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MICROSTRUCTURAL ANALYSIS OF FRESH-CUT RED BELL PEPPER (*CAPSICUM ANNUUM L.*) FOR POSTHARVEST QUALITY OPTIMIZATION

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KEYWORDS

Quality, microstructure, SEM, image analysis.

ABSTRACT

The main objective of this work was to evaluate the microstructure of fresh-cut red bell pepper (*Capsicum annuum L.*) after cutting, when maintained (for 0, 4 and 7 d) under refrigerated storage (2 °C). In order to assess the microstructure of the product, scanning electron microscopy (SEM) was applied; however, to improve this technique, preparation procedures and quantitative image analysis were specifically developed. Since sample preparation affects deeply image quality, three sample preparation procedures (viz. freezing by immersion in liquid nitrogen and subsequent storage at -80 °C, freezing by storage at -80 °C and subsequent freeze-drying, and freezing by immersion in liquid nitrogen and subsequent freeze-drying) were tested. One could also test (with success) a methodology of quantitative image analysis via a panel – a large number of people (N=25) rated the degree of cellular destruction, using a continuous scale (from 1- no cellular destruction to 9-extreme cellular destruction). Statistical analysis of the experimental data revealed that frozen samples exhibited higher cellular destruction than via the other two procedures; no statistically significant differences were observed between these two other procedures. Red bell pepper samples stored for 4 and 7 d presented (as expected) higher degree of cellular destruction than initial day samples. This work allowed one to develop appropriate preparation procedures of sample and quantitative image analysis – that will permit the application of this microscopy technique in future work in this area.

INTRODUCTION

Rationalization of the deterioration processes that take place during postharvest of fresh fruits and vegetables, and in particular of fresh-cut ones, is essential to optimize postharvest quality and maximize shelf-life thereof. Quality optimisation of fresh products is based upon selection and monitorization of quality indicators – viz. sensory (appearance, flavour and texture), nutritional (vitamins, fibre and phytonutrients) and microbial attributes. Textural quality of a food product is one of the most important characteristics in determining consumer preference and product acceptability. Recall that texture is strongly determined by both microscopic and macroscopic cell structure; hence, it is important to study the former and understand the factors that influence it.

Scanning electron microscopy (SEM) is an interesting microscopy technique in terms of application to microstructural studies. However, due to extreme fragility of the living cell structure, sample preparation is a restrictive factor on the final quality of SEM images. Acquisition of quantitative data from SEM

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images is another limitation of said technique. Scarce work has indeed focused to date on the microstructure of fresh and fresh-cut fruits and vegetables [1, 2, 3, 4, 5, 6, 7].

The main objective of this research effort was the application of SEM to evaluate the microstructure of fresh-cut red bell pepper (*Capsicum annuum L.*), after cutting and maintaining (for 0, 4 and 7 d) under refrigerated storage (at 2 °C). In order to achieve this goal, two sub-objectives were specified: i) to optimise the sample preparation procedure prior to analysis of red bell pepper by SEM, and ii) to develop an adequate methodology to quantify SEM images.

MATERIALS AND METHODS

Plant material and experimental design

Fresh red bell peppers (*C. annuum L.*) were obtained just after harvest at a local distribution centre, and duly transported to the Food Process Optimization Laboratory at Escola Superior de Biotecnologia. Fruits were washed, drained and dried with absorbent paper. The core was removed, and rings (width=1 cm) were cut with an electrical food slicer (model CF-7691 from Ufesa, UK). Rings were in turn cut in dices (1x1 cm) with a stainless steel sharp knife in a refrigerated room. All dices were randomly mixed, and divided into three plastic jars covered with a perforated lid. These plastic jars were then stored at 2±0.5 °C and 90-95 % relative humidity.

Image acquisition

Samples of diced red bell pepper for microstructural analysis were taken randomly just after cutting, and by 4 and 7 d of refrigerated storage. The peel of fruit was removed, and the flesh was cut (parallel to peel) into small, oriented blocks (1x5x5 mm) with a razor blade. It was then prepared according to one of the following preparation procedures: i) samples (five replicates) were frozen by storage for 1 d at -80 °C, freeze-dried and then stored at -80 °C until analysis; ii) samples (five replicates) were frozen by immersion in liquid nitrogen, freeze-dried and then stored at -80 °C until analysis; and iii) samples (five replicates) were frozen by immersion in liquid nitrogen, and subsequently stored at -80 °C until analysis.

