

Effect of Supercritical Carbon Dioxide on the Enzymes Inactivation in Single-Strength Carrot Juice

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Abstract The inactivation of Pectin Methylesterase (PME), and Peroxidase (POD), in fresh single-strength carrot juice by supercritical carbon dioxide (SCCD) at 40 °C and a CO₂/juice ratio (w/w) equal to 3 was investigated. In the range of pressure from 10 to 25 MPa and treatment time from 40 to 110 min, both PME and POD were effectively inactivated significantly. The laboratory inactivation tests were performed by using a semi continuous method with a continuous stream of CO₂ fed at constant mass flow rate from the beginning to the end of each experimental test. A semi empirical kinetic model that accounts for a residual equilibrium fraction, $\alpha_{eq,j}$, of POD and PME was used to describe satisfactorily the inactivation behavior. Under the above mentioned experimental conditions the pressure dependence of the kinetic constants, k_j , can be described satisfactorily by a constitutive equation that is a linear function of the reduced pressure, P_r , of carbon dioxide. The numerical values of the two model parameters, calculated by fitting the experimental data in terms of dimensionless residual activity measured at the end of each experimental tests are: 0.04035 min^{-1} , 0.10720 for POD and 0.00345 min^{-1} , 0.08941 for PME.

Keywords Carrot juice, Supercritical CO₂, Enzymes inactivation, Kinetic constants, Residual activity

1. Introduction

Carrot is one of the top-ten most economically important vegetables crops widely cultivated in many parts of the world [1]. Currently, China is the first producer with almost 50% of the global world production. About 60% of world carrots production occurred in Asia, followed by the Europe (20%), Americas (10%), and Africa (4%). From the early 70s to today, the global world production of carrots increased about five times, significantly more than the overall increases in world vegetable production [2]. Carrots are an excellent source of vitamins, dietary fibers, minerals and other phytonutrients like polyacetylenes able to interact with carotenoids in preventing the growth of colon cancer cells and coronary hearth disease [3, 4]. These properties make carrots of particular interest as a source of juice prepared ready to drink and stimulate the industrial production of enhanced shelf-life (ESL) carrot juice (CJ) of comparable quality. However, since CJ is thermal sensitive, the application of a number of non-thermal preservation technologies have been investigated during the last two

decades as substitute of the thermal treatment [5-10]. Among others, the one based on the use of gaseous-high-density carbon dioxide, usually in the supercritical state, (DPCD) appears to be very promising for processing liquid food and juices of fruits and/or vegetables, including fresh CJ [10]. In fact, it can be applied at the temperature of human and animal body, while typical operating pressure values are thirty times smaller than those typical of the treatment at high hydrostatic pressures (HHP). In general, DPCD has potential advantages over other non-thermal processes in terms of both capital and operating costs since it is easy to scale-up to continuous industrial processing operating with recirculating CO₂, thereby avoiding significant loss of the processing fluid and flavours. One of the main objectives in the industrial processing of CJ is to stabilize the cloudiness of the product during storage, while preserving quality parameters such as colour, flavour, viscosity and nutritional characteristics. In this respect, is well recognized, for example, that pectic enzymes and in particular pectin methylesterase (PME) influence viscosity and cloud stability, while peroxidase (POD), characterized by high thermo-stability, is involved in the oxidation of many organic compounds, leading to deterioration in flavour, colour, nutritional and functional properties [9-11]. Therefore, the inactivation of indigenous PME and POD in fruits, vegetables and derived beverages

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has been widely investigated but, in many case, this has been done using model aqueous solutions neglecting, in this way, the effect of decreasing pH and interaction with other substances present in the natural matrix [12-14]. The purpose of this work is to quantify and to simulate the capability of DPCD in inactivating PME and POD in fresh, single-strength CJ obtained from fresh carrots.

