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PROCESSING OF PRE-COOKED FROZEN BRUSSELS SPROUTS: HEAT TRANSFER MODELLING AS RELATED TO ENZYME INACTIVATION AND QUALITY STABILITY

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Graphical abstract
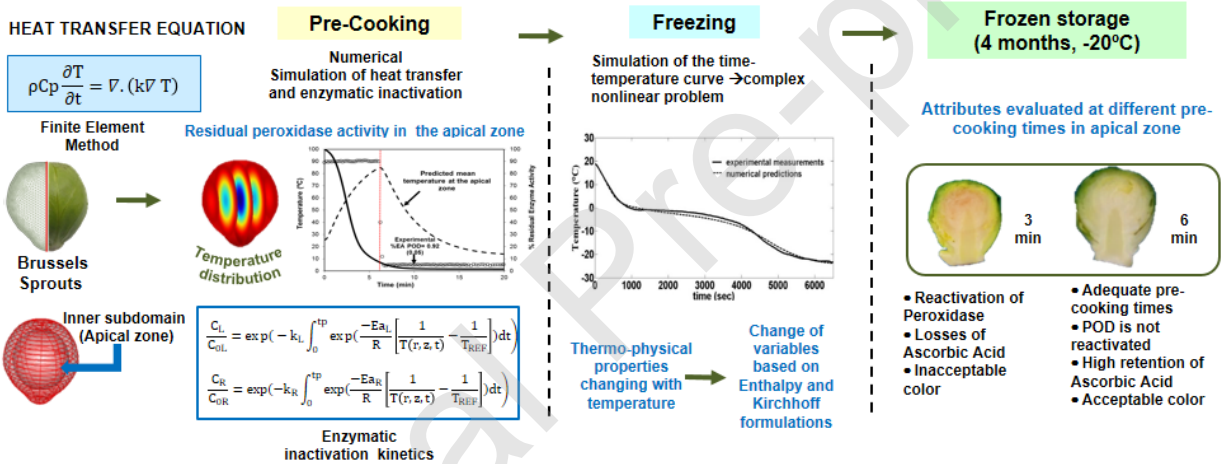
Highlights

- Heat transfer numerical simulation using finite elements for process optimization.
- The specific heat of the vegetable and heat transfer coefficients were experimentally determined
- Enzymatic inactivation kinetics of peroxidase was modeled in pre-cooking stage.
- Quality parameters (color, ascorbic acid content, sensory analysis) were described.
- Residual enzyme activity was related to quality after frozen storage.

ABSTRACT:

The finite element numerical method was applied to simulate heat transfer processes (heating, cooling and freezing) in irregular domains during the production of precooked frozen Brussels sprouts; the effect of temperature on thermo-physical properties in the partial differential

equation, and experimental heat transfer coefficients were considered. During precooking, heat transfer was coupled to the inactivation kinetics of thermo-labile and thermo-resistant fractions of peroxidase. The temperature distribution, and enzyme activity in the vegetable domain were modeled; these simulations were validated, obtaining a satisfactory agreement between the predicted values and: i) the experimental time-temperature curves, ii) the residual enzyme activity of peroxidase measured in the vegetable.

The simulation of the time-temperature curve during the freezing process is a complex nonlinear problem that requires knowledge of the thermo physical properties changing with temperature. A change of variables based on Enthalpy and Kirchhoff formulations, was adopted for the numerical solution. The effects of precooking times (3,6,10 minutes) on quality parameters of the frozen vegetable (Browing Index, textural properties, residual peroxidase activity, ascorbic acid content) were measured in the samples after freezing and storage during 4 months at -20ºC. The pre-cooking time was optimized to get acceptable quality parameters avoiding peroxidase reactivation during frozen storage.'

Keywords: Pre-cooked frozen vegetables; Peroxidase inactivation kinetics; Finite elements; Mathematical modeling; Process optimization.

Abbreviations

AA   Ascorbic Acid
Ea   Activation energy
EA   Enzymatic activity
EC   Enzyme Commission number
FEM   Finite Element Method
LOX   Lipoxygenase
PC   After the pre-cooking and cooling stage
PCFS   Precooked, frozen and stored
POD   Peroxidase
PPO   Polyphenoloxidase
RMSE   Root mean square error
SD   Standard Deviation

Nomenclature

C   Enzyme concentrations
CG   Global capacitance matrix
C_p Specific heat J/(kg ºC)
C_{pap} Apparent specific heat J/(kg ºC)
D   Diameter
E   Kirchhoff function (W/m)
FG   Global force vector
Fm   Maximum puncture force (N)

H   Volumetric enthalpy (J/m^3)

h   Surface heat transfer coefficient ($W/m^2\ ^oC$)

k   Thermal conductivity ($W/m\ ^oC$)

KG   Global conductance matrix

MG   Global convective matrix

N   Vector containing the shape functions

N_j Shape function j

S   Stiffness (N/mm)

t   Time (s)

T   Temperature (^oC)

T_{ext} External fluid temperature (^oC)

T_f Initial freezing temperature (^oC)

T_{ref} Reference temperature (^oC)

v   Fluid velocity (m/s)

x   Mass fraction

xb   Unfrozen water fraction (g of bound water/g of total mass)

Greeks letters

Δt Time interval (s)

ε   Porosity

ρ   Density (kg/m^3)

Ω   Domain

α   Isoenzyme fraction

β Volumetric expansion coefficient (K^{-1})

μ   Viscosity (Kg/m s)

Subscripts

0   Initial

b   Bound water

e   Element

e1   Border element

h   Mass fraction of ice

L   Thermo-labile isoenzyme

R   Thermo-resistant isoenzyme

w   Water

Superscripts

i   Component

s   Solid matrix

T   Transpose of a vector or a matrix

1. INTRODUCTION

Brussels sprouts (*Brassica olareacea* L. g*emmifera*) have numerous beneficial compounds such a considerable amount of vitamin C (ascorbic acid) which have made them attractive for consumers (Gebhardt and Thomas, 2002). These vegetables that belong to the mustard family (Brassicaceae) have the characteristic feature of containing naturally occurring glucosinolates. Foods containing glucosinolates, (cruciform vegetables) exhibit anti-inflammatory, antioxidant,

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chemoprotective, anticancer, and immune system–enhancing properties (Glade and Meguid, 2015; Herr et al., 2013).

Brussels sprouts exports totaled US$181.8 million in 2017; the value of exported Brussels sprouts increased by 0.9% from 2016 to 2017. The five countries that exported the highest dollar value worth of Brussels sprouts during 2017 were: Netherlands (40.9% of total Brussels sprouts exports), Mexico (28.9%), United States(12.8%), Belgium (5.2%), Canada (2%) (Workman, 2018).

The frozen vegetable market in 2017 was valued at $26,479.0 million, and is expected to reach a total of $38,845.7 million by 2025, registering a compound annual growth rate (CAGR) of 5.0% from 2018 to 2025 (Sumes and Deshmukh, 2018).

It is widely known that enzyme activity causes loss of quality and irreversible deterioration in raw and underblanched vegetables (Müftügil, 1984). These detrimental effects include discoloring, loss of flavor and development of off-odors during frozen storage.

In order to commercialize frozen Brussels sprouts a previous thermal treatment is needed in order to inactivate certain enzymes responsible for quality and nutrient losses. The precooking stage in Brussels sprouts involves a thermal treatment and a cooling process; afterwards they are submitted to freezing and cold storage.

Enzymes such as lipoxygenase, peroxidase and polyphenoloxidase are oxidoreductases present in vegetable tissues.

The presence of polyphenoloxidase (PPO; EC 1.14.18.1) produces browning that is associated to mechanical and physical stresses that occur during the post-harvest process and storage. PPO in presence of oxygen generates the oxidation of phenolic compounds such as *o*-diphenols; this reaction produces *o*-quinones, which subsequently polymerize with others *o*-quinones, phenolic substances, proteins, and/or amino acids to produce brown pigments (Murasaki-Aliberti et al., 2009). Since Brussels sprouts do not suffer mechanical stress, PPO is not usually selected as an index of the precooking stage.

Lipoxygenase (LOX; EC 1.13.11.12) catalyzes the bioxygenation of polyunsaturated fatty acids containing a cis,cis-1,4-pentadiene unit to form conjugated hydroperoxydienoic acids. It has been linked to the development of off-flavors and odor production, loss of pigments such as carotenes and chlorophylls, and destruction of essential fatty acids (Szymanowska et al., 2009).

Peroxidase (POD; EC 1.11.1.7), is a glycoprotein whose primary function is to oxidize phenolic compounds in presence of H_2O_2. POD is a hemeprotein that catalyzes the nonenzymatic, peroxidative degradation of unsaturated fatty acids yielding volatile and flavorful carbonyl

compounds that may contribute to oxidized flavor (Rodriguez-Saona, Barret and Selivonchjck,1995; Richardson and Hyslop, 1985). It has been reported that POD is responsible not only for off-flavors but also for browning discoloration and nutritional damage (Lagrimini et al., 1993). POD generates a dark coloring when present in a vegetable tissue and it is usually the enzyme of choice when trying to monitor the process, since it facilitates the quantification of the enzyme throughout the thermal processing. However, its complete inactivation may result in overblanching (Williams et al., 1986).

Pérez-Calderón et al. (2017) determined the thermal inactivation kinetic parameters of LOX and POD in Brussels sprouts and broccoli, showing a biphasic behavior with thermo-labile and thermo-resistant fractions of isoenzymes. In that work the authors reported that after thermal treatment and frozen storage, POD was the enzyme that exhibited the highest influence on quality deterioration of frozen Brussels sprouts.

The numerical simulation and optimization of each stage during the production of precooked frozen Brussels sprouts is useful for food engineers since it minimizes energy costs by accurately determining precooking and freezing times. The precooking and freezing processes can be described by heat transfer phenomena and the required input data are thermo-physical parameters as well as surface heat transfer coefficients.

