Texture Analysis of Freeze Dried Banana Applying Scanning Electron Microscopy Combined with Image Analysis Techniques

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***Abstract*—The aim of the present research was to evaluate the impact of freeze drying on texture properties of banana *cv* Cavendish. Freeze drying was performed at different rates (T_1= 7h (-40 ℃)/24h (40 ℃); T_2= 7h (-40 ℃)/48h (40 ℃); T_3= 18h (-40 ℃)/24h (40 ℃) and T_4 = 18h (-40 ℃)/48h (40 ℃)). Microstructure was analyzed using a Scanning Electron Microscopy (SEM); Surface texture analysis by Grey Level Co Matrix Analysis (GLCM) and water absorbing capability and porosity by conventional techniques. Micrographs performed at 250 and 500 times magnification revealed that T_1 and T_2 showed higher porous size structure with larger and irregular cavities, and higher rehydration process was observed in T_1 and T_2 when it was related to T_3 and T_4. A higher rehydration process is due to a porous network with permeable barriers. Significant difference (*P*<0.05) were obtained for texture parameters, water absorbing capability and porosity. Hardness and roughness decreased in T_3 and T_4. These results suggest that prediction of texture parameters in banana *cv*. Cavendish can be performed easily by processing the surface and cross section images with SEM and GLCM methods.**

***Index Terms*—image analysis, quality, surface analysis techniques, freeze drying, GLCM**

I. Introduction

Banana is one of the most important high sugar tropical fruit crops grown in many countries. It is highly perishable and it is a bulky fruit which requires processing it into a more stable and convenient form due to it is susceptible to quality deterioration.

Traditionally bananas are dried by sun as slices or by conventional dehydration [1]. Benefits in dried bananas are reduction in weight and volume; minimizes packaging, storage and transportation cost, it also enables storability of the product under ambient temperature, it is easy to handle and can be easily incorporated during food formulation and preparation. Drying is a complex process accompanied by physical and structural changes. There is a continuous change in the dimensions during drying as a result of water removal and internal collapse of the particulates [2] and due to this, undesirable quality appears such as browning, leathery texture, losses of nutritive values, etc. [3], [4]. 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 [5], [6].

An interesting alternative for analyzing the surface of food products and quantifying appearance characteristics is to use computerized image analysis techniques [7]. 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 morpho-colorimetric 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 [8].

Grey Level Co-Occurrence Matrix (GLCM) is probably one of the most frequently cited methods for the texture analysis of images. The texture of an image corresponds to the spatial organization of pixels in the image and the Co-Occurrence Matrix describes the occurrence of grey level between two pixels separated in the image by a given distance. GLCM results in the calculation of up to 14 textural features which can be expected to represent the textural characteristics of the image studied [9].

When microscopy 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 [10]. 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.

Due to the stated points above mentioned, the aim of the present research was to evaluate the impact of freeze drying on texture properties of banana *cv* Cavendish applying Scanning Electron Microscopy with Image Analysis techniques.

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II. Materials and Methods

A. Sample Preparation and Freeze Drying Cycle

Green *cv*. Cavendish dessert bananas (FB) were obtained at a local farmer at their green mature stage. Samples were cross sectional sliced as chips with a porcelaine knife into 10 mm thick sections and then peeled.

Freeze drying process was carried out in a pilot plant freeze dryer supplied with four trays designed by an Industrial constructor (Rificor, Argentina). Process was performed freezing at -40 ℃ and drying at 40 ℃ applying different cycles: T_1= 7h (-40 ℃)/ 24h (40 ℃); T_2=7h (-40 ℃)/ 48h (40 ℃); T_3= 18h (-40 ℃)/ 24h (40 ℃) and T_4 = 18h (-40 ℃)/ 48h (40 ℃) under a chamber pressure of 0.346 Pa. Freeze dried (FD) samples were vacuum packaged, individually identified and stored in a dark place at room temperature until analysis.

B. Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was used for the observation of the microstructure of fresh (FB), FDB and FDRB. Samples were cross sectioned using a scalpel; the cut was always performed in the same direction. In order to analyze FDRB, rehydration was performed with tap water at 98 ℃. The duration of rehydration process was fixed in 6 min as after that time, there was no more absorption of water by the sample.

