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EFFECT OF CONVECTIVE DRYING ON TOTAL ANTHOCYANIN CONTENT, ANTIOXIDANT ACTIVITY AND CELL MORPHOMETRIC PARAMETERS OF STRAWBERRY PARENCHYMAL TISSUE (*Fragaria x ananassa Dutch*)

EFECTO DEL SECADO CONVECTIVO EN EL CONTENIDO TOTAL DE ANTOCIANINAS, ACTIVIDAD ANTIOXIDANTE Y CAMBIOS MORFOMÉTRICOS DE CÉLULAS DE PARÉNQUIMA DE FRESA (*Fragaria x ananassa Dutch*)

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Abstract

The effect of convective drying of strawberries (*Fragaria x ananassa Dutch*) on the contents of anthocyanins (AcyTot) and antioxidant capacity (TEAC), as equivalents of Trolox, was evaluated as well as its relation to cell morphological changes in the mesocarp by means of determining the variations in Area (A), Perimeter (P), Feret Diameter (Fe) and Fractal Dimension of parenchymal tissue cells. A decrease in anthocyanin content caused by drying at 60, 70 80 and 90 °C and 1 m/s airflow was observed. ACyTot and TEAC showed correlation (r = 0.784). The decrease in ACyTot and TEAC in samples dehydrated at 60 °C, was associated to the decrease in values of A, P, Fe and FD found in samples dried at this temperature whereas no changes in A, P and Fe were found in samples dried at 70-90 °C in relation to those observed at 60 °C. The cell contour resulted smoother after high-temperature drying, as indicated by the decrease in FD. The antioxidant activity related to changes in ACyTot and TEAC took place at progressively higher levels in samples that changed morphology in the same proportion among each other.

Keywords: strawberries, convective drying and morphological parameters, total anthocyanin content, antioxidant capacity.

Resumen

Se estudió el efecto del secado convectivo de fresas (*Fragaria x ananassa Dutch*) sobre el contenido de antocianinas (AcyTot) y la capacidad antioxidante en equivalentes de Trolox (TEAC) y su relación con los cambios morfométricos de las células del mesocarpo mediante variaciones de Área (A), Perímetro (P), Diámetro de Feret (Fe) y dimensión Fractal (FD) de las células. Se observó una disminución de contenido de antocianinas por efecto del secado a 60, 70, 80 y 90 °C y velocidad de aire de 1 m/s. ACyTot y capacidad antioxidante (TEAC) mostraron correlación entre ellas (r = 0.784). La disminución de ACyTot y TEAC de muestras deshidratadas a 60 °C fue asociada con la disminución de los valores de A, P, Fe y FD, mientras que no se encontraron cambios apreciables en muestras deshidratadas en el intervalo de 70-90 °C en los parámetros A, P y Fe con respecto a lo observado a 60 °C. El contorno de las células resultó más liso en las muestras deshidratadas en el intervalo superior de temperatura (70-90 °C), indicado por la disminución de la FD. La actividad antioxidante relacionada con cambios de ACyTot y TEAC tuvo lugar en grados progresivamente mayores en muestras que mostraron cambios morfológicos similares entre sí.

Palabras clave: fresas, secado convectivo, parámetros morfológicos, antocianinas totales, actividad antioxidante.

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1 Introduction

Strawberries are commercial fruits with a high level of acceptability given their sensory attributes and attractive, characteristic red color which is mainly due to the presence of anthocyanins that in conjunction to flavonoids and ascorbic acid, constitute a rich source of antioxidants and help in the prevention of affections related to oxidative stress (Basu A. *et al*. 2010; Cardenas-Sandoval B.A 2012). This fruit has a short shelf-life due to respiration rate, softening and microbial spoilage and is widely consumed in fresh state but a great proportion of its production is processed in the form of juice, jams, canned, refrigerated, frozen, and dehydrated product either in the form of a powder or as a whole fruit which can be added to dairy and bakery products and breakfast cereals (Seeram *et al*. 2005).

