j|} \qquad \text{(Eq. 3)}$$

$$\mathrm{ASM} = \sum_{i=0}^{N-1}\sum_{j=0}^{N-1}\mathrm{Pd},\theta\,(i,j)^{\,2} \qquad \text{(Eq. 4)}$$

$$\mathrm{COR} = \frac{[\sum_{i=0}^{N-1}\sum_{j=0}^{N-1}(ij)P(i,j)]-\mu_x\mu_y}{\sigma_x\sigma_y} \qquad \text{(Eq. 5)}$$

where ux, uy, σ_x, and σ_y are the means and standard deviations of p_x and p_y

2.5 Water Activity and Porosity

Water Activity (a_w) and Porosity (P) was performed in freeze dried samples. a_w was performed using a Water Activity Meter (AquaLab 4TE, USA) and P was analyzed using a Stereopycnometer (Quantachrome multipycnometer Model MVP-1, USA) with an accuracy of 0.001 cm^3, utilizing helium gas. All experiments were carried out by triplicate.

2.6 Solid soluble content

Solid soluble content (SSC) of stored peaches were measured using a hand-held refractometer (Atago Co., PR-1 Brix-Meter, Tokyo, Japan). The exuded juice from the total peach flesh was used as a sample. The value of SSC of each sample was obtained by averaging three evaluations of the sample.

2.7 Statistical Analysis

Regression equations and correlation coefficients (R^2) were performed. Significant differences between values were determinated by Tukey Test. A *P* value of 0.05 was used to verify the test significance. All statistical tests of this experiment were conducted using SPSS-Advanced Statistics 13 software (SPSS Inc, Chicago, IL).

3. Results and discussion

3.1 Scanning Electron Microscopy

Micrographs taken from cross sectional cut of P, FDP and FDRP were performed at 250, 500 and 1000 times magnification. Micrographs of P at T_0, T_3 and T_4 were smooth, flat, uniform and regular; showing an organized structure without gaps (**Figure 1**). Structures in FDP showed that cell walls were dehydrated, separated and partially fragmented. Fragility of cell walls appeared; especially tearing of cell walls from their base. High pore size structure with larger and irregular cavities was observed until T_3 (**Figure 2**). Lower pore size and higher pore amounts were observed at T_4 (**Figure 2**). FDP showed that the pores mainly were not uniformly distributed, this can be attributed to its tissue, containing different types of cells having different size, shape and orientation with different cell wall thickness and strength. During freezing the growth of an ice crystal ruptures, pushes and compresses cells. This process is influenced by the strength of the cell walls (Mousavi *et al*., 2007). Pores and cavities are left after sublimating the ice crystals from the matrix. The ice crystals will grow in the cell direction creating elongated pores.

FDRP showed that surfaces were smooth, flat, uniform and regular until T_3, similar to P_3; T_4 showed changes as it was compared to P_4 (**Figure 3**). In freeze drying process, higher porous size helps to maintain the structure without the deformations that are inevitable in other drying methods, allowing a fast rehydration process due to that water easily reoccupies the empty spaces.

A general view of all micrographs showed that lower porous size was observed at T_4; porous size and amount was similar from T_0 to T_3. Porosity seemed to be gradually dispersed due to a fast and good rehydration process in freeze dried samples until T_3 and similar microstructure was observed in P and FDRP from T_0 to T_3.

3.2 Water activity and porosity

Significant differences between a_w values (P<0.0001) were obtained for FDP ($a_{w\,T0}$=0.31; $a_{w\,T1}$ =0.24; $a_{w\,T2}$=0.23; a_{wT3}=0.20; a_{wT4}=0.13). Lower values of a_w generate secondary reactions that may affect attributes. Significant differences between porosity values (P<0.0001) were observed. Mean values of FDP revealed that porosity increased among storage (P_{T0}=79.8; P_{T1}=81.9; P_{T2}=82.1; P_{T3}=82.9; P_{T4}= 92.8). Lower pore size and higher amounts of pores was observed at T_4. In freeze drying, the porosity degree has influence on texture and rehydration ability, when the size of the air cells in porous material are larger, it allows a fast rehydration due to the fact that water easily enters and reoccupies the empty spaces (Oikonomopoulou *et al*., 2011; Leelayuthsoontorn and Thipayarat, 2006).

