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Research Article

Enzymatic Pretreatment Effects on the Drying Process in Terms of Kinetics, Physical and Structural Aspects of the Vegetable Carrot *Daucus carota*

[1, 2, 3]Wafaa Sahyoun, [1, 2, 4]Sabah Mounir and [1]Tamara Allaf
[1]ABCAR-DIC Process, BP12053, 17010 La Rochelle Cedex 01,
[2]University of La Rochelle, Intensification of Industrial Eco-Processes, Laboratory of Engineering Science for Environment LaSIE-UMR-CNRS 7356, 17042 La Rochelle, France
[3]Department of Biology, Faculty of Sciences, Lebanese University, Tripoli, Lebanon
[4]Department of Food Science, Faculty of Agriculture, Zagazig University, 44115 Zagazig, Egypt

Abstract: This study is part of a large comparative study of various pre-treatments used to improve the technological abilities of plants. This study is specifically designed to systematically analyze the intensification of vacuum drying of carrots using cellulase, pectinase and macerozyme as a way to change both structure and functional behavior. These enzymatic pretreatments resulted in major changes in porosity, drying time, capacity and rehydration kinetics and water activity. However, their effects were fundamentally different from physical pretreatments generally used to increase technological capability of drying carrots, such as Freezing/Thawing (FT) instantaneous controlled pressure drop (DIC), or Simple Steaming (SS). Thus, in enzymatic pretreatments no correlation was observed in terms of structure and functional characteristics. This completely contrasts with FT, DIC and SS as thermal/texturing pretreatments, where we found that functional properties strictly depend on structural modifications. Thus thermal/texturing pretreatment operations involve systematically a high correlation between porosity and the ability to dehydration and rehydration. The changes induced by the enzyme pretreatments were closely linked to irreversible microscopic changes of the polymer chains.

Keywords: Carrot, cellulase, enzyme pretreatment, macerozyme, pectinase, vacuum drying

INTRODUCTION

Carrot (*Daucuscarota sativus*), a root vegetable of *Apiaceae* (*Umbelliferae*) family, is a seasonable vegetable that is cultivated worldwide (Debs-Louka *et al*., 1996). Carrot possesses many virtues since it is an excellent source of β-carotene, a precursor of vitamin A, protecting cells from free radicals which may damage the basic cell structure of healthy cells. Carrot is rich in antioxidants such as α-carotene, β-carotene, phytochemicals, glutathione, calcium, phosphorus. They all play a major role in protecting cells from free radicals damage that attack cell structure of healthy cells (Gamboa-Santos *et al*., 2013).

Many industrial processes have been applied to preserve carrots including drying. Drying is an important industrial process applied to extend the shelf life of fruits and vegetables overcoming the problem of their seasonality (Barat and Grau, 2016). Carrot drying has been extensively addressed in terms of modeling and study of drying kinetics (Erenturk and Erenturk, 2007; Mulet *et al*., 1989). Several pretreatments are performed upstream of the drying process to better preserve carrot properties resulting from dehydration process. Those include blanching (Negi and Kumar Roy, 2001), freezing and thermo-mechanical pretreatment (Debs-Louka *et al*., 1996), ultrasound (Gamboa-Santos *et al*., 2013; Chen *et al*., 2016) Enzymatic treatment is one of the potential pretreatment which is widely applied in various industrial processes (Kuhad *et al*., 2011; Sharma *et al*., 2013). Most enzymes used in food industry are enzymes that act on cell wall; these enzymes are used to improve juice production in terms of quantity and quality, they act to modify fruit purees viscosity, to enhance maceration, to improve food texture and impact the aroma and flavor and volatile properties of fruits and vegetables.

Commercially available enzymes include pectinase, an enzyme that breaks down pectin, The latter is a compound found as the first part of the plant cell walls; it is in the middle lamella formed during

Corresponding Author: Dr. Tamara Allaf, ABCAR-DIC Process, BP12053, 17010 La Rochelle Cedex 01, La Rochelle, France, Tel.: +33671559599

cytokinesis (Sharma *et al*., 2013). Other group of enzymes comprises cellulases, which act on cellulose degradation. Cellulases include 3 different groups of enzymes endo-(1,4) β-D-glucanase (EC 3.2.1.4), exo-(1,4) β-D-glucanase (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) (Bayer *et al*., 2004). Pectinases and cellulases have important application as a part of mixture of macerating enzymes complex consisting of cellulases, xylanases and pectinases used for food processing. Enzyme macerating mixture is frequently used since it intensifies industrial process yields and performances without additional capital investment. For instance, the macerating enzymes increase cloud stability and texture and decrease viscosity of the nectars and purees from tropical fruits such as mango, peach, papaya, plum, apricot and pear. Infusion of enzymes such as pectinases and β-glucosidases improve texture, flavor and aroma properties of fruits and vegetables by reducing excessive bitterness of citrus fruits.

As far as we know, the potential use of enzymatic pretreatment in the drying process has not been addressed before. Here, we report the first study that explores the effect of pectinases, cellulases and macerozymes on the drying process of the carrot.

MATERIALS AND METHODS

Sample preparation: Fresh carrot (*Daucuscarota L. var. Nantesa*) as raw material was purchased from a popular market, in Picardie-France; with initial water content of 87.3% wet basis (wb) or 6.69 g H_2O/g dry basis (db). Carrots were stored at 4-6°C till used. They were washed, peeled before treatment and cut into slices of 0.5 cm in terms of thickness after removing spikes and collars at the same proportions.

Enzymatic pre-treatment: All samples were steam blanched for 12 min at 0.15 MPa prior to the enzymatic treatment in order to facilitate the enzymes penetration. For all enzymes, design of experiments was performed to determine the optimum incubation conditions for each enzyme in term of pH, temperature, stirring velocity and time as shown in Table 1. Enzymatic reactions were conducted by incubating 200 g of carrots with suitable enzymes in a buffer consisting of sodium acetate in predetermined conditions as mentioned below.

Cellulase: "ONOZUKA-R-10" of *Trichoderma viride* (EC 3.2.1) (Cat. No. 16419) was purchased from SERVA (10 g of 0.85 U) (HEIDELBERG, Germany). Cellulase is able to decompose natural (e.g. filter paper) as well as modified celluloses (e.g., carboxymethyl cellulose). It hydrolyses 1, 4-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Conditions for a light action on the cell wall were optimized by measuring the activity from 0.2 to 0.8 mg/mL in 100 mL of 1%-carboxymethyl cellulose prepared in 0.1-M sodium acetate solution (Table 2). Reactions were stopped by immersion in boiling water for 10 min. Released sugar was dosed according to BOEHRINGER method (Massiot *et al*., 1987, 1988).

Pectinase S: Pectinase S from *Aspergillus niger* was purchased from SERVA (Heidelberg, Germany) with a pectinase activity of 0.9 U/mg. Conditions were setup by measuring enzymatic reactions on 0.4% of polygalacturonic acid in 0.05 M sodium acetate with enzymatic concentrations ranging from 0.15 to 0.25 mg/g of substrate. Reactions were stopped by incubation in boiling water for 10 min (Table 2). Released sugars were measured according to the method of Dinitrosalicylate (DNS) (Mandels *et al*., 1976; Schwald *et al*., 1988).

Macerozyme R10: Macerozyme R10 issued from *Rhizopus sp* was purchased from SERVA (EC.3.3.1.15) (Heidelberg, Germany). This complex enzymatic mixture owns activities of pectinase (0.5 U/mg), cellulase (0.1 U/mg) and hemicellulase (0.25 U/mg). Treatment conditions for this mixture were fixed using pectinase activity (as reference) as mentioned above (Table 2).

Vacuum drying: After various enzymatic pretreatments, samples were washed with buffer to eliminate enzyme traces. Subsequently the samples were drained and dried under vacuum until obtaining the desirable level of final water content. Vacuum drying was carried out under conditions of 1300 Pa as absolute pressure and 80°C as temperature of heating plate according to Sahyoun *et al*. (2016); the exact experimental procedures of vacuum drying were applied to provide a comparison with other thermo-mechanical pretreatment operations used in the previous study including freezing/thawing FT, instant controlled pressure drop DIC and simple steaming SS.