2. Materials and Methods

2.1. Materials

PGI (protected geographical indication) carrots (*Daucus Carota* L.) officially named "Carotadell" Altoiano del Fucino" were taken from a local farmer that also operated a large carrots and vegetables juices industrial plant. Carrots were stored at 2°C for a time never longer than three days until used for producing the amount of juice required for making a laboratory experimental test. To this purpose, after washing with chlorinated water, rinsing with tap water, and peeling, the juice was obtained using a juice extractor (MULINEX FRUTTI-PRO XL). Then, the juice having a pH about 6 was filtered through a 4-layers cheesecloth, divided in three parts and suddenly used for analysis and for processing. The third portion was bottled, sealed and stored in a refrigerator at about 2 °C to be used as reference sample in the evaluation of ESL.

2.2. Enzyme Assay

Peroxidase activity was assayed by the Pyrogallol test [15] with minor modifications, at pH=6 and 20 °C, using 20 g of homogenized sample previously filtered on filter paper. Pectin Methylsterase activity was assayed by evaluating the carboxylic groups that are released as a result of the reaction of the indigenous PME on added Pectin (P9135 Sigma). Titration occurs automatically (pH-Stat option, Metrohm) by recording the amount of 0.1 N NaOH supplied over time, which is necessary to maintain pH at 7.5, at 30 °C. 10 g of homogenized sample previously filtered on filter paper mixed with 200 mL of NaCl solution and adjusted to pH = 7.5 with 0.1 N NaOH, were used.

2.3. Experimental Apparatus and Procedure

The main component of the system (Figure 1) is a 0.250 L AISI 329 stainless still autoclave (NOVA-WERKE, Zurich, Switzerland) designed to operate up to 70 MPa at 773 K with three holes on the head which could be removed before the beginning and at the end of each experimental test. The reactor was equipped with a variable speed magnetic stirring system and a gas diffuser to achieve an intimate contact between the liquid inside the vessel and the CO₂. A membrane high-pressure metering pump (flow rate: 0-3 kg/h; P_{max} = 35 Mpa; MILTON ROY) was used for feeding the reactor with liquid CO₂ at a predetermined constant rate, continuously monitored by a mass-flow-rate (MICROMOTION, D6) located at the exit of the expansion

vessel. The desired operating temperature was obtained by inserting the autoclave into an electrical oven equipped with two heating resistances (Wallow, MT03L3AP-3000, 200 V, 1000 W) and an automatic temperature control system (CAL Controls, 3300). A type J thermocouple connected to one of the three holes available on the head with an high-pressure pipe having an internal diameter of 0.001 m, was inserted into the reactor in order to continuously detect the temperature inside the cell with an accuracy of ± 0.2 K, while the internal pressure was continuously monitored by a pressure transducer (HAENNI, EDR430), fitted on the reactor head, with an accuracy of 0.15%. To start a run, the cleaned reactor was hermetically closed, heated at the desired temperature, evacuated and filled with 0.190-0.220 L of CJ taken from the refrigerator. Then, the pump of the inlet CO₂ liquid stream was operated to pressurize the cell; when the predetermined pressure value was reached, the high-pressure metering valve located on the exit line of CO₂ was opened in order to keep a constant CO₂/CJ = 3 (w/w) with the help of the above mentioned mass flow-meter. At the end of each run the reactor was slowly depressurized and suddenly opened to recover the treated CJ that was collected in two sterile glass bottles, refrigerated at 2 °C and then analysed.

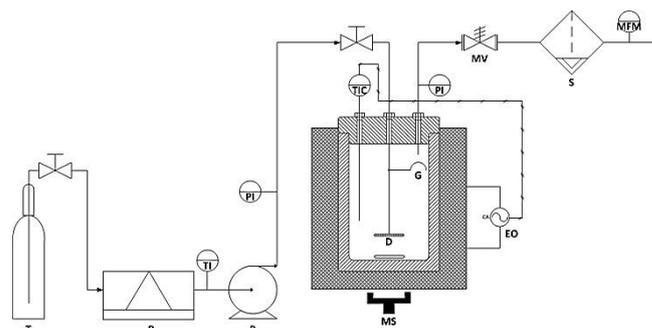


Figure 1. Schematic diagram of the experimental apparatus (T: CO₂ Tank; R: Refrigerator; P: High pressure pump; MV: Micrometric valve; EO: Electric oven; MS: Magnetic stirrer; S: Gas-Liquid separator; TI: Temperature indicator; PI: Pressure indicator; TIC: Temperature controller; MFM: Mass flow meter; D: CO₂ diffuser; G: Splashguard)

