University POLITEHNICA of Bucharest

Doctoral School Chemical Engineering and Biotechnologies

Supercritical fluid extraction of bioactive compounds from tomatoes

Ph.D. Thesis Summary

Supervisor:

Prof. dr. eng. Costin Sorin Bîldea

Student:

Eng. Mihaela Popescu

Bucharest

2023

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Keywords: carotenoids, lycopene and β-carotene, UV-VIS spectrometry, isosbestic point, absorbance ratio factor, simultaneous quantification methods, tomatoes, tomato drying,drying kinetics, mathematical modelling, carotenoids degradation, bioactive compounds, antioxidant compunds, plant seeds, ω-fatty acids, supercritical fluid extraction, extraction enhancement, modifiers, supercritical carbon dioxide, green solvents, economic analysis.

CHAPTER 1 MAIN CONTRIBUTIONS AND LITERATURE REVIEW

1.1 Motivation

Nowadays, the global trends are oriented to a healthy lifestyle based on increased consumption of fruits and vegetables, high quality foods and the introduction of nutritional supplements into the diet to prevent various diseases and to prolong life. The consumption of nutritious, less processed food containing natural additives recovered from plants using green methods is necessary for a healthy lifestyle.

The main factors that motivated the research of the tomato plant are the high global production (ranking second among all the plants), the significant amount of tomato pomace obtained industrially as waste and leading to disposal problems, the quality of this by-product in terms of bioactive compounds as carotenoids (mainly lycopene), and fatty acids (mainly ω6-linoleic acid), and the high added-value of natural lycopene with many applications in food, pharmaceutical and cosmetic industries. In Romania, tomatoes represent an available plant resource being highly cultivated, consumed and processed, even in households.

Lycopene is the carotenoid with the highest antioxidant activity that is used in food, supplements and cosmetics to prevent oxidation reactions associated with the occurrence of many diseases. This compound cannot be synthetized by the human body and the diet is the only source it can be obtained through. Recovery of this compound from tomatoes is possible through the extraction process. Supercritical fluid extraction using carbon dioxide as green solvent is a method suitable for recovering lycopene from tomatoes with the purpose of being used as additive in food, cosmetic or pharmaceutical industries without the need for further purification or decontamination steps. Moreover, the supercritical fluid extraction method is environmentally friendly.

Based on these considerations, this thesis was focused on the recovery of bioactive compounds such as lycopene, β-carotene and essential fatty acids from tomatoes using green extraction and optimization of operating parameters to obtain high quality products to be used in the food, cosmetic or pharmaceutical industries.

1.2 Thesis overview

The thesis includes five chapters that can be read independently. The main results were already published in 4 articles. Main areas studied in this thesis are the simultaneous quantitative determination of lycopene and β-carotene in tomato extracts, the determination of optimal drying conditions (method and temperature) of tomato sample and the determination of optimal extraction

conditions (green method, green solvent, temperature, time, pressure, solvent flow rate, raw material type) to obtain high quality extracts with less degradation of target compounds.

Chapter 1 presents a short literature study focused on main areas explored within this thesis concerned in supercritical fluid extraction of bioactive compounds from tomatoes. The study concerns natural target carotenoids (lycopene and β-carotene) sources and benefits, quali-quantitative analysis of carotenoids in plant extracts, drying step applied as a pretreatment of tomato samples before extraction to remove the moisture to prevent the sample spoilage and to increase the extraction efficiency, extraction methods applied on tomatoes to recover carotenoids and natural carotenoids possible applications in food, cosmetics and pharmaceutic industries.

Chapter 2 presents the research regarding the simultaneous analysis of lycopene and β-carotene. An algorithm to evaluate and improve the UV–VIS spectrophotometric method for simultaneous quantification of lycopene and β-carotene found in mixture, as tomato extracts is presented. Also, a solvent selection algorithm was developed to use less amount of toxic solvent, hexane. Acetone was mixed with hexane (Hx), obtaining acetone:hexane (1:1, v/v) mixture (Ac:Hx) as solvent for sample solubilization. The solvent was selected by comparison of solubilization property of both Hx and Ac:Hx solvents through UV-VIS analysis of tomato extracts. The results showed that Ac:Hx conducted to tomato sample absorbances higher than those measured with Hx, for the same tomato extracts. The solubilization efficiency of Ac:Hx is higher by 8 – 13 % compared to Hx. The calibration curves of pure lycopene and β-carotene in Ac:Hx using ten points in 0.5 – 5 mg/L concentration range were determined and statistically validated using regression analysis, homogeneity of variances, repeatability and intermediate precision tests. The maximum absorption peaks of lycopene and β-carotene in Ac:Hx were identified and the absorption coefficients of pure lycopene and β-carotene in this mixture at the wavelengths corresponding to their absorption maxima were determined. Using stock solutions of pure compounds in Ac:Hx, thirteen synthetic mixtures of lycopene and β-carotene with known concentration between 1 – 4 mg/L were prepared to be used as reference. Based on pure carotenoids and their mixture spectral data analysis, four physical models with different assumptions were presented. The simultaneous quantification of lycopene and β-carotene algorithm comprise in four methods as *Wavelengths groups method* (WGM), *Absorption factor method* (AFM), *Absorbance subtraction method* (ASM) and *Isosbestic point method* (IPM) which were further divided in other versions depending on the considered assumptions. An isosbestic point of pure lycopene, β-carotene and their mixture was identified at 461 nm. The concentration equations were formulated based on considered assumptions regarding the choice of characteristic wavelengths group for lycopene and β-carotene quantification (six wavelengths groups WG_1 – WG_6 are analyzed), the spectra overlapping property, the presence of isosbestic point or the contribution of each compound to mixture

absorbance through proposed absorbance factors to improve the concentrations equations accuracy. Finally, seventeen methods were analyzed as WGM–I–WG_1, WGM–I–WG_2, WGM–I–WG_3, WGM–I–WG_4, WGM–I–WG_5, WGM–II–WG_1, WGM–II–WG_2, WGM–II–WG_3, WGM–II–WG_4, AFM–I–WG_3, AFM–II–WG_3, AFM–III–WG_3, ASM–I–WG_6, ASM–II–WG_6, ASM–III–WG_6, IPM–I–WG_6 and IPM–II–WG_6. Methods validation is performed using thirteen synthetic mixtures and tomato residue extracts based on calibration data of carotenoids in Ac:Hx, parity charts, percentage errors and statistical analysis by equivalence tests for concentrations sets and methods, homogeneity of variance test for concentrations sets and Student test. The results showed that one of the isosbestic point methods (labeled IPM–II–WG_6) gives the best correlation from statistical point of view, with medium accuracy errors, below 5 %, for simultaneous quantification of lycopene and β-carotene in a mixture.

Chapter 3 presents the research regarding the drying pretreatment applied to tomato samples based on two studies.

The first study named "**Effect of drying processes on lycopene recovery from tomato peels**" presents the evaluation of the influence of three drying methods, five drying temperatures in 50 – 120 °C range and of the moisture content on lycopene recovery from dried tomato peels of Crystal variety. Tomato peels were subjected to three drying methods as oven drying, vacuum-oven drying (at a constant pressure of 0.9 bar) and hot-air drying at six different temperatures of 50 °C, 70 °C, 80 °C, 100 °C and 120 °C at the same drying time of 5 h. The influence of the drying method, drying temperature and drying time on the mass and moisture content of the tomato peels were analyzed and the variations of moisture and mass were plotted and discussed. The Soxhlet extraction method with Ac:Hx as solvent was used to recover lycopene from dried tomato peels. The lycopene quantification from the extracts was achieved through UV-VIS spectrometry method. Based on the dried tomato peels aspect, the extraction yields and on the calculated lycopene concentrations in the extracts, the influence of the drying method and drying temperature on the quality of the dried products were analyzed. The experimental data was compared to other literature results. The results showed that the hot-air drying method is recommended to be used for drying of tomato peels from Crystal variety for 5 h at a temperature of 80 °C which is associated with the best aspect of tomato peels, the lowest moisture content of 20.16 % and the highest amount of lycopene.

The drying temperature effect on tomato samples was further analyzed in the second study named "**Mathematical modeling of thin-layer drying kinetics of tomato peels: influence of drying temperature on the energy requirements and extracts quality**" which presents the mathematical modeling of thin-layer drying kinetics of tomato peels, from Rila variety, subjected to hot-air drying method at six different temperatures in 50 – 75 °C range to determine the

optimum drying temperature in terms of specific energy requirements and dried sample quality expressed by the lycopene and β-carotene concentrations from dried sample extracts. Tomato peels were subjected to hot-air drying at six different temperatures of 50 °C, 55 °C, 60 °C, 65 °C, 70 °C and 75 °C until similar final moisture of 6 – 7 %. The specific energy requirements of the drying processes were also calculated to determine the optimum drying temperature from an economic perspective. Based on drying experimental data, ten different thin-layer mathematical models as one theoretical diffusion model (*Fick's second law of diffusion Model*), three semi-theoretical models derived from Newton law of cooling (*Newton Model*, *Page Model* and *Modified Page Model*) and six semi-theoretical models derived from Fick's second law of diffusion (*Henderson and Pabis Model*, *Modified Henderson and Pabis Model*, *Midili Model*, *Logarithmic Model*, *Two term Model* and *Two term exponential Model*) were used to predict the drying behavior using the estimated drying kinetic parameters. The validation of the drying mathematical models to evaluate the models fitting accuracy with the drying experimental data was performed using three statistical parameters as the coefficient of determination R^2, the reduced chi-square χ^2 and the root mean square error RMSE. The theoretical diffusion model was described in details using an illustrated physical model and a mathematical model developed based on considered assumptions. The effective moisture diffusivity coefficients and the activation energy for hot-air drying of tomato peels in 50 – 75 °C temperature range were calculated. The influence of the drying temperature on the samples quality was determined based on samples aspect, extraction yields and carotenoids concentrations calculated in the dried sample extracts. Dried tomato peels were subjected to Soxhlet extraction using Ac:Hx as extraction solvent and the carotenoids contents were calculated for the extracts using UV-VIS spectrometry method. Using the experimental determinations, two degradation models of lycopene and β-carotene concentrations with the drying temperature were formulated and the carotenoids concentrations were predicted in 50 – 110 °C temperature range. The experimental and predicted results were consistent to literature data. The results showed that using hot-air drying method, a drying temperature of 50 °C for tomato peels of Rila variety is recommended to obtain high quality extracts, with a minimum energy consumption and degradation of the sample, for a final moisture content of 6 – 7 %.

Chapter 4 presents the research regarding the carotenoids extraction from different types of tomato samples and contains two studies.

The first study named "**Valuable natural antioxidant products recovered from tomatoes by green extraction methods**" presents the valorization of tomatoes using green extraction to obtain value-added products rich in carotenoids and polyunsaturated ω-fatty acids (ω-PUFA). For this purpose, tomatoes from Rila variety were used to prepare three samples as tomato slices, tomato

pomace and tomato seeds. Tomato seeds were subjected to Soxhlet extraction with hexane to recover tomato oil. For tomato slices and pomace samples two extraction processes were used as Soxhlet extraction (SE) and supercritical fluid extraction (SFE). For SE method, two green solvents, bioethanol and ethyl acetate were analyzed. For SFE method, green solvent, carbon dioxide (CO_2), and two extraction parameters sets at 400 bar extraction pressure, 70 °C extraction temperature, 9 kg/h CO_2 flow rate and 10 h extraction time (set 1) and 450 bar, 70 °C, 11 kg/h and 10 h (set 2) were analyzed. The extracts obtained from the SFE method were centrifuged to isolate three SFE fractions as solid oleoresin, oil and liquid. The extraction efficiencies of SE and SFE methods were determined by evaluating the solvent affinity, the seed content of the samples, the extraction pressure and the solvent flow rate influence on the extraction yields. The oil extracted from tomato seeds was transesterified using an acid-catalyzed procedure to obtain fatty acids methyl esters (FAME). The analyses of the prepared samples (FAME, SE raw extracts, SFE raw extracts, solid oleoresin, oil and liquid) were performed using five methods. The GC-MS method was used to determine the FAME profile of the tomato seed oil. The UV-VIS method was used for the qualitative and quantitative analysis of the lycopene and β-carotene in all the extracts. The FT-IR method was used for the qualitative analysis of the carotenoids and oils in the centrifuged SFE fractions. The Folin-Ciocalteu method was applied to determine the total phenolic content in the SE and SFE raw extracts from tomatoes and the DPPH method was applied to calculate the antioxidant activity of all the extracts. Finally, an economic analysis at three scale-up capacities (1:10:100 kg dried pomace/batch) of the SE and SFE processes was performed to estimate the extraction process profitability to produce four valuable extracts using dried tomato pomace as raw material, as SE and SFE raw extracts, solid oleoresin and oil due to their carotenoids and FAME contents and superior antioxidant activity and phenolic content. The results showed that the supercritical fluid extraction method at 450 bar, 70 °C and 11 kg/h flow rate using green CO_2 and tomato pomace is recommended to obtain two value-added products as lycopene-rich solid extract and pigmented oil rich in carotenoids (lycopene and β-carotene) and ω-PUFA (ω6-linoleic acid) because of the obtained extracts' quality and positive effects on both the environment and human health. Also, with a plant capacity higher than 100 kg, greater profitability is realized.

The research continued with the determination of optimal extraction parameters in supercritical process in the second study named "**Carotenoids recovery enhancement by supercritical CO_2 extraction from tomato using seed oils as modifiers**" that presents the improvement of the quantity and quality of the extracts recovered from tomato slices by adding 20 w/w % seeds as modifiers. The design of experiments was used to determine the optimal extraction parameters. Tomatoes from Rila variety were prepared to obtain tomato slices and tomato seeds. Three types of seeds were used as modifiers, tomato seeds, camelina seeds and hemp seeds. Three steps were

involved in this research: evaluation of carotenoids solubility in vegetable oils, the extraction of carotenoids from tomatoes samples using modifiers and the analysis of extraction optimal operating parameters. The Soxhlet extraction method with Ac:Hx was used to recover the vegetable oils from the three types of seeds and to determine the extraction yields. Vegetable oils were subjected to transesterification reactions to obtain FAME and the analysis of these compounds was performed using GC-MS method. The solubility of the lycopene and β-carotene from tomato slices in obtained vegetable oils was checked using maceration and the analysis of carotenoids in the extracts was performed using UV-VIS spectrometry method. The SFE method with CO_2 was applied to extract carotenoids from tomato slices enriched with seeds as modifiers and to determine the extraction efficiency, using as extraction parameters pressure of 450 bar, temperature of 70 °C, CO_2 flow rate of 11 kg/h and extraction time of 10 h. The SFE extracts were centrifuged to separate the solid and oil fractions. The influence of the seeds addition on the SFE extraction process, extraction yields and extracts compositions were investigated based on the extraction curves, extracts compositions in fractions obtained after centrifugation and the separated SFE fractions compositions in lycopene and β-carotene determined with UV-VIS method. Further, the Box-Behnken method was used to determine the optimal extraction parameters for SFE extraction with seed oils as modifiers based on fifteen extraction experiments. The chosen factors were the extraction pressure of 350, 400 and 450 bar, the type of the seeds as tomato, camelina and hemp seeds and the CO_2 flow rate of 9, 11 and 13 kg/h. Chosen responses were the extraction yields of the oil and solid fractions separated from the SFE extracts, the total carotenoids content in the oil fraction and the lycopene content in the solid fraction. The effects of the selected factors on chosen responses were determined using four second-order polynomial models and illustrated using response surface plots. The mathematical models were validated with statistical analysis, using the coefficient of determination and the lack-of-fit test and the determination of the optimal extraction parameters was performed using response surface plots and the desirability function. Finally, the quality of the extraction products was analyzed using UV-VIS, GC-MS and DPPH methods to determine the carotenoids and ω-PUFA contents and the antioxidant activities. The results showed that by using SFE as an environmentally friendly extraction technique under optimal conditions of 450 bar, 70 °C, 13 kg/h and two vegetal samples with large productivity as tomatoes and camelina seeds, two value-added natural products are obtained: a solid oleoresin enriched in lycopene, which can be used as a natural colorant or additive in the food industry, and tomato and camelina oil enriched in carotenoids (lycopene and β-carotene) and ω-PUFA (ω3-linolenic acid, ω6-linoleic acid) which can be used as such for consumption.

Chapter 5 presents the main conclusions of the experimental research conducted in this thesis and presents some future perspectives in the field of supercritical CO_2 extraction of bioactive compounds from tomatoes. Future research is oriented in two directions. The first direction is the scale-up analysis and simulation of supercritical extraction process to separate value-added products from tomatoes mixed with seeds as modifiers. The second direction is oriented to the experimental analysis of supercritical CO_2 extraction from tomato pomace enriched with seeds and mathematical modelling of the extraction process.