Assessments of samples: Quality attributes of final product including functional and structural

Table 1: Incubation conditions of carrot with different enzymes according to Massiot *et al*. (1987, 1988)

Conditions	Cellulase: CE	Pectinase: PE	Macerozyme: MA
pH	4.4	4	4.5
Stirring Velocity (t.p.m)	100	100	100
Temperature (°C)	40	35	45
Time (min)	30	30	20

Table 2: Enzymatic pretreatment conditions of carrot

			Mg/g blanched carrot			
Trial identification		Buffer	Cellulase	Pectinase	Macerozyme	Remarks
Blanching		0	0	0	0	12 min at 1.5 bar steam
Buffer	TP1C	0.1	0	0	0	
	TP005	0.05	0	0	0	
	TP001	0.01	0	0	0	
Cellulase	CE1	0.01	1.58	0	0	Buffer for Cellulase
	CE2	0.01	0.4	0	0	
	CE3	0.01	0.1	0	0	
	CE4	0.01	0.05	0	0	
	TP001	0.01	0	0	0	
Pectinase	PE1	0.01	0	1	0	Buffer for Pectinase
	PE2	0.01	0	0.2	0	
	PE3	0.01	0	0.05	0	
	TP001P	0.01	0	0	0	
Macerozyme	MA1	0.01	0	0	2.5	Buffer for Macerozyme
	MA2	0.01	0	0	2	
	MA3	0.01	0	0	1	
	MA4	0.01	0	0	0.1	
	TP001M	0.01	0	0	0	
Combining enzymes	CiP	0.01	0.05	0.05	0	Simultaneous Cellulase/Pectinase under cellulase conditions
	CiM	0.01	0.05	0	0.1	Simultaneous Cellulase/Macerozyme under cellulase conditions
	Cpi	0.01	0.05	0.05	0	Simultaneous Pectinase/Cellulase under pectinase conditions
	PiM	0.01	0	0.05	0.1	Simultaneous Pectinase/ Macerozyme under pectinase conditions
	CMi	0.01	0.05	0	0.1	Simultaneous Cellulase/Macerozyme under Macerozyme conditions
	PMi	0.01	0	0.05	0.1	Simultaneous Pectinase /Macerozyme under Macerozyme conditions
	TP1C2	0.01	0.05	0.05	0	Buffer for Pectinase followed by Buffer for Cellulase
	P1C2	0.01	0	0	0	Pectinase followed by Cellulase under respective buffer conditions

characteristics and the concentration of the main compounds were assessed. Functional properties represent the solid-water interaction such as dehydration time, rehydration capacity and kinetics and water activity, while physical/structural characteristics include the various densities of product in addition to absolute porosity and absolute or relative expansion ratio. In this study lipid and carotene contents in vacuum dried carrot were also assessed.

The determination of various types of density was achieved following the methods of Sahyoun *et al*. (2016). The apparent density ρ_{app} has concerned the mass of dry matter reported to the whole volume of each sample including its pores and material; we determined it using buoyancy effect and its values strictly depended on pretreatment and drying processes, mainly because of shrinkage phenomenon. The intrinsicdensity $\rho_{intrinsic}$ was measured using argon-pycnometer method. In the case of carrot, we assumed that the measurements of various samples with three repetitions each had their average and standard deviation as the value and errors, respectively of intrinsic density of the carrot samples:

$$\rho_{intrinsic} = 1258 \pm 8\ kg/m^3$$

From these various amounts of apparent densities and intrinsic density, relative expansion ratio ε_{rel} and absolute expansion ratio -ε_{abs}, as well as absolute porosity ratio ϑ_ were calculated as following:

$$\varepsilon_{abs} = {\rho_{intrinsic}}/{\rho_{app}} \quad (1)$$

$$\varepsilon_{rel} = {\varepsilon_{abs,tr}}/{\varepsilon_{abs,rm}} = {\rho_{app,rm}}/{\rho_{app,tr}} \quad (2)$$

$$\vartheta = 1 - {\rho_{app}}/{\rho_{intrinsic}} \quad (3)$$

where, rm indicates raw-material and tr indicates treated sample.

Determination of water content: Briefly, water content of carrots was determined following Karathanos method (Karathanos *et al*., 1990), the weight of samples before and after drying was recorded using a laboratory drying oven (UFE 400) at 70°C overnight. The

determination of water content was triplicated. Water content (W) was expressed by g H_2O/g dry basis db.

Determination of lipid and carotene content: Lipid and carotene contents were determined following the method of Sahyoun *et al*. (2016):

Preparation of samples: For fresh and pretreated carrots: 10 g of sample (1.67 g dry matter), were ground with food blender for 3 min. Usually the grinding was subsequently completed by a mortar to obtain fine powder.

For dried carrot samples (which underwent various pretreatments and vacuum drying): 2 g (1.85 g dry matter) were ground in food blender for 3 min:

- 50 mL of distilled water were added to powdery sample and let to stand 60 min at room temperature protecting them from sunlight.
- This mixture was added to 30 mL of chloroform/methanol (2:1) and, then, to 20 mL of pure chloroform.
- The mixture constantly protected from sunlight was combined to 100 mL of distilled water by stirring and then decanting into a funnel.
- The remaining organic phase was dried over anhydrous sodium and then filtered through Whatman filter paper n°0.4. Subsequently, the filter was rinsed with 10 mL of chloroform and solvent traces were evaporated using a rotary vacuum evaporator at 30°C and absolute pressure of 1200 Pa.
- The residual lipids were taken up in 10 ml of hexane and preserved at -20°C.

Separation and identification of lipid compounds were performed using combining thin layer chromatography on silica 60 (Merck F254, 0.25 mm layer thickness, Massachusetts, USA) with visualization by a variety of reagents, including mixture of aldehyde, acetic acid and sulfuric acid. Carotenoids were estimated by specific colorimetry by considering the absorbance at 450 nm for lipid extract in a methanol/chloroform solvent. The measurements were performed using spectrometry (SPECTRONIC GENEYS 5). They were compared to a reference ranged between 0 and 5 g/L of commercial carotene (SIGMA).

Therefore, gas chromatography separation and analyses were conducted according to Chaveron and Adenier (1980) method using GIRDEL, series 3000 (Rheinfelden, Germany) equipped with a flame ionization detector and a capillary column of fused silica packed with apolar phase DB1 JW (polydimethylsiloxane, internal diameter: 0.32 mm, 7 m, temperature maximum: 350°C). The chromatograph was connected to an integrator (Waters 746, Millipore, Massachusetts, USA). The operating conditions were the following: 1-bar Helium as carrier gas; 375°C and 10°C/min as injection needle temperature value and rate, respectively. All lipids were thus separated into different classes, including: free fatty acids, free sterols, diglycerides, esterified sterols and triglycerides. The identification of free sterols, tocopherols, diglycerides and triglycerides provided by co-injection with the respective commercial control compounds as internal standards and their quantification was achieved by adding to the total lipid extract a well-known amount of cholesterol.

The absorbance at 450 nm of the lipid extract in a solvent methanol/chloroform (Wolff, 1968) was measured by spectrometry (SPECTRONIC GENEYS 5; Pont-Saint-Pierre, France) and compared to a reference ranged from 0 to 5 g/L of commercial carotene (SIGMA) lipids separated by chromatography and then measured by spectrophotometry.

Microstructure analysis using Scanning Electron Microscopy (SEM): Dried samples were directly processed by SEM, while fresh samples were maintained in a desiccator of silica gel for 48 h prior to SEM analysis. Gold nanoparticles were used as staining and samples were observed with a JEOL JSM 840 electron microscope.

RESULTS AND DISCUSSION

The impact of enzymatic pretreatment using three different enzymescellulase, pectinase and macerozyme was evaluated in order to analyze its effect on cell wall permeability and carrot drying. Enzyme pre-treatments were performed separately, simultaneously and sequentially. Various pre-operating conditions and operating conditions were used. Pre-operating conditions defined the optimum enzymatic parameters concerning pH, temperature, velocity and incubation time (Table 1). Enzyme concentrations varied through the operating conditions and ranged from 0.05 to 2.5 mg/g of steam blanched carrots.

Impact of enzymatic pretreatment on drying time:

Impacts of single enzyme pretreatment: We first evaluated carrot pretreatment with buffer control media in the absence of enzymes. Results show an effect of the ionic strength of the reaction buffer as it reduced the drying time. Figure 1 and 2 illustrate the drying time as function of buffer or enzyme concentrations. Vacuum drying time decreased when buffer concentration increased but enzymatic pretreatment with different concentrations remain more effective. To exclude that the effect seen during enzymatic pretreatment is due to the buffer effect, the reaction buffer concentration was kept constant during all reactions at 0.01M.