2.4. Kinetics of Enzymes Inactivation

It is reasonable to assume that at 40 °C the kinetics of enzymes inactivation is not affected by the temperature [16] at least for the time required for the treatment with supercritical CO₂ (SCCD), while it is reasonable to expect that the degree of inactivation is affected by the treatment time and by the value of the operating reduced pressure of CO₂. Furthermore, the presence of a labile and a stable fraction of the enzymes exposed to different inactivation treatments was observed and reported in the literature along with convincing argumentations for a number of fruits, vegetables, and derived products including CJ, and for a number of enzymes, including POD and PME [11-13]. The following semi empirical model, eq. 1, for the kinetics of inactivation of indigenous POD and PME in single strength CJ, accounts for all the above mentioned considerations.

$$a_j = a_{eq,j} + (1 - a_{eq,j})e^{(-k_j \cdot P_r \cdot t)} \quad j = POD \text{ or } PME \quad (1)$$

Where:

a_j is the dimensionless residual activity of POD or PME defined as $A_j/A_{0,j}$;

$a_{eq,j}$ is the dimensionless residual activity of POD or PME after prolonged treatment (equilibrium conditions) defined as $A_{eq,j}/A_{0,j}$;

A_j is the residual activity of POD or PME (Units/g) after t min of treatment;

$A_{0,j}$ is the activity of POD or PME (Units/g) in the untreated single strength carrot juice;

$A_{eq,j}$ is the residual activity of POD or PME (Units/g) after prolonged treatment (equilibrium conditions);

k_j is the kinetics constant (min^{-1}) of POD or PME at 40 °C and $\text{CO}_2/\text{CJ} = 3$ (w/w);

P_r is the operating reduced pressure of SCCD.

Equation 1 was obtained by the fundamental equation representing the POD or PME conservation law in combination with: a) a first-order fractional kinetic model for enzymes inactivation; b) a linear dependence with the pressure of the kinetic constant at 40 °C and a $\text{CO}_2/\text{CJ} = 3$ (w/w). More particularly the following plain constitutive equation:

$$f_j(P) = k_j \cdot P_r \quad j = POD \text{ or } PME \quad (2)$$

was selected instead of using the Eyring equation as suggested by several authors [17] to express the dependence of the rate constant by the pressure at constant temperature. The main motivation for this choice is that, unlike the HHP treatment, it is reasonable to think that the inactivation of the enzymes and/or microorganisms with DPCD is mainly related to its solubility in the CJ and to its solvent capability. Using Eq. 2 implies that the influence of the temperature on POD and PME inactivation is negligible when the temperature is equal or below to 40 °C, and the effect of the CO_2 is negligible when its reduced pressure is significantly lower than one or, the CO_2 is not sufficiently dense. The two adjustable parameters in eq. 1 are $a_{eq,j}$ and the k_j of POD or PME. It is worth to underline that, while $a_{eq,j}$ is a single value (one for POD and one for PME), $A_{eq,j}$ (Units/g) is proportional to $A_{0,j}$ (Units/g), which is related to the carrots used to prepare the juice in terms of variety, seasonality, duration and mode of storage, manufacturing technology of the juice, and others.