FB and FDRB samples were gradually dehydrated in an ethanol series (25%, 50%, 75%, and at 100%, 10 min each), once in acetone (100%, 10 min) and then dried. All solvents used in these experiments were of high purity and purchased from Sigma–Aldrich®.

Samples were mounted on holders and coated with gold [11]. 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 that must be controlled during the acquisition of images; therefore, the values of these parameters were kept constant for each magnification during the process of image acquisition.

C. Grey Level Co-occurrence Matrix and Image Texture Analysis

Image analysis was measured at 1000 time magnifications in FDB and FDRB samples. Eighteen images of 1024x800 pixels were captured using an Scanning Electron Microscopy and stored as bitmaps in a gray scale with brightness values between 0 and 255 for each pixel constituting the image. The size of each sample (region of interest: 122x122 pixels) was the same for all the evaluated magnifications. Texture parameters (energy (ASM), contrast (CON), correlation (COR), homogeneity (HOM) and entropy (ENT)) were calculated on SEM images using the Grey level Co-occurrence Matrix method (GLCM). The textural feature angular second moment also called ASM, measures the texture uniformity or orderliness of an image [12]. ASM values indicate more directional uniformity in the image. The textural feature CON is a measure of the intensity contrast between a pixel and its neighbor over the whole image. It measures the local variation in the GLCM. CON can be seen as dynamic range of grey level or sharpness of edges. The range of CON lies between [0 (size (GLCM,1)-1)2]. Furthermore, contrast is 0 for a constant image [13].

The textural feature COR is a measure of how correlated a pixel is to its neighbour over the whole image. Its range lies between −1 and +1. Also, the correlation is +1 or −1 for a perfectly positively or negatively correlated image. Correlation measures the joint probability of occurrence of pixel pairs of GLCM. The textural feature ENT shows how often a pixel with grey-level (greyscale intensity) value i occurs horizontally adjacent to a pixel with the value j [14]. ENT is a statistical measure of randomness that can be used to characterize the texture of the input image. ENT is higher when all entries in p (i, j) are of similar magnitude and small when the entries in p (i, j) are unequal. HOM shows the level of uniformity on the image. High values of HOM show improvement of uniformity and smoothness of the image. Five image texture features (Correlation (COR), Energy (ASM), Homogeneity (HOM), Entropy (ENT) and Contrast (CON)) were calculated using MATLAB 8.4 (The Math Works, Inc., MA, USA).

D. Porosity and Water Absorbing Capability

Porosity (P) in FDB was analyzed using a Stereopycnometer (Quantachrome multipycnometer Model MVP-1, USA) with an accuracy of 0.001 cm3, utilizing helium gas as described by [15]. All experiments were carried out in triplicate.

Water absorbing capability (WAC) was performed as follows: 25 g of freezed dried samples from each treatment were weighed and rehydrated in distilled water (98 ± 1 ℃) until 60, 120, 240 and 360 sec, then drained over a mesh for 30 s to eliminate the superficial water. The WAC was recorded as gram of water retained per gram of dried sample. All experiments were carried out in triplicate. WAC values obtained were used to analyze microstructure, texture and porosity in FDRB.

E. Statistical Analysis

Significant differences between means were determinate by Tukey’s Test. A P value of 0.05 was used to verify the significance of the tests. All statistical test of this experiment were conducted using SPSS-Advanced Statistics 13 software (SPSS Inc, Chicago, IL).

III. Results and Discussion

A. Scanning Electron Microscopy

Micrographs taken of cross sectional cut of FB, FDB and FDRB at 250 and 500 times magnification are shown in Fig. 1, 2 and 3. Micrographs of FB were smooth, flat, uniform and regular; showing an organized structure without gaps (Fig. 1).

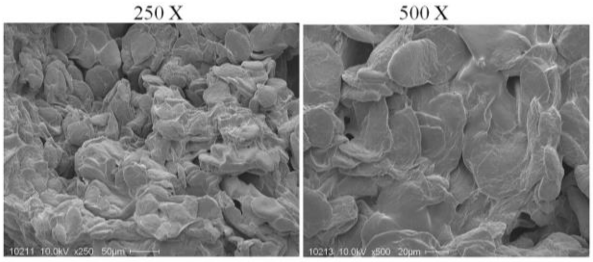

Figure 1. Scanning micrographs performed at 250 and 500 time ́s magnification of cross sectional banana without treatment.