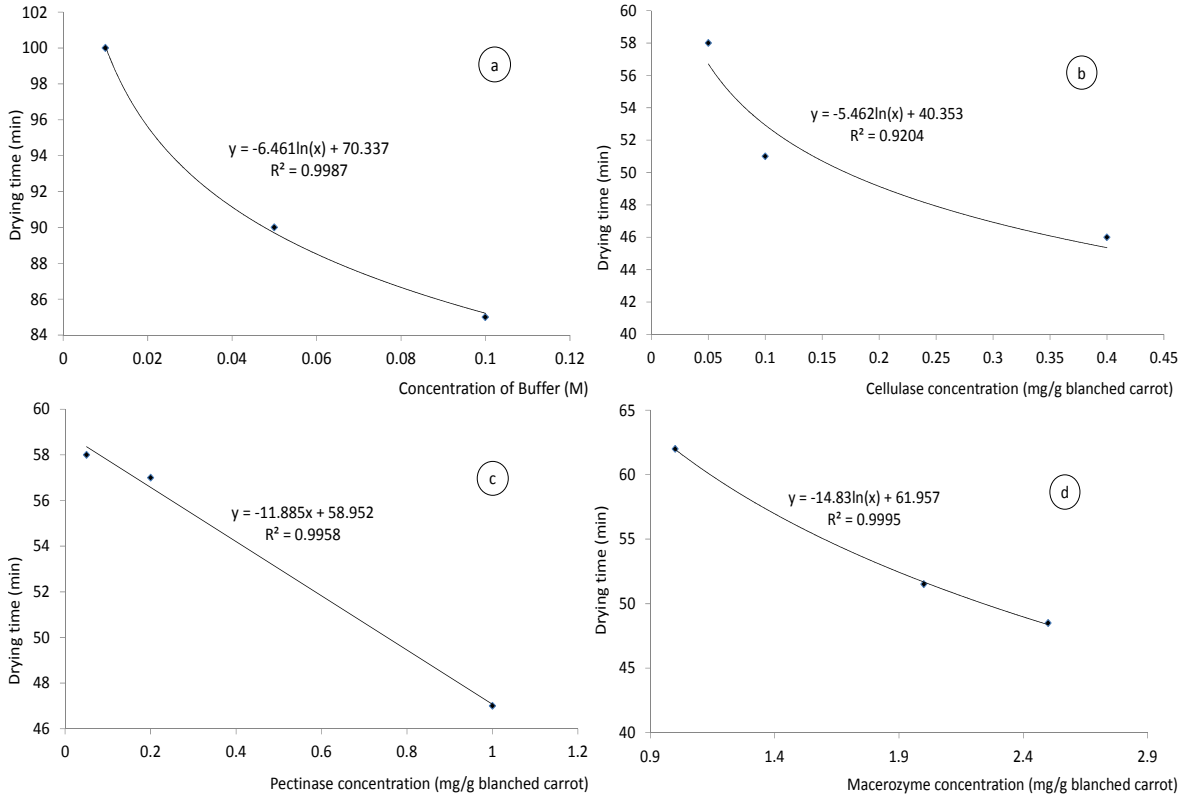


Fig. 1: Impacts of concentrations of reaction buffer; (a): Cellulase; (b): Pectinase; (c): and Macerozyme; (d): on vacuum drying time

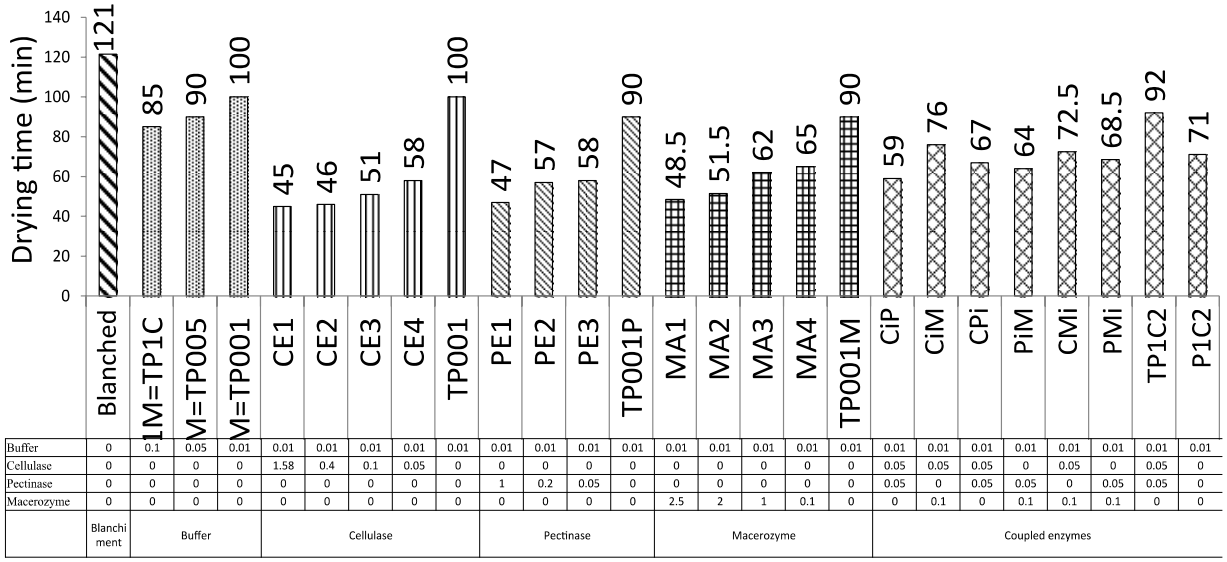


Fig. 2: Impact of enzymatic pretreatment on vacuum drying time

Enzymatic pretreatment prior to vacuum drying resulted in decreasing required drying time compared to steam blanching. The observed decrease in drying time correlated with enzymes concentration; as a higher enzyme concentration resulted in shorter drying time.

The impact of enzymatic pretreatment using cellulose and pectinase was similar; the drying time was decreased by 52% using 0.05 mg enzyme/g steam blanched carrot. This was found to be 63 and 61% using 1.58 mg and 1 mg as concentration of cellulase and pectinase respectively (Table 1).

Macerozyme concentration had slightly lower impact, with a decrease in vacuum drying time that ranged from ~47% to 60% for 0.1 to 2.5 mg of

Table 3: Comparison between effects of single enzyme and simultaneously combined enzymes in terms of drying time

Responses	CE4	PE3	MA4	CiP	CiM	CPi	PiM	CMi	PMi	P1C2
Drying time (min)	58	58	65	59	76	67	64	72.5	68.5	71
Effect on drying time (%)	52%	52%	47%	51%	37%	45%	47%	40%	44%	42%
Synergism effect SE (%)				-102%	-125%	-126%	-114%	-120%	-80%	-124%

Table 4: Global comparison between various enzymatically treated carrots in terms of apparent density, ε_{abs} (absolute expansion ratio) and ϑ(porosity factor)

Trial identification	Apparent density (kg m^{-3})	Absolute expansion ratio ε_{abs}	Porosity % ϑ
Control	1150	1.09	8%
Blanching	680	1.85	46%
Buffer 0.05 M	460	2.73	63%
Buffer 0.01 M	384	3.28	69%
Cellulase	513±6	2.45±0.03	59.0±0.1%
Pectinase	521±31	2.43±0.14	58.6±2.5%
Macerozyme	469±35	2.70±0.19	62.7±2.8%
Simultaneous enzymatically treatment	476±37	2.66±0.22	62.1±3.0%

macerozyme/g steam blanched sample, respectively. It is worth mentioning that we did not use higher concentration of macerozyme, to get similar decline in vacuum drying time as that observed in the case of cellulase and pectinase.

Synergistic effect of combined enzymes: Successive enzymatic pretreatment such as P1C2 (0.05 mg pectinase followed by 0.05 mg cellulase under their respective incubation conditions) led to a decreasein drying time by 23% compared TP1C2 (buffer for pectinase followed by buffer for cellulase), which confirm the efficiency of enzymatic pretreatment compared to the buffer pretreatment without enzymes as mentioned above.

However, in several cases, it is worth mentioning that synergistic effect was absent (nil) or even negative. Indeed, synergistic effect SE of two enzymes can be quantified through the following equation:

$$SE = \left(E_{1+2} - (E_1 + E_2)\right)/(E_1 + E_2)/2 \qquad (4)$$

where, E1 and E2 are their respective effects.

Combined double enzymatic treatments were performed prior to vacuum drying, as given in Table 3: CiP, CPi, CiM, CMi, PiM, PMi and TP1C2. Simultaneous incubation of enzymes was performed as follow: CiP and CPi (simultaneous Cellulase and Pectinase; under Cellulase and Pectinaseconditions, respectively); CiM and CMi (simultaneous Cellulase and Macerozyme; under cellulase and macerozyme conditions, respectively); PiM and PMi (simultaneous Pectinase and Macerozyme; under Pectinase and Macerozyme conditions, respectively).

Results shown in Table 3 highlight a general antagonistic effect when the enzymes were combined. For the combination CiP, no additional impact was seen on vacuum drying time; the effect was similar to the effect seen for single pretreatment with cellulase (CE4) or pectinase (PE3). This is revealed by SE~-100%.

The highest antagonistic effects were obtained from CPi, CiM and CMi (~SE = -125%).

Enzymatic pretreatment effect on physical properties of the dried product:

Densities and porosity: Table 4 shows the effect of enzymatic pretreatments on physical characteristics of carrot; bulk density, absolute expansion ratio and porosity.

We observed that blanching led to a decrease in bulk density by 41% compared to fresh carrot. On the other hand, buffer pretreatment led to a significant density decrease by 60 and 67% for 0.05 and 0.01 M buffer concentrations respectively. Enzymatic pretreatment resulted in a reduction in bulk density value whatever the type of enzymes used. Cellulase and pectinase had the same impact on bulk density, which decreased by 55% compared to fresh carrot and by around 25% compared to steam blanched carrot (Table 4). Whereas, Macerozyme showed a slight increase in bulk density decreasing of carrot, it was decreased by 59% and 31% compared to fresh and steam blanched carrot, respectively. The combination between the enzymes didn't imply any synergistic effect on bulk density, which decreased only with macerozyme. When comparing enzymatic pretreatments, no significant difference was observed in bulk density taking in consideration the standard deviation between values (Table 4).

Concerning the absolute expansion ratioε_{abs}, we found that all the pretreatments had a significant effect on this property; it was increased by 70% for steam blanched carrot compared to fresh sample. However buffer pretreatment had more impact on the absolute expansion; indeed the obtained value increased by 150% and 200% when compared to fresh carrot for a 0.05 and 0.01 buffer concentration respectively (Table 4).

The absolute expansion ratio for enzymatically pretreated carrot samples was generally lower than those of buffer pretreatment; it was increased by 125% for samples pretreated by buffer used for the incubation of cellulase or pectinase and by 148% for samples pretreated by macerozyme, compared to fresh sample.