2.5. Statistical Analysis

To validate eq. 1, the values of the Percent Average Absolute Error (PAAE) defined by:

$$PAAE_j = 100 (1/N) \sum_{i=1}^N (|a_{i,j}^{exp} - a_{i,j}^{cal}| / a_{i,j}^{exp}) \quad (3)$$

along with the values of the Maximum Percent Error (EPMAX_j) and the coefficients of determination (R^2_j) were determined for $j = POD \text{ or } PME$. Moreover, for validation of eq. 1, the bias factors $B_{f,j}$ and the accuracy factors $A_{f,j}$ were also calculated to compare the experimental values $a_{i,j}^{exp}$ with the predicted values $a_{i,j}^{cal}$ given by the model.

$B_{f,j}$ and $A_{f,j}$ [18, 19] were calculated by eqs. 4 and 5, respectively.

$$B_{f,j} = 10^{(1/N) \sum_{i=1}^N \log(a_{i,j}^{cal} / a_{i,j}^{exp})} \quad (4)$$

$$A_{f,j} = 10^{(1/N) \sum_{i=1}^N |\log(a_{i,j}^{cal} / a_{i,j}^{exp})|} \quad (5)$$

The model parameters $a_{eq,j}$ and k_j (min^{-1}) were calculated along with their significance at 95% confidence, by fitting eq. 1 to the experimental data, $a_{i,j}^{exp}$.

3. Results

Table 1. Values of the parameters of the enzymatic inactivation model, $a_{eq,j}$ and k_j , ($j = POD \text{ or } PME$) and corresponding significance at 95% confidence

Enzyme	$a_{eq,j}$	k_j
POD	0.10720±0.01314	0.04035±0.00472
PME	0.08941±0.02632	0.00345±0.00148

Table 2. Experimental and calculated values of the dimensionless residual activity, $a_{i,j}$, of POD and PME along with the percent error

Enzyme	a_i^{exp}	a_i^{cal}	Percenterror
POD	0.2100	0.2074	1.25
	0.1787	0.1834	2.66
	0.1324	0.1101	16.88
	0.1160	0.1085	6.49
	0.0783	0.1078	37.58
	0.1124	0.1075	4.41
	0.1039	0.1072	3.19
PME	0.8406	0.8270	1.61
	0.7821	0.7770	0.64
	0.6522	0.6272	3.83
	0.5175	0.5406	4.46
	0.5125	0.5406	5.49
	0.4250	0.4070	4.23

Table 3. Values of PAAE_j, EPMAX_j, R^2_j , $B_{f,j}$, and $A_{f,j}$ for POD and PME as defined in Statistical Analysis section

Enzyme	PAAE	EPMAX	R^2	B_f	A_f
POD	10.35	37.58	0.89	1.01	1.10
PME	3.38	5.49	0.98	0.99	1.03

The following least square function was minimized to parameterize the kinetic model (eq. 1).

$$S(a_{eq,j}, k_j) = \sum_{i=1}^N (a_{i,j}^{exp} - a_{i,j}^{cal})^2 \quad j = POD \text{ or } PME \quad (6)$$

The results were reported in Table 1. The values of the calculated $a_{i,j}$ for POD and PME were reported in Table 2 along with the corresponding experimental values and with the corresponding percent errors. All the statistical

parameters described in the previous section were also calculated and reported in Table 3 to quantify the performance of the kinetic model. As can be seen the results are satisfactory for both POD and PME. Furthermore, Figure 2 shows graphically that the calculated values of $a_{i,j}$ compare reasonably well with the corresponding experimental values in the whole range of the experimental

operating parameters. Finally, to better show the effect of the pressure of the supercritical carbon dioxide stream and of the treatment time on the POD and PME inactivation, the calculated behaviour of a_j were reported in Figures 3 and 4, along with the experimental data. As can be seen a reduction greater than 90% can be obtained for the enzymatic activity of POD and of about 70% for PME.