Anthocyanins are a very important group of water soluble polyphenols-flavonoids which give typical pink, red, blue and violet color to fruits and vegetables depending on source of the product and which due to their chemical structure, their color changes with pH, co-pigmentation (determined by the presence of other pigments), number and space-orientation of hydroxyl and metoxyl groups, temperature and light (Cerezo *et al*. 2010).

Convective drying or dehydration is a food processing operation in which water is removed from a solid or liquid product by the action of heat in a dedicated piece of equipment (dryer) in which a stream of hot air is passed through the product in such a way so as to transfer heat and remove water from it (Brennan, 2011). By elimination of moisture, microbial spoilage and biochemical and physical deteriorative processes are slowed or almost stopped and the consequent decrease in weight and very often of volume, reduces storage, packing and transport associated costs. Typical solid-food drying temperatures are around 40-90°C and time of exposure to heat may also cause deteriorative phenomena which must be carefully evaluated and controlled by suitable quality. Chemical changes during dehydration include, loos of heat-sensitive products including vitamins and other nutrients, unfavorable reactions between components as well as enzymatic inactivation (Civello *et al*. 1997; Lohachoompol *et al*. 2004; Alonzo-Macías *et al*. 2013; Brennan, 2011) whereas physical changes include, formation of crust, migration of water soluble compounds (creation of concentration gradients), shrinking and deformation, textural changes as well as discoloration of tissues (Contreras *et al*. 2008; Brennan, 2011; Cordenunsi *et al*. 2003). It has also been reported that macroscopic changes related to dehydration, are related to tissue and cellular damage (Campos-Mendiola *et al*. 2007; Tapia-Ochoategui *et al*. 2011).

A careful selection of drying operating conditions and evaluation of heat-damage to products is a very important issue in food processing and quality assurance practice (Alonzo-Macías *et al*. 2004). Low drying temperatures are associated to long drying times and slow moisture diffusion rates so shrinking and deformation are favored whereas high temperatures may cause marked crust formation and intense sensory and nutritional heat associated changes (Doymaz, 2008). Evaluation of shrinking and deformation at different scales may be carried out by means of Digital Image Analysis (DIA) which is a tool that may be used in the assessing of structures and provide quantitative information on microstructural properties so as to differentiate between objects that have been subjected to different processes or treatments (Barletta and Barbosa-Canovas, 1993; Alvarado-González *et al*. 2012; Domínguez-Fernández *et al*. 2012). Parameters such as Area (A), Perimeter (P), Feret Diameter (Fe) are indicators of size of the analyzed objects and one important parameter related to irregularity that may also be calculated by DIA is the Fractal Dimension (FD) REF. The word fractal comes from the word *fractus* (in Latin) that means broken, and its corresponding verb *frangere* meaning breaking. This term and concept was proposed by Mandelbrot (1982) to characterize non-linear, temporal phenomena or to describing objects having a variable degree of irregularity. FD may be estimated by using various algorithms among which the box counting method is widely used (Chanona *et al*., 2003).

The aim of this work was to determine the effects of convective drying on cell disruption of strawberry samples and the relation of such morphological changes, evaluated by means of DIA in the form of variations of A, P, Fe and FD, with the differences observed in AcyTot and TEAC.

2 Material and methods

2.1 Raw material

Fresh strawberries were purchased in a local market of Mexico City. Such fruits had homogeneous

characteristics of color and weight (12 ± 2 g each) and were stored at 4 °C until analysis. All fruits were absent of visible damage and/or microbial contamination, and were chosen by following the Mexican official standard NMX-FF-062-1987.

2.2 *Preparation of raw material for experiments*

Strawberries were rinsed with tap water and sanitized by submersion into a solution of AgCl in water of 15 mg/L concentration. The fruits were kept at 4 °C. Each specimen was stored for less than 48 h before being used for any analysis.

The samples used for drying kinetics were longitudinally cut into slices of 2 ± 0.2 mm thickness (Mitutoyo Micrometer, Japan) by using an electric food slicer (Eura-250, Mexico). Portions of 10-12 g of strawberry slices were put on a stainless steel mesh in order to determine their moisture content before the convective drying processing (Doymaz, 2008).