The finite element method (FEM) is a numerical method that allows to obtain the time temperature curve at each point in the irregular domain (vegetable) as the precooking and freezing process evolves.

During the precooking stage (which includes thermal treatment and a cooling process) the coupling of the enzyme inactivation kinetics is of great importance in order to determine the degree of residual enzyme activity of peroxidase, and to correlate this information with quality parameters such as browning index and ascorbic acid content.

Literature reports concerning the numerical modeling of protein inactivation coupled with mass and energy transfer during skim milk production have been reported Jakulski et al., (2017). This model used CFD technique to establish the thermal degradation of components (proteins) during the process, determining the average concentration of protein in powders after spray drying.

There is scant published literature on numerical simulations of precooking and cooling of Brussels sprouts coupled with enzyme inactivation kinetics of POD considering the irregular shape of the vegetable.

Additionally, in the simulation of the freezing process the phase change transition of water into

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ice in the foodstuff leads to thermo-physical properties that are strongly temperature dependent, establishing a highly nonlinear mathematical problem. A change in the variables of the heat transfer partial differential equation in terms of Enthalpy and Kirchhoff formulation enables the determination of freezing times using finite elements. Previous publications showed that the numerical method led to an excellent convergence and a satisfactory agreement between experimental measurements and numerical predictions in different food systems (Dima et al., 2014; Santos et al., 2010; Santos and Lespinard 2011).

The objectives of the present work are: a) to determine experimentally the heat transfer coefficients and the specific heat of Brussels sprouts in a wide range of temperatures that include precooking , cooling and freezing stages; b) to numerically simulate by using the finite element method (FEM) the time-temperature curves during precooking stage (thermal treatment and cooling) of Brussels sprouts; c) to calculate the residual enzyme activity after precooking stage by coupling the enzyme inactivation kinetics of POD and the heat transfer program ; d) to validate the mathematical simulation with experimental time-temperature curves and residual POD enzyme activity; e) to simulate using FEM, the freezing stage of precooked Brussels sprouts by reformulating the heat transfer equation in terms of enthalpy and Kirchhoff functions; f) to determine the optimum conditions to obtain high quality frozen precooked Brussels sprouts, analyzing the effect of precooking times on residual POD activity, browning index and ascorbic acid content, after frozen storage.

2. MATERIALS AND METHODS

2.1 Vegetable samples and proximate composition

Brussels sprouts (*Brassica oleracea* L*. var gemmifera*) were purchased from agricultural producers of field-grown in the horticultural zone of La Plata city (Buenos Aires Province, Argentina) between the months of May and June 2017, 2018. The average weight of the sprout heads was 17.8 ± 0.9 g. Moisture content was determined by the indirect method of drying the sample in a vacuum oven LiTekvo model DZF-6030A (LiTekvo Instruments, Minhang District, Shanghai, China) at 70 ºC and reduced pressure of 100 mmHg to a constant dry weight (AOAC, 2010). The crude protein content was determined by Kjeldahl method (AOAC, 2010) using an appropriate factor for these vegetables (6.25) to convert total nitrogen to crude protein (Lisiewska and others 2009). Fat was determined using the Soxhlet extraction method with ethyl ether (AOAC, 2010). Ash content was obtained by calcination of the samples in a muffle at 550 °C for 8 hours (AOAC, 2010). Carbohydrates and fibers were determined by difference.

2.2 Thermal treatment and cooling of the vegetables. Precooking Stage

Several Brussels sprouts were heated in a stirred thermostatic bath Techne Model FTE-10DDC (Bibby-scientific Ltda, Stone Staffordshire, United Kingdom) recording temperature vs. time curves using thermocouples placed axially at the center inside the product. To avoid flotation the Brussels sprouts were placed in a canister attached to sinkers. The water temperature of the thermostatic bath was fixed at 80, 85, or 90 ^{o}C during each experiment.

After specific times (3, 6, or 10 minutes) the vegetables were immediately placed in an ice-water bath to stop the heating process. This is the usual process recommended by Barbosa-Cánovas et al. (2005). The thermocouples (Type T Copper/Constantan) used in the experiments were connected to an acquisition system (TESTO175, TESTO AG, Germany) where the thermal histories were stored.

To determine the coordinates of the thermocouples in the Brussels sprouts the vegetable was maintained at a fixed position vertically; a caliper was used to measure diameters and heights of all the vegetables to locate the central point. At the opposite end of the stem a small needle was inserted until reaching the half-height of sample. The needle enabled the thermocouple to be easily inserted in the Brussels sprout with minimum radial movement recording the temperature history in a defined point. When the experiment was finished the vegetable was cut and the position of the thermocouple was measured again to evaluate eventual displacements in the vegetables

2.3 POD inactivation during the precooking stage of vegetables

The enzyme activity measurements consist of mixing a vegetable extract with a specific substrate for each enzyme. Determination of the enzymatic activity (EA) of POD was assessed spectrophotometrically according to the method described by Morales-Blancas et al. (2002) by mixing 0.120 mL of enzyme extract with 3.5 mL of the substrate solution. Enzyme inactivation assays were carried out by immersing vegetable extracts in a thermostatic water bath Techne Model FTE-10DDC (Bibby-scientific Ltda, Stone Staffordshire, United Kingdom) at three different temperatures (75, 80, 90 °C), with contact times ranging between 50-400 s. Afterwards, samples were immediately immersed into an ice-water bath (0 °C) to stop the enzyme inactivation process, minimizing the cooling time.

The extract and substrate for POD inactivation assay was thoroughly described in Pérez-Calderón et al. (2017). Brussels sprouts showed a biphasic behavior of POD, which indicates that isoenzymes with different thermal stability are present within the vegetable. This biphasic

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behavior of the POD was also observed for Broccoli (Morales-Blancas et al., 2002).

The thermal inactivation kinetic parameters such as the rate constants for the labile (k_L) and resistant (k_R) fractions, at different temperatures and activation energies (Ea) were previously determined and reported by Pérez-Calderón et al. (2017).The reported parameters of POD thermal inactivation in Brussels sprouts were: Ea_L= 62.5 kJ/mol, (labile fraction) Ea_R= 56.3 kJ/mol (resistant fraction). The kinetic inactivation rate constants at 80°C were $k_L = 4.24 \times 10^2$ s^{-1}, $k_R = 5.37 \times 10^3$ s^{-1}, and the initial fraction of the heat-resistant enzyme was α_R = 25.02%

2.4 Freezing experiments and frozen storage

Several samples of precooked and cooled vegetables were placed in a tunnel freezer with circulating air in order to get similar operating conditions to those of the industry. The time-temperature curves at several points inside the product were recorded using a temperature acquisition device (Testo 175, Testo AG, Germany). The cooling air velocity in the tunnel freezer was measured using a hot wire anemometer (TSI model 1650). Afterwards the samples were maintained frozen during 4 months at -20 °C.

2.5 Mathematical Modeling and Numerical Simulations

The two-dimensional axial symmetric domain of the Brussels sprout was built from images of the samples using the Image Processing Toolbox, MATLAB 6.5 (MathWorks, Natick, Massachusetts), where the contour of the image was approximated with a B–Spline curve; this curve was transformed into a solid object.

Fig. 1a shows an example of a Brussels sprout irregular contour that was approximated as a solid of revolution having a symmetry plane at r=0, therefore cylindrical coordinates were implemented. Several Brussels sprouts were simulated and for each vegetable digital photographs were taken to obtain the irregular bidimensional domain; the range of diameters and heights were 29-39 mm and 30-41 mm, respectively.

By importing this geometry the software generated the discretization into finite elements (bidimensional mesh of the domain) that enables the numerical simulations. The finite element method was applied to simulate the thermal treatment (precooking and cooling stage) and freezing process of a 2D axial symmetric geometry.

A subdomain representing the apical zone of the vegetable was defined in order to simulate the average enzyme activity in the inner region that enables the comparison of predicted and experimental enzyme activities. The diameter of apical zone was 26.6 mm ±1.8 mm. Fig. 1b shows the 3D domain and the inner subdomain corresponding to the apical zone.

2.5.1 Precooking stage.

In the precooking stage that includes the thermal treatment and cooling of the vegetables before the freezing process, the thermo-physical properties do not suffer abrupt changes with temperature in the range 1-95 ºC. The partial differential equation that represents the energy transfer is as follows:

$$\rho C_p p \frac{\partial T}{\partial t} r = \frac{\partial}{\partial r}\left(kr\frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(kr\frac{\partial T}{\partial z}\right) \tag{1}$$

This equation is valid in the domain Ω being T the temperature, t the time of the process, ρ density, k the thermal conductivity, C_p the specific heat of the foodstuff, r and z the geometric coordinates.

The initial and boundary conditions are the following:

$$T = T_0 \qquad\qquad t = 0 \qquad \text{in } \Omega \tag{2}$$

$$\left(\frac{\partial T}{\partial z}.n_z + \frac{\partial T}{\partial r}.n_r\right)k = hj\left(T_{ext,j} - T\right) \qquad t \geq 0 \qquad \text{in } \partial\Omega_1 \tag{3}$$

$$\left(\frac{\partial T}{\partial z}.n_z + \frac{\partial T}{\partial r}.n_r\right)k = 0 \qquad t \geq 0 \qquad \text{in } \partial\Omega_2 \tag{4}$$

where $\partial\Omega_1$ corresponds to the convective interface and $\partial\Omega_2$ to the symmetry axis, n_r and n_z are normal outward vector components at the radial and axial axis, respectively, n is the outward-pointing normal unit vector at the surface. T_0 is the initial temperature, h_j is the surface heat transfer coefficient, when j=1 the h corresponds to the heating stage (stirred hot water bath) and when j=2 the h corresponds to the cooling stage (a stagnant ice-water bath), $T_{ext,j}$ is the external fluid temperature (j=1 temperature of the stirred hot water bath, j=2 temperature of the ice-water bath). The T_{ext} and h change simultaneously when the vegetable finishes the thermal treatment and enters the cooling stage at a specific time period (3, 6 or 10 minutes).