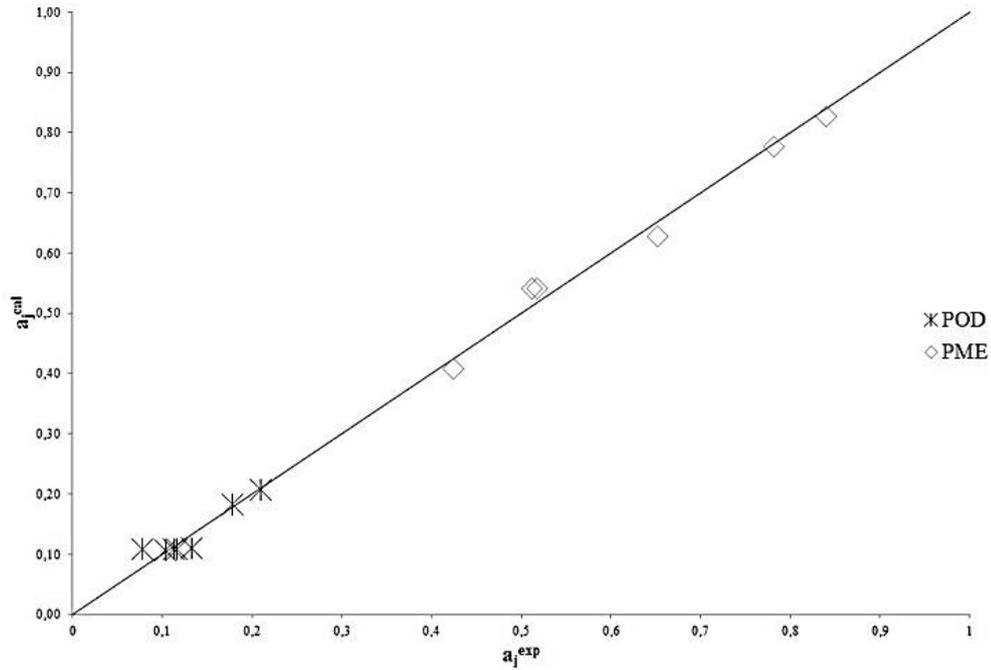


Figure 2. Experimental versus calculated values of the dimensionless residual activity, a_i , of POD and PME

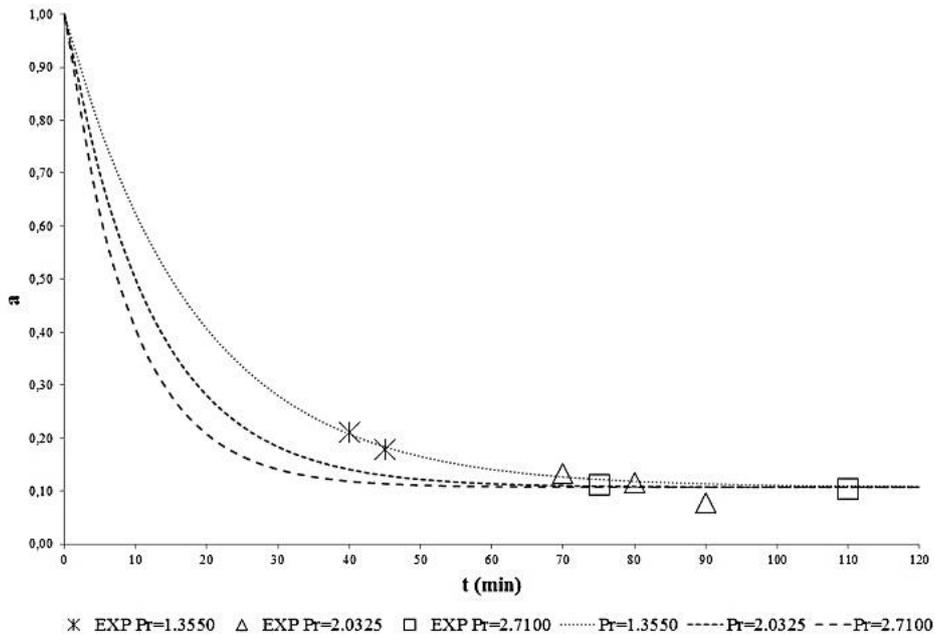


Figure 3. Effect of supercritical CO₂ stream pressure and duration of treatment on POD inactivation