2.3 *Extraction and quantification of total anthocyanin content*

The extraction and quantification of the total content of anthocyanins was performed by using the method reported by Abdel-Aal and Huel (1999). Maximum absorbance wavelength for these compounds was determined as 515 nm by means of a wavelength scanning from 472 to 560 nm. Absorbance was read for each prepared dilution and the following equation was used to calculate the total content of anthocyanins (AcyTot) in the strawberry samples:

$$AcyTot = \frac{Abs}{\varepsilon}\left(\frac{Vol}{1000}\right)MW \times 10^6 \qquad (1)$$

Where:
AcyTot = total concentration of anthocyanins in mg/kg, expressed as pelargonidin-3-glucosid.
A = absorbance at 515 nm.
ε = molar absorptivity (pelargonidin 3-glucosid = 15600 L cm^{-1} mol^{-1}).
MW = Molecular weight of the pelargonidin-3-glucoside (433.2 g/mol).
Vol = End volume of dilution.

2.4 *Determination of antioxidant activity by the DPPH method*

The antioxidant activity of the extract was determined by applying the methodology of Ramos *et al*. (2008), and using the method of DPPH. The DPPH (2,2-diphenyl-2-picryl-hidrazil) radical has an unpaired electron and a blue-violet color which changes to pale yellow when it reacts with an antioxidant substance. Color change is measured spectrophotometrically at 517 nm (Ramos *et al*., 2008). The results of the assay were expressed relative to milimolar Trolox per kg of dry weight in terms of Trolox equivalent antioxidant capacity (TEAC).

2.5 *Drying of samples*

Drying was performed by using a hot-air experimental tunnel dryer (Gumeta-Chávez, 2009), by using 1 m/s airflow, parallel to sample, at 60, 70, 80 and 90 °C. A total of 12.5 ± 1.5 g of sample were used for each drying curve. Drying was carried out in duplicate and was determined by means of a gravimetric oven method AOAC (1993). The four drying kinetics were reported as the graphical relationship between the ratio of moisture content at any given time and the initial moisture (X/X_0) and drying time.

2.6 *Preparation of samples for microscopy observations*

Strawberry samples, both dehydrated and non-dehydrated, were cut for observation under light optical microscope (Eclipse, Nikon, Japan) by using a microtome-cryostat (International Equipment Company). Thickness of cuts was 10 μm.

2.7 *Microscopy observations and digital image analysis*

Images of the strawberry samples, both before and after drying, were captured by means of a digital camera (Nikon Digital SightDS-2Mv, TV Lens 0.55XDS, Japan) coupled to the light microscope at 600x magnification. All images were captured with a resolution of 1280 × 960 pixels (3 μm/pixel) and stored with JPEG format in a personal computer. A total of 20 fields were observed for each sample.

The images were analysed by using the software ImageJ 1.48 g (National Institutes of Health, Bethesda, USA). The analysis consisted of segmentation of the image by using the *Threshold* tool of the software, after transforming the image to 8 bits (grayscale). The segmented images were converted to binary (black and white) format to obtain morphometric parameters (A, P and Fe) of strawberry cells (Gumeta-Chávez,

2009) as well as their FD by using the FracLac plugin (Karperien, 2004).

2.8 Statistical analysis

One-way ANOVA for repeated measures tests (OneWay RM ANOVA) were carried out by using the software SigmaStat 3.5 (Systat Software Inc., USA). A Student-Newman-Keuls test was used for comparison of means. The level of significance for each test was $2\alpha = 0.05$.

3 Results and discussion

3.1 Drying kinetics

The four drying kinetics of sliced strawberries are presented in Figure 1. Initial water content for all samples was 92.2 ± 0.4% wet basis which is similar to values reported for this fruit (Doymaz, 2008). It was possible to observe that all samples reached equilibrium moisture content within 60-90 min. Higher temperatures resulted, as expected, in shorter drying times. Shape of drying curves and drying times are typical of operating conditions used (Brennan, 2011). Final moisture contents found were (dry basis): 2.8% (drying air temperature, 60 °C), 1.42% (drying air temperature, 70 °C), 1.63% (drying air temperature, 80 °C) and 0.91% (drying air temperature, 90 °C).