The numerical model was solved using the COMSOL Multiphysics 3.5b software incorporating the thermo-physical properties, surface heat transfer coefficients, external fluid temperatures. Specific sentences were coded in Matlab language inside COMSOL environment in order to describe the consecutive stages of the precooking process (thermal treatment and cooling

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stage). These sentences consist of programing the simulation of the thermal treatment which occurs during a specific period of time at an external temperature (ranges of T_{ext}= 80-90 ºC) and with an h value that corresponds to a stirred thermostatic water bath (forced convection). After these pre-cooking times (3, 6 or 10 minutes) the program switched to the cooling process which requires a new Text (ranges of T_{ext}= 0-5 ºC) with an h value for the cooling bath (water-ice), which is under natural convection conditions. The program allowed to describe the temperature curve at each point of the vegetable during heating and subsequent cooling, considering non-uniform temperature distribution inside the vegetable when it starts to cool down.

2.5.2 Coupling enzyme inactivation kinetics with the heat transfer numerical program

First order kinetics, representing the thermal inactivation rate of each iso-enzyme (heat labile and heat resistant) is described as follows:

$$\frac{dC_L}{dt} = -k_L C_L \tag{5}$$

$$\frac{dC_R}{dt} = -k_R C_R \tag{6}$$

where C_L y C_R are the enzyme concentrations of the heat labile and heat resistant fraction, respectively, and k_L and k_R are the heat labile and heat resistant reaction constants, respectively.

The concentration of the enzyme at t=0 and at a given time t can be written as follows:

$$C_0 = C_{0R} + C_{0L} \tag{7}$$

$$C = C_R + C_L \tag{8}$$

where C_{oL} and C_{oR} are the initial heat labile and heat resistant enzyme activities, respectively at time t :

$$\frac{C}{C_0} = \frac{C_R+C_L}{C_{0R}+C_{0L}} = \left(\frac{C_L}{C_{0L}}\right)\frac{C_{0L}}{C_{0R}+C_{0L}} + \left(\frac{C_R}{C_{0R}}\right)\frac{C_{0R}}{C_{0R}+C_{0L}} = \left(\frac{C_L}{C_{0L}}\right)(1-\alpha_R) + \left(\frac{C_R}{C_{0R}}\right)(\alpha_R) \tag{9}$$

By integrating Eq. (5)-(6) and using the Arrhenius equations the following is obtained:

$$\frac{C_L}{C_{0L}} = \exp(-k_L \int_0^{tp} \exp(\frac{-Ea_L}{R}\left[\frac{1}{T(r,z,t)} - \frac{1}{T_{REF}}\right])dt) \tag{10}$$

$$\frac{C_R}{C_{0R}} = \exp(-k_R \int_0^{tp} \exp(\frac{-Ea_R}{R}\left[\frac{1}{T(r,z,t)} - \frac{1}{T_{REF}}\right])dt) \tag{11}$$

where Ea is the activation energy, k is the reaction constant; subscripts R (resistant) or L (labile) correspond to the resistant or labile fractions. T_{REF} is the reference temperature given in (K) which in this work was T_{REF} =353.15 K, R is the gas constant (8.31439J/mol K). T(r,z,t) is the temperature (K) as a function of time, at a given position inside the vegetable; this function is calculated using the numerical finite element program for all the time steps and radial and axial positions. By incorporating T(r,z,t) the temperature profile can be associated with an enzyme activity distribution in the Brussels sprout as the thermal treatment evolves.

The average enzyme activity in the whole Brussels sprout was calculated using the following equations:

$$\overline{C_L} = \frac{\int_v C_L(r,z,t)dV}{\int_v dV} \tag{12}$$

$$\overline{C_R} = \frac{\int_v C_R(r,z,t)dV}{\int_v dV} \tag{13}$$

The average overall enzyme activity is given by:

$$\overline{C} = \overline{C_R} + \overline{C_L} \tag{14}$$

by applying Eqs. (12)-(13) the average enzyme activity for the heat labile and heat resistant fractions were calculated at any time during the precooking stage. All the integrals were calculated numerically; time discretization was implemented using trapezoidal rule; for the volume integrals the quadrature for the standard triangular elements was used (Hughes, 1987)

2.5.3 Freezing stage

During the phase change transition in the freezing process the thermo-physical properties are strongly dependent on temperature. This constitutes a highly non-linear mathematical problem. The heat conduction equation in Cartesian coordinates with phase change transition can be written as follows:

$$\rho(T)c_p(T)\frac{\partial T}{\partial t}r = \frac{\partial}{\partial r}\left(k(T)\ r\frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k(T)\ r\frac{\partial T}{\partial z}\right) \quad \text{in} \quad \Omega \tag{15}$$

k(T) is the thermal conductivity, $C_p(T)$ the apparent specific heat, and $\rho(T)$ the density (Carslaw and Jaeger, 1959). The boundary and initial conditions are:

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$$\left(\frac{\partial T}{\partial z} \cdot n_z + \frac{\partial T}{\partial r} \cdot n_r\right) k = h\ (T_{ext} - T) \qquad t \geq 0 \qquad in \qquad \partial\Omega_1 \qquad (16)$$

$$\left(\frac{\partial T}{\partial z} \cdot n_z + \frac{\partial T}{\partial r} \cdot n_r\right) k(T) = 0 \qquad t \geq 0 \qquad in \qquad \partial\Omega_2 \qquad (17)$$

$$T = T_0 \qquad t = 0 \qquad in \qquad \Omega \qquad (18)$$

where n_r and n_z are the normal unit vector components in cylindrical coordinates , h in this case corresponds to the surface heat transfer coefficient of the tunnel freezer.

By performing the following change of variables:

$$H(T) = \int_{T^*}^{T} \rho(T).C_p(T)dT \qquad (19)$$

$$E(T) = \int_{T^*}^{T} k(T)dT \qquad (20)$$

where H is defined as the volumetric specific enthalpy (Comini et al., 1974), E is the Kirchhoff function that represents the thermal conductivity integral (Comini et al., 1974; Fikiin, 1996), and T* is a reference temperature that corresponds to a zero value of H and E. These functions are monotonic increasing and continuous because C_p, k, and ρ are positive and bounded. Consequently, there exists a one to one mapping between the enthalpy/Kirchhoff values and the temperature variable.

By combining Eq. (15), Eq. (19) and Eq. (20), and the initial and boundary conditions represented by Eq. (16)-(18) the following equations were obtained:

$$\frac{dH}{dt} = \nabla^2 E \qquad (21)$$

With initial and boundary conditions as follows:

$$H = H_0 \qquad in \qquad \Omega \qquad a\ t=0 \qquad (22)$$

$$-(\nabla E \cdot n) = h(T - T_{ext}) \qquad in \qquad \partial\Omega_1 \qquad a\ t>0 \qquad (23)$$

$$-\nabla E \cdot n = 0 \qquad in \qquad \partial\Omega_2 \qquad a\ t>0 \qquad (24)$$

Applying the finite element technique the following system of ordinary differential equations was solved:

$$CG \cdot \frac{dH}{dt} + FG \cdot T(H) + KG \cdot E(H) = m \qquad (25)$$

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where:

$$CG = \sum_{e=1}^{ne} \int_{\Omega_e} (N^T r N) d\, \Omega_e \qquad \text{is the global capacitance matrix}$$

$$KG = \sum_{e=1}^{ne} \int_{\Omega_e} (B^T r B) d\, \Omega_e \qquad \text{is the global conductive matrix}$$

$$FG = \sum_{s=1}^{ns} \int_{\Omega_s} (N^T h r N) d\, \delta\Omega_s \qquad \text{is the global convection matrix}$$

$$m = \sum_{s=1}^{ns} \int_{\partial\Omega_s} (N^T h r T_{ext}) d\, \delta\Omega_s \qquad \text{is the global thermal load vector}$$

H, E, and T are the nodal values of enthalpy, the Kirchhoff function, and temperature, respectively. N is the vector of dimensions [1x4] containing the shape functions (Nj) with j=1-4 for the reference tetrahedron element, Nt is the transpose vector (dimension 4x1), e is the total number of elements, e1 is the number of boundary elements, Ω_e is the integration domain, $\partial\Omega_s$ is the boundary integration domain. The matrix B (dimension 3x4) is defined as follows:

$$B = \begin{bmatrix} N_{1r} & N_{2r} & N_{3r} \\ N_{1z} & N_{2z} & N_{3z} \end{bmatrix} \text{ where } N_{ir} = \frac{\partial N_i}{\partial r}, N_{iz} = \frac{\partial N_i}{\partial z} \text{ for } i=1-3$$

This system was solved using the standard Matlab 6.5 routines ODE (Ordinary Differential Equations) which minimizes the computational efforts of the numerical algorithm.

2.6 Thermo-physical properties of the Brussels sprouts

The thermophysical properties of the vegetable samples during the precooking stage were considered constant in the temperature range 1-90⁰C. The presence of air in the vegetable was considered and the air volume fraction (ε) was determined. This air fraction was used to evaluate the effective thermal conductivity by using Maxwell equation for heterogeneous systems. Freezing involves the phase change transition of water into ice as time elapses. The dependence of the thermophysical properties. $k(T)$, $\rho(T)$ and $C_p(T)$ are necessary to simulate the heat transfer inside the vegetable as time elapses.