Table 5: Capacity of Rehydration versus time (g H_2O/100 g db)

Rehydration time:		1 min	10 min	20 min
Blanching		192	514	660
Cellulase	CE1	200	465	518
	CE2	128	351	391
	CE3	177	435	472
	CE4	222	444	522
	TP001	135	361	391
Pectinase	PE1	172	394	419
	PE2	182	435	477
	PE3	180	432	468
	TP001P	177	448	524
Macerozyme	MA1	162	393	451
	MA2	144	363	397
	MA3	172	411	436
	MA4	200	467	502
	TP001M	246	559	611
Combined enzymes	CiP	198	499	526
	CiM	206	438	468
	Cpi	228	515	577
	PiM	210	506	554
	Cmi	174	406	440
	Pmi	260	590	623
	TP1C2	143	381	436
	P1C2	167	418	464

The same absence of synergistic effect was also observed when two enzymes were combined; the absolute expansion ratio was increased by 144% as average value compared to fresh carrot.

As to porosity factor ϑ, the minimum (8%) and maximum (69%) values were recorded for fresh carrot (control) and 0.01 M buffer concentration pretreatment respectively, followed by macerozyme or combined enzymatic pretreatment (~62%). Similar effect was observed when cellulase or pectinase was used as enzymatic pretreatment; porosity factor ϑ was found to be around 60% (Table 3).

Rehydration capacity: Rehydration kinetics reflect the quantity of water absorbed by the dry matter versus time. Table 5 shows the results of rehydration kinetics for steam blanched and various pretreated carrot samples.

Steam blanched carrot showed the lowest value of rehydration capacity during the first minute of rehydration (192 g H2O/100 g db). While, the highest value (260 g H2O/100 g db) was observed for the combination PMi (0.05 mg pectinase and 0.1 mg macerozyme/g steam blanched carrot under incubation conditions of macerozyme), followed by enzymatic pretreatment by cellulase CE4 (0.05 mg cellulase/g steam blanched carrot).. On the other hand, the successive enzymatic pretreatment (P1C2) revealed insignificant impact on rehydration capacity along the rehydration duration; where the increase in water capacity was 17, 10 and 6%, respectively during 1, 10 and 20 min respectively compared with TP1C2 (only buffer).

Comparing the buffer pretreatments, the buffer used for macerozyme incubation (0.01M) showed the highest values of rehydration capacity with time (1, 10 and 20 min) compared to other buffer pretreatments (Table 5). The rehydration capacity after 1 min and 10 min of rehydration were increased by 35 and 15%, respectively for the enzymatic combination PMi (0.05 mg pectinase and 0.1 mg macerozyme/g steam blanched carrot under the incubation conditions of macerozyme compared to steam blanched carrot.

Water activity: Table 5 shows the results of sorption isotherm of different pretreated carrot samples, for a_w ranged from 0.1 to 0.8 and water content ranged from 2.72 to 16.09 g/100 g db.

For all enzymatic pretreatments, we observed a discrete higher water activity than the buffer as well as the blanched control. It is worth highlighting that drying time did not follow similar evolution; moreover, no correlation was observed between these two functional characteristics. This means that vacuum drying, which is mainly controlled by internal diffusion of water within the matter, does not depend on water activity (Massiot *et al*., 1987).

Synergistic effect was observed when macerozyme combined with pectinase; PiM (0.05 mg pectinase and 0.01 mg macerozyme/g steam blanched carrot under the incubation conditions of pectinase). Water content was lower than the separate pretreatments. On the other hand, successive enzymatic pretreatment had an impact similar to one enzyme's effect.

For different sorption isotherms, two empirical models water content db versus water activity were used: GAB and Peleg models (Table 6 and 7). Both presented a very interesting R^2 close to 99.99%.

Table 6: Water content versus water activity for different enzymatic pretreated carrots

a_w:		0.1	0.2	0.3	0.4	0.5	0.6	0.8
Blanching		2.715	5.429	8.144	10.446	11.858	13.27	16.093
Cellulase	CE1	2.336	4.672	7.007	9.343	11.505	13.599	17.788
	CE4	2.192	4.384	6.576	8.768	10.993	13.261	17.796
	TP001	2.172	4.344	6.516	8.688	10.898	13.164	17.697
Pectinase	PE1	3.343	6.686	10.024	12.832	15.64	18.448	25
	PE3	2.483	4.966	7.448	9.931	12.414	14.897	19.862
	TP001P	2.326	4.653	6.979	9.305	11.45	13.518	17.653
Macerozyme	MA1	2.394	4.788	7.181	9.575	11.784	13.954	18.293
	MA4	2.221	4.441	6.662	8.882	11.042	13.139	17.333
	TP001M	2.192	4.383	6.575	8.766	11.008	13.315	17.928
Combined	CiP	2.013	4.025	6.038	8.05	10.065	12.155	16.335
enzymes	CiM	2.114	4.228	6.342	8.456	10.613	12.89	17.443
	CPi	1.985	3.971	5.956	7.942	9.927	12.072	16.373
	PiM	1.817	3.635	5.452	7.27	9.087	10.904	14.539
	CMi	2.091	4.182	6.293	8.364	10.455	12.546	16.728
	PMi	2.181	4.361	6.542	8.723	10.904	13.084	17.446
	TP1C2	1.913	3.826	5.74	7.653	9.566	11.608	15.767
	P1C2	1.94	3.88	5.82	7.76	9.7	11.71	15.76

Table 7: Statistical results obtained from the empirical GAB (Guggenheim-Andersen-de Boer) model: $X_e = \frac{a.b.c.a_w}{[(1-c.a_w)(1-c.a_w+b.c.a_w)]}$ where a is expressed by g H_2O/100 g db

Treatment identification	Parameters of the GAB model					
	a (g H_2O/100 g db)	b	c	R^2	$RMSE$	χ^2
Blanching	41	3871	0	0.998	0.289	0.083
TP005	54	2	0	1.000	0.014	0.000
CE1	221	203	0	1.000	0.054	0.003
CE4	62	2	0	1.000	0.011	0.000
TP001	54	2	0	1.000	0.014	0.000
BE1	135	70	0	1.000	0.105	0.011
PE3	336	2	2	1.000	0.001	0.000
TP001P	180	16	0	1.000	0.058	0.003
MA1	193	9	0	1.000	0.051	0.003
MA4	228	5	0	1.000	0.029	0.001
TP001M	51	2	0	0.212	0.017	0.000
CiP	43	2	0	1.000	0.012	0.000
CiM	37	2	0	1.000	0.023	0.001
CPi	29	2	0	1.000	0.024	0.001
PiM	221	2	0	1.000	0.001	0.000
CMi	137	2	0	1.000	0.007	0.000
PMi	264	2	0	1.000	0.001	0.000
TP1C2	27	2	0	1.000	0.017	0.000
P1C2	38	2	0	1.000	0.010	0.000

Table 8: Peleg Model of water activity a_w ($X_e = k_1 a_w^{n_1} + k_2 a_w^{n_2}$): Statistical results obtained from various enzymatic pretreatments, where k_1 and k_2 are expressed in g H_2O/100 g db

Treatment identification	Parameters of the Peleg model						
	k_1	n_1	k_2	n_2	R^2	RMSE	X^2
Blanching	9.78	0.76	9.78	0.76	0.9933	0.60	0.35
TP005	1.51	2.04	20.85	0.98	1.0000	0.01	0.00
CE1	11.06	0.95	11.06	0.95	0.9998	0.11	0.01
CE4	11.14	1.01	11.14	1.01	1.0000	0.04	0.00
TP001	11.08	1.02	11.08	1.02	1.0000	0.05	0.00
BE1	14.82	0.92	14.82	0.92	0.9996	0.20	0.04
PE3	12.41	1.00	12.41	1.00	1.0000	0.00	0.00
TP001P	10.97	0.95	10.97	0.95	0.9998	0.12	0.02
MA1	11.37	0.96	11.37	0.96	0.9998	0.11	0.01
MA4	10.81	0.98	10.81	0.98	0.9999	0.06	0.00
TP001M	11.23	1.02	11.23	1.02	1.0000	0.06	0.00
CiP	10.22	1.01	10.22	1.01	1.0000	0.04	0.00
CiM	10.94	1.03	10.94	1.03	0.9999	0.08	0.01
CPi	10.26	1.03	10.26	1.03	0.9998	0.09	0.01
PiM	9.09	1.00	9.09	1.00	1.0000	0.00	0.00
CMi	10.45	1.00	10.45	1.00	1.0000	0.01	0.00
PMi	10.90	1.00	10.90	1.00	1.0000	0.00	0.00
TP1C2	9.87	1.03	9.87	1.03	0.9998	0.09	0.01
P1C2	19.22	1.00	0.79	3.44	1.0000	0.01	0.00

Table 9: Lipid and carotene contents for different enzymatic pretreated carrots (p: pretreated, dh: dehydrated)

		mg/100 g db			
Treatment identification		PL + DG (p)	PL + DG (dh)	Carotene (p)	Carotene (dh)
Blanching		2.59	0.77	0.77	0.52
Cellulase	CE1	3.45	1.559	0.7696	0.798
	CE4	3.87	2.31	0.638	0.394
	TP001	5.29	2.55	0.82	0.35
Pectinase	PE1	2.75	1.93	0.45	0.17
	PE3	4.68	0.2	0.58	0.33
	TP001P	8.89	0.06	0.8	0.34
Macerozyme	MA1	4.4	1.58	1.63	1.34
	MA4	2.91	0.63	1.39	0.979
	TP001M	8.92	3.51	1.237	0.8326
Combined enzymes	CiP	6.336	3.803	1.24	0.4
	CPi	2.57	2.48	0.43	0.32
	PiM	10.75	9.4	1.23	0.61
	PMi	11.46	6.26	1.2	1.1
	TP1C2	4.8	1.2	1.66	0.87
	P1C2	4.01	0.19	2.42	0.88

Effect of enzymatic pretreatment on lipid content of the product before and after drying: Table 8 shows lipid and carotene availabilities in various pretreated and vacuum dried carrot samples. In the present study, lipid content was quantified as phospholipids and diacylglycerol (PL+DG). The obtained results show significant decrease in lipid and carotene contents in all carrot samples after enzymatic pretreatment and/or vacuum drying compared to fresh pretreated carrot.