3.2 Total content of anthocyanins

In Table 1, total anthocyanin content expressed as pelargonidin-3-glucoside for fresh and dried strawberries is shown. Values for fresh strawberries are within the range of those reported by Lopes da Silva *et al*. (2007), who found values equivalent to approximately 1000-6000 mg/kg (dry basis). Fresh samples had an average ACyTot content of 2298.29 mg/kg (dry basis). It is possible to observe that drying caused a decrement in anthocyanins of 37.04, 50.17, 52.91 and 55.32% for drying at 60, 70 80 and 90 °C respectively. It is noteworthy that as from drying times: 35, 35, 45 and 50 min for drying at 90, 80, 70 and 60 °C respectively, and given that linearity of kinetics decreased at those drying times, temperature of the samples should have increased, approaching the dry-bulb temperature of the air. The loss of anthocyanins due to both conventional and freeze-drying methods were reported by Wojdylo *et al*. (2009). Results obtained in this work for dried slices at 60 °C are slightly lower than those reported by Alonzo-Macías *et al*. (2013) for whole strawberries dried at 50 °C. Differences may be due to variety of strawberries used and to the fact that whole fruits have a better protection of phytochemicals during heat treatments and to the lower temperatures used in reference work. Long exposure times to hot drying air and severity of thermal treatments have been related to loses of free phenols in hot air-dried freeze dried samples as compared to freeze-dried vegetables (Hung and Duy, 2012). Therefore, it is possible to consider a direct relationship between the intensity of convective drying and the extent of anthocyanin loss, and this is consistent with the findings reported by Kwok *et al*. (2004). Although anthocyanin content is decreased due to the exposure to high temperatures, the pigment that is retained becomes concentrated in a reduced volume. As well, the conditions of convective drying (high temperature and the presence of oxygen) may promote the activity of polyphenol oxidase, which results in browning (Howard *et al*., 1996).

Table 1. Total anthocyanin content of dried strawberries at indicated temperatures and corresponding decrement in relation to original content of fresh strawberries of 2298.29 (mg/kg dry basis)*

Drying Temperature (°C)	AcyTot (mg/kg dry basis)*	Decrement AcyTot (%)
60	1447.04 ± 3.56	37.04
70	1145.20 ± 2.88	50.17
80	1082.23 ± 4.32	52.91
90	1026.81 ± 6.54	55.32

* Expressed as pelargonidin-3-glucosid.

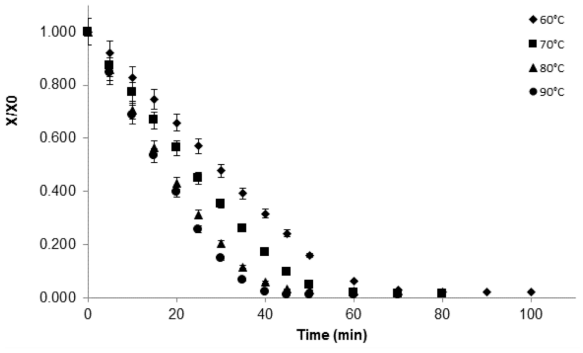

Fig. 1. Drying kinetics for slices of strawberries at different temperatures of drying air.

Table 2. Antioxidant activity[a] of dried strawberries at indicated temperatures decrement in relation to original TEAC of fresh strawberries of 89.7 ± 3.98 (mM eq. Trolox/ kg dry basis)

Drying Temperature (°C)	TEAC (mM eq. Trolox/kg dry basis)
60	55.29 ± 0.67
70	54.35 ± 0.55
80	51.72 ± 3.10
90	48.14 ± 2.10

[a]Data are expressed as mean ± SD calculated from three repetitions as mM eq. Trolox/kg dry basis.