CHAPTER 2 UV-VIS SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS QUANTIFICATION OF LYCOPENE AND β-CAROTENE

Abstract

UV–VIS spectrophotometric analysis is one of the cheapest and most handy methods used for carotenoids quantification in solutions. According to Lambert-Beer's law, carotenoids concentrations in a mixture are computed using mixture absorbances and characteristic absorption coefficients for pure compounds. This work proposes four methods for simultaneous quantification of two carotenoids using mixture spectral data, physical models of pure and mixtures spectra in acetone-hexane mixture (Ac:Hx), different wavelengths groups for absorbance of each carotenoid, the spectra overlapping property and isosbestic point presence. Two absorbance factors defined to improve the concentrations equations accuracy and different assumptions in terms of wavelengths groups and contribution of each carotenoid at mixture absorbance are considered. These methods are validated using lycopene and β-carotene synthetic and two tomato residues extracts. IPM–II–WG_6 method gives the best correlation from statistical point of view, with medium accuracy errors, for simultaneous quantification of lycopene and β-carotene in a mixture.

2.2.8 Physical models

The physicals model are based on pure carotenoids (*X, Y*) and their mixture (*M*) spectra, obtained by scanning stock solutions with different compositions. The maximum absorbances of X and Y carotenoids ($A^{pureY}{}_{\lambda 1}$, $A^{pureX}{}_{\lambda 2}$) are identified at λ_1 and λ_2 wavelengths. The mixture absorbances ($A^{M}{}_{\lambda 1}$, $A^{M}{}_{\lambda 2}$) are measured at the same wavelengths. X, Y and M spectra can totally or partially overlap.

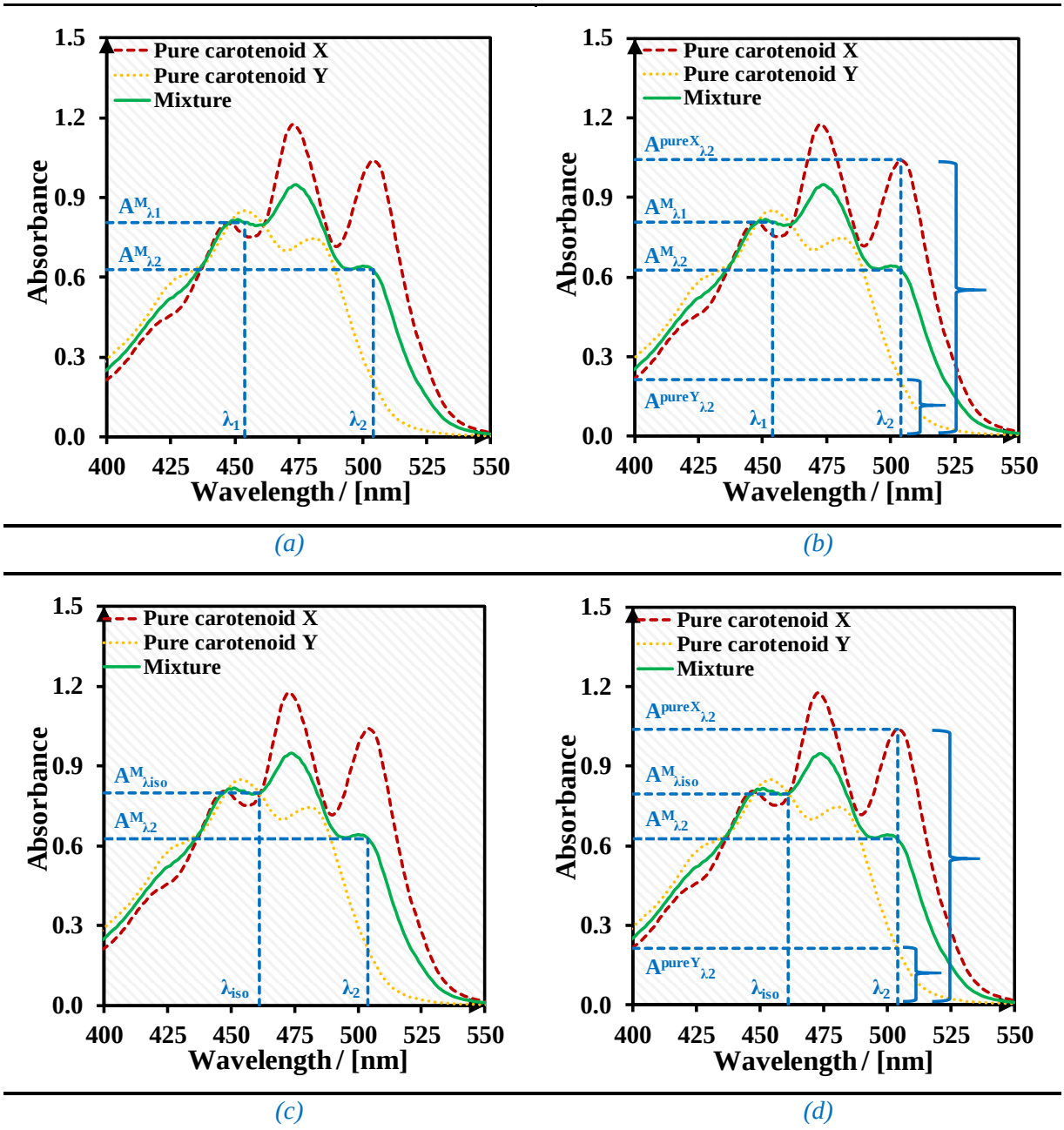


Figure 2.4 Physical models for: (a) WGM–I, WGM–II and AFM–I methods; (b) AFM–II, AFM–III methods; (c) ASM–I and IPM–I methods; (d) ASM–II, ASM–III and IPM–II methods; (X concentration – 4 mg/L, Y concentration – 4 mg/L, Mixture concentration – 4 mg/L)

Four physical models are presented in *Figures 2.4a–d*, according to the form of overlapping spectra: partially (*Figure 2.4a*), totally (*Figure 2.4b*), partially with isosbestic point (*Figure 2.4c*), totally with isosbestic point (*Figure 2.4d*). If there is an overlapping of spectra at one wavelength, a ratio of absorbances of both pure compounds can be defined and used for concentrations calculation of the mixture. Groups of wavelengths and maxima absorptions are identified and different assumptions are formulated using these physical models.

2.3.2 Calibration curves

For calibration curves, L_1 – L_{10} (lycopene solubilized in Ac:Hx) and B_1 – B_{10} (β-carotene solubilized in Ac:Hx) stock solutions with concentration range 0.5 – 5 mg/L were prepared. Five replicates of samples, at ten concentration levels for each analyte were scanned by UV–VIS spectrometer. Absorption spectra present bands with maximum absorbances at 426 nm, 454 nm, 480 nm wavelengths for β-carotene and 447 nm, 473 nm, 504 nm for lycopene. Moreover, these pure carotenoids present an isosbestic point at 461 nm.

Table 2.8 Regression analysis results for lycopene and β-carotene calibration

Carotenoid	λ / [nm]	Slope b ± SD_b	Intercept a ± SD_a	Determination coefficient, R^2	Response standard deviation, SD_y	F test; Significant *F*
Lycopene	447	0.198 ± 0.002	0.012 ± 0.004	0.9996	0.287	**; <0.05
β-carotene		0.188 ± 0.001	0.020 ± 0.003	0.9999	0.356	**; <0.05
Lycopene	473	0.294 ± 0.002	0.009 ± 0.013	0.9992	0.233	**; <0.05
β-carotene		0.173 ± 0.000	0.009 ± 0.002	0.9999	0.453	**; <0.05
Lycopene	504	0.261 ± 0.002	0.011 ± 0.004	0.9998	0.186	**; <0.05
β-carotene		0.051 ± 0.000	0.005 ± 0.001	0.9997	0.078	**; <0.05
Lycopene	461	0.194 ± 0.001	0.012 ± 0.002	0.9999	0.293	**; <0.05
β-carotene		0.194 ± 0.001	0.013 ± 0.002	0.9999	0.294	**; <0.05
Lycopene	426	0.113 ± 0.001	0.009 ± 0.002	0.9998	0.163	**; <0.05
β-carotene		0.142 ± 0.001	0.010 ± 0.003	0.9996	0.133	**; <0.05
Lycopene	454	0.187 ± 0.001	0.012 ± 0.004	0.9997	0.270	**; <0.05
β-carotene		0.205 ± 0.005	0.014 ± 0.013	0.9996	0.316	**; <0.05
Lycopene	480	0.253 ± 0.002	0.012 ± 0.007	0.9994	0.368	**; <0.05
β-carotene		0.182 ± 0.001	0.011 ± 0.003	0.9998	0.170	**; <0.05

*SD – standard deviation, F – Snedecor's F test, significant F – probability for level of significance α = 0.05.** no statistically significant difference in absorbances sets values can be observed.*

The regression analysis was used to determine the linear dependency of absorbances with analyte concentration for seven different wavelengths for each carotenoid. Calculated regression parameters of each calibration curve (slope and intercept) are presented in *Table 2.8*. For all calibration curves, slopes between 0.051 and 0.294, determination coefficients between 0.9992 and 0.9999 and significant *F* below 0.05 were identified. Calibration curves of lycopene and β-carotene at isosbestic wavelength (461 nm) have the same slope and intercept. For the other groups of wavelengths, there are differences between the slopes.

2.3.3 Absorbance factors

Using calibration curves of pure compounds, two absorbance factors $F^{\lambda 1}{}_{\lambda 2}$ are defined at 454 – 504 nm and 461 – 504 nm. Their calculated values are used in ASM and AFM methods to describe lycopene contribution in the mixture (*Table 2.9*).

Table 2.9 Calculated absorbance factors for lycopene and β-carotene quantification

Factor type	Wavelengths / [nm]		Target mixtures	Value ± SD
	λ_1	λ_2		
Absorbance factors ($F^{\lambda 1}{}_{\lambda 2}$)	454	504	$M_1 – M_{13}$	0.716 ± 0.003
	461	504	$M_1 – M_{13}$	0.741 ± 0.003

Absorbance ratio factor $R^{X/Y}{}_{\lambda 2}$ is evaluated using equation (*2.2*), as the ratios of absorbances at 504 nm wavelength of pure lycopene and β-carotene synthetic samples solubilized in Ac:Hx, in 0.5 – 5 mg/L concentration range.

$$R_{\lambda 2}^{X/X} = \frac{A_{\lambda 2}^{pureX}}{A_{\lambda 2}^{pureY}} \qquad (2.2)$$

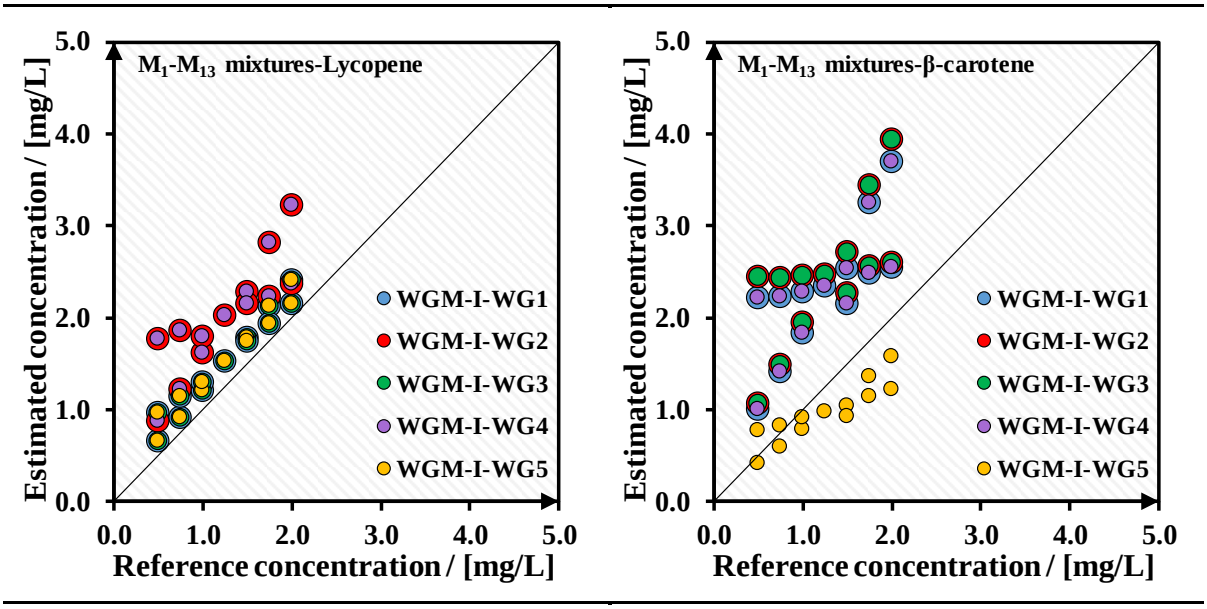
Using regression analysis, the slope of linear regression of these values vs. concentrations ratios C_X/C_Y is calculated as $r^{X/Y}{}_{\lambda 2} = 4.813$. This ratio factor can be computed for any sample solubilized in Ac:Hx, considering the slope $r^{X/Y}{}_{\lambda 2}$ in (equation (*2.4*)).

$$R_{\lambda 2}^{X/X} = r_{\lambda 2}^{X/X} \cdot \frac{A_{\lambda 1}^{M} \cdot a_{\lambda 2}^{Y} - A_{\lambda 2}^{M} \cdot a_{\lambda 1}^{Y}}{A_{\lambda 2}^{M} \cdot a_{\lambda 1}^{X} - A_{\lambda 1}^{M} \cdot a_{\lambda 2}^{X}} \qquad (2.4)$$

2.3.6 Concentration calculation using WGM methods

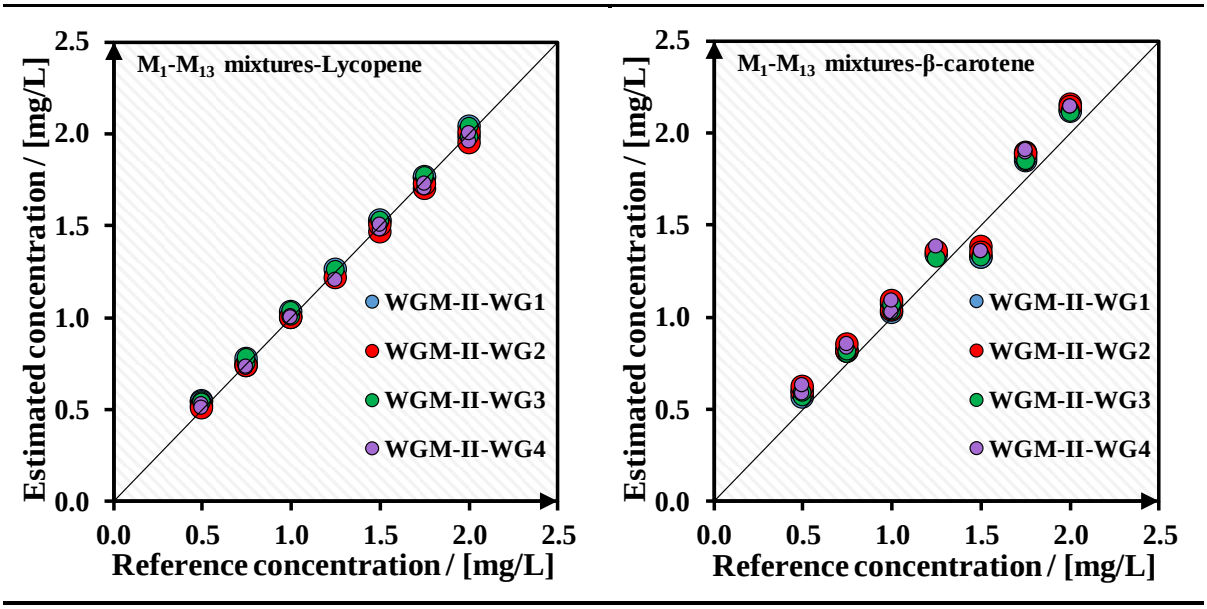
Concentrations of X and Y are estimated based on method assumptions and wavelengths groups (*WG*). The equations contain only data from spectra of pure carotenoids and mixtures absorbances. Evaluated concentrations vs. reference concentrations are plotted as parity charts in *Figures 2.9a,b* and *Figures 2.10a,b* for WGM–I and WGM–II methods.