2.6.1 Density

The density (ρ) of unfrozen Brussels sprouts was determined by measuring the mass using a Mettler Toledo AB-204 (Switzerland) laboratory balance. Afterwards the vegetable was submerged in a laboratory-graduated cylinder filled with water and the displaced volume

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corresponded to the volume of the solid object. Density was calculated by the relationship mass/volume (in triplicate). Since the vegetable contains air, the sample was wrapped in aluminum foil attached to a sinker in order to fully immerse the object; the volume of the foil and lead were measured for calculation purposes.

In porous materials with porosity ε (air volume / total volume), the density can be described by the following equation:

$$\frac{(1-\varepsilon)}{\rho} = \sum_{i=1}^{n} \frac{x_i}{\rho_i} = \frac{1}{\rho_s} \tag{26}$$

Where: ρ is the global density of the vegetable containing air, ρ_i is the density of each component i (water, carbohydrates, ash, protein, and lipids) that constitutes the solid matrix. The density of the solid matrix (ρ_s) was calculated using the model proposed by Choi and Okos (1986):

$$\rho_s(T) = \frac{1}{\sum \frac{x_i}{\rho_i}} \tag{27}$$

where ρ_s (T) is the density of the solid as a function of temperature and ρ_i is the density of component, where i corresponds to water, carbohydrates, ash, lipids, or proteins.

The fraction "x_i" corresponds to the mass fraction of each component. Choi and Okos (1986) proposed the following expressions for the determination of density of each component:

ρ water = 997.18 + 3.1439 × 10^{-3} T − 3.7574 × 10^{-3} T^2

ρ carbohydrates = 1599.1 − 0.31046 T

ρ protein = 1330 − 0.5184 T

ρ lipid = 925.59 − 0.41757 T

ρ ash = 2423.8 − 0.28063 T

where the ρ are expressed in kg/m^3 T in °C in the range −40 and 150°C.

The porosity ε of the vegetable was calculated from Eq. 26 by knowing ρ and ρ_s values. The ρ as a function of T in the freezing range temperatures was estimated considering the ice content that is formed as the temperature decreases. The ρ of ice was calculated as follows (Choi and Okos, 1986): ρ ice = 916.89-0.13071× 10^{-1}T.

2.6.2 Specific heat

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Specific heat of Brussels sprouts in the entire range of temperatures (thermal treatment and freezing process) and the latent heat of ice melting were measured by using a Differential Scanning Calorimeter (DSC) model Q100 TA Instruments, (New Castle, Delaware, USA). Samples were enclosed in sealed aluminum pans. An empty pan was used as a reference sample. Pans were heated at 2°C/min from -50 to 100 °C with isothermal periods at the initial and final temperatures. In order to measure the specific heat three scans were carried out: one for the sample, one for a standard (sapphire), and one for the empty sample pan. In these scans the reference holder contains an empty pan. Distilled water was also scanned using the same program to verify the equipment calibration. The specific heat was calculated following the ASTM E1269-11 (2011) procedure and McNaughton and Mortimer (1975) recommendations.

The latent heat of melting (ΔH_m) was determined as indicated by Roos (1986) by integrating the peak of the melting curve; this value was used to estimate the unfrozen water fraction in the food material. The temperature integration limits of the peak were chosen when a clear separation between curve and base line was detected.

Unfrozen water fraction (xb), was considered as the difference between total water content and the amount of frozen water in the deep frozen material. The fraction of frozen water was obtained from the ratio between the latent heat of melting determined for the food material and the heat of melting of pure water.

The ice content as a function temperature (at $T < T_f$) was estimated using the equation proposed by Miles et al. (1983):

$$x_h = (x_w - x_b)\left(1 - \frac{T_f}{T}\right) \tag{28}$$

where x_h is the mass fraction of ice, T_f is the initial melting point of the product, x_w is the mass fraction of water in the foodstuff, and x_b is the mass fraction of bound water.

2.6.3 Thermal conductivity

To determine the effective thermal conductivity (k) of the vegetable (heterogeneous system containing air) during precooking stage, Maxwell equation was applied using air as the disperse phase in the solid continuous phase (Rahman and Said Al-Saidi, 2009; Pietrak and Wisniewski, 2015).

$$k = kc\left(\frac{2kc + kd - 2\varepsilon(kc - kd)}{2kc + kd + \varepsilon(kc - kd)}\right) \tag{29}$$

where kc corresponds to the thermal conductivity of the continuous phase (solid matrix), kd is the thermal conductivity of the disperse phase (air) and ε the porosity. The thermal conductivity of the solid matrix (continuous phase) kc of the Brussels sprout was estimated using the following equation (Choi y Okos, 1986):

$$kc(T) = \sum x_i^{v} \cdot k_i(T) \tag{30}$$

where: k_i is the thermal conductivity of component i which corresponds to the proximate composition of the vegetable (water, carbohydrates, ash, protein, and lipids); x_i^{v} corresponds to the volume fraction of each component. In order to calculate the k of each component the following equations dependent on the temperature T ($^{\circ}$C) were used:

k water $= 0.57109 + 1.7625 \times 10^{-3}T - 6.7036 \times 10^{-6}T^2$
k carbohydrates $= 0.20141 + 1.3874 \times 10^{-3}T - 4.3312 \times 10^{-6}T^2$
k protein $= 0.17881 + 1.1958 \times 10^{-3}T - 2.7178 \times 10^{-6}T^2$
k lipids $= 0.18071 - 2.7604 \times 10^{-3}T - 1.7749 \times 10^{-7}T^2$
k ash $= 0.32961 + 1.4011 \times 10^{-3}T - 2.9069 \times 10^{-6}T^2$

The conductivity as a function of temperature during the freezing stage was estimated considering the ice that is formed as temperature decreases. The conductivity of ice as a function of temperature was determined as follows (Choi and Okos, 1986): kice $= 2.2196 - 6.2489 \times 10^{-3}T + 1.0154 \times 10^{-4}T^2$

2.7. Heat transfer coefficients (h)

2.7.1 Precooking stage: Thermal treatment and cooling in ice-water bath
To estimate the heat transfer coefficient, an aluminum spherical object was built using similar dimensions as the Brussels sprouts (Radius= 0.025 m) and immersed in the same water bath, used for the experiments with the vegetables (temperature range: 80-90 $^{\circ}$C). A thermocouple was inserted at the center of object to sense the time-temperature history during the thermal treatment in the stirred thermostatic bath and the ice-water bath. The following thermo-physical properties of aluminum were considered: k = 178.35 W/m K, ρ = 2700 kg/m^3, C_p = 908.53 J/kg K. The lumped heat transfer method was applied based on the resolution of the following ordinary differential equation, which assures an almost instantaneous uniform temperature profile due to the high thermal conductivity of the metal:

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$$\rho C_p V \frac{dT}{dt} = hA \, (\, Ts - T_\infty) \tag{31}$$

where: ρ is the density, C_p the specific heat, V the volume, A the surface of the metal sphere. Ts is the temperature of the solid and T_∞ the external temperature of the bath (stirred hot water or water-ice bath).

The analytical solution of Eq. (31) (Sablani, 2009) is as follows:

$$\frac{T - T_\infty}{Ti - T_\infty} = \exp \left[\frac{-hA \, t}{\rho \, V \, C_p} \right] \tag{32}$$

where Ti is the initial temperature of the sphere. After applying the natural logarithm of the experimental dimensionless temperature a linear regression as a function of time allowed to obtain the h value of the system from the slope of the straight line.

Moreover, the h values were calculated using forced convection literature correlations for spheres during the thermal treatment. The following dimensionless parameters were estimated:

Reynolds: $Re = \frac{\rho v D}{\mu}$ (33)

Nusselt: $Nu = \frac{hD}{k}$ (34)

Prandtl: $Pr = \frac{C_p \mu}{k}$ (35)

where D= diameter (m); k= thermal conductivity of the external fluid (water) (W/m K); ρ= density (Kg/m^3); v= fluid velocity (m/s); μ=viscosity (Kg/m s); C_p= specific heat (J/Kg K). For the cooling stage, natural convection literature correlations were used where the Nusselt number depends on the Grashof (Gr) and Prandtl numbers:

$$Gr = \frac{D^3 \rho^2 g \beta \Delta T}{\mu^2} \tag{36}$$

where β= is the volumetric expansion coefficient (K^{-1})

2.7.2 Freezing stage

In order to estimate the h value (W/m^2 K) in a tunnel freezer the correlation proposed by Earle (1988) was used, in which the measurement of the cooling air velocity is required. For air

velocities v < 5m/s the equation is:

$$h = 5.7 + 3.9 \cdot v \tag{37}$$

where v is the air velocity in m/s. The air velocity of the cooling air was measured using a hot wire anemometer (TSI model 1650). The tunnel freezer used in the experiments was pilot-scale.

2.8 Initial freezing temperature

The initial freezing temperature T_f of the vegetable was experimentally determined from the freezing curves. T_f was measured by using thermocouples inserted at the core of the vegetables by applying the tangent method (Fennema et al., 1973).

2.9 Enzyme activity and quality attributes of Brussels sprouts during frozen storage

2.9.1 Enzyme activity

The enzyme activity (EA) of POD after the pre-cooking and cooling stage (PC; samples), and in precooked, frozen and stored samples (PCFS) was measured at the apical zone of the Brussels sprout. This zone corresponds to an inner spherical sample of the vegetable (Fig. 1b).The %EA was experimentally measured according to the technique described in Section 2.3 and calculated as the ratio between the EA of the sample submitted to a specific thermal processing time and that of the raw sample. The EA was related to the quality attributes such as color, texture, and ascorbic acid content.