In Fig. 2, FDB showed that the sublimation of the ice crystals grown within the banana left a dried matrix representing a fingerprint of the ice crystals sizes and shapes. FDB structure appeared organized showing gaps; cell walls were dehydrated and separated for T_1, T_2, T_3 and T_4 (Fig. 2). When freeze drying cycles were compared, higher porous size structure with larger and irregular cavities was observed for T_1 and T_2; T_3 and T_4 showed lower porous size with lower and irregular cavities. FDB showed that the pores 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 [16]. 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.

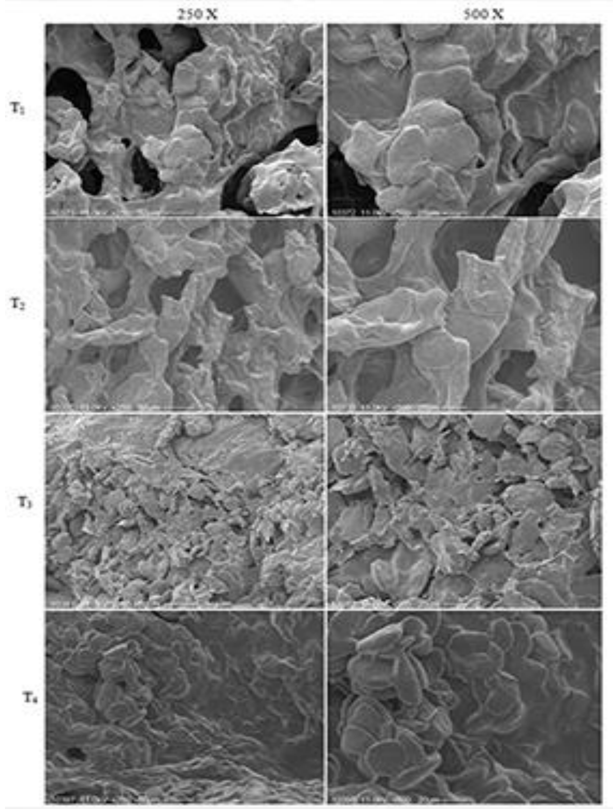

Figure 2. Scanning micrographs performed at 250 and 500 time ́s magnification of cross sectional freeze dried banana at different freeze drying cycles: T_1= 7h (-40 ℃)/ 24h (40 ℃); T_2= 7h (-40 ℃)/ 48h (40 ℃); T_3= 18h (-40 ℃)/ 24h (40 ℃) and T_4 = 18h (-40 ℃)/ 48h (40 ℃).

FDRB showed that surfaces were smooth, flat, uniform and regular (Fig. 3), similar to FB. In freeze drying process, high porosity 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 [17]. A general view of all micrographs showed that a higher porous size was obtained when freeze drying cycles was performed at T_1 and T_2. It also revealed that FB and FDRB had similar structure, showing that porosity seemed to be gradually dispersed due to a fast and good rehydration process after freeze drying.