Applying both forms of WGM method, with WG_1 – WG_5 wavelengths groups, estimated carotenoids concentrations are higher than reference concentrations. Regardless used method, the β-carotene errors are significantly higher than those obtained for lycopene quantification. However, the percentage errors are over 10 %, which means that the assumptions of these methods are not justified, although results based on these assumptions are reported by some authors [5,10,14]. Only one method, WGM–II–WG_3 led to acceptable percentage errors between 1.51 – 5.15 % for lycopene and 7.25 – 11.28 % for β-carotene. This method considers WG_3 wavelengths group (λ_1 = 454 nm, λ_2 = 504 nm), usually used for lycopene and β-carotene quantification.



(a) *(b)*

Figure 2.9 Estimated vs. reference concentrations with WGM–I method for simultaneous quantification of: (a) lycopene; (b) β-carotene



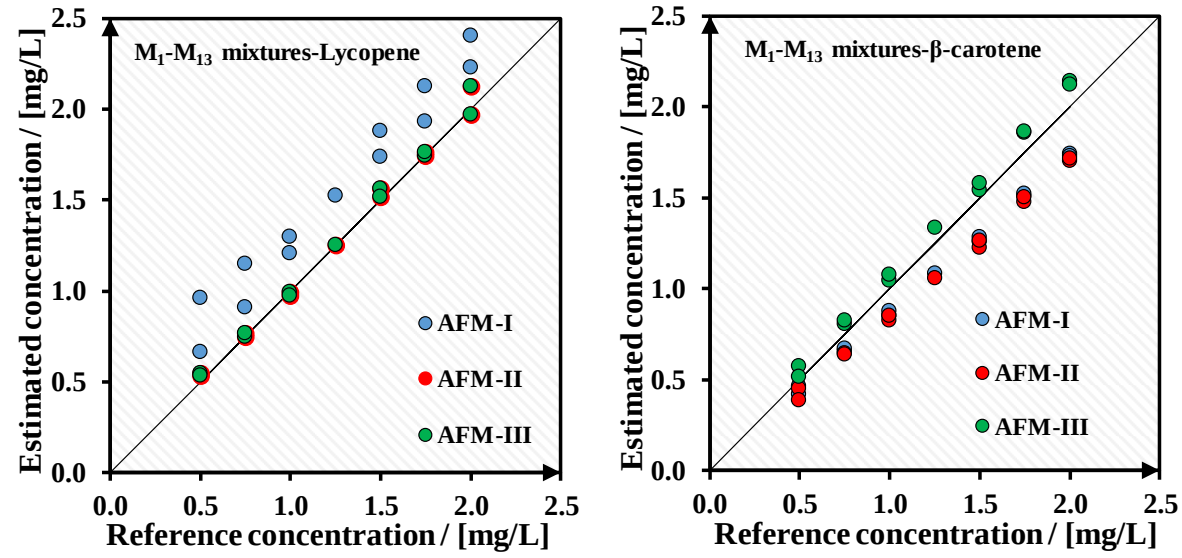
(a) *(b)*

Figure 2.10 Estimated vs. reference concentrations with WGM–II method for simultaneous quantification of: (a) lycopene; (b) β-carotene

2.3.7 Concentrations calculation using AFM, ASM and IPM methods

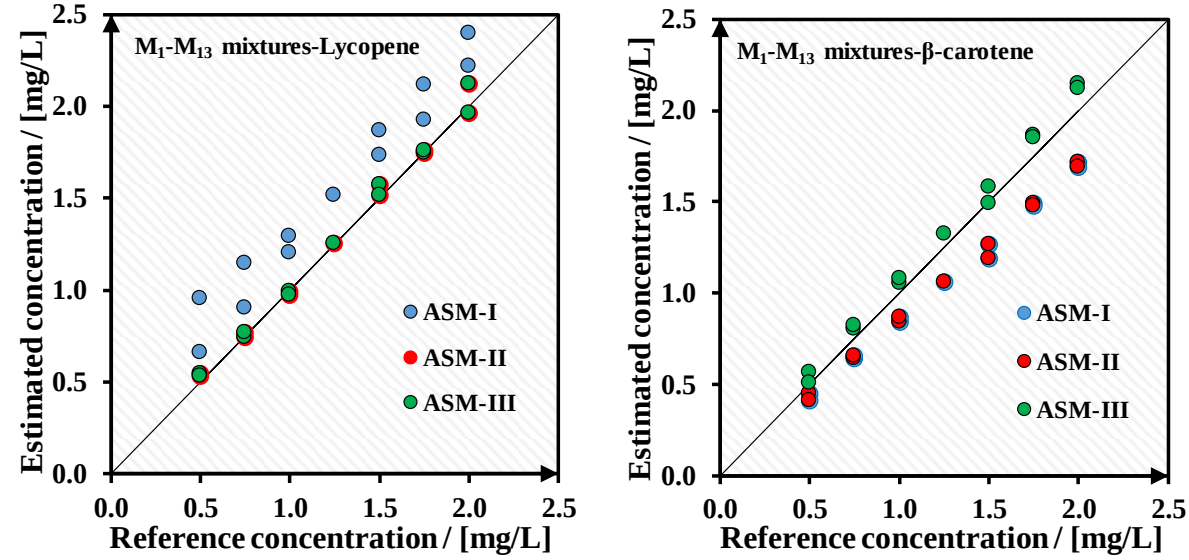
In *Figures 2.12–2.14*, the parity graphs for estimated concentrations using proposed methods (AFM, ASM and IPM) vs. reference concentrations of thirteen synthetic mixtures are presented. High deviations are observed for the first form of each method. Calculated concentrations with second and third form of AFM, ASM and IPM methods present smaller deviations from the reference values, especially those estimated based on $R^{X/Y}{}_{\lambda 2}$ factor. Additionally, there is a good correlation of lycopene concentrations with the reference values, compared to β-carotene. First

form of each method, with simplified assumptions led to higher errors (percentage errors over 10 % for both lycopene and β-carotene compounds). Between AFM methods, the lowest errors below 3.63 % for lycopene and 6.72 % for β-carotene were obtained using AFM–III–WG_3 method. For ASM methods, the lowest errors were obtained using ASM–III–WG_6 method, with values below 3.74 % for lycopene and 6.50 % for β-carotene.



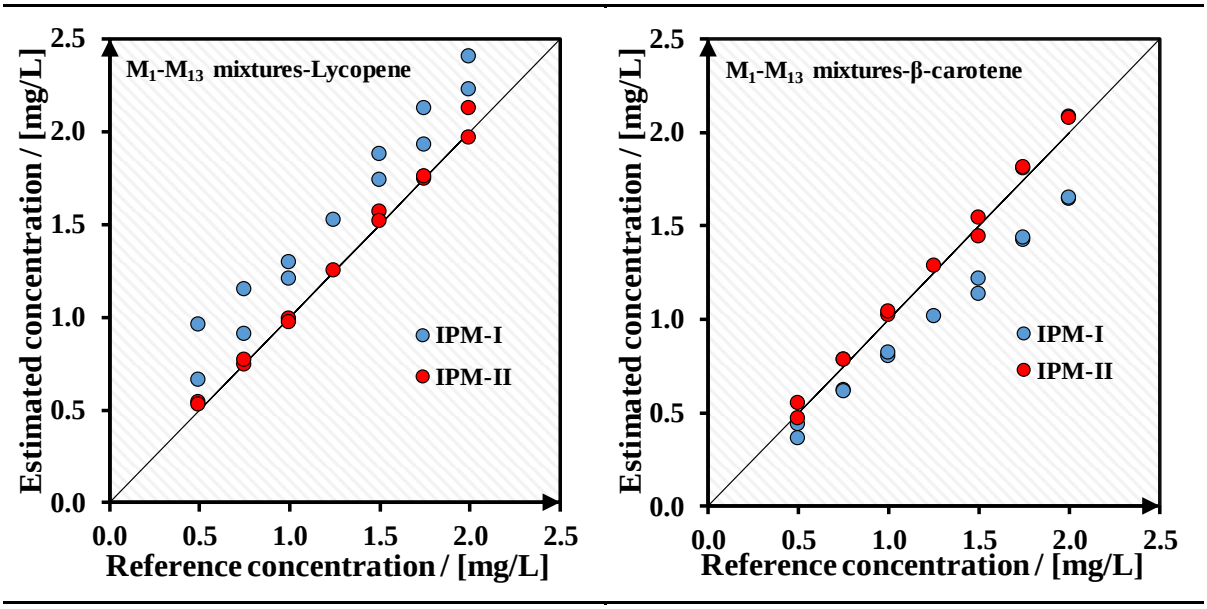
(a) *(b)*

Figure 2.12 Estimated vs. reference concentrations with AFM methods for simultaneous quantification of: (a) lycopene; (b) β-carotene



(a) *(b)*

Figure 2.13 Estimated vs. reference concentrations with ASM methods for simultaneous quantification of: (a) lycopene; (b) β-carotene



(a) *(b)*

Figure 2.14 Estimated vs. reference concentrations with IPM methods for simultaneous quantification of: (a) lycopene; (b) β-carotene

Finally, acceptable percentage errors below 5 % (3.74 % for lycopene and 4.79 % for β-carotene) were obtained with IPM–II–WG_6 method, which considers $R^{X/Y}{}_{\lambda 2}$ value and absorbance values at 461 nm and 504 nm, for simultaneous estimation of lycopene and β-carotene concentrations.

2.2.12 Isosbestic point method (IPM)

This method can be applied to determine carotenoids concentrations if two conditions are fulfilled. The spectra of pure carotenoids with the same concentration (*X*, *Y*) and their mixture (*M*) present an isosbestic point and the spectrum of X is extended more than Y. Physical models are presented in *Figures 2.4c,d*, while mathematical models are presented in *Table 2.5*. Concentrations are computed using only spectral data for pure and mixtures of carotenoids solutions. Two forms are presented if the contribution of Y at λ_2 is considered or not.

2.2.12.1 Isosbestic point method (IPM–I)

This method is based on concentrations calculation of total carotenoids (*TC*) at izosbestic point (λ_{iso}), of X at a maximum absorption of lycopene at which β-carotene has no contribution (λ_2) and Y as the difference between TC and X concentrations. The assumptions of this method are the same as ASM–I method.

2.2.12.2 Modified isosbestic point method (IPM–II)

This method considers the same assumptions as AFM–III and ASM–III methods and X concentration in mixture is calculated using $R^{X/Y}{}_{\lambda 2}$, mixture absorbance and absorption coefficient of X at λ_2. The equations for X and Y concentrations in mixture are presented in *Table 2.5*.

Table 2.5 IPM models for simultaneous carotenoids quantification in a mixture

IPM-I	IPM-II
Model assumptions:	***Model assumptions:***
$\lambda_1 = \lambda_{iso} \rightarrow (X+Y)$	$\lambda_1 = \lambda_{iso} \rightarrow (X+Y)$
$\lambda_2 = \lambda_{max,X} \rightarrow (X)$	$\lambda_2 = \lambda_{max,X} \rightarrow (X+Y)$
$A_{\lambda iso}^{M} = A_{\lambda iso}^{pureX} = A_{\lambda iso}^{pureY}$	$A_{\lambda iso}^{M} = A_{\lambda iso}^{pureX} = A_{\lambda iso}^{pureY}$
$a_{\lambda iso}^{M} = a_{\lambda iso}^{pureX} = a_{\lambda iso}^{pureY} = a_{\lambda iso}^{X=Y}$	$a_{\lambda iso}^{M} = a_{\lambda iso}^{pureX} = a_{\lambda iso}^{pureY} = a_{\lambda iso}^{X=Y}$
$A_{\lambda iso}^{M} = A_{\lambda iso}^{X} + A_{\lambda iso}^{Y}$	$A_{\lambda iso}^{M} = A_{\lambda iso}^{X} + A_{\lambda iso}^{Y}$
$A_{\lambda 2}^{M} = A_{\lambda 2}^{X}$	$A_{\lambda 2}^{M} = A_{\lambda 2}^{X} + A_{\lambda 2}^{Y}$
	$R_{\lambda 2}^{X/X} = r_{\lambda 2}^{X/X} \cdot \frac{A_{\lambda 1}^{M} \cdot a_{\lambda 2}^{Y} - A_{\lambda 2}^{M} \cdot a_{\lambda 1}^{Y}}{A_{\lambda 2}^{M} \cdot a_{\lambda 1}^{X} - A_{\lambda 1}^{M} \cdot a_{\lambda 2}^{X}}$
Model equations:	***Model equations:***
$C_{TC}^{M} = A_{\lambda iso}^{M} / a_{\lambda iso}^{X=Y}$	$C_{TC}^{M} = A_{\lambda iso}^{M} / a_{\lambda iso}^{X=Y}$
$C_{X}^{M} = A_{\lambda 2}^{M} / a_{\lambda 2}^{X}$	$C_{X}^{M} = \left(A_{\lambda 2}^{M} \cdot R_{\lambda 2}^{X/Y}\right) / \left[a_{\lambda 2}^{X} \cdot \left(R_{\lambda 2}^{X/Y} + 1\right)\right]$
$C_{Y}^{M} = C_{TC}^{M} - C_{X}^{M}$	$C_{Y}^{M} = C_{TC}^{M} - C_{X}^{M}$

CHAPTER 3 THE INFLUENCE OF DRYING PARAMETERS ON BIOACTIVE COMPOUNDS RECOVERY FROM TOMATOES BY EXTRACTION

I. EFFECT OF DRYING PROCESSES ON LYCOPENE RECOVERY FROM TOMATO PEELS

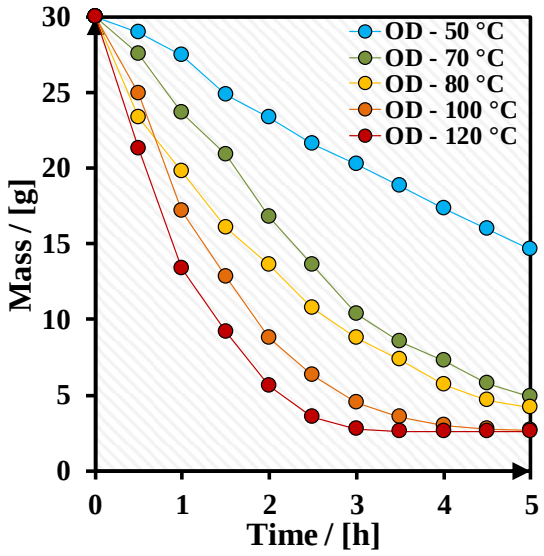
Abstract

The aim of this work is to analyze the effect of three drying processes (oven drying OD, vacuum oven drying VOD and hot air drying HAD) of tomato peels from Crystal variety in terms of moisture content and lycopene recovery. The drying process is performed for 5 h, at temperatures between 50 °C and 120 °C. The influence of the drying temperature is observed on the dried tomato peels aspect, which changes from bright red to dark brown at drying temperatures higher than 80 °C, and also in the moisture content of the samples which decreases with temperatures increase. The highest moisture is observed at oven-dried tomato peels, while the lowest value is found in hot-air-dried tomato peels. The highest amount of lycopene, 34 mg/100 g fresh tomato peels (329 mg/100 g dried tomato peels), is extracted from tomato peels dried using hot-air drying method, at the temperature of 80 °C which is associated with the lowest moisture content of 20.16 %.

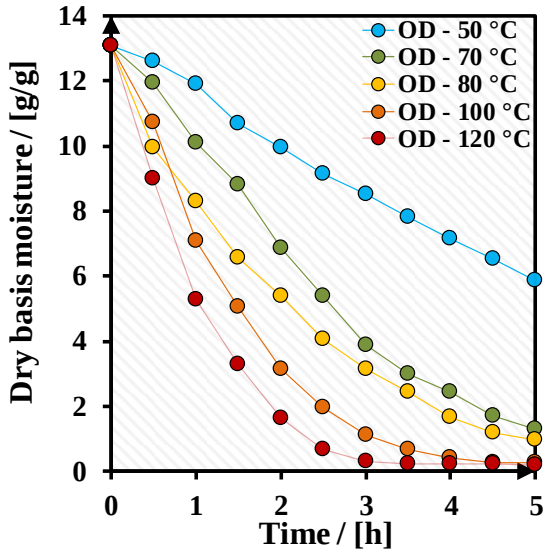
3.3.1 Influence of drying method on moisture content

Figures 3.2a,c,e show the change of the sample mass during the drying process performed in dryers of different types (OD – *Figures 3.2a*; VOD – *Figures 3.2c*; HAD – *Figures 3.2e*) and at different temperatures. The initial mass of the sample was 30 g.

In OD the mass transfer of water from solid to vapor state takes place by natural convection. Thus, complete drying is achieved in 3.5 h at 120 °C and in 5 h at 100 °C. In VOD, as the evaporation potential is increased due to the vacuum, complete drying is achieved in shorter time, namely 3 h (at 120 °C) or 4 h (at 100 °C). During HAD, the air flows over the solid material and drives the water vapors, improving the mass transfer rate. As a result, the method is more efficient, complete drying requiring only 2 h at 120 °C or 3.5 h at 100 °C.



