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Dear Editor,

Please find enclosed the manuscript entitled *Extraction of polyphenols from grape skins and defatted grape seeds using subcritical water: experiments and modeling*, submitted for publication in *Food Research International*, special issue on *Recovery and Utilization of Valuable Compounds from Food Processing By-products*.

The work is original and unpublished and is not being considered for publication elsewhere.

I also enclose the relevant files:

- Highlights.doc
- Manuscript.doc
- Table 1.doc
- Table 2.doc
- Figure captions.doc
- Figure 1.tiff
- Figure 2.tiff
- Figure 3.tiff
- Figure 4 for web.tiff (color version for the web)
- Figure 4 for print.tiff (black and white version for print)
- Figure 5 for web.tiff (color version for the web)
- Figure 5 for print.tiff (black and white version for print)

Yours sincerely

Luca Fiori

Assistant professor of Chemical Engineering Fundamentals

***Highlights (for review)**

Highlights

- Polyphenols extracted from grape skins and defatted seeds with subcritical water
- Operative conditions: 10 MPa, 80-120 °C, 2-5 mL/min, 2 h
- Polyphenols yield: higher at high temperature and low solvent flow rate
- Polyphenols yields: higher for grape seeds (123.9 mg/g) than for skins (76.7 mg/g)

Extraction of polyphenols from grape skins and defatted grape seeds using subcritical water: experiments and modeling

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ABSTRACT

Polyphenols were extracted from grape skins and defatted grape seeds (cultivar: *Pinot Nero*) by using subcritical water in a semi-continuous mode. Extractions were performed at a pressure of 10 MPa, at three different temperatures (80, 100 and 120 °C) and with two water flows (2 and 5 mL/min). For both skins and defatted seeds, total polyphenol (TP) yield significantly increased with temperature: for skins from 44.3±0.4 to 76.7±2.8 mg/g, while for defatted seeds from 44.2±2.4 to 123.9±0.7 mg/g when the temperature increased from 80 to 120 °C. TP yield decreased with flow rate at constant temperature. The extraction kinetics was simulated by two-site kinetic model. The adjustable parameters of the models were calculated by best fitting procedures with experimental data: they resulted in good agreement with literature values. The model fitted the experimental kinetics curves in a satisfactory way with root mean square error (RMSE) in the range of 10^{-2}-10^{-1} and percent average absolute relative deviation (AARD) of 0.5-4%.

Keywords: subcritical water extraction, kinetics models, grape seeds, grape skins, polyphenols

1. Introduction

The wine-making process generates substantial volume of solid by-products consisting of skins, stalks and seeds in different proportions. Researches in the past few decades have shown that the possibility of valorizing these by-products for the recovery of oil, phenolic compounds, and fibers are immense (Shrikhande, 2000). Usually, grape seeds are sold to the oil extraction industry and more recently they are asked for by food, cosmetic and pharmaceutical sectors for their use as a source of antioxidants (Fiori et al., 2014). The wine-making by-products are rich in polyphenols (Palma & Taylor, 1999; Bail, Stuebiger, Krist, Unterweger, & Buchbauer, 2008; Casas et al., 2010; Casazza, Aliakbarian, Mantegna, Cravotto, & Perego, 2010; Aliakbarian, Fathi, Perego, & Dehghani, 2012). There are thousands of compounds identified as polyphenols, the main classes including flavonoids, phenolic acids, tannins and stilbenes (Ignat, Volf, & Popa, 2011). These compounds exhibit wide range of bioactivities as antioxidants, antimicrobials, neuro-sedative, anti-inflammatory, anti-viral, anti-cancer, anti-ulcer, anti-carcinogenic, and anti-mutagenic (Palma & Taylor, 1999; Casazza et al., 2010; Aliakbarian et al., 2012). Therefore, the isolation of polyphenols from grape marc (wine industries by-product) can be an additional source of revenue besides its use as feedstock in ethanol production and grape seed oil extraction (Monrad et al., 2014).