Freeze-dried samples were mounted on stubs, and observed by low vacuum SEM using a JSM-5600LV instrument (from JEOL, Japan), operated at an accelerating voltage of 15 kV. Frozen bell pepper samples were mounted on stubs in a cold stage, coupled to SEM equipment at -22 ^{o}C (so as to maintain the frozen state), and observed by low vacuum SEM. Images were taken in representative parts of sample, and observed at a magnification of 50x.

Image analysis

A methodology to analyze SEM images, based on techniques used in sensory analysis with a trained panel, was developed [8]. Before assessment of images by panellists, the following three steps took place: i) identification of descriptor and definition of scale; ii) evaluation of discriminatory and reproducibility capacities of panellists; and iii) selection of panel. The selected descriptor was cellular destruction and the scale was a continuous 1-9 intensity rating one (1=absence of cellular destruction, 3=very little, 5=moderately, 7=very much and 9=extreme cellular destruction). After these three preliminary steps were taken, the following methodology was chosen for assessment by the panellists, of the images taken from red bell pepper samples at different combinations of time of storage after cutting and sample preparation procedures: i) select a set of nine images representing all combinations of conditions; ii) code each image with a three digit-number; iii) prepare an electronic document with each image and the corresponding evaluation scale in independent pages; iv) send by e-mail the file to the selected panellists; and v) ask them to rate each image independently, and return the file duly filled in.

Statistical analysis

The experimental results were subject to two-factor analysis of variance (ANOVA), followed by application of Bonferroni test to detect differences (at a significance level of 5%) using the SPSS software (v. 12.0 for Windows, from SPSS, USA).

RESULTS AND DISCUSSION

Selected scanning electron micrographs of fresh-cut red bell pepper, according to different preparation procedures and time after cutting, are presented in Fig. 1. Apparent differences were observed between preparation procedures; in Fig. 1C, a dendritic structure was formed during ice microcrystal formation inside the cells. Differences in microstructural effects associated with time of storage after cutting are also shown in Fig. 1; samples cut and stored for 7 d showed a great degree of cell decompartmentation and collapse, reflected by poor definition of cell walls — as compared with samples taken just after cutting. Tissue softening likely involved cell separation and cell breakage [9]. This fact is in agreement with the decrease in instrumental firmness, from 18±5 N just after cutting to 14±2 N by 7 d under refrigeration.