(a)



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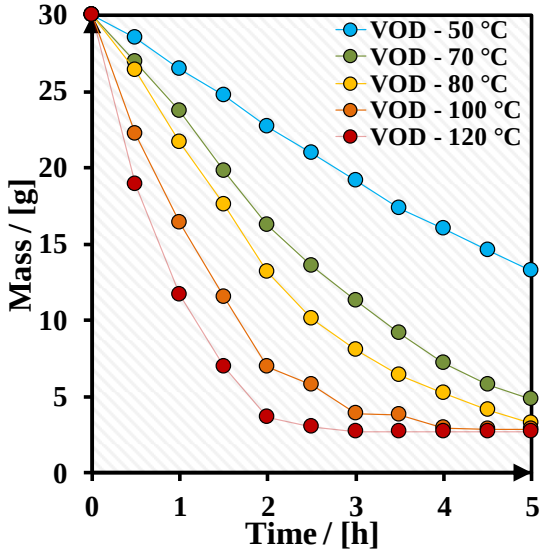

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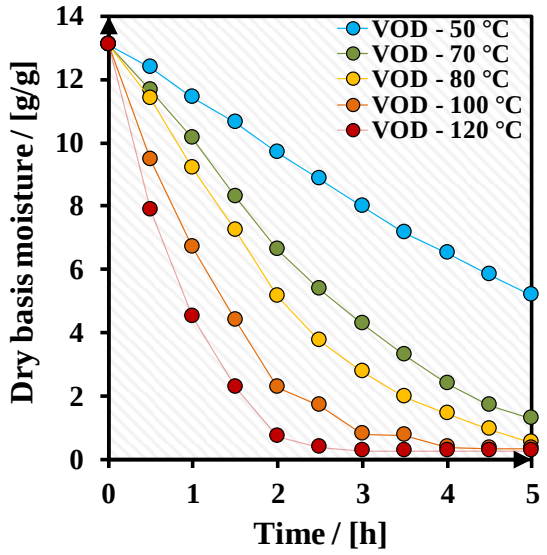

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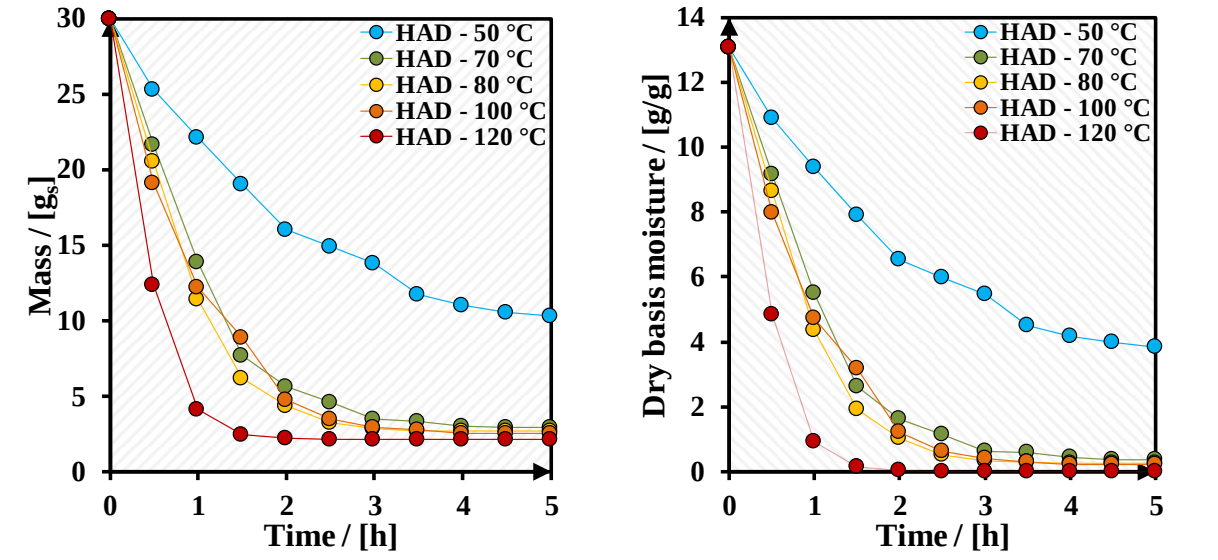


(e) *(f)*

Figure 3.2 The variation of mass and moisture content of tomato samples: (a) mass variation in OD process; (b) dry-basis moisture variation in OD process; (c) mass variation in VOD process; (d) dry-basis moisture variation in VOD process; (e) mass variation in HAD process; (f) dry-basis moisture variation in HAD process

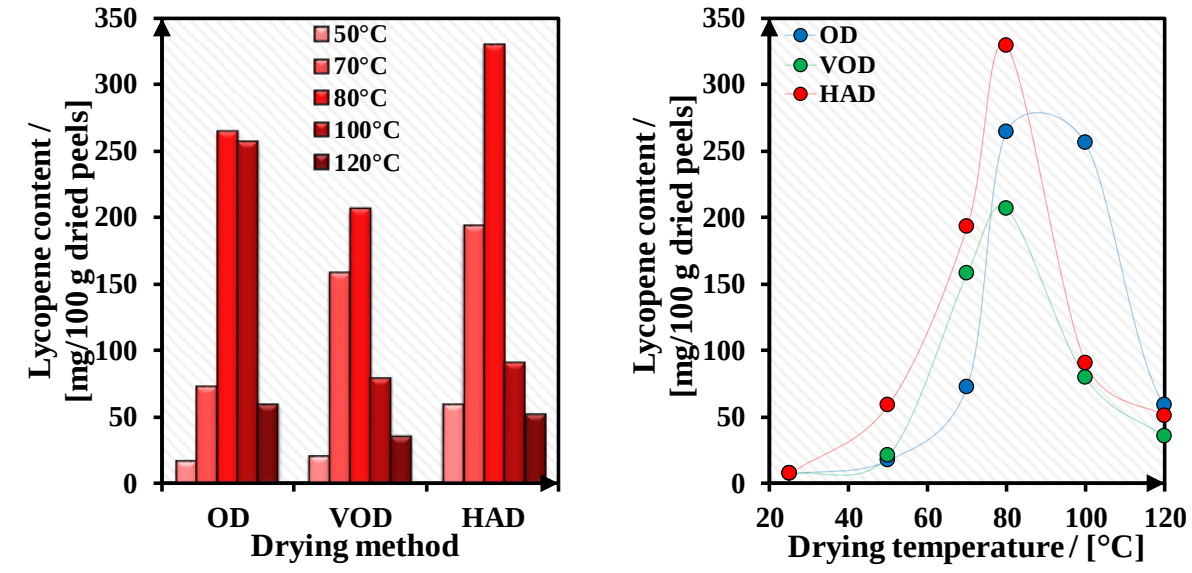
As expected, the drying is faster at higher temperature. When the drying is performed at low temperature (50 °C), after 5 h, the mass of dried peels is about 13 – 14 g (OD and VOD) and 10 g (HAD). During the initial period (about 1 h at 120 °C), the rate of water removal is constant. *Figures 3.2b,d,f* show the moisture content (M_{DB}) using the three drying methods as OD – *Figures 3.2b*, VOD – *Figures 3.2d* and HAD – *Figures 3.2f*. After 5 h of drying at 50 °C, the moisture content (M_{DB}) is 5.88 g/g (OD), 5.20 g/g (VOD), and 3.85 g/g (HAD). These values correspond to removal of 55 % (OD), 60 % (VOD), and 70 % of the initial amount of water. A similar trend is observed at other temperatures.

3.3.4 Influence of drying method on the amount of recovered lycopene

Figures 3.6a,b present the amount of lycopene recovered from tomato peels relative to dried (*Figure 3.6a*) and fresh samples (*Figure 3.6b*), which varies with the drying method between 17 – 329 mg/100 g dried peels.

For OD tomato peels the highest lycopene amount of 264 mg /100 g dried tomato peels was obtained from tomato peels dried at 80 °C, while the lowest amount of 17 mg/100 g of dried tomato peels was obtained from tomato peels dried at 50 °C. For VOD tomato peels the highest lycopene amount of 207 mg/100 g dried tomato peels was obtained from tomato peels dried at 80 °C, while the lowest amount of 21 mg/100 g of dried tomato peels was found in tomato peels dried at 50 °C. For HAD tomato peels the highest lycopene amount of 329 mg/100 g dried tomato peels

was obtained from tomato peels dried at 80 °C, while the lowest amount of 51 mg/100 g of dried tomato peels was found in tomato peels dried at 120 °C.



(a) *(b)*

Figure 3.6. Lycopene content during drying of tomato peels: (a) variation with the drying method; (b) variation with the drying temperature

II. MATHEMATICAL MODELLING OF THIN-LAYER DRYING KINETICS OF TOMATO PEELS: INFLUENCE OF DRYING TEMPERATURE ON THE ENERGY REQUIREMENTS AND EXTRACTS QUALITY

Abstract

Drying is the most popular technique used at industrial level as pretreatment step of samples subjected to extraction process to valorize the natural products as carotenoids with high nutraceutical importance from tomatoes. Tomato drying implies high energy consumption due to the high moisture content and limiting drying temperatures are necessary to avoid carotenoids degradation. To scale-up the Rila tomato peels drying process, ten thin-layer mathematical models derived from Fick's second law of diffusion are formulated based on experimental data for six different temperatures (50 – 75 °C) and validated by statistical analysis. Based on R^2, χ^2 and root mean square error (*RMSE*), the best predicted values were calculated using Two term model compared with Fick's second law of diffusion model which present small deviations at lower temperatures. Experimental data on extract quality (carotenoids content) were used to predict the effect of drying temperature on lycopene and β-carotene content and two degradation models were formulates. Increasing the drying temperature from 50 °C to 110 °C a degradation of 94 % for

lycopene and 83 % for β-carotene was predicted. Using hot-air drying method, the recommended drying temperature for Rila tomato peels is 50 °C to avoid the carotenoids degradation with specific energy consumption of 56.60 kWh/kg tomato peels.

3.7.6.1 Theoretical diffusion model of drying

Diffusion physical model. For tomato peels sample, the physical model of moisture diffusion is illustrated in *Figure 3.8*. The tomato peels bed in the form of a regular parallelepiped slab with geometric characteristics as the cross sectional area A and the thickness L is placed in a hot air environment, in isothermal conditions ($T = ct$). The moisture diffuses from the center of the slab to the surface, in both top and bottom directions (z *axis*). At the initial moment ($t = t_0$) the moisture ratio (M_0) is maximum, then when the diffusion starts ($t > t_0$), the water concentrates on the surface and the core of the slab becomes dry. Inside of the tomato peels bed, an element of volume with the thickness Δz is considered. The moisture flux entering in this volume is $J\big|_z$, while the moisture flux leaving the volume is $J\big|_{z+\Delta z}$. The diffusion is one-dimensional because there is a concentration gradient only along the z-axis.

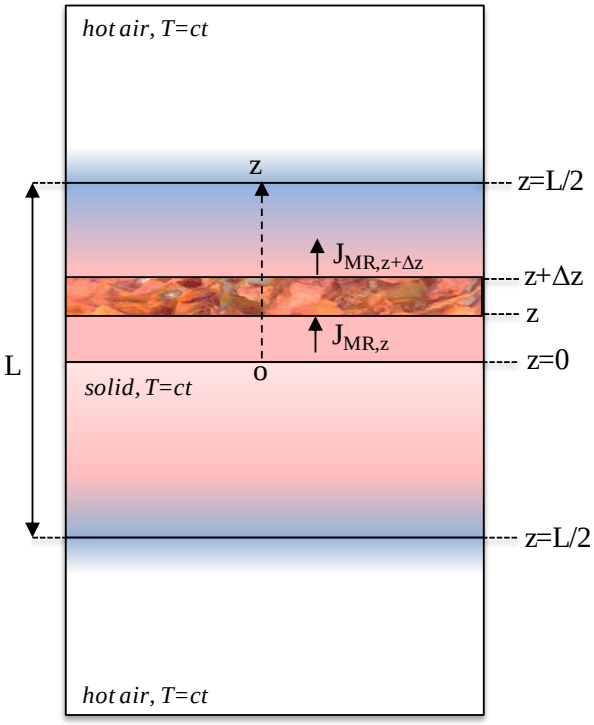


Figure 3.8 Diffusion physical model at time $t > t_0$ (L – the slab thickness / [m], J – the moisture mass flux / [kg/(m^2·s)], z – the diffusion direction, T – the drying temperature [°C])

Diffusion mathematical model. The model assumptions consider that a) the moisture is uniformly distributed into the solid sample; b) negligible external resistance for mass transfer; c) isothermal conditions for convective drying with hot air; d) the shrinkage effect of the solid during

drying is neglected e) the moisture concentration at both slab surfaces are equal. The analytical solution for the diffusion, equation (*3.20*), is given for the initial and boundary conditions, and for the considered assumptions in the falling rate period and proposed slab geometry of the tomato peels as a sum of infinite Fourier series [34]:

$$MR = \frac{8}{\pi^2}\left[e^{-\frac{\pi^2 \cdot D_{eff} \cdot t}{4(L/2)^2}} + \frac{1}{9} \cdot e^{-\frac{9 \cdot \pi^2 \cdot D_{eff} \cdot t}{4(L/2)^2}} + \frac{1}{25} \cdot e^{-\frac{25 \cdot \pi^2 \cdot D_{eff} \cdot t}{4(L/2)^2}} + ... \right] \cdot \qquad (3.20)$$

where $\frac{D_{eff} \cdot t}{(L/2)^2}$ is the Fourier number. For long drying time, Fourier number is higher than 0.2 and only the first term is significant, thus the equation (*3.20*) can be simplified in (*3.21*) form, without affecting the accuracy of the model's prediction [32]:

$$MR = \frac{8}{\pi^2} \cdot e^{\frac{\pi^2 \cdot D_{eff} \cdot t}{4(L/2)^2}} \qquad (3.21)$$

3.7.6.2 Semi-theoretical models of drying

For fitting the experimental drying data, semi-theoretical models were also chosen to describe the drying behavior of tomato peels.

Table 3.3 Thin-layer mathematical models for the drying of tomato peels

Model name	Model equation*
Theoretical Diffusion Model	
Fick's second law of diffusion	$MR = 8/\pi^2 \cdot \exp(-(\pi^2 D_{eff} t)/4/(L/2)^2)$
STM–N Models	
Newton	$MR = \exp(-kt)$
Page	$MR = \exp(-kt^n)$
Modified Page	$MR = \exp(-(kt)^n)$
STM–F Models	
Henderson and Pabis	$MR = a \cdot \exp(-kt)$
Modified Henderson and Pabis	$MR = a \cdot \exp(-kt) + b \cdot \exp(-gt) + c \cdot \exp(-ht)$
Midili	$MR = a \cdot \exp(-kt^n) + b \cdot t$
Logarithmic	$MR = a \cdot \exp(-kt) + b$
Two-term	$MR = a \cdot \exp(-k_1 t) + b \cdot \exp(-k_2 t)$
Two term-exponential	$MR = a \cdot \exp(-kt) + (1 - a) \cdot \exp(-kat)$

** MR is the dimensionless moisture ratio; D_{eff} / [m^2/s] is the effective diffusivity coefficient; L / [m] is the slab thickness; k, k_1, k_2, g, h / [s^{-1}] are the drying constants; a, b, c, n are the dimensionless model constants; t / [s] is the drying time.*

Semi-theoretical models are divided in two categories as semi-theoretical models derived from Newton's law of cooling (STM–N) and semi-theoretical models derived from Fick's second law of diffusion (STM–F), as presented in *Table 3.3*.

3.8.1 Experimental drying results

In *Figure 3.9a* is illustrated the variation of the moisture content of the tomato peels during hot-air drying (HAD) at six different temperatures in 50 – 75 °C range, until each sample reaches a final average wet-basis moisture of around 6.42 ± 0.30 %wt., which corresponds to a final moisture ratio of 0.014 ± 0.001. To avoid the carotenoids degradation during drying process of tomatoes, the moisture of the sample should be less than 10 % [46] and higher than 4.6 % [47]. The initial average wet-basis moisture of used tomato peels was 82.63 ± 1.51 %, in the range reported by other authors as 79.13 % [8], 80 % [25] or 80 – 85 % [48]. As it can be seen, the drying time increases from 6 to 11 h with a HAD temperature increase of 5 degree in the 50 – 75 °C range, for a similar final moisture of dried tomato peels sample.