The highest contents of (PL+DG) and carotene in enzymatic pretreated fresh carrot samples compared to steam blanched sample were found in the combination PMi (0.05 mg pectinase and 0.01 mg macerozyme/g steam blanched carrot under the incubation conditions of macerozyme) and P1C2 [(0.05 mg Pectinase (under incubation conditions of pectinase) followed by 0.05 mg cellulase (under incubation conditions of cellulase conditions)]; 11.46 g oil/100 g db and 2.42 mg carotene/100 g db respectively. However, the lowest content of (PL+DG) and carotene content were recorded for CPi (0.05 mg Pectinase and 0.05 mg cellulase under incubation conditions of pectinase); 2.57 g oil/100 g db and 0.43 mg carotene/100 g db respectively.

After vacuum drying, the highest content of (PL+DG) and carotene in enzymatic pretreated was observed for the combination PiM (0.05 mg Pectinase and 0.1 mg Macerozyme under incubation conditions of macerozyme) and MA1 (2.5 mg Macerozyme); 9.4 g oil/100 g db and 1.34 mg carotene/100 g db, respectively.

Whereas the lowest content was found in samples pretreated by PE2 (0.2 mg Pectinase) and PE1 (1mg Pectinase); 2 g oil and 0.17 mg carotene/100 g db respectively.

Concerning the buffer media, we found that they had a strong impact on the availability of lipids in pretreated fresh carrot before vacuum drying (Table 9). While after vacuum drying, the impact of these buffers was not identical; the highest and lowest values of lipids content were found to be 3.51 and 0.06 g oil/100 g db respectively for buffers TP 0.01M (under incubation conditions of macerozyme) and TP0.01P (under incubation conditions of pectinase). Moreover, whatever the enzyme's incubation conditions in the buffer, no significant variation in carotene content was observed.

Moreover, although P1C2 (successive enzymatic pretreatment) and CiP and CPi (combination between cellulase and pectinase) contained the same concentration of cellulase and pectinase (0.05 mg for each/g steam blanched carrot), we observed an increase in carotene content in P1C2 pretreated steam blanched fresh carrot sample.

Effect of enzymatic pretreatment on microstructure: The effect of enzymatic pretreatment on microstructures was further evaluated. Pretreatment with the control buffer media without enzyme show very little cellular changes compared to the blanched control (Fig. 3). Cellulase pretreatment allowed cell walls in xylem to be extended and in phloem to be collapsed with a disappearance of the intercellular boundaries (Fig. 4). After drying, xylem cells were best defined with dilated walls, more frequent meatus, cracks and small holes in the cell walls.

In both cases of cellulase and pectinase pretreatments, cell deformation increased with the enzyme concentration and xylem was more resistant to deformation than the phloem, while cell deformation rate correlated with the drying time. After drying, cells got more defined appearance particularly, the xylem cell walls were more dilated and the presence of meatus in xylem increased cell separations (Fig. 5).

The cellulase-pectinase mixture brought more changes to the xylem while improving the preservation of phloem. Similarly, a mixture of both polygalacturonase activities allowed the largest cell walls to be expanded at both xylem and phloem.

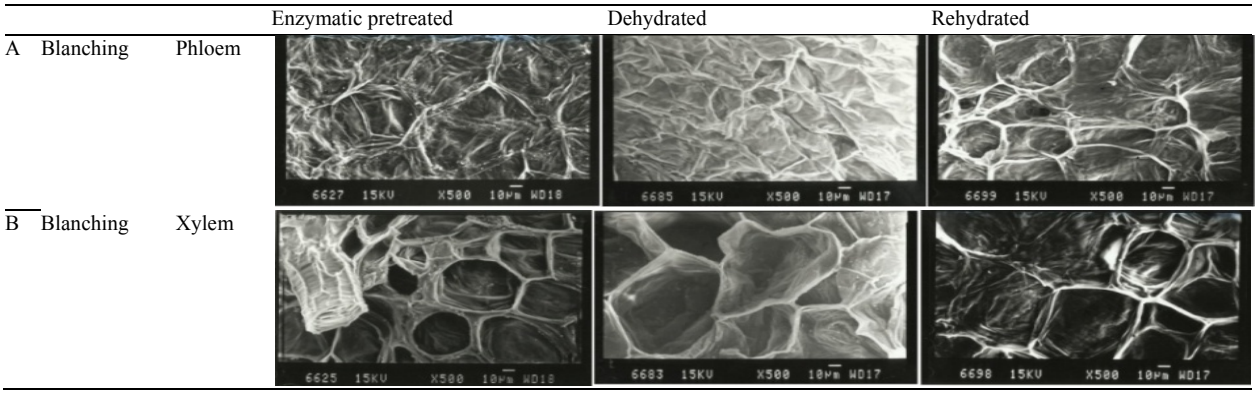


Fig. 3: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (Blanching)

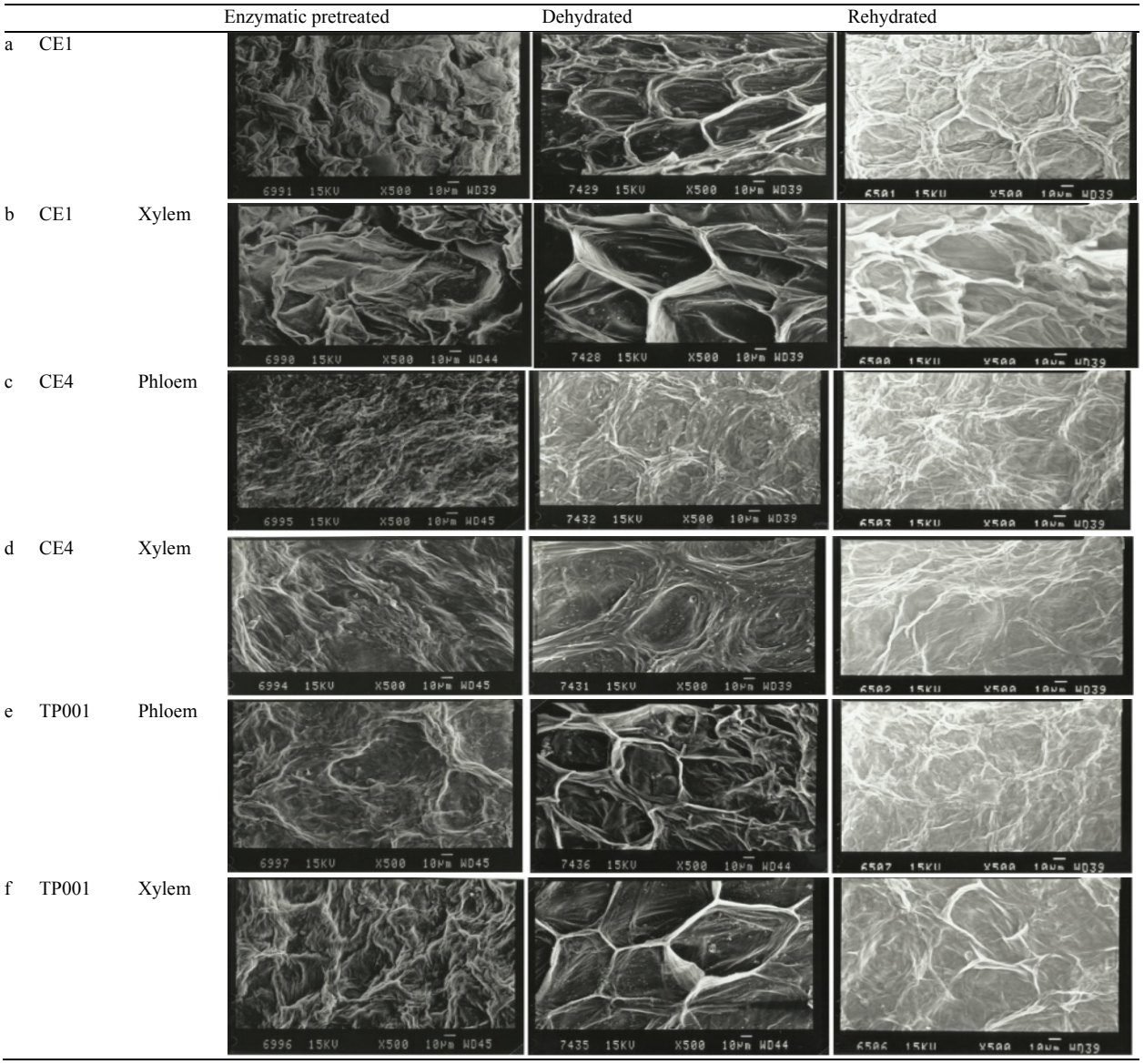


Fig. 4: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (Cellulase)