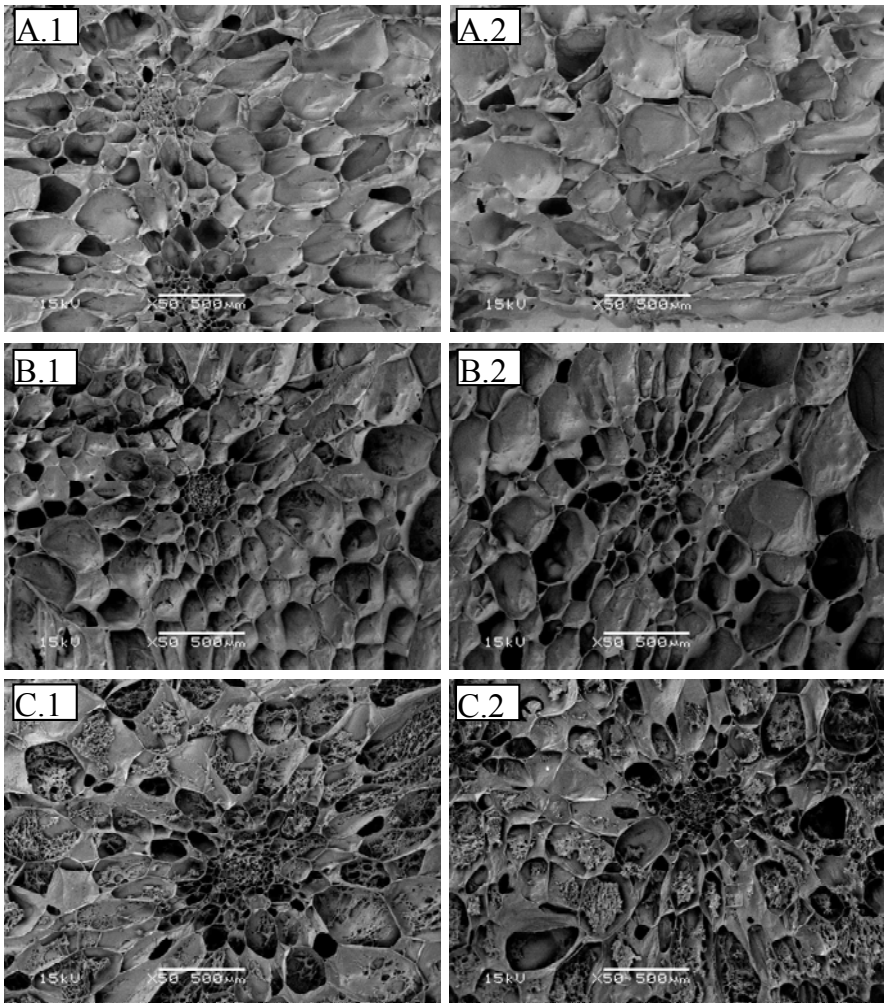

Fig. 1. Scanning electron micrographs of fresh-cut red bell pepper, according to different preparation procedures: (A) sample frozen in liquid nitrogen and freeze-dried; (B) sample frozen at -80 °C and freeze-dried; and (C) sample frozen at -80 °C, (1) just after cutting the product and (2) by 7 d under refrigeration.

Statistical analysis of the experimental data generated by the panel pertaining to SEM images indicated that there are microstructural differences between pepper samples from the distinct preparation procedures (p=0.001) and time after cutting under refrigerated storage (p=0.001); however, the interaction between these two factors was not statistically significant (p=0.051). Frozen samples presented higher cellular destruction than the other two procedures, and no statistical significant differences were observed between these two (Fig. 2). Red bell pepper samples stored for 4 and 7 d presented (as expected) higher degree of cellular destruction than their initial sample counterpart (Fig. 2).

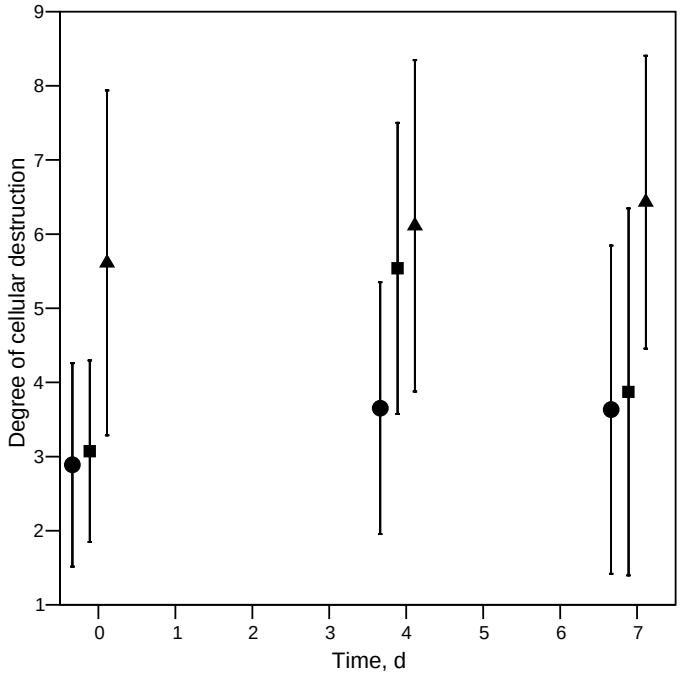

Fig. 2. Degree of cellular destruction of red bell pepper after cutting, over time under refrigerated storage (average±standard deviation), according to different preparation procedures: (●) sample frozen in liquid nitrogen and freeze-dried; (■) sample frozen at -80 °C and freeze-dried; and (▲) sample frozen at -80 °C.

CONCLUSIONS

SEM was successfully applied in the microstructural study of fresh-cut red bell pepper; it provided a clear visualization of microstructural changes over time after cutting under refrigerated storage. Red bell pepper samples stored for 4 and 7 d presented (as expected) higher degree of cellular destruction than samples at 0 d. Samples for SEM prepared via freeze-drying produced better quality images than frozen samples. The methodology applied, which was based on panel evaluation of SEM images, proved a valuable tool to obtain quantitative parameters. This work allowed optimization of the preparation procedures of sample, and development of a successful methodology for quantitative image analysis – which will eventually permit application to related work in other food matrices.

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