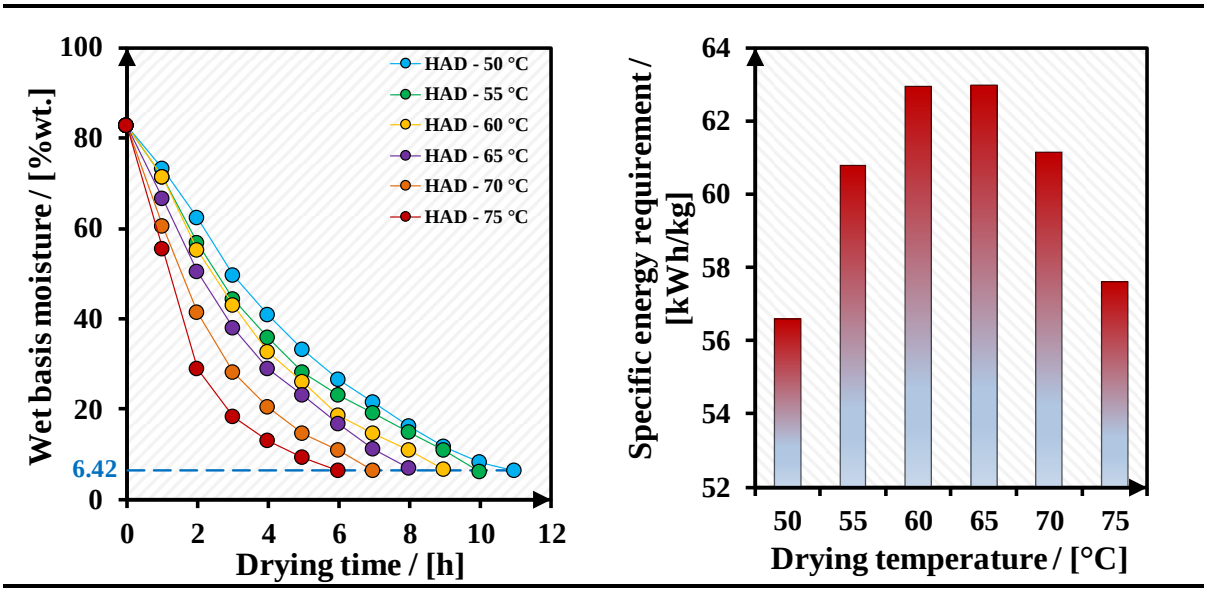


Figure 3.9 Tomato peels hot-air drying (HAD) characteristics variation with the drying temperature: (a) wet-basis moisture; (b) specific energy requirements.

In *Figure 3.9b* is presented the variation of the specific energy requirement for tomato peels hot-air drying with the drying temperatures. The specific energy requirement values varied between 56.60 – 63.00 kWh/kg peels, with minimum value for 50 °C. The drying specific energy is influenced by the temperature-time combination. The decrease of the specific energy requirement after the temperature of 65 °C may be associated with the decrease of the drying time, because at temperatures of 70 °C and 75 °C the drying time is 7 and 6 h, respectively.

3.8.2 Determination of effective moisture diffusivity and activation energy

The effective moisture diffusivity coefficient D_{eff} values were determined by fitting experimental data at six different drying temperatures, from the slope of linear regression of the falling rate period moisture ratio data and slab thickness of 10 mm. For tomato peels drying, calculated D_{eff} values varied between $1.01 \cdot 10^{-9}$ – $1.53 \cdot 10^{-9}$ m^2/s in the drying temperature range of 50 – 75 °C, increasing with the drying temperature. The pre-exponential factor and the activation energy (E_a) for diffusion coefficient at different temperatures were determined. *Figure 3.10* presents the linear relationship between ln(D_{eff}) and the inverse of the drying temperature ($1/T$) that has high correlation, R^2 value of 0.9706 and low values of χ^2 and *RMSE* as 0.0010 and 0.0259, respectively. The estimated value of E_a for HAD drying of tomato peels is 16.27 kJ/mol and falls within the general range of 14.42 – 43.26 kJ/mol reported for drying of for fruits and vegetables [28].

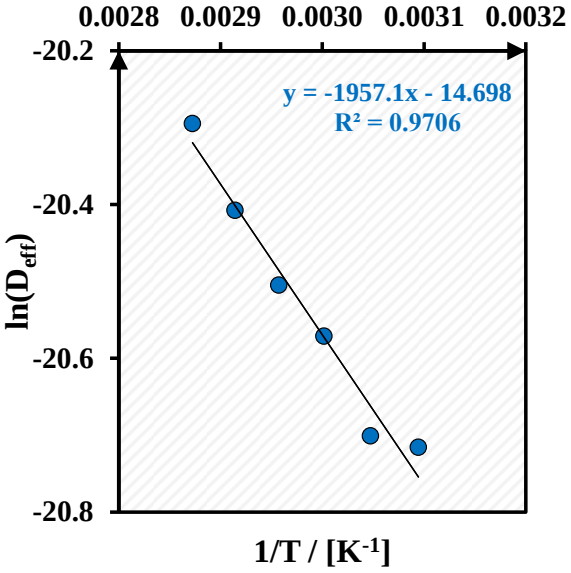
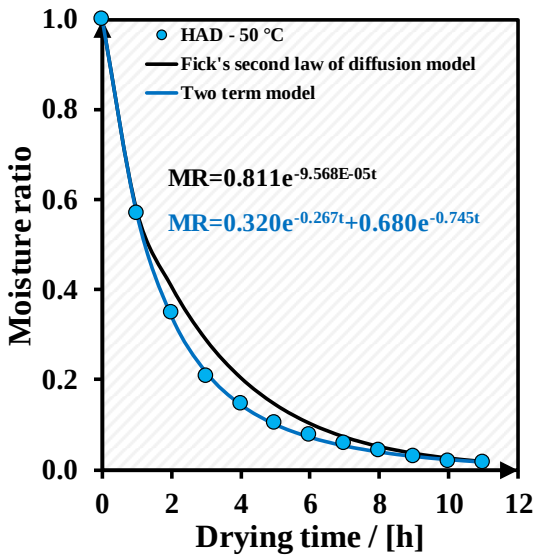


Figure 3.10 Estimation of the pre-exponential factor D_0 and activation energy E_a for moisture diffusivity coefficient.

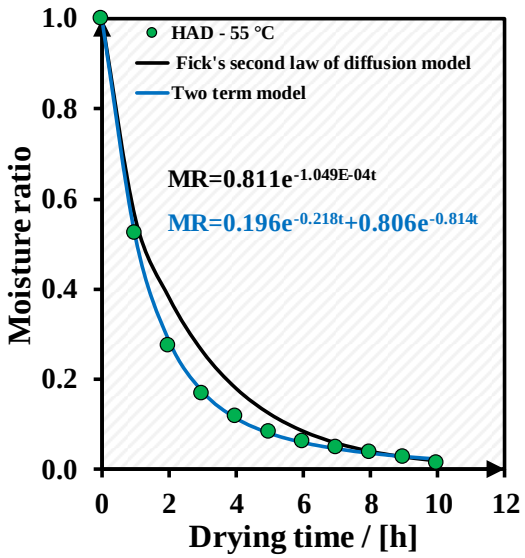
3.8.3 Drying kinetic parameters and models validation

Moisture ratio data obtained from the drying experiments at different temperatures were fitted using ten thin-layer drying mathematical models to determine which model adequately fits the experimental data of tomato peels drying with HAD method, to obtain samples with similar final moisture. In *Figures 3.11a–f* are illustrated the variation with drying time of the experimental moisture ratios and predicted values with Fick's second law of diffusion and Two-term models, for temperatures between 50 – 75°C. The most suitable model was chosen based on the highest R^2 and lowest χ^2 and *RMSE* values. R^2 values show a good prediction for all the models (R^2 between 0.9590 – 0.9999). A slight deviation was observed in the area of higher drying temperatures (75 °C) for Fick's second law of diffusion model which consider the diffusion coefficient (R^2 between

0.9432 – 0.9999). For this model, χ^2 is less than 0.01 for temperatures below 70 °C and for semi-theoretical models (STM-N and STM-F) χ^2 is lower than 0.002 for all the drying temperatures. Based on statistical values, the semi-theoretical model named Two-term fits with higher goodness experimental data (R^2 between 0.9995 – 0.9999, χ^2 between $9.4655 \cdot 10^{-6}$ – $7.7173 \cdot 10^{-5}$ and *RMSE* between 0.0013 – 0.0068) for tomato peels drying kinetics in 50 – 75 °C temperature range. The drying curves have a similar trend to exponential functions decreasing with drying time. The plots show that the Two-term model best fits the experimental moisture ratios for all drying temperatures. The Fick's second law of diffusion model prediction presents deviation towards the experimental data, mostly at higher temperatures.



(a)



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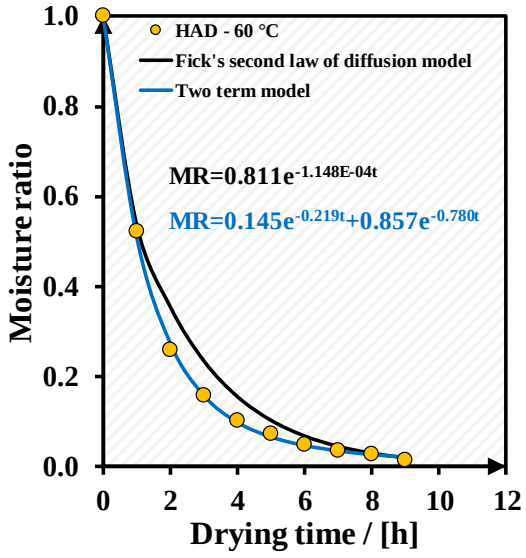

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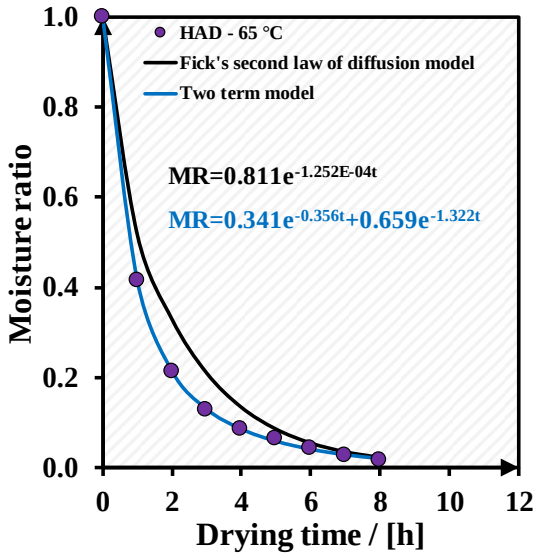

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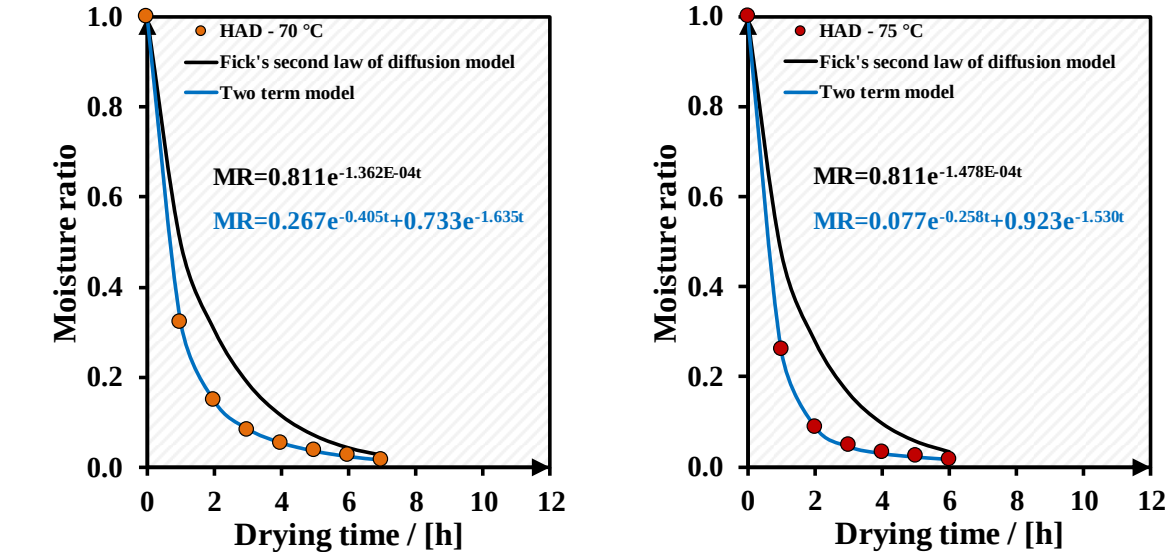
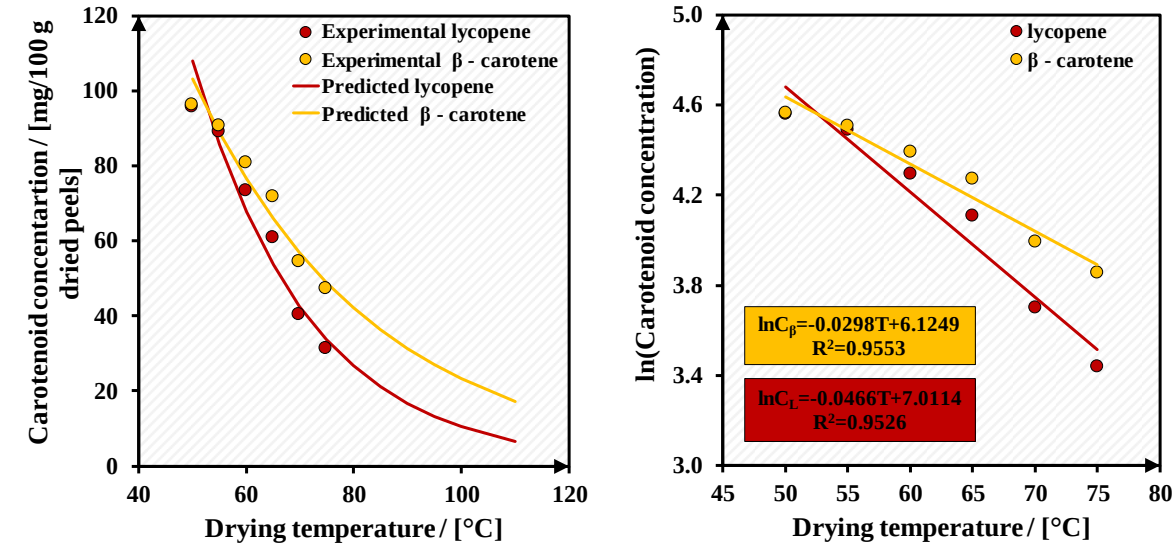


(e) *(f)*

Figure 3.11 Experimental vs. predicted drying data with Fick's second law of diffusion and Two-term models at different temperatures: (a) 50 °C; (b) 55 °C; (c) 60 °C; (d) 65 °C; (e) 70 °C; (f) 75 °C

3.8.4 Carotenoids degradation

In *Figure 3.13a* is presented the variation of lycopene and β-carotene contents from dried tomato peels with the drying temperature. Both compounds present high concentrations of around 96 mg/100 g dried peels at 50 °C and low values of 31 mg lycopene and 47 mg β-carotene/100 g dried peels at 75 °C. A small degradation of 5 % takes place up to 55 °C, regardless carotenoid compound. Between 60 – 75 °C the lycopene and β-carotene degradation increases from 21 % to 67 % and from 16 % to 51 %, respectively.



(a) *(b)*

Figure 3.13 Carotenoids concentrations vs. drying temperature: (a) carotenoids concentration experimental/predicted; (b) carotenoids degradation model

Based on the experimental data regarding the lycopene and β-carotene contents from tomato peels dried at different temperatures, two degradation models were formulated using regression analysis with good values of statistical parameters as R^2 higher than 0.95 and low values of χ^2 and *RMSE*. Using the equations of the models, illustrated in *Figure 3.13b*, the lycopene and β-carotene amounts in dried peels were predicted for 50 – 110 °C temperature range, as it can be noticed in *Figure 3.13a*. Following the degradation curves, it seems that at 110 °C the final lycopene and β-carotene contents were 6.59 mg/100 g dried peels and 17.23 mg/100 g dried peels, respectively, a degradation of 94 % being obtained for lycopene and of 83 % for β-carotene. The degradation of β-carotene is about 10 % higher than that of lycopene, this behavior being also observed in other studies [57,60].