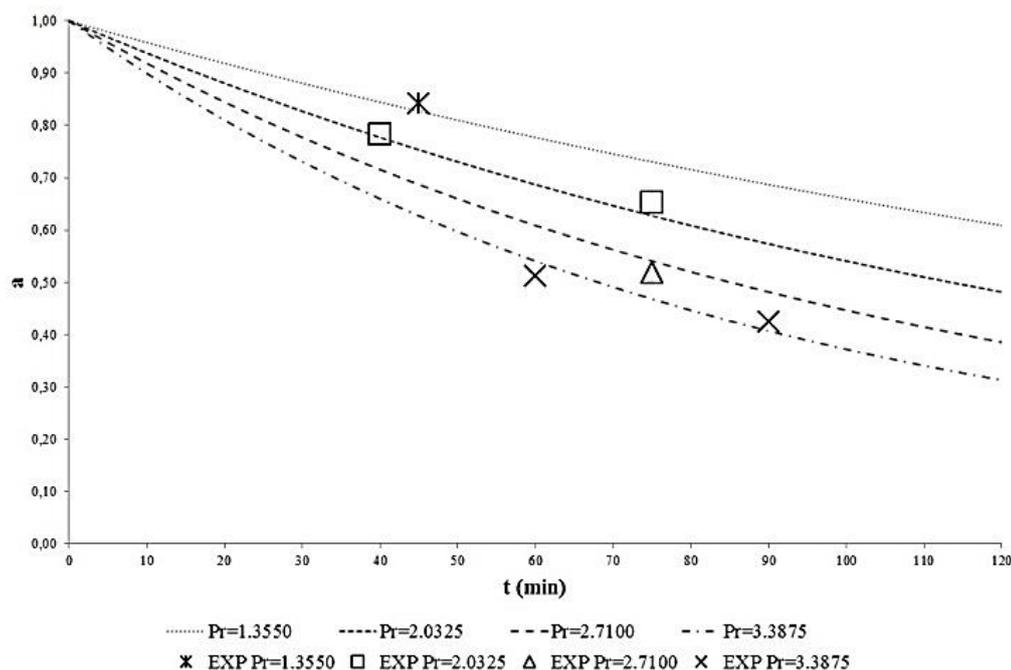


Figure 4. Effect of supercritical CO₂ stream pressure and duration of treatment on PME inactivation

4. Conclusions

The activity of indigenous POD and PME in fresh single-strength carrot juice can be significantly reduced by applying DPCD treatments. The experimental results obtained with a semi-continuous laboratory apparatus operated at 40 °C, CO₂/CJ = 3 (w/w), pressures from 10 to 25 MPa, and treatment time from 40 to 110 min show that the labile fraction of the POD activity was completely inactivated, while the labile fraction of the PME activity was reduced of about 80%. The enzymes activity decreases with increasing CO₂ stream pressure and treatment time; this behaviour is quantitatively described by a semi-empirical, fractional, first-order kinetic model. Further work is required to optimize the operating process parameters using a continuous pilot plant with recirculating CO₂.