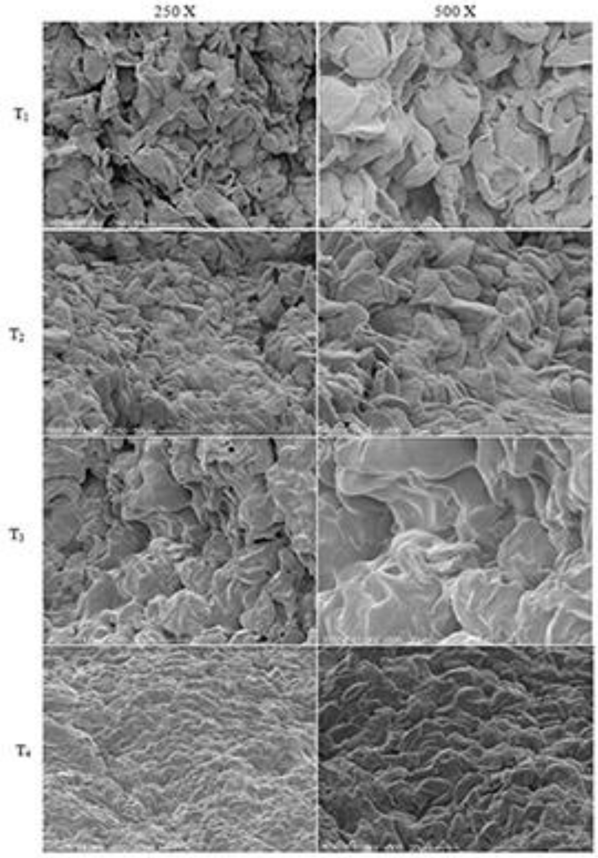

Figure 3. Scanning micrographs performed at 250 and 500 time ́s magnification of cross sectional freeze dried rehydrated banana at different freeze drying cycles: T_1= 7h(-40 ℃)/ 24h (40 ℃); T_2= 7h (-40 ℃)/ 48h (40 ℃); T_3= 18h (-40 ℃)/ 24h (40 ℃) and T_4 = 18h (-40 ℃)/ 48h (40 ℃).

B. Porosity and Water Absorbing Capability

Significant differences ($P<0.0001$) were obtained for porosity values (P1=81.44, P2=83.23, P3= 87.3, P4=91.14) for FDB among different freeze drying cycles. In freeze drying, high porosity (higher amounts of pores) helps to maintain the structure without the deformations that are inevitable in other drying methods. The degree of porosity also has influence in texture and rehydration ability, when the size of the air cells in porous material are bigger, it allows a fast rehydration due that water easily enters and reoccupies the empty spaces [18]. During subsequent freezing and freeze drying the ice sublimation creates pores; the amount of pores (porosity) is related to the water uptake and is higher when the water uptake is increased. The porous structure is also easily enters and reoccupies the empty spaces [18]. During subsequent freezing and freeze drying the ice sublimation creates pores; the amount of pores (porosity) is related to the water uptake and is higher when the water uptake is increased. The porous structure is also influenced by the freezing process and a cooling procedure with a high undercooling procedure leads to smaller ice crystals and a larger inner surface. Due to the high porosity the freeze dried cell suspension there is a high specific surface area; this influences the sorption behavior as well as the rehydration process [19], [20]. When porosity was related to SEM images, results revealed that T_1 and T_2 had higher size pore and less amounts of pores appeared; pore amount increases during freeze drying cycles.

Higher WAC values (Fig. 4) were obtained at T_1 (R^2= 0.987) and T_2 (R^2= 0.980); lower WAC values at T_3 (R^2= 0.951) and T_4 (R^2= 0.970) in FDB. Decrease in WAC is due to smaller ice crystals formed, leading to smaller pore sizes, which in turn gave considerably higher values of surface area which decreases rehydration in samples.

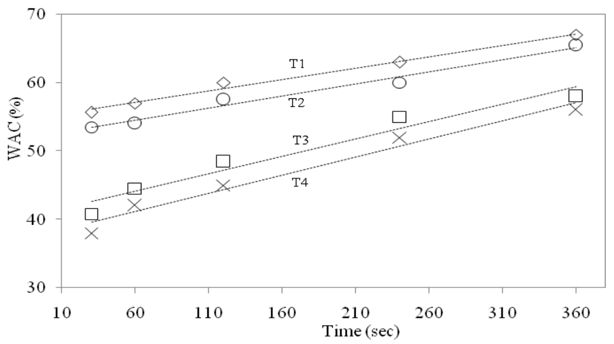

Figure 4. Water absorbing capability of freeze dried banana at different freeze drying cycle: T1= 7h (-40 ℃)/ 24h (40 ℃); T2= 7h (-40 ℃)/ 48h (40 ℃); T3= 18h (-40 ℃)/ 24h (40 ℃) and T4 = 18h (-40 ℃)/ 48h (40 ℃).

Therefore, SEM micrographs with porosity and WAC confirm the based microstructure discussion presented above. Results revealed that s porosity and rehydration depend on the freeze drying cycle.

C. Image Texture Analysis

Table I shows image texture values for FDB and FDRB. Significant differences (*P*<0.05) were obtained for ASM, CON, ENT, HOM and COR.

FDB showed that T_3 and T_4 had higher COR, ASM, ENT, HOM and lower values of CON when they were related to T_1 and T_2. On the other hand, FDRB showed that T_3 and T_4 had higher COR, ASM and HOM values while ENT and CON decreased.

COR indicates the linearity of the image; for an image with large areas of similar intensities, a high value of correlation is measured. ENT takes low values for smooth images. ASM represents the smoothness of an image, when ASM is high the image has very similar pixels. CON is a measure that shows the difference from one pixel to others close to it representing a measure of local gray variations; the softer the texture the lower the contrast, which is due to lower pixel value difference between two neighbors [21]. Lower CON values are related to softness and higher ASM values to lower roughness. Increases in hardness can be due to losses in moisture and increase in roughness to losses in moisture and to higher porosity attributed to the freeze drying process [22].