During subsequent freezing and drying the ice sublimation creates pores; the amount of pores is related to the water uptake and is higher when water uptake is increased. The pore structure is also influenced by the freezing process; a high undercooling procedure leads to smaller ice crystals and a larger inner surface. Due to the high porosity the freeze dried cell suspension creates a high specific surface area; influencing this fact the sorption behavior as well as the rehydration process (Mounir, 2015).

When porosity was related to SEM images, results revealed that T_0 to T_3 exhibited higher pores size and less amounts of porous; lower porous size and higher amounts of porous were obtained at T_4. Therefore, SEM micrographs with porosity confirmed the based micro-structure discussion presented above.

3.3 Colour analysis

Significant differences (P<0.0001) (**Table 1**) were obtained for colour parameters in P and FDRP. Data revealed that, lightness decreased gradually from T_0 to T_4 in P and FDRP; decreases in lightness are related to the darkening of the fruit and linked to the browning of the surface due to the enzymatic browning (Rocha and Morais, 2003). On the other hand, *a** and *b** values increased in P and in FDRP, this can be attributed to the various pigments present in the skin and flesh tissue of fruit which, in the case of FDRP differences among colour parameters may be assigned to the drying process.

For consumer acceptance a ripe or 'ready to eat' peach is defined when flesh firmness is approximately 0.9–1.4 kgf and 'ready to sale´ firmness below 2.7–3.6 kgf. (Crisosto and Neri, 2006). Mitchell et al. (1991) reported that firmness was well correlated with background colour; a more mature fruit shows better skin color, flavor, higher soluble solids concentration and lower titratable acidity than less mature fruit. Byrne et al. (1991) reported that in different genotype of peaches, *a** value was also well correlated with firmness.

Results mainly revealed that colour values in P were higher than FDRP and that decreases in lightness were linked to darkening of the fruit.

Table 1 Colour parameters of peaches *cv. Snow Giant*

3.3 Solid soluble content

Statistical differences (P<0.0001) were obtained for SSC during storage. Data showed that SSC content increased among storage. (T_0=11.0, T_1=11.2, T_2=11.8, T_3=12.4, T_4=13.9 °Brix). Decrease in acidity and increase in sucrose concentration contributes to a peach with better sensory acceptability. Peaches testing below 10° Brix usually are not satisfying to consumers, being optimal peaches those that reach (11.4-14) ° Brix, for consumer's acceptance (Meredith *et al.*, 1989).

According to the authors above mentioned and to Perkins–Veazie *et al.* (1999), optimal consumer´s acceptance is produced when SSC is between (11.4-14) ° Brix, results revealed that stored peaches had optimal SSC for consumers acceptance from T_2 to T_4. Changes in the amount of SCC can be due to changes in constituents of SSC such as ratio of glucose/fructose, organic acids during storage (Javanmardi and Kubota, 2006) and to moisture content (Romano *et al.*, 2011).

3.5 Image Texture Analysis

Significant differences (P<0.05) were obtained for image texture analysis, ASM, CON, ENT and COR (**Table 2**). P samples showed that ASM, CON and ENT value increased from T_2 to T_4 which are related to roughness, hardness and uniformity. FDRP showed increases of ASM, CON and COR from T_2 to T_4, related to hardness, linearity and uniformity and increases from T_3 to T_4 for ENT related to roughness.

When P and FDRP were compared, P samples revealed to have higher ASM and lower ENT, CON and COR values than FDRP. A higher ASM value means represents the smoothness of an image, when ASM is high the image has very similar pixels. ENT values is related to smoothness, lower ENT values the lower is smooth in the image. COR indicates the linearity of the image; for an image with large areas of similar intensities, a high value of correlation is measured. CON is a measure that shows the difference from one pixel to others close to it representing a measure of local grey variations; the softer the texture the lower the contrast, which is due to lower pixel value difference between two neighbors (Laddi *et al*., 2013; Koc et al., 2008; Zheng *et al*., 2006).