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REFERENCES

- [1] Simon, P.W., Freeman, R.E., Vieira, J.V., Boiteux, L.S., Briard, M., Nothnagel, T., Michalik, Young-Seok Kwon, B. 2008. Carrot. In: Jaime Prohens & Fernando Nuez (eds.). Handbook of Plant Breeding. Vol. 2, NY: Springer. pp. 327–357. ISBN 978-0-387-74108-6.
- [2] FAOSTAT Database, Food and Agricultural Organization of the United Nations, 2013.
- [3] Purup, S., Larsen, E., Christensen, L.P., 2009. Differential Effects of Falcarinol and Related Aliphatic C17-Polyacetylenes on Intestinal Cell Proliferation., *J. Agric. Food. Chem.* 57 pp. 8290—8296.
- [4] Griep, L.M.O., Verschuren, W.M.M., Kromhout, D., Geleijnse, J.M., Ocke, M.C. 2011. Colours of fruit and vegetables and 10-year incidence of CHD, *Br. J. Nutr.* 8, pp. 1-8.
- [5] Zhang, H.Q., Barbosa-Cánovas, G.V., BalaBalasubramaniam, V.M., Dunne, C.P., Farkas, Yuan, D.F. 2011. *Nonthermal Processing Technologies for Food*, Wiley-Blackwell and IFT Press.
- [6] Barba, F.J., Esteve, M.J., Frigola, A. 2012. High Pressure Treatment Effect on Physicochemical and Nutritional Properties of Fluid Foods During Storage: A Review, *comprehensive Reviews in Food Science and Food Safety* 11, pp. 307-322.
- [7] Hammad, A.A., Abd El-Khalek, H.H., Youssef, K.A., Abd El-Kader, R.M. 2013. Microbiological, Nutritional and Sensorial Changes in Fresh Carrot Juice Preserved by Irradiation, *Food Science and Quality Management* 11, pp. 61-69.
- [8] Schultz, A.K., Anthon, G.E., Dungan, S.R., Barrett, D.M. 2014. Effect of Pectin Methyltransferase on Carrot (*Daucus Carota*) Juice Cloud Stability, *J. Agric. FoodChem.* 62, pp. 1111-1118.
- [9] Zhu, L., Wang, Y., Hu, X., Wu, J., Liao, X. 2009. Effect of high pressure carbon dioxide on the quality of carrot juice, *Innovative Food Science & Emerging Technologies*, 10, pp. 321–327.
- [10] Balaban M.O. and Ferrentino, G. 2012. *Dense Phase Carbon Dioxide: Food and Pharmaceutical Applications*,

Wiley-Blackwell.

- [11] Quintão-Teixeira, L.J., Soliva-Fortuny, R., Mota Ramos, A., Martín-Belloso, O. 2013. Kinetics of peroxidase inactivation in carrot juice treated with pulsed electric fields, *J. Food Sci.*, 78, pp. E22-E228.
- [12] Ly-Nguyen, B., Van Loey, A.M., Smout, C., Eren Özcan, S., Fachin, D., Verlent, I., Vu Truong, S., Duvetter, T., Hendrickx, M.E. 2003. Mild-Heat and High-Pressure Inactivation of Carrot Pectin Methylesterase: A Kinetic Study, *J. Food Science* 68, pp. 1377-1383.
- [13] Zhou, L., Zhang, Y., Hu, X., Liao, X., He, J. 2009. Comparison of the inactivation kinetics of pectin methylesterases from carrot and peach by high-pressure carbon dioxide, *Food Chemistry* 115, pp. 449-455.
- [14] Cullen, P.J., Pankaj, S.K., Misra, N.N. 2013. Kinetics of Tomato Peroxidase Inactivation by Atmospheric Pressure Cold Plasma Based on Dielectric Barrier Discharge, *Innovative Food Science & Emerging Technologies* 19, pp. 153-157.
- [15] Chance, B., Maehly, A.C. 1955. Assay of catalase and peroxidases B. The Pyrogallol test. *Methods in Enzymology* 2, pp. 773-775.
- [16] Truong, T.T., Boff, J.M., Shellhammer, T.H. 2002. Effect of Carbon Dioxide in High-Pressure Processing on Pectinmethylesterase in Single-strength Orange Juice, *J. Food Science* 67, pp. 3058-3062.
- [17] Ly-Nguyen, B., Van Loey, A. M., Fachin, D., Verlent, I., Hendrickx, M.E. 2002. Partial Purification, Characterization, and Thermal and High-Pressure Inactivation of Pectin Methylesterase from Carrots (*Daucuscarota L.*), *J. Agric. Food Chem.* 50, pp. 5437-5444.
- [18] Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology, *J. Appl. Bacteriol.*, 81, pp. 501-508.
- [19] Baranyi, J., Pin, C., Ross, T. 1999. Validating and comparing predictive models, *International Journal of Food Microbiology*, 48, pp. 159-166.