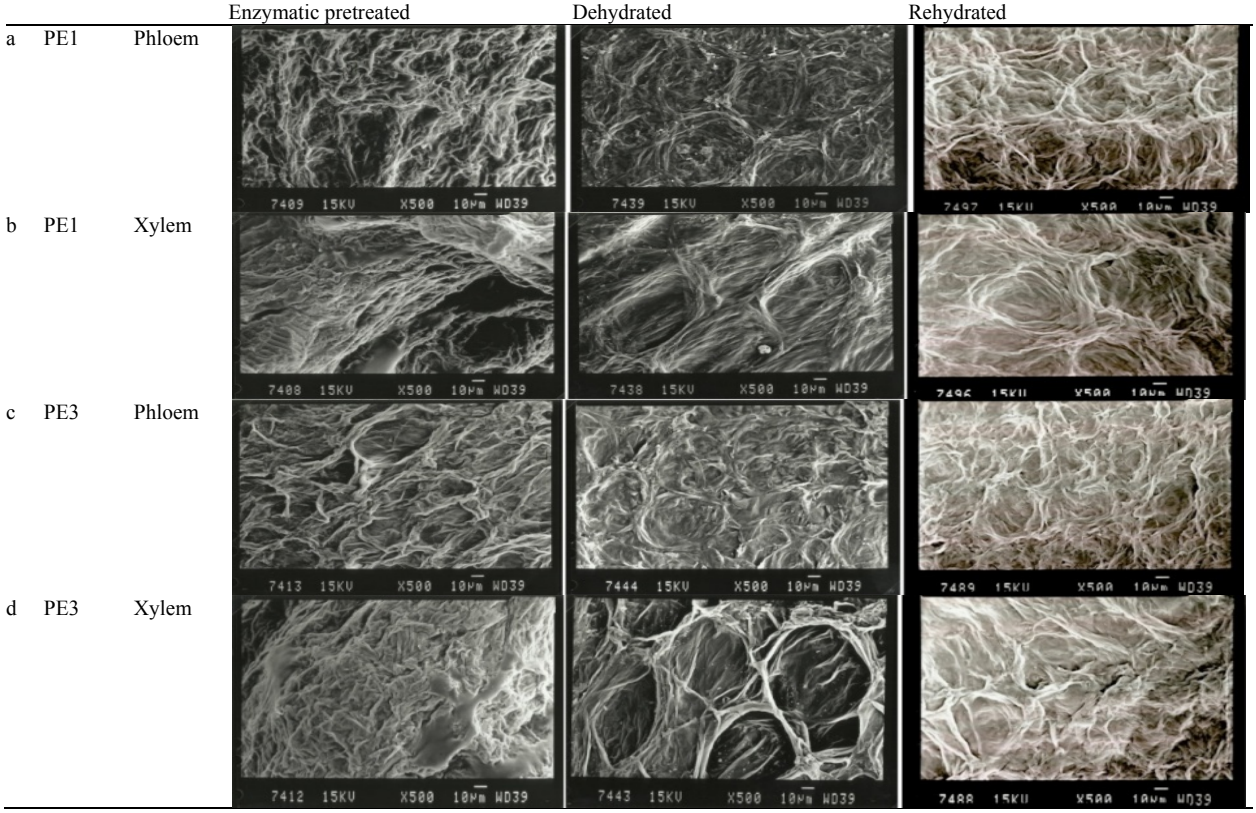
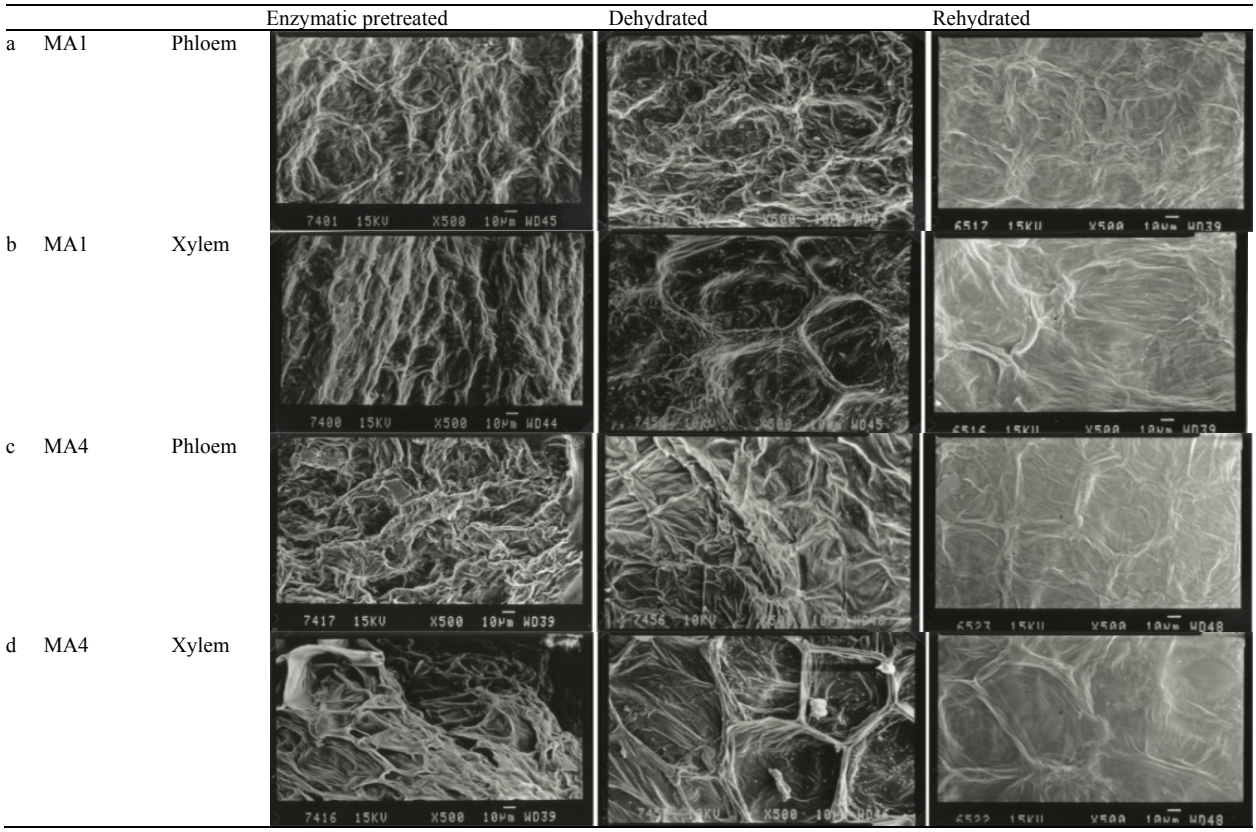


Fig. 5: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (Pectinase)



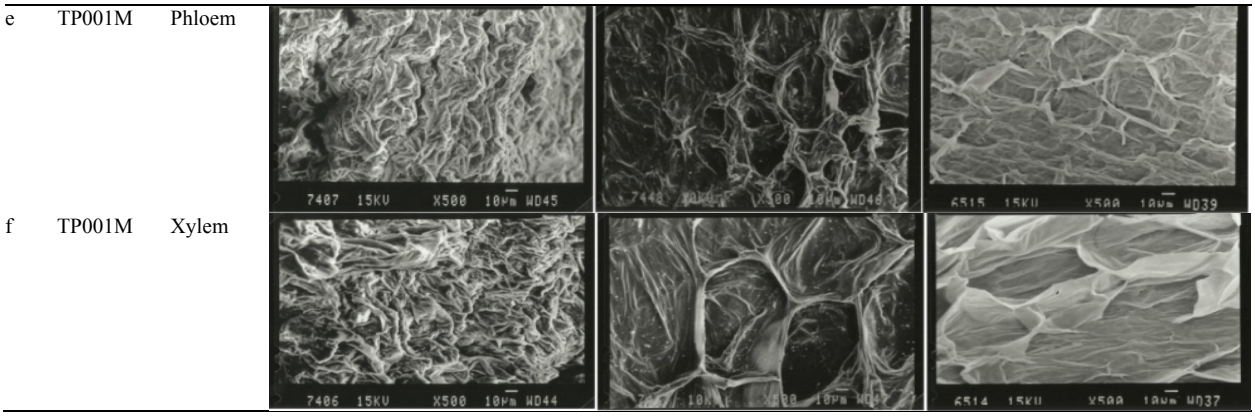


Fig. 6: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (Macerozyme)

Pectinase action was higher in the phloem than in the xylem. This latter was more degraded with CPi (cellulase mixture with pectinase under the conditions of the second). Improving rehydration ability following pectinase pretreatment should be correlated to better preservation and swelling of cellulose fibers. Thus, the cells were more elongated and dilated than the cell walls of dried samples. Moreover, the presence of polygalacturonase activity facilitated the water access to the cellulosic fibers thus improving the product rehydration, however, less than that of blanched product.