Traditionally, polyphenols are extracted from natural products using organic solvents. However, these techniques require long extraction period and result in low yields of extract (Singh & Saldaña, 2011). To overcome these limitations, considerable research efforts are done in the extraction of plant constituents using non-conventional techniques like ultrasonic-assisted and microwave-assisted extraction (Casazza et al., 2010; Bagherian, Zokaee Ashtiani, Fouladitajar, & Mohtashamy, 2011; Barrera Vázquez et al., 2014). Even though these techniques allow improving the extraction yield and reducing the extraction time, they still use conventional solvent and the urge for searching for an environmentally friendly solvent remains challenging. Recently, subcritical water (SW) extraction, also referred as pressurized or low polar water extraction, is emerging as an alternative technique for the extraction of both polar and non-polar compounds (Ramos, Kristenson, & Brinkman, 2002; Herrero, Cifuentes, & Ibanez, 2006; Ong, Cheong, & Goh, 2006; Carr, Mammucari, & Foster, 2011).

SW is defined as water at a temperature between its boiling and critical point where the pressure is regulated in such a way that water remains in the liquid state (Herrero et al., 2012). The technique is getting much attention in the field of extraction, reaction and chromatography (Khajavi, Kimura, Oomori, Matsuno, & Adachi, 2005; Lindquist & Yang, 2011) mainly because water is readily available, non-flammable, non-toxic, low cost, and an environmentally acceptable solvent.

The uses of SW as an extraction solvent for natural products were recently presented by several authors; interesting literature reviews on the topic are also available (Ramos et al., 2002; Herrero et al., 2006; Ong et al., 2006; Carr et al., 2011). Under subcritical conditions, the dielectric constant of water can be tuned by changing the temperature which in turn changes the water polarity. For instance, under standard temperature and pressure (25 ^{o}C and 101 kPa) water is a polar compound with dielectric constant of about 80 (Carr et al., 2011; Herrero et al., 2012); but, when the temperature is increased to about 200-350 °C, the dielectric constant drops to around 20-30, which is similar to the range of dielectric constants of conventional solvents like methanol, ethanol and acetone at room temperature, which makes SW an excellent solvent also for weakly polar compounds.

It is widely reported that the solubility of organic compounds in subcritical water depends on several factors like chain length, type and position of side group, molecular weight, position of hydrogen bonding etc. (Carr et al., 2011). Increase in temperature results in reduction of hydrogen bonding strength in water, which makes the water to behave more like a non-polar compound which in turn increases the solubility of some organic compounds. As polyphenols contain wide range of compounds, the optimum solubility within SW depends on the proper selection of the operating conditions.

In the past few decades, SW has been also used as reaction medium in the degradation of many organic compounds. It is widely believed that the ionization product of water increases by up to three orders of magnitude in going from ambient to near-critical conditions, making it a source of both hydronium and hydroxide ions. As a result of this, chemical reactions can take place without any catalyst in SW (Khajavi et al., 2005). In case of presence of dissolved oxygen, oxidation reactions may also occur (Yang & Hildebrand, 2006; Lindquist & Yang, 2011). A range of reactions of organic molecules occurring in SW are presented in an interesting review by Siskin & Katritzky (2000) in low temperature (≤ 150 °C) natural environments.

In an effort to valorize wine industry by-products, a significant number of research studies has been done recently. Some of these works used SW for the extraction of high added valued compounds; to point out some: García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno (2006) used SW to recover catechins and proanthocyanidins from grape seeds. Aliakbarian et al. (2012) studied SW extraction of phenolic compounds from grape pomace. Bucić-Kojić, Sovová, Planinić, & Tomas (2013) investigated the effect of the temperature on the extraction kinetics of phenolic compounds from grape seeds utilizing as solvent a water-ethanol mixture and operating in batch mode.

In this work, SW extraction of polyphenols from *Pinot Nero* grape skins and defatted seeds was investigated at constant pressure of 10 MPa and flow rate of 2-5 mL/min, under three operating temperatures, namely 80, 100 and 120 °C. The extraction kinetics was modeled and discussed.

2. Material and Methods

2.1 Sample preparation

Pinot Nero grape marc samples were obtained by winemakers located in Northern Italy. At the winery, stalks were separated from seeds and skins. The mixture of seeds and skins was taken to the laboratory and stored at -20 °C before drying. The samples were dried at 55 °C for 48 h, and then skins and seeds were separated by means of vibrating sieves and further cleaned manually and stored in dark under vacuum at ambient temperature. Dried skins and seeds were milled by a grinder (Sunbeam Osterizer blender, Boca Raton, USA) just before extraction. To avoid overheating, the sample was flaked for 10 s, then grinding was halted and the sample was shaken for another 10 s, and the milling process was continued.