2.9.2 Texture

The texture was evaluated by a puncture test performed using a texturometer TA-XT2i (Stable Micro Systems Ltd., Godalming, Surrey, UK). The test was carried out using a 2 mm diameter stainless steel needle probe (P/2), with a velocity of 0.5 mm.s^{-1} and the puncture reached up to 50% of the total height of the sample. From the force deformation curve the following parameters were obtained: (i) maximum puncture force in N (Fm) which corresponds to the first peak and it is related to sample firmness (Olivera et al., 2008), (ii) the slope of force-deformation curve for the processed sample which represents the sample stiffness in N/mm (S) (Nguyen et al., 2010). Textural measurements were performed using six replicates to minimize inherent sample-to-sample biological variations.

The determinations were expressed as relative values (%) calculated as the ratio between texture parameters (Fm and S) of PC and PCFS samples with respect to the raw vegetable.

2.9.3 Color

The surface color of Brussels sprouts at the core was determined in PC and PCFS samples. The change of color was determined using a Minolta colorimeter CR 400 Series (Konica, Osaka,Japan). The CIEL*a*b* scale was used to analyze of results of lightness (L*) and chromaticity parameters: change of the red-green (a* parameter) and yellow-blue (b* parameter). These results were used to determine the Browning Index (%BI) according to Eqs. 37 and 38, which represents the purity of brown color.

$$\%BI = \frac{[100(x-0.31)]}{0.172} \tag{38}$$

$$\text{where } X = \frac{(a^*+1.75\,L^*)}{(5.645\,L^*+a^*-3.012\,b^*)} \tag{39}$$

2.9.4 Ascorbic Acid

Ascorbic Acid (AA) content of fresh vegetables, after the pre-cooking stage (PC samples), and after frozen storage (PCFS samples) were measured at the apical zone, to evaluate the effect of processing conditions on the AA loss. The AA was determined using liquid chromatographic method in a HPLC Waters equipment (Waters, Milford, MA, USA) Waters 1525 Binary HPLC Pump; Detector UV-Waters 2998 fitted with a photo diode array detector and a RP-C18 column 4.6x150 mm, 5 µm (Waters, Milford, MA, USA). This determination consisted of an isocratic elution procedure with UV–visible detection at 245 nm. The samples were grounded taking 0.6 g and mixed with 2.5 ml of an aqueous solution containing 5 g/100 mL metaphosphoric acid (Sigma Aldrich, USA) during 15 min and later centrifuged for 10 min at 2000 rpm in centrifuge Rolco CM 2036 (Rolco, Argentina). Supernatants were collected and filtered through a 0.45-µm Millipore filter (Millipore Corporation, Billerica, MA, USA). The extracts were stored at 4ºC until use.

The employed mobile phase was 0.5 g/100 mL of metaphosphoric acid /acetonitrile (93:7) (Nojavan et al., 2008). A standard ascorbic acid (Sigma Aldrich, USA) solution was used for identification and quantification. An aliquot (20 µL) of the extract was used for HPLC analysis, the chromatograms were analyzed using the software Peakfit v.4. 12.00 (SeaSolve Software Inc, Framningham, MA, USA). The reported results correspond to the mean of 2 determinations for each sample. The residual AA was determined as AA/AA_o, where AA is the ascorbic acid in precooked vegetables after frozen storage (PCFS) and AA_0 the initial AA content in the fresh vegetable.

2.10 Sensory Analysis

A sensory evaluation with a panel of 30 consumers was carried out using a 9 point hedonic scale to determine the degree of liking of the final product (Peryam and Girardot, 1952). The acceptability test was applied and the scale assigned one for "dislike extremely" and nine for "like extremely". Analysis of variance followed by comparisons of means for more than two products (Lawless and Heymann, 2010) was applied to analyze the information. Sensory analysis was based on the evaluation of four samples: F(cooked fresh vegetable, control sample) , PCFS (samples precooked during 3, 6 or 10 minutes at 90ºC , frozen and stored 4 months at -20ºC). The sample F consisted in the fresh vegetable which was washed, rinsed and cooked in water during 10 minutes at 90ºC before consumption. The frozen stored samples (PCFS) were heated similarly at 90°C during 3 min. before consumption. All the samples were analyzed for their global acceptability, evaluating the following attributes: general acceptability, interior color, texture and aroma. Samples were presented in disposable trays that contained the three samples.

2.11 Statistical Analysis

In order to estimate the goodness of fit of the numerical simulation model, experimental and predicted temperatures were considered in each stage (pre-cooking and freezing) and the root mean square error (RMSE) was determined as follows:

$$RMSE = \sqrt{\frac{\Sigma(T_{exp} - T_{pred})^2}{N}} \tag{40}$$

The software Origin Pro 8 (Origin Lab Corporation, Northampon, Ma., U.S.A.) was used for analysis of variance (ANOVA). The analyses were carried out to assess significant differences between the samples; means were compared by a Fisher LSD test using a 95% confidence level (significant difference, P≤0.05).

3.   RESULTS AND DISCUSSION

3.1 Thermo-physical properties and heat transfer coefficients during the precooking stage.

The average proximal composition values of the vegetable and their Standard Deviation (between parentheses) were: 83.54% (SD=0.55) moisture, 3.20% (SD=0.09) protein, 0.36%

(SD=0.02) fat, 1.19 % (SD=0.02) ash, and 11.74% carbohydrate calculated by difference.

The experimental density value of the Brussels sprouts was ρ=880 kg/m^3 (SD=12). The density of the solid matrix was calculated based on the proximal composition using Eq.(27) being 1050 kg/m^3. By applying Eq (26) a porosity (ε) of 16% was obtained.

The thermal conductivity of the solid continuous matrix (without air) kc=0.55 W/m K was estimated using Eq.(30). Using the Maxwell equation (Eq. 29) with a value of porosity ε =16%, and a thermal conductivity of air at 50ºC of 0.02735 W/m K, a value of k= 0.50 W/m K was obtained for the vegetable sample. An average value of the specific heat, C_p=3.86 x10^3 J/kg K was obtained by DSC for the temperature range 1-100 ºC; in this temperature range C_p is almost constant .

The heat transfer coefficients (h) in both stages: a) thermal treatment in a stirred thermostatic bath (forced convection condition) and b) cooling in an ice-water bath (natural convection condition) were determined using an aluminum sphere. In the case of stage a), the average h value was 2262 W/m^{2}K (SD=285.02). Additionally, literature correlations were used to compare these h values (Earle 1988; Nesvadba 2008). The equation used during the thermal treatment for spheres immersed in a fluid under forced convection conditions was:

$$Nu=2+0.6Re^{1/2}Pr^{1/3} \tag{41}$$

The aluminum sphere (D=0.05m) had an initial temperature of 20 ºC and the hot water 90ºC, therefore the film temperature was T_f= 55ºC; the properties of water were considered at T_f (Dinçer and Zamfirescu, 2015). The Prandtl number at a temperature T_f calculated by using Eq. 35 was Pr=3.4. The velocity of the water in the stirred bath was 35 cm/s (SD=4 cm/s). An average Re number was calculated by Eq.(33) obtaining Re=3.74x10^4. Finally the Nu number was calculated using Eq. (34), resulting in 176.92 and h=2300 W/m^{2}K. Applying the correlation (Eq.41) for Brussels sprouts with an average diameter of 0.036m and using literature thermo-physical properties of water the h value was h=2414 W/m^{2}K.

In the case of cooling (the metal sphere was immersed in the ice-water bath) the average h value obtained by using Eq.(32) was h= 494 W/m^{2}K (SD= 50). Moreover, a dimensionless correlation for spheres under natural convection conditions was applied:

$$Nu = 2 + 0.6 \, Gr^{1/4} \, Pr^{1/3} \tag{42}$$

The dimensionless numbers calculated were Gr= 2.8x10^6; Pr= 3.55 and Nu=39, resulting in a

value of h=500 W/m²K, which is close to that obtained experimentally. Using the dimensions of the Brussels sprouts with the natural convection correlation (D=0.036m) the h value obtained was h= 567 W/m²K for the ice-water bath.

3.2 Numerical simulation of thermal histories in the precooking process. Model validation

The 3D object was built by revolution of the bidimensional domain (Fig.1a,b). A triangular mesh using Lagrange elements of order 2 was applied to discretize the domain. The number of elements that constituted the mesh for the whole vegetable was 4664 with a total number of nodal points of 2437. The apical region was discretized using 1786 elements. The use of finer mesh showed no significant effect on the accuracy of the solution. The time discretization scheme used was a Backward Euler Differentiation (minimum order 1 and maximum order 5) with a tuning step having a maximum of 0.1 s and a minimum initial starting value of 0.001s. The absolute and relative tolerances for each integration step were 0.001 and 0.01, respectively.

Operating conditions were modeled, considering two different h values for heating and cooling. This allowed to obtain the time-temperature profile in the entire domain of the vegetable. Fig.2 a) shows one the analyzed cases of the predicted and experimental time-temperature curves during the precooking stage corresponding to a thermocouple position r=0, z= 21.3 mm. Vertical dashed line indicates the time when the thermal treatment stopped and the vegetable was cooled in the ice-water bath. In this figure the experimental conditions were: temperature of the stirred thermostatic bath 90°C, thermal processing time: 6 min; Heat transfer coefficient h =2200 W/m²K for the stirred hot water bath and h =580 W/m²K for the ice-water bath.   An excellent agreement can be observed indicating that the program satisfactorily simulates the time temperature curve of the precooking process. For three replicates RMSE calculated were 1.85, 2.55 y 2.33 °C. As can be observed in the thermal history during the cooling stage, the temperature at the analyzed point increased even though the vegetable was submitted to a low external fluid temperature during the cooling stage.

The predicted mean temperature ($\overline{T}$) of the inner apical zone (interior subdomain) was calculated as follows:

$$\overline{T} = \frac{\int T(r,z,t)dV}{\int dV} \tag{43}$$

where T (r,z,t) are the temperatures at each point of the apical zone. Fig. 2b shows the mean

temperature $\overline{T}$ (dashed line) obtained by applying Eq.(43).