CHAPTER 4 BIOACTIVE COMPOUNDS EXTRACTION FROM TOMATOES

I. VALUABLE NATURAL ANTIOXIDANT PRODUCTS RECOVERED FROM TOMATOES BY GREEN EXTRACTION METHODS

Abstract

Lycopene, β-carotene and ω-fatty acids are major compounds in tomatoes with known antioxidant activity, capable of preventing health disorders. The identification of potential natural sources of antioxidants, extraction efficiencies and antioxidant activity assessments are essential to promote such products to be used in the food, pharmaceutical or cosmetic industries. This work presents four added-value products recovered from tomatoes: pigmented solid oleoresin, pigmented oil and two raw extracts from supercritical and Soxhlet extraction. Different parameters including the matrices of tomatoes, extraction methods, green solvents and operating parameters were varied to obtain extracts with different qualities. Extracts analysis was performed using UV–VIS, FT–IR, GC–MS, Folin–Ciocalteu and DPPH methods. The highest-quality extract was the solid oleoresin obtained from tomato pomace using supercritical CO_2 extraction at 450 bar, 70 °C and 11 kg/h: 1016.94 ± 23.95 mg lycopene/100 g extract, 154.87 ± 16.12 mg β-carotene/100 g extract, 35.25 ± 0.14 mg GAE/g extract and 67.02 ± 5.11 % inhibition DPPH. The economic feasibility of the three extraction processes (1,10 and 100 kg dried pomace/batch as scalability criterion) was evaluated. The most profitable was the supercritical extraction process at the highest capacity, which produces pigmented solid oleoresin and oil with high contents of lycopene valorized with a high market price, using natural food waste (tomato pomace).

4.3.3 Soxhlet extraction

The Soxhlet extraction (*SE*) method's efficiency was checked analyzing two factors, solvent affinity and the seed content of tomato samples. Used green solvents were bioethanol (*1*) and ethyl acetate (*2*), while used tomato samples were slices (*TS*) and pomace (*TP*). From *Figures 4.3a,b*, it can be observed that raw extracts obtained with bioethanol from both types of samples (TS–1–SE, TP–1–SE) were less pigmented, with orange colors, than raw extracts with ethyl acetate (TS–2–SE, TP–2–SE), with red colors, regardless of sample type. The intense red color is associated with a higher carotenoid content [31]. For both types of tomato samples, the extraction was improved when ethyl acetate (*2*) was used, with 40 % for TS and 8 % for TP. Due to the different contents in the seeds of tomato samples and different affinity of both solvents, the calculated extraction efficiencies were between 5.92 ± 0.69 and 13.23 ± 1.14 g extract/100 g dried tomato slices using solvent (*1*) and 8.72 ± 0.93 – 14.33 ± 1.19 g extract/100 g dried tomato pomace using solvent (*2*). These results are similar to other studies: 8.46 % from TP and 4.41 % from tomato peels [1] or 6.5 – 19.3 % from TP [7].

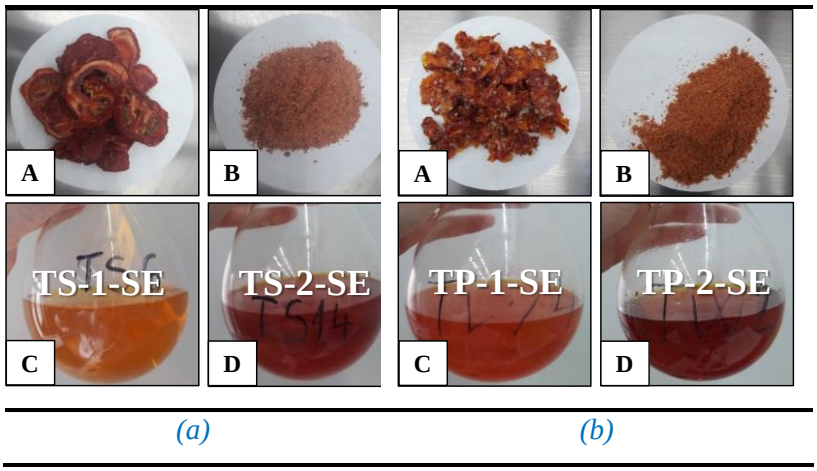


Figure 4.3 Tomato samples and raw extracts obtained with Soxhlet extraction (SE) using extraction solvents (1) bioethanol and (2) ethyl acetate, A – samples before grinding, B – samples after grinding, C – bioethanol extract (1), D – ethyl acetate extract (2: (a) tomato slices (TS); (b) tomato pomace (TP)

4.3.4 Supercritical CO_2 extraction

The seed content of the samples, the extraction pressure and the solvent flow rate were considered as factors that can affect the quality of raw extract obtained with SFE. Two sets of operating parameters were analyzed, set (*1*) at 400 bar, 70 °C and 9 kg/h and set (*2*) at 450 bar, 70 °C and 11 kg/h. By using operating parameter set (*2*) at high values of pressure (450 bar) and CO_2 flow rate (11 kg/h), the extraction was more efficient than operating parameter set (*1*), regardless of the tomato sample. Tomato samples subjected to SFE and extracts are presented in *Figures 4.4*.

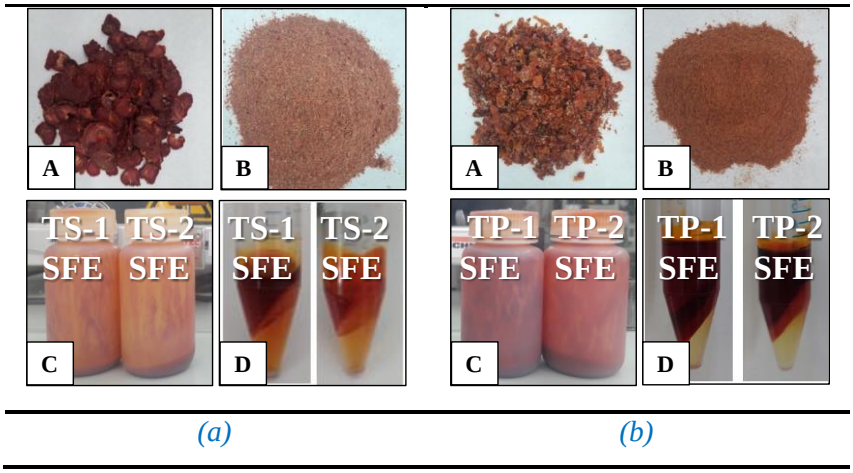


Figure 4.4 Tomato samples, raw extracts and centrifuged fractions obtained with supercritical CO_2 extraction (SFE) using operating parameter set (1) at 400 bar, 70 °C, 9 kg/h and set (2) at 450 bar, 70 °C, 11 kg/h. A – before grinding, B – after grinding, C – operating parameter sets raw extracts (1,2), D – operating parameter sets centrifuged extracts (1,2): (a) tomato slices (TS); (b) tomato pomace (TP)

SFE raw extracts are red and extracts obtained from TP are more pigmented. Regarding the operating parameters, set (*2*) raw extracts had more intense colors. Yields were improved with 21 % for TS and 19 % for TP. Obtained yields were between 5.25 ± 0.79 and 6.64 ± 1.12 g extract/100 g dried TS and between 10.02 ± 1.14 and 12.35 ± 1.55 g extract/100 g dried TP. These values are in line with previous results reported by other authors: 10.3 – 13.4 % from TP [23], 11.4 – 24.6 % from TP [7], 12.51 % from TP and 2.5 % from peels [1]. An increase of 100 % for extraction efficiency was obtained for TP samples, regardless of the operating parameter sets.

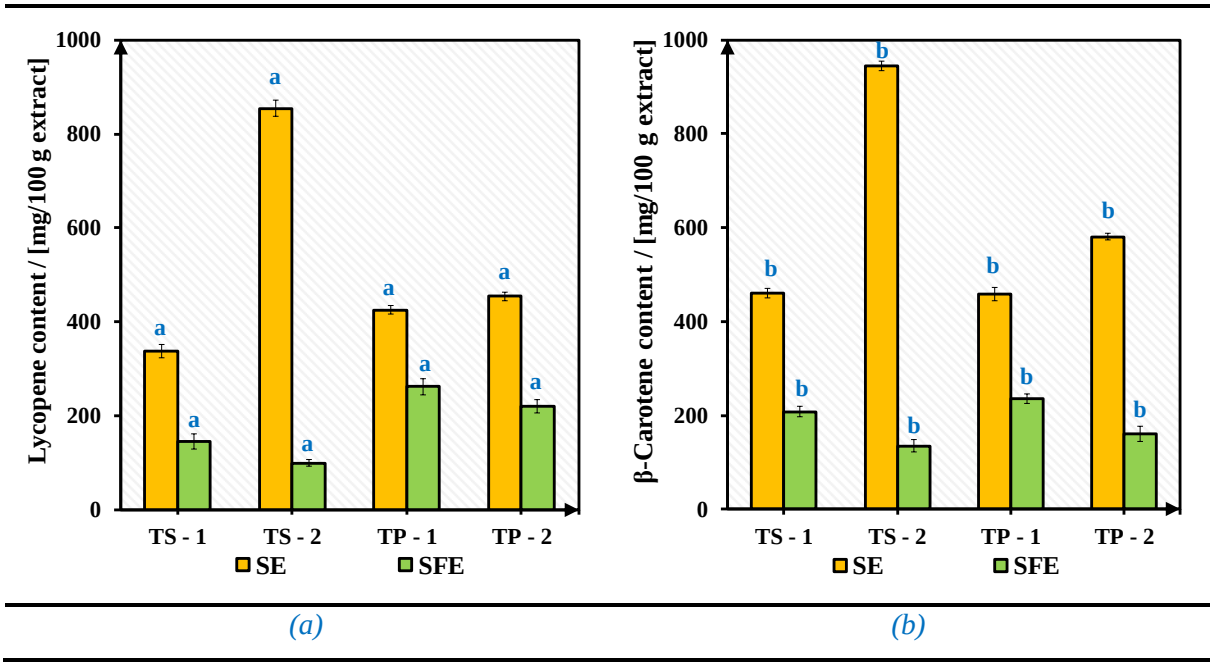
4.3.5 Extracts Centrifugation

The consistency of the raw extracts from SFE is similar to a liquid oleoresin. For an accurate analysis, separation of the raw extracts' phases was necessary to evaluate their quality. Using the centrifugation method, three fractions were obtained. The upper fraction (SFE–A) was red and oily, the middle fraction was a dark red solid oleoresin (SFE–B) and the lower fraction was a yellow liquid (SFE–C) (*Figure 4.4*). The consistency of SFE–B fractions was similar to SE raw extracts. TS raw extracts contained 66 – 73 % SFE–A, 5 – 7 % SFE–B and 20 – 30 % SFE–C fractions, while TP raw extracts had 73 – 77 % SFE–A, 13 – 15 % SFE–B and 11 – 13 % SFE–C. TP raw extracts were more pigmented and contained higher amounts of SFE–A and SFE–B fractions than TS raw extracts.

4.3.7 Carotenoid quantitative analysis of SE and SFE raw extracts

Lycopene and β-carotene contents (mg carotenoid/100 g extract) are shown in Figures 4.6. SE raw extracts contained higher amounts of both carotenoids than SFE raw extracts, regardless of the used extraction solvent (in SE) and operating parameters (in SFE). Lycopene contents of SE and

SFE raw extracts varied between 336.77 ± 14.05 and 854.50 ± 7.51 mg/100 g extract and between 99.41 ± 5.72 and 261.70 ± 6.66 mg/100 g extract, respectively. β-carotene contents were between 459.06 ± 6.46 and 945.00 ± 10.87 mg/100 g extract and between 134.77 ± 13.28 and 236.11 ± 10.17 mg/100 g extract. Extraction solvents influence the carotenoids' recovery due to their polarity. The descending order of the used green solvents' polarity is bioethanol > ethyl acetate > carbon dioxide [27]. Ethyl acetate (2) raw extracts had higher content of both lycopene (854.50 ± 7.51 mg/100 g extract from TS and 454.64 ± 8.76 mg/100 g extract from TP) and β-carotene (945.00 ± 10.87 mg/100 g extract from TS and 580.96 ± 9.51 mg/100 g extract from TP) than bioethanol (1). For the SFE method, higher carotenoid contents were obtained using operating parameter set (1) at lower values of pressure and CO_2 flow rate. TP raw extracts had a higher amount of oil; hence, the extracted carotenoids were dissolved in a larger amount of liquid than the carotenoids extracted from TS, which led to an extract diluted in tomato seed oil.



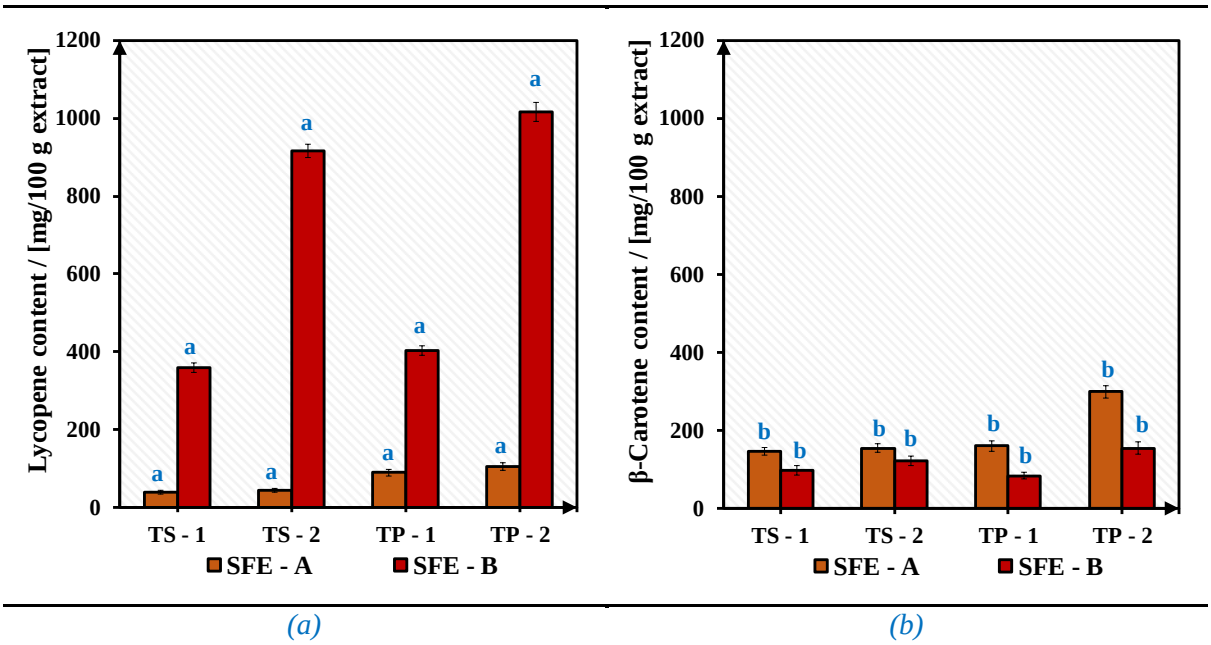
(a) *(b)*

Figure 4.6 Carotenoid content of SE and SFE raw extracts obtained from tomato slices (TS) and tomato pomace (TP), using as extraction solvents (1) bioethanol and (2) ethyl acetate and as operating parameters set (1) at 400 bar, 70 °C, 9 kg/h and set (2) at 450 bar, 70 °C, 11 kg/h: (a) lycopene; (b) β-carotene.

4.3.10 Carotenoid quantitative analysis of SFE fractions

The carotenoid contents of SFE fractions in terms of lycopene and β-carotene content, obtained through the UV–VIS method, are presented in *Figures 4.9*. The lycopene content of the SFE–A and SFE–B fractions was between 38.74 ± 0.18 – 105.02 ± 0.40 mg/100 g for the SFE–A fraction and 359.66 ± 1.84 – 1016.94 ± 8.95 mg/100 g for the SFE–B fraction. The results for β-carotene were between 146.24 ± 4.95 – 299.06 ± 5.64 mg/100 g for the SFE–A fraction and 83.96 ± 8.04 –

154.87 ± 6.12 mg/100 g for the SFE–B extract. SFE–B fractions had higher carotenoid contents than SFE–A fractions and their values are in line with the SE extracts. Regarding operating parameters, although the carotenoid content in SFE raw extracts obtained with set (*1*) was higher, analyzing SFE–B fractions, it seems that set (*2*) was more efficient, regardless of the type of tomato sample. Carotenoid yields are positively correlated with pressure and flow rate, these parameters playing a significant role for carotenoid recovery with SFE [7,23,25]. For the carbon dioxide solvent, at lower pressures (*set 1*), the polarity of supercritical CO_2 is comparable to hexane, while, at high pressures (*set 2*) with chloroform, and carotenoids are more soluble in chloroform than in hexane [8].



(a) *(b)*

Figure 4.9 Carotenoid content of SFE fractions (SFE–A and SFE–B) obtained from tomato slices (TS) and tomato pomace (TP) with operating parameter set (1) at 400 bar, 70 °C, 9 kg/h and set (2) at 450 bar, 70 °C and 11 kg/h: (a) lycopene; (b) β-carotene.