3.3 Antioxidant activity

In Table 2, the results of the assay were expressed relative to milimolar Trolox per kg of dry weight, *i.e.*, in terms of Trolox equivalent antioxidant capacity (TEAC). Initial TEAC was 55.03 ± 0.76 and progressively decreased with drying. A decrement ($p < 0.05$) in the TEAC is, in general, associated to loss of anthocyanins. It has been reported that antioxidant activity of these fruits is greatly due to anthocyanins and other phenolic compounds (Cárdenas *et al*., 2012; Wojdylo *et al*., 2009). The resulting decrements of TEAC were: 38.36, 39.40, 42.34 and 46.34 mmolEq Trolox/g dry sample for drying at 60, 70 80 and 90 °C, respectively, in relation to fresh samples. Hence, decrements varied from 22.9 to 32.9% for samples obtained at drying air temperatures of 60 and 90 °C, respectively. Decrements in antioxidant activity by effect of temperature have been reported by Wang and Lin (2000), Wang *et al*. (2002) and Mori *et al*. (2007).

The antioxidant capacity followed the same trend observed for the total content of anthocyanins. The higher the temperature, the lower the content of anthocyanins, as reported by Wojdylo *et al*. (2009). Drying, due to heat damage and reduction in moisture content, greatly influenced the decrease in antioxidant activity. It was interesting to observe that strawberry samples after drying preserved the characteristic color of fresh samples, which may indicate that anthocyanin molecules were not totally degraded. Similar results were found by Alonzo-Macías *et al*. (2013), even by using non-thermal processes to dehydrate strawberry samples (such as freeze-drying) the effect of reducing moisture content has significant effect on the decrement of antioxidant activity of this type of compounds.

3.4 Image analysis

Original and binarized optical microscope images corresponding to fresh and dehydrated samples of strawberry mesocarp are presented in Figure 2. Parenchymal tissue damage due to cell disruption can be observed in dried samples and its quantitative assessment by means of area, perimeter, Feret's diameter and fractal dimension of cells are reported in Figures 3-6. A contraction or shrinking of cells due to dehydration was detected. Significant ($p < 0.05$) higher values for A, P and Fe were found between cells of dried tissue at 60 °C and those for 70, 80 and 90 °C. Fractal dimension, on the other hand, significantly decreased ($p < 0.05$) at 90 °C while there were not found differences ($p > 0.05$) within the lower temperature range (60-80 °C). For all cases, a significant decrease ($p < 0.05$) of morphometric parameters was observed between original and dehydrated samples. A reduction of 74.2, 49.8, 50.7 and 3.0 % were found for A, P, Fe and FD respectively for samples dried at 60 °C (lowest temperature), which indicated a marked morphological damage for all dehydrated samples in relation to initial one, except for irregularity of cell morphology (evaluated as FD) in which a slight decrement of this value associated to an smoothener effect of dried tissue was observed in all drying experiments which resulted to be more intense in samples dehydrated at 90 °C, probably due to the pressure that the expansion of the water when changing to vapor phase is exerted in the interior of the parenchymal tissue. Results indicated a higher level of shrinking when applying 70-90 °C drying air temperature which may be due to the fact that not apparent crust formation was observed in all cases so that size decrement associated with crust and low shrinking did not seem to take place.

3.5 Anthocyanins, antioxidant activity and drying

A correlation test was carried out for ACyTot and TEAC of dried slices of strawberries. Results showed a good correlation ($r = 0.784$). Linear relationships between these parameters have been found by Alonzo-Macías *et al*. (2013). Decrement in ACyTot and TEAC in samples dehydrated at 60 °C, were associated to the marked decrease in values of A, P, Fe and FD found in samples dried at this temperature.