In general image texture analysis revealed that hardness and roughness decreased in FDB and in FDRP when freeze drying cycle at T_3 and T_4 was applied. Higher hardness and roughness was obtained when freeze drying cycle was applied using T_1 and T_2. The textural characteristics can be associated with the composition and structure of the cell walls, likely caused by physical and structural modifications of the bananas tissue inducing viscoelastic behavior; this is due to that moisture content decrease in freeze drying [19].

TABLE I. TEXTURE ANALYSIS OF FREEZE DRIED AND FREEZE DRIED REHYDRATED BANANA CV. CAVENDISH AT DIFFERENT FREEZE DRYING CYCLE

Sample	Parameter	T_1	T_2	T_3	T_4	RSME
FDB	COR	0.68^{c}	0.68^{c}	0.84^{a}	0.75^{b}	0.822
	ASM	0.23^{c}	0.16^{d}	0.30^{b}	0.68^{a}	0.979
	ENT	6.06^{c}	5.43^{d}	7.00^{a}	6.38^{b}	0.938
	HOM	0.83^{c}	0.84^{c}	0.88^{b}	0.93^{a}	0.877
	CON	0.39^{a}	0.37^{a}	0.26^{b}	0.25^{b}	0.854
FDRB	COR	0.76^{b}	0.76^{b}	0.80^{a}	0.80^{a}	0.856
	ASM	0.24^{b}	0.17^{c}	0.28^{a}	0.28^{a}	0.678
	ENT	6.57^{b}	6.80^{a}	6.38^{c}	6.12^{d}	0.734
	HOM	0.86^{c}	0.84^{d}	0.87^{b}	0.90^{a}	0.675
	CON	0.35^{a}	0.31^{b}	0.26^{c}	0.26^{c}	0.795

*Small letters in the same row indicate that means are significantly different (P<0.0001) related to freeze drying cycle (Tukey ́s Test). B= Banana; FD= Freeze dried; FDR= Freeze dried rehydrated; T1= 7h (-40 ℃) /24 h (40 ℃); T2= 7h (-40 ℃) /48h (40 ℃); T3= 18h (-40 ℃)/ 24 h (40 ℃); T4= 18h (-40 ℃)/ 48h (40 ℃).RSME= Root mean square error. COR= Correlation; ASM= Energy; ENT= Entropy; HOM=Homogeneity; CON= Contrast.

IV. CONCLUSIONS

When freeze drying cycles T_3 and T_4 were applied results revealed that porosity increased and texture parameters such as hardness and roughness decreased in freeze dried rehydrated banana. T_1 and T_2 revealed increases in hardness and roughness when freeze drying was applied. A higher rehydration process was observed in T_1 and T_2 due to a higher porous size when it was related to T_3 and T_4.

When Scanning Electron Microscopy was combined with images analysis texture characteristics could be predicted by processing the surface and cross section images of the product. These results suggest the relevance of image analysis, due to that prediction of quality parameters in banana *cv*. Cavendish can be performed easily as a quantitative and non-invasive technique. Anyway, it would be recommendable to investigate other cultivars to increase the number of samples in future research in order to establish a general trend and to improve the research.

REFERENCES

[1] A. Etsey, E. Sakyi-Dawson, S. Sefa-Dedeh, E. Afoakwa, K. Tano-Debrah, and G. Annor, “Effects of cowpea fortification and the

level of ripeness of plantain on the nutritive value of plantain based snack foods," *Afr. J. Biotechnol.*, vol. 6, pp. 799-802, 2007.

[2] W. Senadeera, B. Bhandari, G. Young, and B. Wijesinghe, "Modelling dimensional shrinkage of shaped foods in fluidized bed drying," *J. Food Process Preserv.*, vol. 29, pp. 109-119, 2005.

[3] G. Echeverría, A. Cantín, M. Ortiz, I. López, J. Lara, and A. Graell, "The impact of maturity, storage temperature and storage duration on sensory quality and consumer satisfaction of 'Big Top' nectarines," *Sci. Hort.*, vol. 190, pp. 179–186, 2015.