Carotenoid distribution in tomato extracts was also analyzed. Lycopene/β-carotene mean ratios from TS in SE and SFE raw extracts were 45/55 and 42/58. In SFE–A and SFE–B fractions, these ratios were 22/78 and 83/17, respectively. For TP in SE and SFE raw extracts, mean ratios were 46/54 and 55/45, while from SFE–A and SFE–B fractions, their values were 43/57 and 85/15. These ratios show that lycopene content is higher in SFE–B fractions and SE raw extracts, which are not diluted by oil, as in SFE raw extracts and SFE–A fractions.

4.3.14 Economic analysis

To estimate the extraction process profitability, three plant capacities (1, 10 and 100 kg dried pomace/batch) were analyzed to produce four valuable extracts with antioxidant activity using dried tomato pomace as a raw material: SE raw extract (TP–2–SE), SFE raw extract (TP–2–SFE),

solid oleoresin (TP–2–SFE–B) and oil extract (TP–2– SFE–A). In *Figure 4.10*, is presented the profit ratio (€/kg dried tomato pomace/batch) for three scale-up capacities. For a small-capacity plant (1 kg dried pomace/batch), no profit is obtained for the SFE process and the SE process is more profitable (profit ratio between 1.46 and 4.52 €/kg dried tomato pomace/batch). By increasing the processing capacity of the plant (100 kg dried pomace/batch), the SFE process becomes more profitable, especially for obtaining two products: pigmented solid oleoresin concentrated in lycopene (TP–2–SFE–B) and pigmented oil extract rich in carotenoids (TP–2–SFE–A) with profit ratio 4.92 €/kg dried tomato pomace/batch.

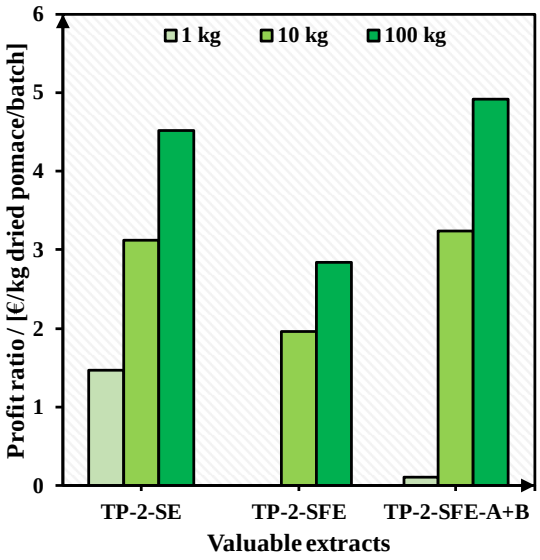


Figure 4.10 Profit ratio estimation for four valuable extracts (TP–2–SE, TP–2–SFE, TP–2–SFE–A and TP–2–SFE–B) and three scale-up capacities (1:10:100 kg dried pomace/batch)

II. CAROTENOIDS RECOVERY ENHANCEMENT BY SUPERCRITICAL CO_2 EXTRACTION FROM TOMATO USING SEED OILS AS MODIFIERS

Abstract

This work aims to improve the quantity and quality of extracts from tomato slices (TS) by enhancing the recovery of the carotenoids from the solid matrix to the solvent using 20 w/w% seeds as modifiers and supercritical CO_2 extraction with optimal parameters. Tomato (*TSM*), camelina (*CSM*) and hemp (*HSM*) seeds were used as modifiers due to their quality (polyunsaturated fatty acids content of 53 – 72 %). A solubility of ~ 10 mg carotenoids/100 g of oil was obtained for CSM and HSM, while, for TSM, the solubility was 28 % higher. An increase in the extraction yield from 66.00 to 108.65 g extract/kg dried sample was obtained in the following order: TSM < HSM < CSM. Two products, an oil rich in carotenoids (203.59 mg/100 g

extract) and ω3-linolenic acid and a solid oleoresin rich in lycopene (1172.32 mg/100 g extract), were obtained using SFE under optimal conditions (450 bar, 70 °C, 13 kg/h and CSM modifier), as assessed by response surface methodology (RSM).

4.7.10 Design of experiments

The Box–Behnken experimental design (*BBD*) was used in this study to evaluate the effects of three selected factors ($k = 3$) with three levels ($p = 3$), including the extraction pressure (350, 400 and 450 bar), seed type (TSM, CSM and HSM) and CO_2 flow rate (9, 11 and 13 kg/h). Four responses, such as the SFE−A (oily extract) yield, SFE−B (solid extract) yield, total carotenoid content in SFE−A and lycopene content in SFE−B, were proposed to evaluate the effects of these three factors. Considering three replicates in the central point and BBD method, the number of experiments was 15, with each experiment being performed in duplicate. The selected factors and responses with their coded and uncoded levels are presented in *Table 4.9* and *Table 4.10*.

Table 4.9 Independent variables (factors) for the BBD experimental design

Independent variables	**Symbol**	**Range of coded levels of variables**		
		Low	**Medium**	**High**
		−1	**0**	**+1**
Extraction pressure / [bar]	X_1	350	400	450
Seeds type	X_2	TSM	CSM	HSM
CO_2 flow rate / [kg/h]	X_3	9	11	13

Table 4.10 Dependent variables (responses) for the BBD experimental design

Dependent variables	**Symbol**
SFE-A yield / [g/100 g dried sample]	Y_1
SFE-B yield / [g/100 g dried sample]	Y_2
SFE-A carotenoids / [mg/100 g extract]	Y_3
SFE-B lycopene / [mg/100 g extract]	Y_4

To predict the effect of each factor that affects the chosen responses of the SFE process using tomato slices, four second-order polynomial models (equation (*4.2*)) for k independent variables (X_1, X_2 and X_3) were developed:

$$Y_i = \beta_{0,i} + \beta_{1,i} \cdot X_1 + \beta_{2,i} \cdot X_2 + \beta_{3,i} \cdot X_3 + \beta_{4,i} \cdot X_1 \cdot X_2 + \beta_{5,i} \cdot X_2 \cdot X_3 + \beta_{6,i} \cdot X_1 \cdot X_3 + \beta_{7,i} \cdot X_1^2 + \beta_{8,i} \cdot X_2^2 + \beta_{9,i} \cdot X_3^2 \quad (4.2)$$

where X_1, X_2 and X_3 are the selected factors and β_{ji} are the intercept ($\beta_{0,i}$), linear ($\beta_{1,i}$, $\beta_{2,i}$ and $\beta_{3,i}$), quadratic ($\beta_{4,i}$, $\beta_{5,i}$ and $\beta_{6,i}$) and interaction ($\beta_{7,i}$, $\beta_{8,i}$ and $\beta_{9,i}$) coefficients that described the factors'

effects on the chosen responses (Y_i). Statistical validation of the models was performed using analysis of variance (*ANOVA*) to verify that the models correctly described the relationship between factors and responses. The accuracy of the models was checked through the coefficient of determination (R^2) and *Lack of fit* test. Finally, the determination of the optimal conditions was accomplished using the desirability function and response surface plots. Response surface plots were generated using the function of two factors and keeping the third factor constant [34].

4.8.2 Extraction of carotenoids with vegetable oils

To assess the solubility of carotenoids in the three selected oils, the maceration method was used. After the same extraction times, three oily extracts (*TSO–TS*, *CSO–TS* and *HSO–TS*) were obtained and changes in the oils' colors were observed: TSO–TS changed from orange to red, CSO–TS changed from yellow to orange and HSO–TS changed from green to brown (*Figures 4.13a,b*). These changes in colors were associated with the carotenoid content extracted from TS, even if the vegetable oils were also pigmented. The lycopene recovery with TSO was higher, with 21 % than CSO and with 26 % than HSO.

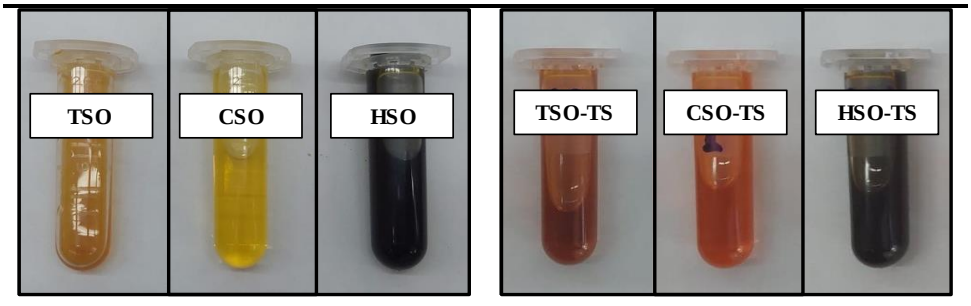


Figure 4.13 Carotenoids' solubility in vegetable oils (TSO, CSO and HSO): (a) vegetable oils; (b) extracts from TS

4.8.6 Optimal parameters for SFE extraction with seed oils as modifiers

The extraction yields at different experimental runs varied between 2.97 and 9.14 g/100 g dried sample for the SFE–A fraction (oily extract) and between 0.22 and 2.81 g/100 g dried sample for the SFE–B fraction (solid extract). For the extracts' compositions, the total carotenoid contents in the oily fractions varied between 86.17 and 206.15 mg/100 g extract, while the lycopene content in the solid fractions was between 659.17 and 1212.68 mg/100 g extract.

In *Table 4.16*, the regression coefficients of four quadratic models that adequately described the effects of independent variables $X_1 - X_3$ through the chosen $Y_1 - Y_4$ responses of the SFE process are presented. For all of the responses, it was observed that the extraction pressure (X_1) and CO_2 flow rate (X_3) had positive effects, while the seed type (X_2) had a complex effect, being positive for the Y_1 and Y_2 responses and negative for the Y_3 and Y_4 responses. The use of CSM led to high

extraction yields and lower carotenoid concentrations, while the use of TSM led to higher recovery of carotenoids and lower yields. Thus, RSM analysis confirmed the importance of the type of seeds used as modifiers in carotenoid recovery from tomato slices through the SFE process.

For the statistical validation of the proposed models, the *Lack of fit* test values were evaluated for a confidence level of $\alpha = 0.05$ (5 % risk is considered significant). Coefficients with $p > 0.05$ were considered insignificant from a statistical point of view and were removed from the model. Additionally, the precision of the predictive models was also verified by the coefficient of determination (R^2). The R^2 values were higher than 0.96, indicating a good fit between the experimental and predicted data.

Table 4.16 Models' regression coefficients

Statistical data	**Y_1 = SFE–A yield**	**Y_2 = SFE–B yield**	**Y_3 = SFE–A carotenoids**	**Y_4 = SFE–B lycopene**
β_0 (Intercept)	6.893	1.688	160.690	918.419
β_1 (X_1)	1.531	0.547	40.398	166.686
β_2 (X_2)	0.902	0.373	−22.978	−108.097
β_3 (X_3)	0.626	0.252	5.735	46.287
β_4 (X_1X_2)	*	*	5.312	−27.241
β_5(X_2X_3)	*	*	*	*
β_6 (X_1X_3)	*	*	*	12.235
β_7 (X_1^2)	*	*	−8.237	21.640
β_8 (X_2^2)	−1.832	−0.830	*	−31.374
β_9 (X_3^2)	*	*	*	9.627
***df* Lack of fit**	8	3	7	4
***p*-value Lack of fit**	0.565	0.154	0.056	0.053
***df* Pure error**	2	2	2	2
Pure error	0.068	0.014	4.830	4.793
R^2	0.983	0.961	0.968	0.999
Adjusted R^2	0.976	0.889	0.950	0.998
Predicted R^2	0.942	0.861	0.922	0.985

** Statistically insignificant ($p > 0.05$) at the $\alpha = 0.05$ level of significance.*

Response surface graphs between two factors keeping the third factor constant were generated to analyze the effect of them on the four responses.

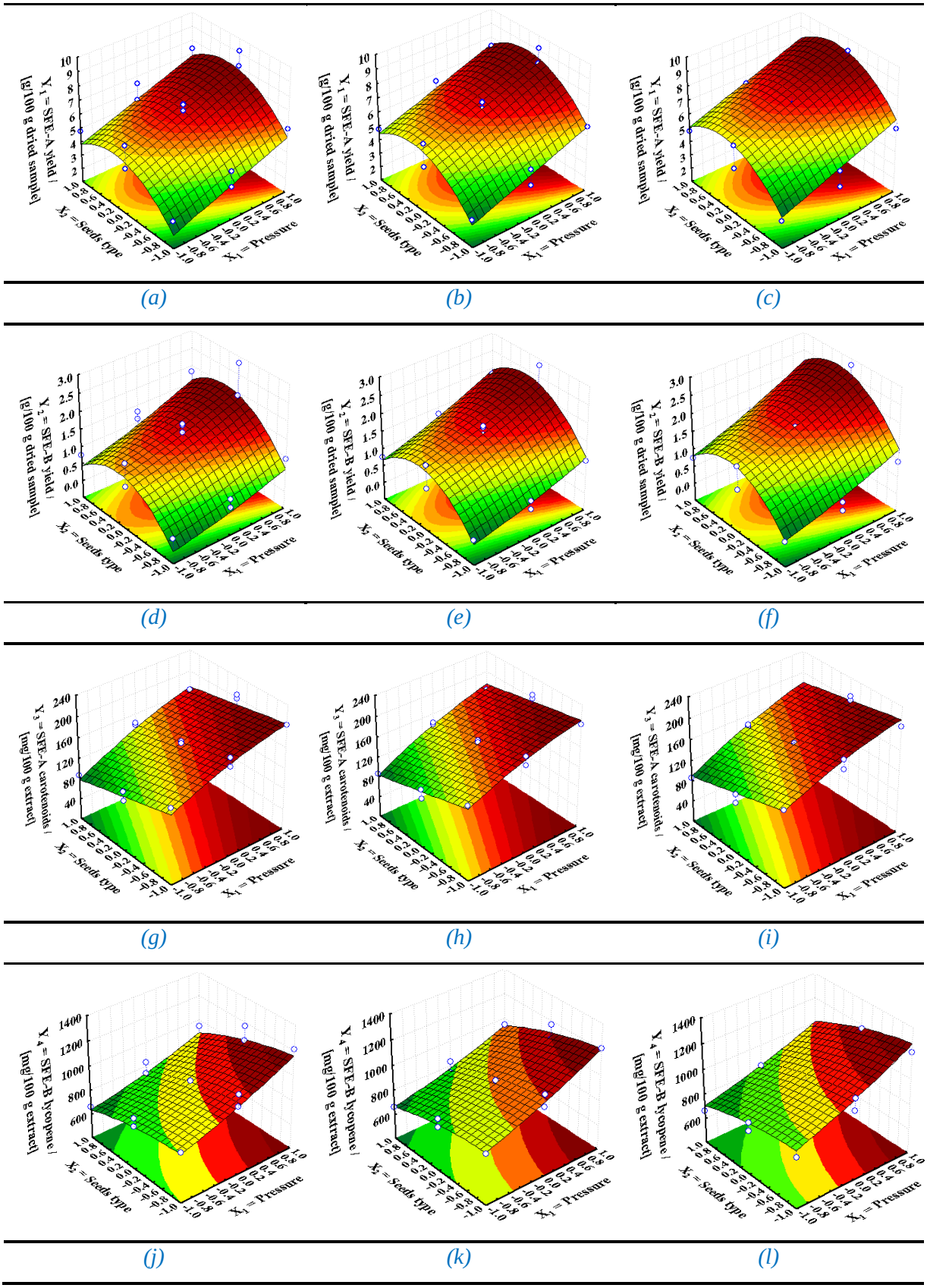


Figure 4.19 Response surface plots of responses for X_1 and X_2 effects when X_3 factor is constant at X_3=–1 (left), X_3=0 (middle) and X_3=+1 (right): (a-c) Y_1 = SFE–A yield; (d-f) Y_2 = SFE–B yield; (g-i) Y_3 = SFE–A carotenoid; (j-l) Y_4 = SFE–B lycopene

In *Figures 4.19a–l* are shown the response surface plots of the $Y_1 - Y_4$ responses when X_3 factor, the CO_2 flow rate, is kept constant to illustrate the effects of X_1 (the extraction pressure) and X_2 (the seeds type) factors. In *Figures 4.19left* plots, the CO_2 flow rate has the minimum value of 9 kg/h ($X_3 = -1$), in *Figures 4.19middle* plots the medium value of 11 kg/h ($X_3 = 0$), while in *Figures 4.19right* plots the maximum value of 13 kg/h ($X_3 = +1$) is used.