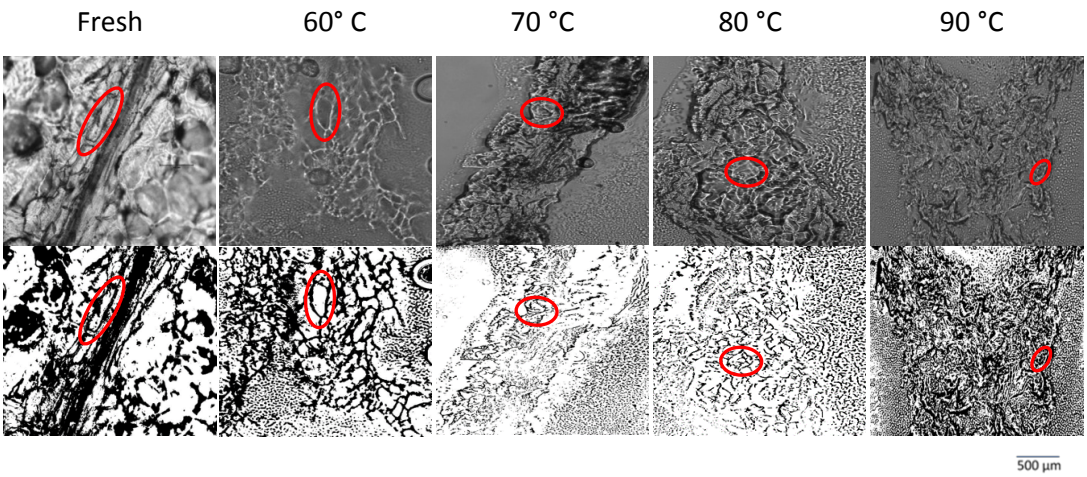

Fig. 2. Light microscopy images (top row) and the corresponding binarization (bottom row) of strawberry parenchymal tissue samples, both fresh and dehydrated, at different drying air temperatures. Examples of cells measured by digital image analysis are pointed out.

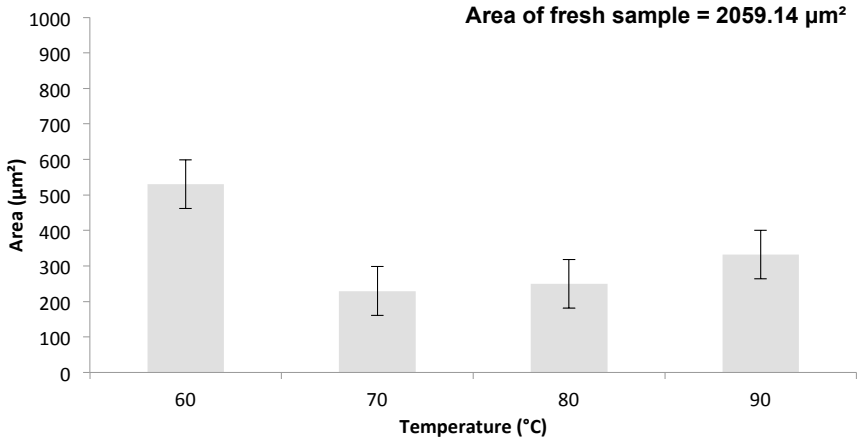

Fig. 3. Final area of parenchymal tissue cells dried at different temperatures.

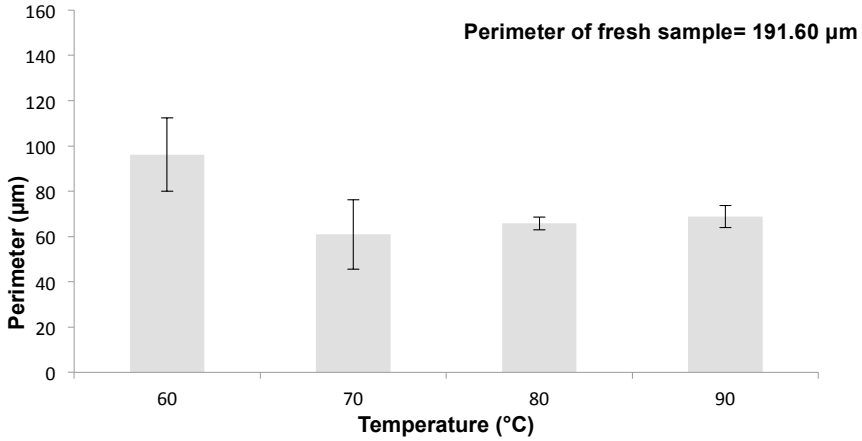

Fig. 4. Perimeter of parenchymal tissue cells dried at different temperatures.