In terms of phloem lipid (PL+DG), pectinase caused higher hydrolysis and greater loss than cellulase. CPi (mixture of cellulase and pectinase under the conditions of the latter) PiM (pectinase mixture macerozyme under pectinase conditions) and PE3 (low concentration of pectinase) implied less important values in lipid and pigment contents.

Macerozyme allowed xylem cells to get an expansion higher than the controls but lower than the pectinase samples. Modification resulted from processing brought the affected phloem and the xylem to a similar level. The structure was only preserved at low enzyme concentrations (Fig. 6). This allowed drying time to increase.

Phloem was slightly more preserved by low concentration of macerozyme from the pectinase. This resulted in better preservation of carotenoids trapped in the cytoplasm. A mix of both polygalacturonase activities from pectinase and macerozyme increased the loss of carotenoids in a greater change in the phloem. Moreover, drying facilitated this carotenoid loss and pigments deterioration because of heat exposure.

The textural changes induced from successive, simultaneous, or separate applications of two enzymes (pectinase and cellulase) implied the same reduction of drying time (Fig. 7 to 9). This was less important than that gathered by processing at higher enzyme concentrations of pectinase or cellulase following the pretreatment. After drying, the cell walls of enzymatically treated samples were more

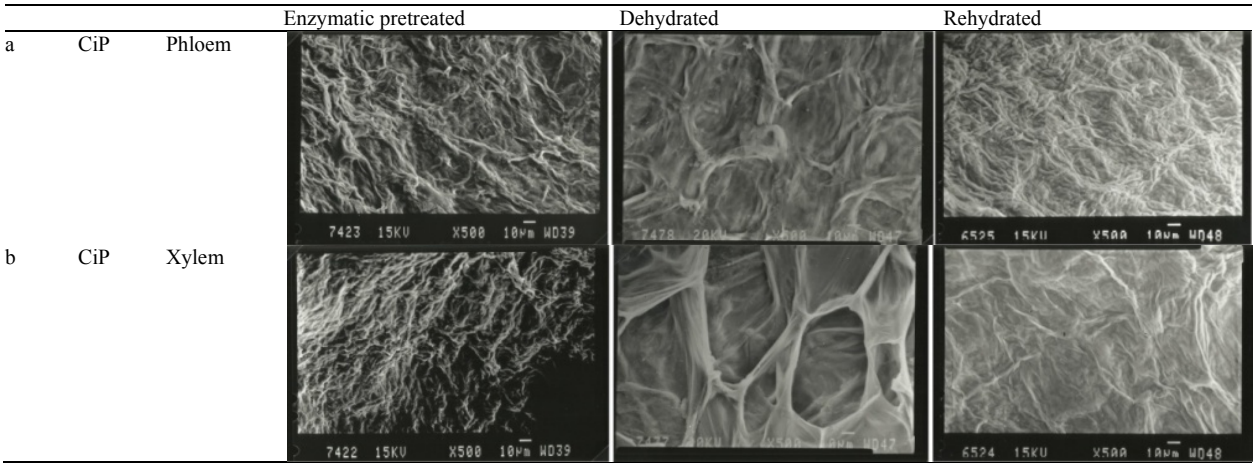


Fig. 7: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (cellulase-pectinase mixtures under cellulase conditions)

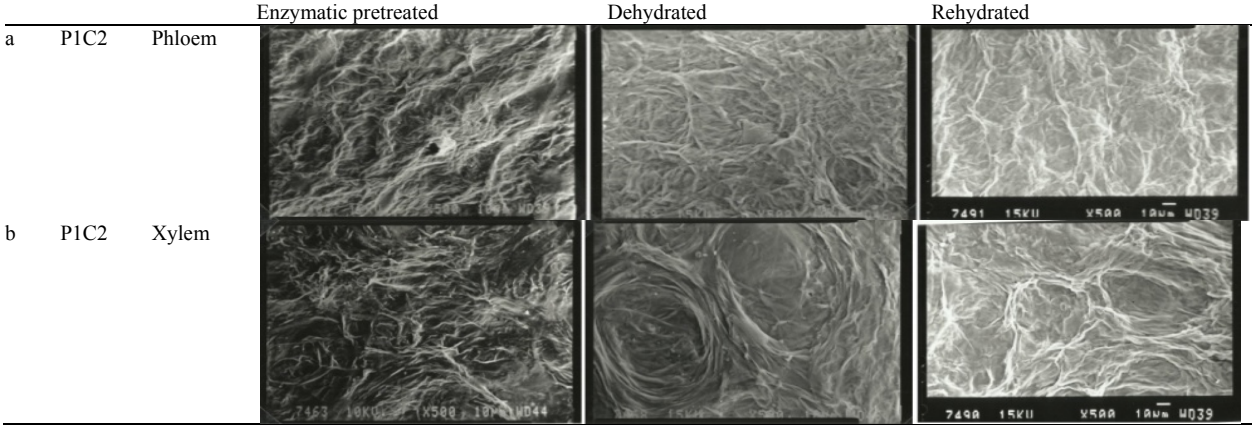


Fig. 8: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (pectinase followed by cellulase)

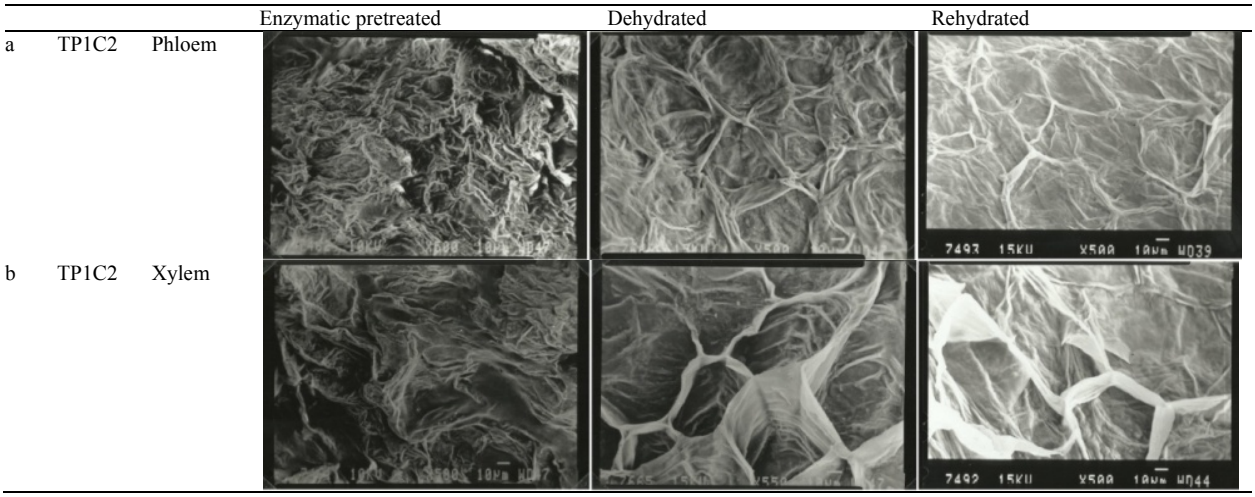


Fig. 9: Effect of various enzymatic pre-treatments, followed by vacuum drying and by rehydration on microstructure of phloem and xylem (buffer for pectinase followed by buffer for cellulase)

dilated than controls with the presence of some meatus in the xylem.

Increasing the treatment time furthermore for one hour could explain the sensitivity to changes of phloem cells relative to that caused by low enzyme concentrations.

Product rehydration ability pretreated shows only a slight increase compared to the buffer. Thus, after 4 min of rehydration, cell walls were more collapsed in the phloem and thicker in xylem. This slight modification from the buffer slightly improved the rehydration capacity, which however were kept below the blanched control.

DISCUSSION

Correlation between functional and structural parameters: Enzymatic pretreatment resulted in a global behavior deeply different than the one of physical pretreatment such as simple steaming SS, freezing/thawing FT, or Instant controlled pressure drop (DIC) texturing as operated by Sahyoun *et al*. (2016). Indeed, the various thermal/texturing pretreatment operations systematically implied a variation of the number and size of pores.

Thus, in the different cases of SS, FT and DIC, the evolution of drying or rehydration times greatly depended on porosity with correlation coefficient of -0.9 and 0.7, respectively (Table 9). On the opposite, variations induced by enzyme pretreatment should be closely related to an irreversible change of the polymer chains. Therefore, this results in different evolution types of the functional behavior of drying and rehydration. No correlation was found between these functional parameters and the structure revealed through porosity.