Fig. 2c shows the predicted temperature distributions in different sections of the whole vegetable during the thermal treatment in the stirred hot water bath during 6 min and the subsequent cooling process.

3.3. Coupling the heat transfer process with enzyme inactivation kinetics and comparison of predicted values with enzyme activity measurements

During heating and cooling stages enzyme inactivation occurs; this amount of inactivated enzyme should be quantified to avoid overcooking and losses of thermolabile compounds such as ascorbic acid. The enzyme inactivation kinetics was coupled to the main heat transfer program allowing to estimate the residual enzyme activity at each position and time during the process. In order to compare the experimental and predicted enzyme activity of the Brussels sprout at the apical zone, the mean temperature calculated by Eq. (43) was used. The average enzyme activity at the apical zone was calculated in this subdomain as a function of the mean temperature ($\overline{T}$) during the thermal treatment and cooling stages.

Fig.2b shows the numerical predictions of the average POD enzyme activity and the $\overline{T}$ vs. time curve obtained for the apical zone. As can be observed during the cooling process the apical region remained at a high temperature for an additional time, increasing the inactivation of POD. Table 1 shows the effect of thermal treatment times (3, 6, 10 minutes) on experimental and predicted Enzyme activity (%EA) of POD in the apical and external regions of the Brussels sprouts.

The enzyme activity (%EA) measurements required the extraction of POD in a finite volume of vegetable, because it cannot be assigned to a single point such as in the case of temperature.

Therefore the apical zone was defined as the internal sphere in the vegetable where the %EA was evaluated (Fig. 1b), corresponding the external zone to the outer region. Differences between experimental and predicted values of %EA can be justified considering two important reasons: i) the reported data of %EA correspond to volumetric average values and ii) the biological variability of the tested samples.

Fig. 3 shows the numerical predictions for the enzyme inactivation of the thermo-labile and thermo-resistant isoenzymes within the entire vegetable as a function of time for a bath temperature of 90ºC. From these results, the residual enzyme activity after the precooking process was determined, being this value the initial % EA of Brussels sprouts for the freezing process.

3.4 Numerical simulation of the freezing process.

The thermo-physical properties C_p and k of the vegetable in the entire range of temperatures are shown in Fig. 4. These data allowed to estimate the Kirchhoff and Enthalpy functions. The initial freezing temperature was -1⁰C, following the procedure reported by Fennema et al. (1973). The bound water fraction in wet basis obtained using the DSC thermogram was x_b=0.1843 (g of bound water/g of total mass).

The volumetric specific heat was calculated by multiplying the $\rho(T)$ by the $C_p(T)$, and further numerically integrated using Eq. (19) to obtain the enthalpy function H(T) (Fig 5 a). The same procedure was carried out with k(T) in order to obtain the Kirchhoff function (E(T)) according to Eq. (20) (Fig 5 b); Fig. 5 c) shows the function E(H).

The surface heat transfer coefficient in the tunnel freezer was in the range 15-20 W/m²K (the average air flow velocity was 2.5m/s). For Brussels sprouts an h value of 19 W/m²K gave the best fit to the experimental measurements.

In order to validate the model, the experimental time-temperature curves were compared with the numerical predictions (in triplicate). Fig. 5d shows the numerical results and experimental measurements indicating the excellent performance of the numerical code to simulate the freezing process. The RMSE calculated was 0.965°C. Numerical simulation allows to determine time temperature histories and freezing times as a function of initial temperature of the sample, refrigerant fluid temperature and h values.

3.5 Quality attributes after thermal treatment and frozen storage

3.5.1 Texture analysis

Maximum force (Fm) and stiffness (S) values for the raw vegetable were 26.80N, 12.18 N/mm respectively. Relative texture values with reference to the raw vegetable, considering the tested pre-cooking times, for PC (precooked samples) and PCFS (precooked, frozen and stored samples) are shown in Table 2. Both relative measurements, the percent maximum puncture force (%Fm) and relative stiffness (%S) decreased after each stage of the process for each treatment time (3, 6, 10 min) in all the samples. Significant differences are observed among the samples submitted to the tested heating times (P≤0.05). During thermal processing and freezing, a gradual tissue softening was produced and these irreversible changes are caused by tissue fractures and mechanical damage of the cell walls generating water leaching (Kidmose and Martens, 1999; Paciulli et al., 2015).

3.5.2. Sensory analysis, enzymatic browning and ascorbic acid content after frozen storage as affected by residual POD enzyme activity

The residual enzyme activity of POD at the apical zone of the Brussels sprouts was determined in PC and PCFS samples; three precooking times at 90⁰C (3, 6, 10 min) were evaluated in each case. Fig. 6a shows the image of the apical region of Brussels sprouts corresponding to: frozen untreated samples (FU) and PCFS that were thermal treated during 3,6 and 10 min

FU and the PCFS samples with an insufficient thermal treatment time (3 min) (Fig. 6a) showed the formation of a brown color at the center of the vegetable that is not accepted by the consumer; therefore this short thermal treatment was excluded from the sensory analysis.

Experiments carried out with fresh vegetables that were frozen without a precooking stage (FU) showed a 79% (SD=1.2) increase in their EA% accompanied with a marked decrease of AA content (21% (SD=5.6)) after a 4 months storage at -20⁰C; these results showed the fact that enzymes maintain their activity during frozen storage, and a precooking stage is needed in order to adequately store these vegetables.

Figs 6 b and c corresponding to PC samples (unfrozen precooked vegetable) and PCFS samples (precooked samples stored during 4 months at -20⁰C) respectively, show the results of Ascorbic acid content (%AA), Browing index (%BI), Peroxidase enzyme activity (%EA POD) at the apical region, as functions of precooking times (3, 6 and 10 min).

Fig. 6b showed that for the tested precooking times (3, 6, and 10 min) the values of residual POD activity were: 7.3% (SD=0.9), 0.92% (SD=0.05) and 0.2% (SD=0.03) respectively. These values complied with the conditions of a residual enzyme activity ranging between 7.5-11% for POD in Brussels sprouts suggested by Williams et al. (1986) in order to obtain a stabilized vegetable with optimum quality (precooked vegetable unfrozen).

However, as can be observed in Fig. 6c for a precooking time of 3 min the %EA after frozen storage (sample PCFS) showed a marked increase of POD (83.5 % (SD=1.6) for the apical region that can be attributed to enzyme reactivation as was reported by Pérez-Calderón et al. (2017). Therefore it can be concluded that a residual %EA above 5% led to the reactivation of the enzyme after frozen storage, and the target upper limit of %EA<5 should be established in order to assure the final quality and nutritional value of the product

This enzyme reactivation (POD) in samples pre-cooked for 3 min, led to a decrease of the AA content (%AA= 23.2% (SD=2.1)) after frozen storage 4 months at -20⁰C (Fig. 6c) because the presence of POD could promote the rapid oxidation of AA (Brewer and Begum, 2003). Gökmen (2010) reported that degradation of AA was high in the case of peas that were subjected to

insufficient thermal treatment to inactivate enzymes, due to the action of oxido-reductases enzymes such as ascorbic oxidase and POD.

AA is thermolabile, and has antioxidant activity; it is an important biocomponent in sensory and nutritional attributes, therefore it is used as a quality parameter. Its concentration decreases during frozen storage, depending on conditions, such as temperature, oxygen content, light and time (Torregrosa Verdú, 2006).

The %BI for a 3 min thermal treatment correlates with the POD reactivation showing an increase of 10% in this parameter after frozen storage (Fig. 6c). For PCFS samples with heating times of 6 and 10 minutes, values of %BI were acceptable and no significant differences were found (P≥0.05); however for 10 min treatment %AA was markedly lower compared to a 6 min processing time. Murcia et al. (2000) reported a drastic decrease in the AA content for broccoli by increasing the exposure time in the pre-cooking treatment. After 15 minutes at 90ºC, AA losses comprised between 40 and 50%. One of the causes of AA decrease is the breakdown of plant tissues by the action of heating in water, generating changes in cells permeability and leaching of this compound (Olivera et al., 2008).

Gonçalves et al. (2011) studied the degradation kinetics of AA in broccoli, and they observed that under isothermal storage conditions (-18ºC) the most drastic changes in AA content occurred during the first 55 days. Similar results were reported for peas, spinach, green beans and okras (Giannakourou and Taoukis, 2003). González-Hidalgo et al. (2018) found that for broccoli pre-cooked for 2 minutes at 97ºC and frozen at -20ºC, the percentage of AA after frozen storage was around 69%.

Comparing the results of the tested precooking times it can be inferred that the optimal heating time is 6 minutes since it maintained the highest AA value in the apical zone of the vegetable after frozen storage(AA=73.1%) with an acceptable browning index BI=35% (SD=4.70).

The sensory analysis included the evaluation of the general acceptability; the obtained scores of the precooked, frozen and stored vegetables were: 6.6 (SD=1.1), for PCSF samples heated during 6 minutes, and 6.9 (SD=2.4) for those heated during 10 min, in comparison to the cooked fresh vegetable F (Score 6.7,(SD=2.1)).The sensory analysis indicated that consumers did not detect differences between the precooked frozen vegetable and the fresh one.

4.   CONCLUSIONS

The finite element method was applied to simulate heat transfer phenomena that are involved during the production of precooked frozen Brussels sprouts (heating, cooling and freezing). The

coupling of subroutines that describe the inactivation of peroxidase in these vegetables was carried out during the precooking process which involves the thermal treatment and a cooling stage. The software Comsol Multiphysics 3.4b was implemented with specific Matlab subroutines in order to determine the enzyme activity of the thermolabile and thermo-resistant fractions of POD at each position and time during the process, considering the irregular shape of the vegetable.