According to image texture analysis, FDRP hardness and linearity increased from T_2 to T_4 and roughness from T_3. Increases in hardness and roughness are due to losses in moisture and to a higher porosity. The textural changes in freeze drying process especially hardness and roughness can be associated with the composition and structure of the cell walls, likely caused by physical and structural modifications of the peaches tissue inducing viscoelastic behavior; this is due to that moisture content decrease in storage and when freeze drying is applied.

Bai *et al* (2003) reported that moisture content decreased in apples when drying methods was applied; at higher drying rates or longer storage apples turned to be more rigid and harder; roughness appeared and the formation of thick crust on the surface appeared.

Image analysis parameters revealed that texture changes are influenced by storage and drying processes. Optimal quality for texture parameters in peach snacks is T_2; after this stage when samples are freeze dried and then rehydrated, texture parameters revealed that samples turn to be harder, roughness and undesirable texture quality appears.

Table 2 Image texture values of peach *cv. Snow Giant*

4. Conclusions

In order to increase consumption of peach and to decrease wasting fruit, freeze drying methods is an excellent option to increase shelf life of peach and to maintain its quality. In order to have good quality in peach snacks it was necessary to reach an initial approach to the effect of freeze drying on the fruit storing. On the other hand, Image Analysis was a technique that allowed reaching a quick approach on texture parameters on colour and physicochemical parameters to evaluate quality.

Results revealed that after 12 days (T_3) of storage and then freeze drying, peach snacks showed lower pores size and higher pores amounts, which affected rehydration process leading to a harder sample. Colour was also affected leading to a darkener fruit.

This approach to quality showed that a way to increase peach consumption and decrease the fruit waste caused by a wrong processing after ripening and a shorter shelf life, is to apply freeze drying process in stored peaches until 12 days, due to the fact that after 12 days of storage samples get harder, darker and undesirable quality appears. Snacks are good options because they are free fat snacks and can contribute to a better healthy life. In the future additional studies involving other cultivars, physicochemical analysis, etc. will be considered to assess to improve the research.

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Figure legends

Figure 1 Scanning micrographs performed at 250, 500 and 1000 X of peach (P) cv *Snow Giant* at T_0, T_3 (12 days) and T_4 (16 days).

Figure 2 Scanning micrographs performed at 250, 500 and 1000 X freeze dried peach (FDP) at T_3 (12 days) and T_4 (16 days).

Figure 3 Scanning micrographs performed at 250, 500 and 1000 X freeze dried rehydrated peach (FDRP) at T_3 (12 days) and T_4 (16 days).

Table 1 Colour parameters of peaches *cv. Snow Giant*

Parameters	P					*P*-value	RSME
	T_0	T_1	T_2	T_3	T_4		
*L**	85.03[a]	84.20[b]	83.96[c]	82.34[d]	82.55[d]	0.0001	0.23
*a**	1.02 [e]	1.15[d]	1.85[c]	2.70[b]	5.45[a]	0.0001	0.16
*b**	43.11[e]	45.01[d]	47.50[c]	48.17[b]	51.57[a]	0.0001	0.04
Parameters	FDRP					*P*-value	RSME
	T_0	T_1	T_2	T_3	T_4		
*L**	80.33[a]	80.20[a]	78.96[b]	75.51[c]	72.05[d]	0.0001	0.15
*a**	0.91[e]	1.02[d]	1.35[c]	2.51[b]	4.39[a]	0.0001	0.08
*b**	40.02[e]	42.56[d]	45.09[c]	46.63[b]	49.73[a]	0.0001	0.25

*Small letters in the same row indicate that means are significantly different (*P*<0.0001) related to treatment (Tucky´s Test). P= peach without treatment; FDR= Freeze dried rehydrated. RSME=Root mean square error; *L**= Lightness; *a**= red to green; *b**= yellow to blue. $T_{0=}$ non stored; T_1= 4 days stored; T_2= 8 days stored; T_3= 12 days stored; T_4= 16 days stored.