[4] S. Devahastin and C. Niamnuy, "Modelling quality changes of fruits and vegetables during drying: A review," *Int. J. Food Sci. Tech.*, vol. 45, pp.1755–1767, 2005.

[5] A. Mujumdar and C. Law, "Drying technology: Trends and applications in postharvest processing," *Food Bioprocess Tech.*, vol. 3, pp. 843–852, 2010.

[6] J. Pedreschi, M. Domingo, and P. Moyano, "Development of a computer vision system to measure the color of potato chips," *Food Res. Int.*, vol. 39, pp. 1092–98, 2006.

[7] F. Mendoza, R. Lu, A. Diwan, H. Cen, and B. Bailey, "Integrated spectral and image analysis of hyperspectral scattering data for prediction of apple fruit firmness and soluble solids content," *Postharvest Biol. and Tech.*, vol. 62, pp. 149–160, 2012.

[8] S. Kono, I. Kawamura, S, Yamagami, T. Araki, and Y. Sagara, "Optimum storage temperature of frozen cooked rice predicted by ice crystal measurement, sensory evaluation and artificial neural network," *Int. J. Refrig.*, vol. 56, 165–172, 2015.

[9] M. Saini, J. Singh, and N. Prakash, "Analysis of wheat grain varieties using image processing-A review," *IJSR*, vol. 3, pp. 490-495, 2014.

[10] F. Pieniazek, A. Sancho, and V. Messina, "Texture and color analysis of lentils and rice for instant meal using image processing techniques," *J. Food Process Preserv.*, vol. 65, no. 3, p. 310, 2015.

[11] V. Messina, F. Pieniazek, A. and Sancho, "Effect of different freeze drying cycle in Semimembranous and Gluteus Medius bovine muscles: Changes on microstructure, colour, texture and physicochemical parameters," *Int. J. Food Sci.Tech.*, vol. 51, pp. 1268–1275, 2015.

[12] X. Ou, W. Pan, and P. Xiao, "In vivo skin capacitive imaging analysis by using grey level co-occurrence matrix (GLCM)," *Int. J. Pharm.*, vol. 460, pp. 28–32, 2014.

[13] A. Laddi, S. Sharma, A. Kumar, and P. Kapur, "Classification of tea grains based upon image texture feature analysis under different illumination conditions," *J. Food Eng.*, vol. 115, pp. 226-231, 2013.

[14] M. Karimi, M. Fathi, Z. Sheykholeslam, B. Sahraiyan, and F. Naghipoor, "Effect of different processing parameters on quality factors and image texture features of bread," *Biotechnol. Bioprocess Eng.*, vol. 2, pp. 2-7, 2012.

[15] B. Koc, I. Eren, F. Kaymak, and L. Ertekin, "Modelling bulk density, porosity and shrinkage of quince during drying: The effect of drying method," *J. Food Eng.*, vol. 85, pp. 340–349, 2008.

[16] R. Mousavi, T. Miri, P. Cox, and P. Fryer, "Imaging food freezing using X-ray microtomography," *Int. J. Food Sci. Technol.*, vol. 42, pp. 714–727, 2007.

[17] V. Oikonomopoulou, M. Krokida, and V. Karathanos, "The influence of freeze drying conditions on microstructural changes of food products," *Procedia. Food Sci.*, vol. 1, pp. 647-654, 2011.

[18] P. Leelayuthsoontorn and A. Thipayarat, "Textural and morphological changes of Jasmine rice under various elevated cooking conditions," *Food Chem.*, vol. 96, pp. 606-613, 2006.

[19] S. Mounir, "Texturing of chicken breast meat as an innovative way to intensify drying: use of a Coupled Washing/Diffusion CWD phenomenological model to enhance kinetics and functional properties," *Dry Technol.*, vol. 33, pp. 1369-1381, 2015.

[20] C. Zheng, D. Sun, and L. Zheng, "Recent applications of image texture for evaluation of food qualities—A review," *Trends Food Sci. Tech.*, vol. 17, pp. 113–128, 2006.

[21] N. Wang, J. Daun, and L. Malcomson, "Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas (Pisum sativum)," *J. Sci. Food Agric*, vol 83, pp. 1228-1237, 2003.

[22] H. Kiani and D. Sun, "Water crystallisation and its importance to freezing of foods: A review," *Trends Fod. Sci. Tech.*, vol. 22, pp. 407–426, 2011.

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