All the numerical results were tested against experimental time-temperature curves and enzyme activity of POD in order to validate the model; a good agreement was obtained and the solution of the numerical code showed the temperature distribution and POD activity at the apical and external zones.

By using Enthalpy and Kirchhoff formulations in the partial differential heat transfer equation, the finite element numerical program was able to predict the time-temperature curve during the freezing process and to calculate freezing times. This change in the variables allowed to solve a complex nonlinear problem involving phase change with a marked dependence of the properties with temperature and strong convergence difficulties. The thermo-physical properties and their temperature dependence were measured and considered in the numerical code.

Quality parameters of the frozen vegetable such as Browing Index, textural properties, residual POD activity and ascorbic acid content were analyzed for several precooking times (3, 6 and 10 minutes) after freezing and storage during 4 months period at -20ºC. The sensory analysis confirmed the general acceptability of the precooked frozen product.

From the evaluation of the quality parameters it could be concluded that the optimal processing time which maintained the highest ascorbic acid content with an acceptable browning index in the apical zone of the vegetable was 6 minutes. It was demonstrated that a short heating time (i.e 3 min) was insufficient to avoid POD reactivation after frozen storage.

These results are useful for food processors since the numerical programs applied can be used to predict the optimum processing times in order to obtain a high quality and nutritious vegetables. This work contributes to the knowledge of industrial processing times in the precooked frozen vegetable industry.

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Figure Captions

Figure 1. **a)** Digital photograph of the contour used to generate the vegetable domain (rotating surface that generates the solid of revolution) and the mesh with triangular elements. Dimensions of one of the samples used in the numerical simulations are indicated **b)** Inner subdomain defined to simulate the average enzyme activity in the apical (central) region of the vegetable.
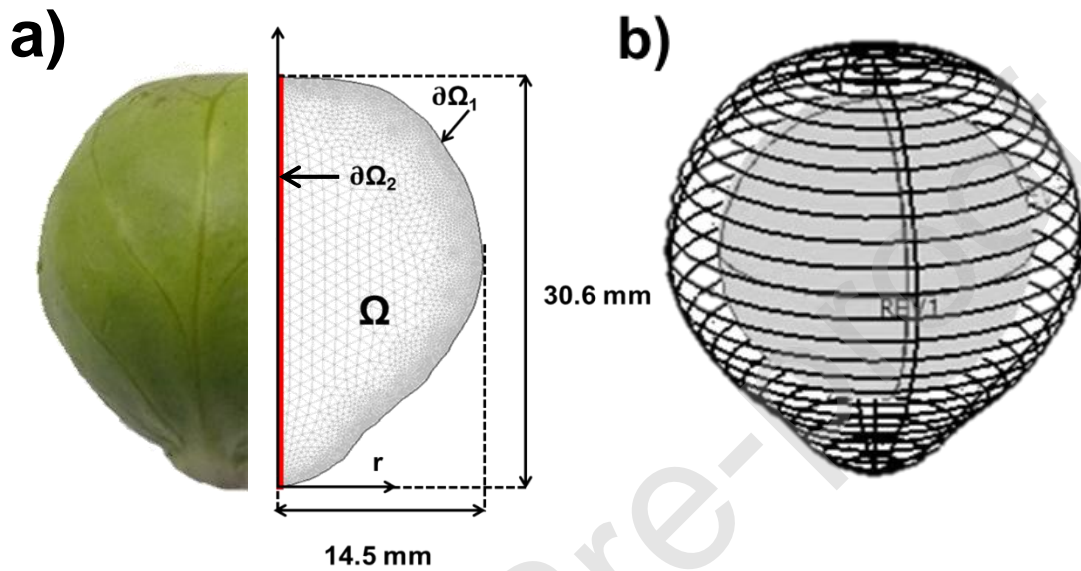
Figure.2 a) Experimental-time temperature measurements (o) and Numerical predictions (- -) at a particular inner point of the vegetable. Thermocouple position r=0, z= 21.3 mm. during the precooking stage. Vertical dashed line indicates the time when the thermal treatment stops and the vegetable is cooled in the ice-water bath. Conditions: Temperature of the stirred thermostatic bath 90⁰C, thermal processing time: 6 min; h =2200 W/m^{2}K for the stirred hot water bath and h =580 W/m^{2}K for the ice-water bath.
b) Numerical predictions as functions of time, at the apical zone. Mean temperature (- -) obtained by applying Eq.(43) and Residual POD activity (——) obtained by coupling the enzyme inactivation kinetics with the heat transfer program. Conditions are the same as described in a). The arrow indicates experimental enzyme activity measured at the apical region after 10 min (n=6).
c). Time temperature distribution of the Brussels sprouts at different processing times. The thermal processing time in the hot stirred bath was 6 minutes.
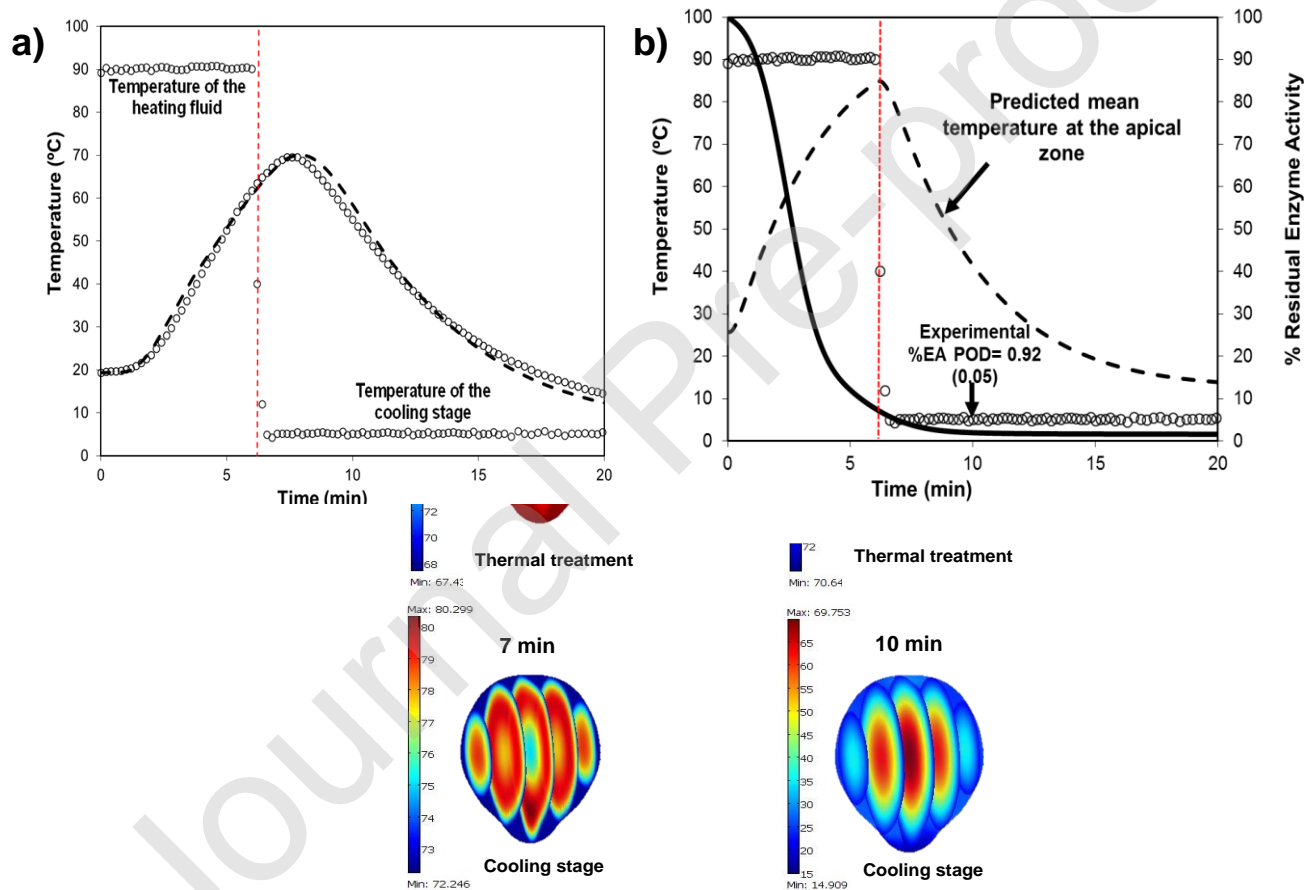
Figure. 3 % Total Enzyme activity (—), thermo-labile fraction (···) and thermo-resistant (- -) for POD
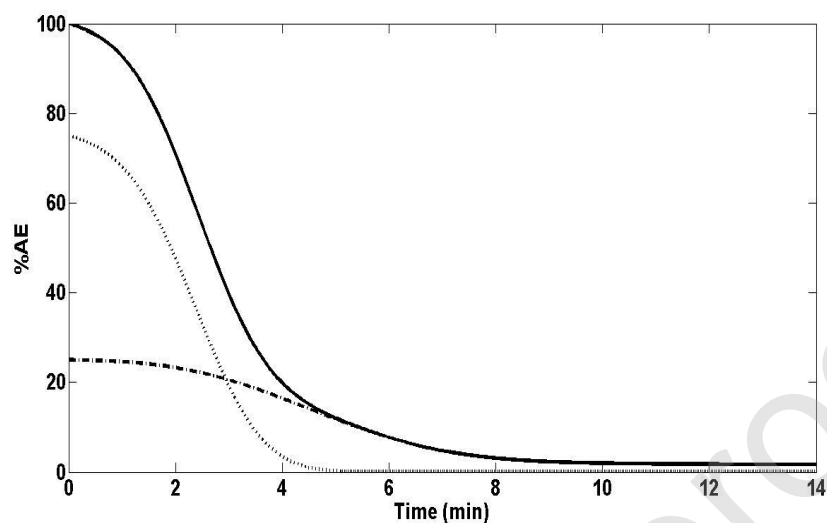