Table 2 Image texture values of peach *cv. Snow Giant*

Sample	Period	Image texture			
		ASM	ENT	CON	COR
P	T_0	0.622[d]	3.753[d]	0.024[d]	0.569[e]
P	T_1	0.783[c]	3.895[c]	0.030[c]	0.658[d]
P	T_2	0.795[b]	4.029[b]	0.031[c]	0.686[c]
P	T_3	0.843[a]	4.031[b]	0.034[b]	0.707[b]
P	T_4	0.841[a]	4.473[a]	0.035[a]	0.765[a]
P-value		0.0001	0.0001	0.0001	0.0001
RSME		0.25	0.30	0.30	0.23
FDRP	T_0	0.393[d]	5.237[d]	0.039[d]	0.837[d]
FDRP	T_1	0.470[c]	5.632[c]	0.072[c]	0.860[c]
FDRP	T_2	0.528[b]	5.633[c]	0.095[b]	0.908[b]
FDRP	T_3	0.539[b]	5.659[b]	0.095[b]	0.911[b]
FDRP	T_4	0.634[a]	5.707[a]	0.108[a]	0.929[a]
P-value		0.0001	0.0001	0.0001	0.0001
RSME		0.40	0.18	0.21	0.19

*Small letters in the same column indicate that means are significantly different (P<0.0001) related to storage (Tucky´s Test). P= peach without treatment; FDR= Freeze dried rehydrated; RSME= Root mean square error. COR= Correlation; ASM= Energy; ENT= Entropy; CON= Contrast. $T_{0=}$ non stored; T_1= 4 days stored; T_2= 8 days stored; T_3= 12 days stored; T_4= 16 days stored.

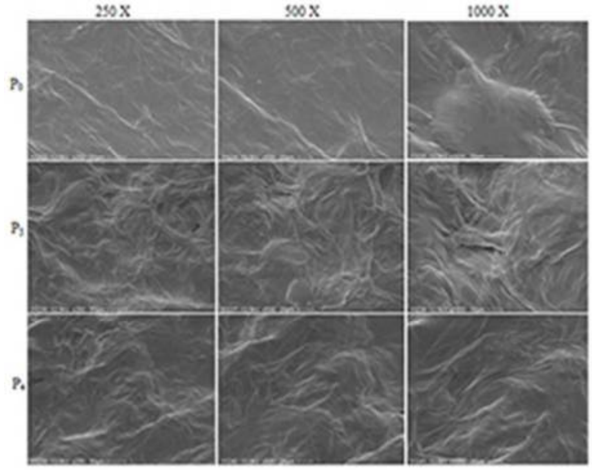

Figure 1 Scanning micrographs performed at 250, 500 and 1000 X of peach (P) cv Snow Giant at T0, T3 (12 days) and T4 (16 days).

105x87mm (72 x 72 DPI)

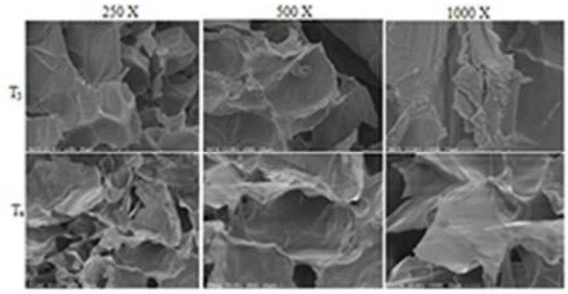

Figure 2 Scanning micrographs performed at 250, 500 and 1000 X freeze dried peach (FDP) at T3 (12 days) and T4 (16 days).

105x59mm (72 x 72 DPI)

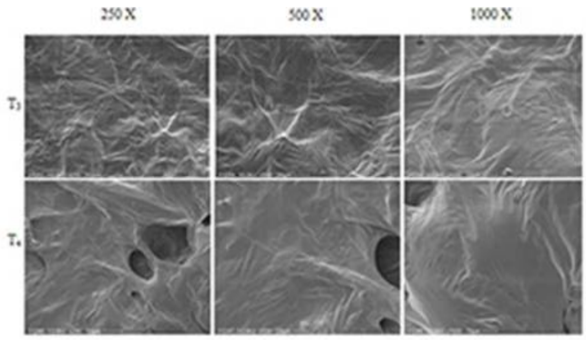

Figure 3 Scanning micrographs performed at 250, 500 and 1000 X freeze dried rehydrated peach (FDRP) at T3 (12 days) and T4 (16 days).

105x59mm (72 x 72 DPI)