This great and significant difference between the enzymatic and texturing by ST, DIC and FT pre-treatments can easily explain the difference between results presented in Table 10 and 11. Indeed, DIC and

Table 10: Global correlations between structural, physical and functional attributes issued from various enzymatic pretreatments

Coef correlation	Buffer (M)	Cellulase concentration	Pectinase concentration	Macerozyme concentration	Drying time
Buffer (M)	1	0	0	0	0
Cellulase	0	1	0	0	0
Pectinase	0	0	1	0	0
Macerozym	0	0	0	1	0
Drying time	0	0	0	0	1
E	0	0	0	0	-1
ρ_{app}	0	0	0	0	0
ε_{abs}	0	0	0	0	0
ϑ	0	0	0	0	0
$W_{1\text{-min}}$	0	0	0	0	0
PL + DG	0	0	0	0	0
Carotene	0	0	0	0	0
aw = 0.1	0	0	0	0	0
GAB (a)	0	0	0	0	0
GAB (b)	0	0	0	0	1
GAB (c)	0	0	0	0	0
Peleg -k_1	-1	0	0	0	0
Peleg- n_1	1	0	0	0	0
Peleg -k_2	1	0	0	0	0
Peleg- n_2	0	0	0	0	0

Coef correlation	Drying time reduction E	Apparent density ρapp	Absolute expansion ratio ε_{abs}	Porosity ratio ϑ	1-min Rehydration W1-min
Buffer (M)	0	0	0	0	0
Cellulase	0	0	0	0	0
Pectinase	0	0	0	0	0
Macerozym	0	0	0	0	0
Drying time	-1	0	0	0	0
E	1	0	0	0	0
ρ_{app}	0	1	-1	-1	0
ε_{abs}	0	-1	1	1	0
ϑ	0	-1	1	1	0
$W_{1\text{-min}}$	0	0	0	0	1
PL + DG	0	0	0	0	1
Carotene	0	0	0	0	1
aw = 0.1	0	0	0	0	0
GAB (a)	0	0	0	0	0
GAB (b)	-1	1	-1	-1	0
GAB (c)	0	0	0	0	0
Peleg -k_1	0	0	0	0	0
Peleg- n_1	0	0	0	0	0
Peleg -k_2	0	0	0	0	0
Peleg- n_2	0	0	0	0	0

Coef correlation	PL + DG (p)	Carotene (p)	aw = 0.1	GAB (a) g H2O/100 g db	GAB (b)
Buffer (M)	0	0	0	0	0
Cellulase	0	0	0	0	0
Pectinase	0	0	0	0	0
Macerozym	0	0	0	0	0
Drying time	0	0	0	0	1
E	0	0	0	0	-1
ρ_{app}	0	0	0	0	1
ε_{abs}	0	0	0	0	-1
ϑ	0	0	0	0	-1
$W_{1\text{-min}}$	1	1	0	0	0
PL + DG	1	1	0	0	0
Carotene	1	1	0	0	0
aw = 0.1	0	0	1	0	0
GAB (a)	0	0	0	1	0
GAB (b)	0	0	0	0	1
GAB (c)	0	0	0	0	0
Peleg -k_1	0	0	0	0	0
Peleg- n_1	0	0	0	0	0
Peleg -k_2	0	0	0	0	0
Peleg- n_2	0	0	0	0	0

Coef correlation	GAB (c)	Peleg -k_1	Peleg- n_1	Peleg -k_2	Peleg- n_2
Buffer (M)	0	-1	1	1	0
Cellulase	0	0	0	0	0
Pectinase	0	0	0	0	0

Table 10: Continue

Coef correlation	GAB (c)	Peleg -k_1	Peleg- n_1	Peleg -k_2	Peleg- n_2
Macerozym	0	0	0	0	0
Drying time	0	0	0	0	0
E	0	0	0	0	0
ρ_{app}	0	0	0	0	0
ε_{abs}	0	0	0	0	0
ϑ	0	0	0	0	0
$W_{1\text{-min}}$	0	0	0	0	0
PL + DG	0	0	0	0	0
Carotene	0	0	0	0	0
aw = 0.1	0	0	0	0	0
GAB (a)	0	0	0	0	0
GAB (b)	0	0	0	0	0
GAB (c)	1	0	0	0	0
Peleg -k_1	0	1	-1	-1	1
Peleg- n_1	0	-1	1	1	0
Peleg -k_2	0	-1	1	1	-1
Peleg- n_2	0	1	0	-1	1

Table 11: Global correlations between Simple Steaming (SS), Instant controlled pressure drop (DIC), Freezing/Thawing (FT) processing conditions and structural, physical and functional attributes

Coef correlation	Freezing time	DIC steam pressure	DIC pressure-drop time	Drying time (min)	Rehydration in 1 min
Freezing time	1			-0.2	0.6
DIC steam pressure	/	1.0	0.0	-0.1	0.1
DIC Pressure drop time	/	0.0	1.0	0.8	-0.8
Drying time (min)	-0.2	-0.1	0.8	1.0	-0.7
Rehydration in 1 min	0.5	0.1	-0.8	-0.7	1.0
ρ_{app}	-0.4	0.0	-0.4	0.9	-0.7
ϑ	0.4	0.0	0.4	-0.9	0.7
PL + DG (p)	-0.8	0.8	-0.2	-0.5	0.4
Carotene (p)	-0.6	-0.3	0.2	0.4	-0.7
a_w= 0.1	0.7	-0.3	-0.1	-0.7	0.4
Coef correlation	Density ρ_{app}	ϑ: porosity ratio	PL + DG (p)	Carotene (p)	a_w = 0.1
Freezing time	-0.4	0.4	-0.8	-0.56	0.7
DIC steam pressure	0.0	0.0	0.8	-0.3	-0.3
DIC Pressure drop time	-0.4	0.4	-0.2	0.2	-0.1
Drying time (min)	0.9	-0.9	-0.5	0.4	-0.7
Rehydration in 1 min	-0.7	0.7	0.4	-0.7	0.4
ρ_{app}	1.0	-1.0	-0.3	0.2	-0.6
ϑ	-1.0	1.0	0.3	-0.2	0.6
PL + DG (p)	-0.3	0.3	1.0	-0.4	0.1
Carotene (p)	0.2	-0.2	-0.4	1.0	-0.5
a_w= 0.1	-0.6	0.6	0.1	-0.5	1.0

FT action is limited to a simple change in structure and porosity (Sahyoun *et al*., 2016; Allaf and Allaf, 2014), modifying diffusivity value; transfers of water within the matrix in both dehydration and rehydration were improved. However, while the pretreatment by low concentration cellulase greatly increased drying kinetics, it implied a little improvement in rehydration. Moreover, in contrast to the effect seen on drying, the rehydration speed and capacity were higher for carrots pretreated with the specific mixture of cellulase-pectinase (CiP).

CONCLUSION

Enzyme pretreatment resulted in significant reduction in drying time. Cellulase provided the greatest reduction and no synergism effect was observed between the cellulase and the two other enzymes pectinases and macerozymes, thus revealing a masking effect usually obtained during simultaneous treatments. The order of pectinase-cellulase application had no significant effect on reducing the drying time. The impact of different enzymes pretreatments applied separately, simultaneously and successively on rehydration parameters of the dried product was assessed. Whatever the type of enzyme we used, no significant improvement of rehydration was observed compared with untreated product. The total rehydration capacity of the latter was systematically greater than that of the enzymatically pretreated products. It is obvious that the deterioration of the structure was generated by the ionic strength of the buffer medium and by the enzyme action. This facilitated the transfer of water inside the product but in no way improved retention. Unlike physical pretreatments, the enzymatic treatment gave no correlation between the porosity and the functional behavior of the product (speed drying and rehydration). Thus, higher porosity did not

necessarily reduce the drying time and improve product rehydration ability. Finally, although the enzyme treatment helped to better reduce the drying time, it resulted in a significant deterioration in the quality through loss of lipids in the reaction medium. It is the cellulase treatment (innermost attack the cell membrane and lipid-polysaccharide bonds) that provided the best reduction of drying time while maintaining the best quality of lipids

The cellulase pretreatment gave the lowest capacity of rehydration. The rehydrated cells were poorly defined and took a better comprehensive look after low concentration hydrolysis (CE4) and by masking the cellulase activity CiM and CiP (cellulase-pectinase and cellulase-macerozyme mixtures under cellulase conditions). The buffer control has the largest rehydration capacity, with cells better defined than the enzymatically pretreated samples.

Finally, note that the higher was the cell deformation, the lower was the lipid content. The latter reached its lowest value at high cellulase concentration. However optimization was obtained after a relatively short drying time coupled with lower cellulase concentration. The assumption of greater direct attack of membrane lipids at high cellulase concentration has been confirmed.

Preservation of carotenoids was correlated with the reduction of the drying time. The carotenoid concentration was especially important when the drying time was reduced. Cellulase acted on cambial and peri-cambial regions, which are rich in fibers, hence preserving the phloem region and showing higher contents in lipids and pigments. Unlike the physical pretreatments by freezing/thawing or by instant controlled pressure drop DIC, low cellular changes enhanced rehydration. PMi (pectinase and macerozyme under the conditions of the second) mixture gave the best rehydration ability and resulted in greater cell expansion. At high enzyme concentration, the cells broke down. Thus they got a better look at low concentration and phloem cell walls thickened and dilated. The enzyme mixtures allowed larger cells to expand more than those obtained with the buffer solution. Better extraction availability was achieved by succession of enzymes. Indeed, the more important the modification of cell walls, the higher the dissemination of carotenoids. For reduced drying time, greater pigment levels were obtained after dehydration; these values were higher than the blanched control.

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