For the extraction yields of the SFE–A and SFE–B fractions, it was presented that an increase in pressure and the use of CSM seeds led to higher yields. Yield values of 7 – 8 g/100 g dried sample for SFE–A and 2 – 2,5 g/100 g dried sample for SFE–B could be obtained using pressures between 400 and 450 bar, (X_1 = 0, 1) and 20 % CSM seeds (X_2 = 0), a mixture of 10 % CSM + 10 % TSM seeds (X_2 = −0.5, 0) or a mixture of 10 % CSM + 10 % HSM seeds (X_2 = 0, 0.5). For the total carotenoid content of SFE–A and lycopene content of SFE–B, an increase in pressure and the use of TSM seeds led to higher amounts of carotenoids. Total carotenoid values of 180 – 200 mg/100 g extract for SFE–A and lycopene values of 1100 – 1300 mg/100 g extract for SFE–B could be obtained using pressures between 375 and 450 bar (X_1 = −0.5, 1) and 20 % TSM seeds (X_2 = −1), a mixture of 10 % TSM + 10 % CSM seeds (X_2 = −1, 0) or a mixture of 10 % TSM + 5 % CSM +5 % HSM seeds (X_2 = −1, 0.5).

Desirability functions are used to determine the optimum values for the varied factors to obtain high values of analyzed responses. It is desirable to obtain both high extraction yields and extracts rich in carotenoids; thus a trade-off is needed. For this purpose, the desirability function was used to obtain the optimum extraction conditions for all responses optimized simultaneously.

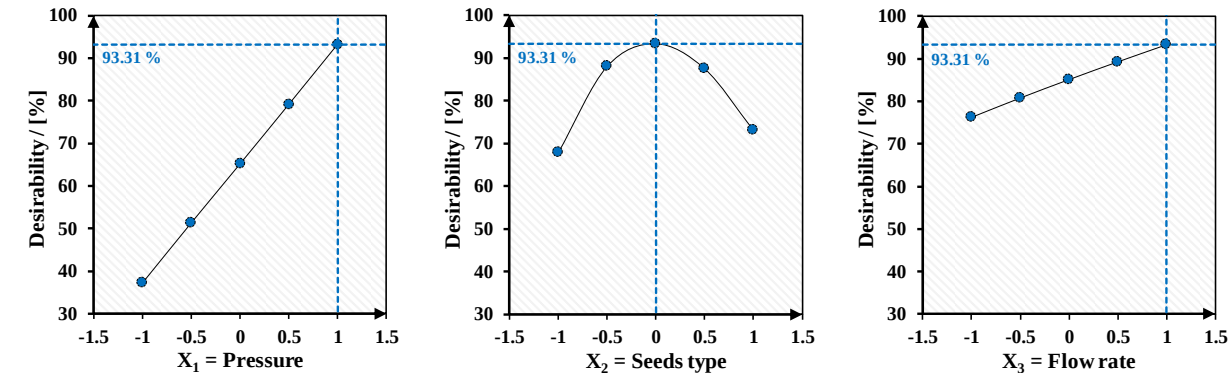


Figure 4.21 Optimal desirability of all the responses: (a) Pressure; (b) Seeds type; (c) Flow rate

In *Figures 4.21a–c*, the optimal desirability plots for the proposed factors (X_1, X_2 and X_3) are presented. A desirability of 93.31 % was obtained when 450 bar, CSM seeds and 13 kg/h were used as the independent variables. Under these conditions, the obtained optimum response values were 8.89 g SFE−A/100 g dried sample, 2.57 g SFE−B/100 g dried sample, 198.49 mg total carotenoids/100 g SFE−A and 1174.90 mg lycopene/100 g SFE−B.

CHAPTER 5 Conclusions

1.3 5.1 Conclusions

The investigation of supercritical carbon dioxide extraction as a green method to recover bioactive compounds from tomatoes was the main goal of this thesis. The study was focused on three major areas necessary to describe and evaluate the supercritical process from tomatoes, such as simultaneous quantification of main carotenoids found in tomatoes, lycopene and β-carotene, the determination of optimal conditions for drying of tomato samples as a pretreatment to increase the extracts quality and the extraction yields, avoiding their degradation, and green extraction of bioactive compounds from tomatoes such as carotenoids (lycopene and β-carotene) and ω-polyunsaturated fatty acids (ω-PUFA) using different types of tomato raw materials mixed with vegetable seeds. 224 bibliographic references were accesed and studied for this thesis. The results are presented in three chapters (Chapter 2, Chapter 3, Chapter 4), with two additional chapters for original contributions (Chapter 1) and conclusions (Chapter 5). The thesis contains 51 figures and 39 tables.

In ***Chapter 2 – UV-VIS spectrophotometric methods for simultaneous quantification of lycopene and β-carotene***, four methods (Wavelengths groups method – WGM, Absorption factor method – AFM, Absorbance subtraction method – ASM and Isosbestic point method – IPM) were evaluated for both carotenoids (lycopene and β-carotene) concentrations calculation starting from Lambert-Beer'law and using the UV-VIS method, due to the property of these compounds which can absorb light in the visible domain. The UV–VIS spectrometry method was used as a quick and easy technique to evaluate the quality of tomato extracts using only spectra measurable data as absorbances of sample mixtures, absorption coefficients of pure compounds determined from calibration curves in acetone:hexane (1:1, v/v) mixture and calculated absorbance factors. The original contributions are based on formulation of the four methods taking into account various assumptions regarding the carotenoids contribution to light absorption, six wavelengths groups for carotenoids calculation, the spectra overlapping property of lycopene and β-carotene, the identified isosbestic point at 461 nm of lycopene and β-carotene and the calculation of absorbance factors. The methods were validated using both synthetic mixtures with known concentrations of target compounds and real tomato mixtures All the experimental data and methods results were validated by statistical analysis. The IPM–II–WG_6 method which considers two wavelengths, one at the isosbestic point (461 nm) and the other at the highest wavelength peak of lycopene (504 nm) showed good correlation with reference data, with medium accuracy errors less than 5 % for

simultaneous quantification lycopene and β-carotene in a mixture and is recommended to be used as an alternative method.

In ***Chapter 3 – The influence of drying parameters on bioactive compounds recovery from tomatoes by extraction***, two studies were performed to evaluate the influence of drying factors on the quality of dried tomato peels of Crystal and Rila varieties. Main contributions were oriented on the evaluation of optimal drying conditions to improve the quality of extracts from dried samples and to avoid the degradation processes of carotenoids during the drying.

The first study (**Chapter 3.I**) was focused on the determination of the influence of three drying methods as oven drying, vacuum-oven drying and hot-air drying, five drying temperatures between in 50 °C and 120 °C at *a constant drying time* of 5 h on the final moisture content of the dried peels, the peels aspect and the amount of recovered lycopene from tomato peels. The used extraction method was Soxhlet extraction with acetone:hexane (1:1, v/v) mixture. The lowest moisture values of the dried peels, the best aspect of the dried peels in terms of color degradation, the highest extraction yields and amounts of lycopene were obtained from tomato peels subjected to hot-air drying method, regardless drying temperature, while the degradation of the peels quality was observed at temperatures higher than 80 °C.

The second study (**Chapter 3.II**) evaluated the influence of drying temperatures between 50 °C and 75 °C at *a constant final moisture* of the samples of 6 – 7 %wt. on the energy consumption and the quality of the final dried product, expressed by the lycopene and β-carotene contents from dried tomato peels. The hot-air drying method and Soxhlet extraction method with acetone:hexane (1:1, v/v) mixture were used for drying and extraction, respectively. Main contributions are the formulation of drying mathematical models based on thin-layer physical model, evaluation of the drying kinetics for hot-air drying of tomato peels using Fick's second law of diffusion and nine different semi-theoretical models and formulation of two degradation models for lycopene and β-carotene in the 50 – 110 °C temperature range based on drying, extraction and analysis experimental data. For tomato peels hot-air drying, Fick's second law of diffusion and Two-term models showed high accuracy in predicting the drying behavior on 50 – 75 °C temperature range, while the carotenoids degradation increases with drying temperature being 83 % higher at 110 ºC than at 50 ºC. Evaluating the energy consumption, the results showed that minimum specific energy consumption is obtained for drying of tomato peels at 50 °C.

The results showed that using hot-air drying method, a drying temperature of 50 °C and a final moisture content of 6 – 7 %wt. for tomato peels of Rila variety, high quality extracts (95.56 mg lycopene and 96.22 mg β-carotene/100 g extract) can be obtained, with minimum energy consumption of 56.60 kWh/kg peels.

The research of ***Chapter 4 – Bioactive compounds extraction from tomatoes*** presents the results of two studies performed to evaluate the quality of the extracts using organic green solvents, supercritical green solvent and modifiers with supercritical green solvent. The research was conducted using Rila variety tomato samples as pomace, seeds and slices.

The first study (**Chapter 4.I**) analyzed the influence of the seeds content of the tomato sample, the type of the green solvent, the extraction method and extraction parameters on extracts quality. Main contributions: the experimental evaluation of extracts quality comparing two types of tomato samples (slices and pomace with different content of tomato seeds), two organic green solvents (bioethanol and ethyl acetate) and one green supercritical solvent (carbon dioxide, CO_2), two extraction methods (Soxhlet and Supercritical fluid extraction) and the type of extraction parameters (pressures of 400 and 450 bar, CO_2 flow rates of 9 and 11 kg/h). The extraction efficiency described by the extraction yields and the quality of the extracts expressed by the lycopene and β-carotene contents, the ω-PUFA content, the antioxidant activity and the total phenolic contents were presented as results of this research. Four natural value-added products rich in bioactive compounds with evaluated quality are presented: a) tomato pomace solid oleoresin Soxhlet extract obtained using ethyl acetate as green extraction solvent, b) tomato pomace liquid oleoresin Supercritical extract, c) tomato pomace solid oleoresin Supercritical extract and d) tomato pomace oil Supercritical extract. The optimal parameters for supercritical CO_2 extraction are 450 bar, 70 ºC and 11 kg/h. By economic evaluation of three scale-up capacities (1:10:100 kg dried pomace/batch) for Soxhlet and Supercritical extraction processes, having as target the four quality products, the greatest profitability can be obtained for plant capacity higher than 100 kg/batch.

The second study (**Chapter 4.II**) aimed to identify the optimal extraction parameters in supercritical process using vegetable seeds as modifiers to improve the extraction efficiency of bioactive compounds as carotenoids and ω-PUFA from tomatoes. Main contributions are: the identification of new solvents which can improve the extraction efficiency as oils extracted from vegetable seeds added to the tomato samples, the evaluation of solubility of the carotenoids in these oils, the determination of optimal parameters of green extraction process and the evaluation of valuable products quality. The research was conducted on tomato slices enriched with 20 % extra seeds (tomato, camelina and hemp seed) as modifiers. Based on Box-Behnken experimental matrix design (15 experiments), the optimal extraction parameters for supercritical carbon dioxide extraction using seed oils as modifiers were identified. Three factors at three levels as the extraction pressure (350 bar, 400 bar and 450 bar), the type of the modifiers (tomato, camelina and hemp seeds) and the CO_2 flow-rate (9 kg/h, 11 kg/h and 13 kg/h) were evaluated to determine their influence on four responses as the extraction yields and carotenoids contents of oil and solid

oleoresin fractions separated through centrifugation from the supercritical extracts. The experimental data were fitted using second-order polynomial models, response surface plots and desirability function. The optimal extraction of 450 bar pressure, 20 % camelina seeds as modifiers and 13 kg/h CO_2 flow rate are recommended to obtain high quality extracts rich in bioactive compounds as lycopene, β-carotene, ω3-linolenic acid and ω6-linoleic acid. Tomatoes and camelina seeds are two vegetal materials with large productivities which can be cultivated in our country. Under these conditions, two natural value-added products rich in bioactive compounds are presented due to their qualities, such as a) tomato and camelina seeds solid oleoresin Supercritical extract and b) tomato and camelina seeds oil Supercritical extract which can be used as natural colorant or additive in the food industry due to the environmentally friendly nature of supercritical carbon dioxide extraction method.

In conclusion, main contributions added by this thesis can be used by food experts to produce new foods (oils rich in carotenoids, ω3-linolenic acid and ω6-linoleic acid) or to improve various food products as bread, spreads, dairy and meat products, juices or beverages with natural products (solid oleoresin rich in lycopene). Also the quality of pharmaceutical and cosmetic formulations or supplemets can be improved using the valuable products presented in this thesis. The quality of these natural products is given by high antioxidant activity due to the significant contents of lycopene, β-carotene, ω-PUFA and phenolic compounds, without toxicity and traces of solvents. These natural products can be industrially obtained by supercritical CO_2 extraction, for plant capacities greater than 100 kg tomato sample/batch. Thesis contributions were presented at 4 international conferences in 5 lectures and 2 posters and published in 4 articles.

PUBLISHED WORK

ISI Journal articles:

1. **Popescu, M.**; Iancu, P.; Plesu, V.; Todasca, M. C.; Bildea, C. S. Effect of different drying processes on lycopene recovery from tomato peels of Crystal variety. *UPB Scientific Bulletin Series B.* **2019**, *81(4)*, 46–58. ISSN: 1454–2331
   Impact Factor = 0.000 (2021)
2. **Popescu, M.**; Iancu, P.; Plesu, V.; Bildea, C.S.; Todasca, M.C. Different spectrophotometric methods for simultaneous quantification of lycopene and β-carotene from a binary mixture. *Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology*. **2022**, *160*, 113238. ISSN: 0023–6438
   Impact Factor = 6.056 (2021)

3. **Popescu, M.**; Iancu, P.; Plesu, V.; Todasca, M.C.; Isopencu, G.O.; Bildea, C.S. Valuable Natural Antioxidant Products Recovered from Tomatoes by Green Extraction. *Molecules.* **2022**, *27*, 4191. eISSN: 1420–3049
   Impact Factor = 4.927 (2021)

4. **Popescu, M.**; Iancu, P.; Plesu, V.; Bildea, C.S. Carotenoids recovery enhancement by supercritical CO_2 extraction from tomato using seed oils as modifiers. *Processes.* **2022**, *10(12)*, 2656. eISSN: 2227–9717
   Impact Factor = 3.352 (2021)

5. **Popescu, M.**; Iancu, P.; Plesu, V.; Bildea, C.S. Mathematical modelling of thin-layer drying kinetics of tomato peels: influence of drying temperature on the energy requirements and extracts quality. Scientific Reports. **2023** ISSN: 2045–2322 – *submitted for publication*
   Cumulative Impact Factor = 14.335 (2021)

International scientific conference oral presentations:

1. **Popescu, M.**; Iancu, P.; Pleșu, V.; Todașcă, M.C.; Bîldea, C.S. Carotenoids recovery from tomato pomace: conventional vs. green extraction method. 21st Romanian International Conference on Chemistry and Chemical Engineering, ***RICCCE 21***, 4–7 September **2019**, Mamaia, Romania

2. **Popescu, M.**; Iancu, P.; Pleșu, V.; Bîldea, C.S.; Todașcă, M.C. Improving the efficiency of the supercritical CO_2 extraction process of hemp seed oil. International Chemical Engineering and Material Symposium, ***SICHEM 2020***, 17–18 September **2020**, Bucharest, Romania

3. **Popescu, M.**; Iancu, P.; Pleșu, V.; Bîldea, C.S.; Todașcă, M.C. Carotenoids green extraction from tomatoes. International Chemical Engineering and Material Symposium, ***SICHEM 2020***, 17–18 September **2020**, Bucharest, Romania

4. **Popescu, M.**; Iancu, P.; Bîldea, C.S.; Pleșu, V.; Todașcă, M.C. The use of supercritical CO_2 extraction in the food industry, Advances in Food Chemistry Conference, ***AdFoodChem 2021***, 15–17 April **2021**, Bucharest, Romania

5. **Popescu, M.**; Iancu, P.; Pleșu, V.; Todașcă, M.C.; Bîldea, C.S. Supercritical CO_2 extraction of plant oils and pigments. 22nd Romanian International Conference on Chemistry and Chemical Engineering, ***RICCCE 22***, 7–9 September **2022**, Sinaia, Romania

International scientific conference poster presentation:

1. **Popescu M.**, Crisciu, A.V., Iancu P., Plesu V., Bîldea C.S., Binary interaction parameters estimation from experimental data for solid-supercritical fluid equilibrium (S-SCFE), Computer Aided Process Engineering, ***CAPE Forum 2018***, 15–17 November **2018,** Bucharest, Romania

2. **Popescu, M.**; Iancu, P.; Isopencu, G.; Bîldea, C.S.; Pleșu, V.; Todașcă, M.C. Antioxidant activities and total phenolic content of tomato residue extracts. Advances in Food Chemistry Conference, ***AdFoodChem 2021***, 15–17 April **2021,** Bucharest, Romania

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