Figure 4. Thermo-physical properties of the vegetable as a function of temperature **a)** specific heat (obtained by DSC) **b)** thermal conductivity.
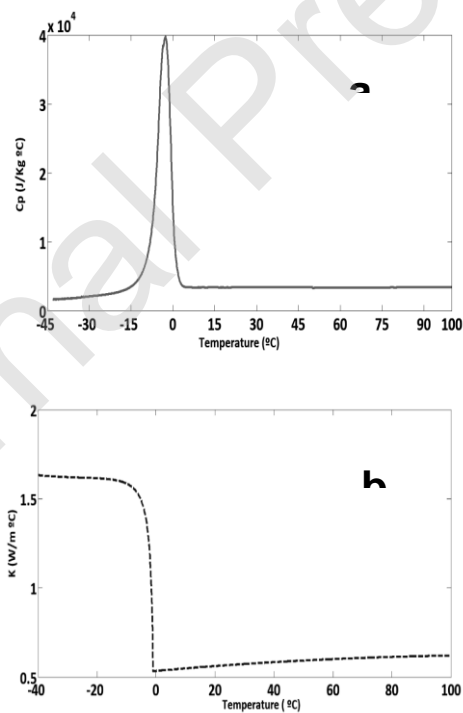

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Figure 5. Functional relationships used in the finite element formulation, **a)** Enthalpy vs. temperature; **b)** Kirchhoff function vs. temperature; **c)** Kirchhoff function vs. enthalpy. **d)** Experimental measurements and numerical predictions of the model during freezing. Thermocouple position r=6 mm, z=23.8 mm. Initial temperature of the vegetable 19.4^{0}C, air tunnel temperature -25ºC.
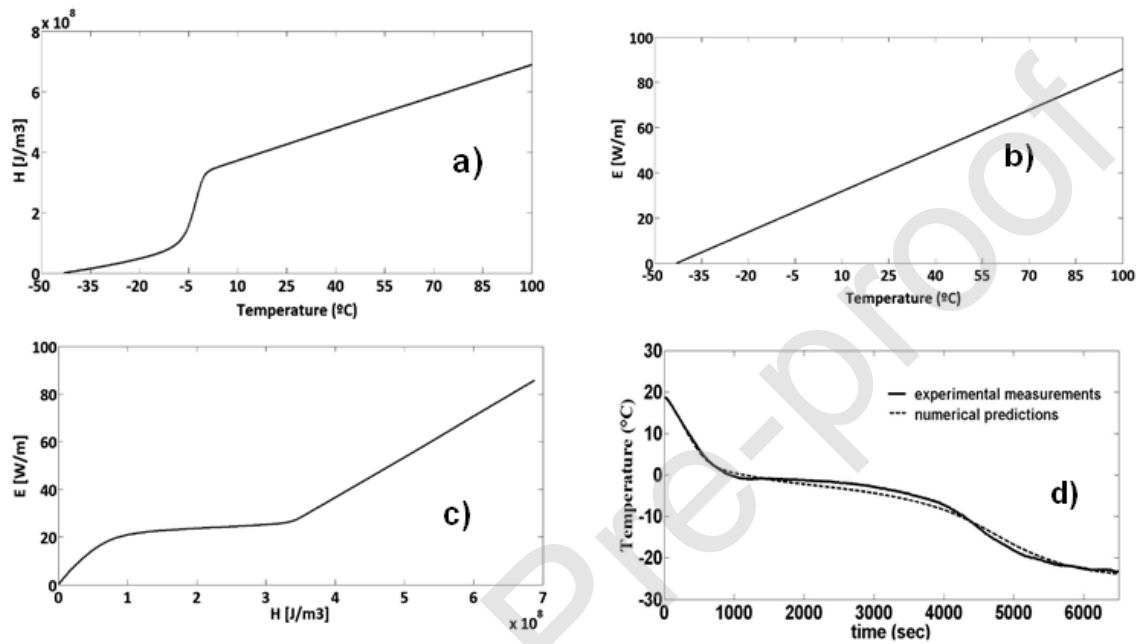
Figure 6. a) Photographs showing the apical zone of Brussels sprouts corresponding to: frozen untreated samples (FU) and precooked samples (PCFS) during 3,6 and 10 min at 90 ºC . All the samples were submitted to a frozen storage of 4 months at -20ºC. Graphs **b)** and **c)** corresponding to PC samples (unfrozen precooked vegetable) and PCFS samples (precooked samples stored during 4 months at -20ºC) respectively, show the results of Ascorbic acid content (%AA),Browing index (%BI), Peroxidase enzyme activity (%EA POD) at the apical region, as functions of precooking times (3, 6 and 10 min). Error bars indicate standard deviation of the average values.
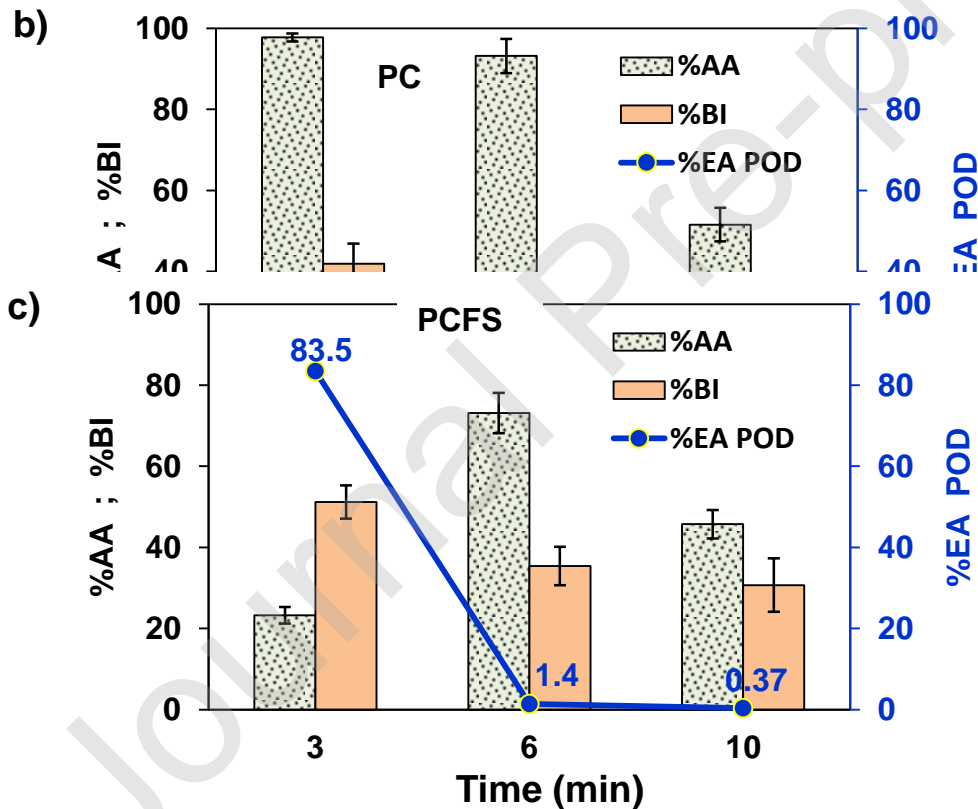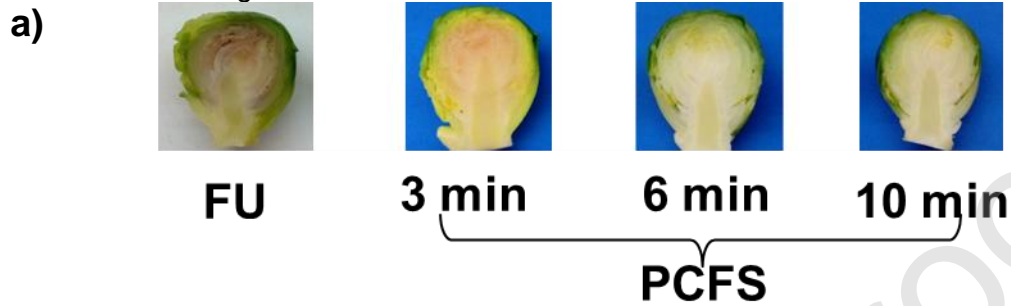
Table 1. Experimental and Predicted Enzyme Activity (%) of POD considering the apical and external regions of the Brussels sprouts. Values between parentheses are standard deviations (n=6)

	APICAL			EXTERNAL	
TIME (min)	**EXPERIMENTAL %EA***	**PREDICTED %EA**		**EXPERIMENTAL %EA***	**PREDICTED %EA**
3	7.3 (0.9)	10.8		2.7 (0.3)	6.47
6	0.92 (0.05)	1.25		0.78 (0.7)	0.90
10	0.12 (0.03)	0.06		0.11 (0.01)	0.05

*Data reported in literature by Perez-Calderon et al. (2015)

Table 2. Effect of pre-cooking times on relative texture parameters with respect to the raw vegetable: Maximum puncture force (% Fm) and stiffness (%S) of pre-cooked and cooled samples (PC) and precooked, frozen and stored samples (PCFS) submitted to different precooking times (3, 6, 10 min). The values between parentheses correspond to standard deviations, (n=6). Different letters in the same column indicate significant differences between the samples (P≤0.05)

Pretreatment heating time (min)	PC		PCFS	
	%Fm	%S	%Fm	%S
3	92.5 [a] (16)	93.4 [a] (5.5)	38.6 (10)	6.9 [a] (1.2)
6	50.1 [b] (7.6)	23.8 [b] (4.2)	30.5 [b] (6.2)	6.4 [b] (0.8)
10	33.9 [c] (8.0)	17.2 [c] (4.5)	22.9 [c] (2.9)	5.1 [c] (0.4)