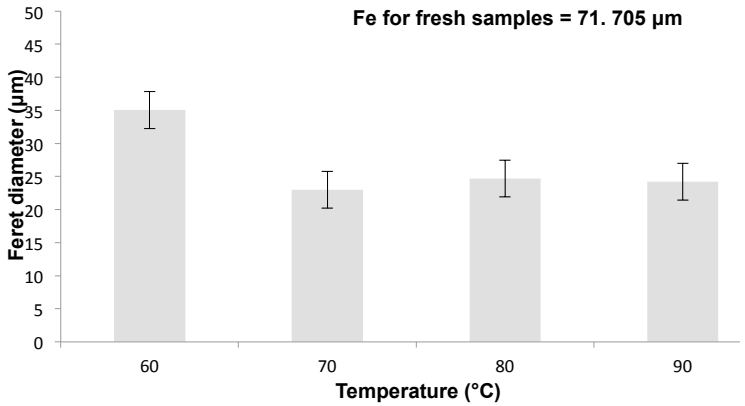

Fig. 5. Feret diameter of parenchymal tissue cells dried at different temperatures.

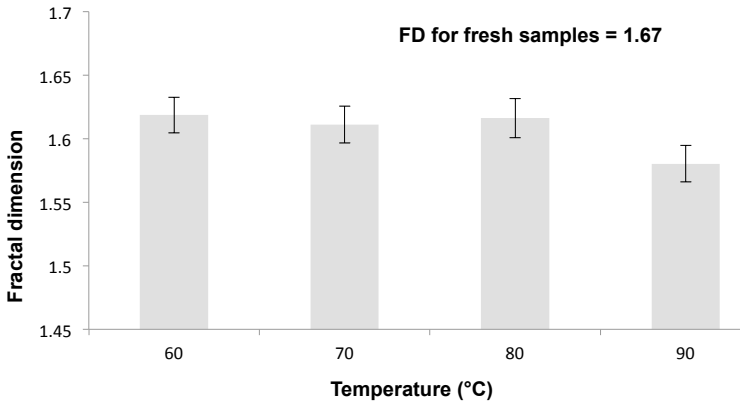

Fig. 6. Fractal dimension of parenchymal tissue cells dried at different temperatures.

Also, observed decrements of ACyTot and TEAC in dried samples at 70-90 °C were associated to A, P, Fe which did not show appreciable changes among each other in relation to those treated at 60 °C which indicated that antioxidant activity related to ACyTot and TEAC changes took place at progressively higher rate in samples that changed morphology in the same proportion (as indicated by A, P and Fe) among each other in relation to initial fresh tissue. A slight tendency towards a smoothener effect at the high temperatures range as indicated by a tendency of FD to decrease was, however, observed.

Samples dried at 70-90 °C had similar values of A, P and Fe while ACyTot and TEAC for each of those samples were progressively lowers. This is, the antioxidant capacity of anthocyanins keeps on decreasing while morphological parameters did not change within the mentioned temperature range. Therefore, the evaluation of morphometric parameters allowed observing that the same changes in cell morphology (cell damage) at temperatures of 70 °C and higher, are not related to the availability of anthocyanins to perform antioxidant activity. Probably, a less aggressive drying treatment (e.g. with lower air temperature or velocity) which were capable to minimize the changes in cell morphology attributes, would generate equally a continuous decrease of antioxidant activity which may be attributed to the moisture content rather than to morphological changes of parenchymal tissue. Therefore, cell disruption of strawberry parenchymal tissue caused by convective drying at temperatures ranging 70-90 °C cannot be considered necessarily related to anthocyanin content or antioxidant capacity.

Conclusions

Drying kinetics showed typical trend with temperature. Faster drying took place at 90 °C than at lower temperatures. ACyTot decreased with drying

temperature as well as antioxidant capacity. Both parameters showed a good correlation between them and for the high temperature range (70-90 °C) these parameters showed a strong linear correlation. A, P and Fe, markedly decreased in relation to values for fresh samples and higher values for A, P and Fe were found between cells of dried parenchymal tissue at 60 °C and those for 70, 80 and 90 °C. Fractal dimension, on the other hand, decreased at 90°C while there were not found differences ($p > 0.05$) within the lower temperature range (60-80°C). Morphological parameters for samples dried at 70-90 °C were similar among each other while ACyTot and TEAC decreased within these temperatures. A tissue smoothing effect when drying at the high temperatures range was observed. At the higher temperature range, cell disruption cannot be considered necessarily related to anthocyanin content or antioxidant capacity.

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