2.2 Defatting of grape seeds

The defatting pre-treatment was done with a supercritical CO_2 equipment (Proras, Rome, Italy) whose design was previously described (Fiori, 2007). Also the procedure utilized was exactly the same as that detailed in (Fiori, 2007). The extractor basket utilized in this study had an internal volume of 0.1 L and was charged with 65 g of milled grape seeds. Pressure, temperature, and CO_2 flow rate were kept constant during the extraction process at 50 MPa, 50 °C, and 8 g/min, respectively. The extraction process was stopped when no more oil was extracted from the matrix, which was thus completely defatted. The resulting oil yield resulted equal to 15.5±0.5 g_{oil}/g_{seeds}. After operation, the particle size distribution of the defatted grape seeds was evaluated by utilizing sieves having different mesh sizes placed in a vibrating device (Automatic Sieve Shaker D406 control, Auckland, New Zealand). From the particle size distribution, the mean particle dimension (Sauter mean diameter) was calculated (Fiori, Basso, & Costa, 2008).

2.3 Subcritical water extraction

In order to perform the SW extractions, the same equipment (Proras, Rome, Italy) previously utilized for defatting the grape seeds was utilized with minor plant modifications as shown in Figure

1. A nitrogen line was connected to the extractor to purge the system before extraction and to de-oxygenate the deionized water utilized as solvent. During the entire SW extraction process, the CO_2 feed line remained closed. The extractor (0.1 L volume) was half filled with glass beads, then further with the substrate to be extracted (2 g), finally with other glass beads till it was completely filled. The extractor was then closed. In order to remove O_2 from the deionized water used as solvent, N_2 was bubbled into the water tank for 15 min while the tank remained open. The oxygen inside the extractor and in the pipe lines was removed by letting N_2 pass through the system for 5 min. During this phase, the back-pressure valve at the extractor outlet was maintained open. After N_2 purging, the back-pressure valve was closed and the extractor temperature control loop was put in auto mode letting the system reaching the desired set point extraction temperature. Then the water was pumped to the extractor by means of a HPLC pump (Gilson, Middleton, USA) – water pump in Fig. 1. As a result of this, the desired pressure was attained. The set point extraction pressure was maintained setting its value as the maximum pressure value of the HPLC pump. The process was kept in static extraction mode for 20 min before back pressure valve was partially opened and dynamic extraction started. The solvent flow rate resulted from the set point value given to the HPLC pump and the back pressure valve opening degree. The water/polyphenols extract was collected every 20 min during the 2 h extraction time. At the end of the extraction time, the water pump was stopped and the solvent inside the extractor was drained out of the extractor. As a result of this procedure, seven samples were collected for each test: six relevant to the dynamic extraction, one relevant to the final drainage of the extractor.

The extracts were concentrated in a rotary evaporator (Heidolph, Schwabach, Germany) at a reduced pressure of 73 mbar, bath temperature of 40 °C and rotation speed of 30 rpm. The concentrates were stored at -20 °C before analysis.

Fig. 1

2.4 Determination of total polyphenol

The total polyphenol (TP) content was determined by a colorimetric method using the Folin-Ciocalteu assay resorting to the same procedure as previously reported (Fiori, de Faveri, Casazza, & Perego, 2009). Measures were carried out at 725 nm using a UV-Vis spectrophotometer, model Lambda 25 (Perkin Elmer, Wellesley, MA) and the calibration curve was made with standard solutions of gallic acid in the range 0.01-1.00 mg/mL. All analyses were performed in triplicate. TP

yield was expressed as milligrams of equivalent gallic acid per gram of dried substrate (mg_{GAE}/g). The method response was described by the linear equation:

$$ABS_{725} = 0.0017TP \quad (1)$$

with $R^2 = 0.9940$.

3. Modeling

The SW extraction kinetics of TP was modeled by the so-called "two-site kinetic model". The literature reports that this model was applied to the SW extraction of essential oil from savory (Kubátová, Jansen, Vaudoisot, & Hawthorne, 2002) and *Z. Multiflora* (Khajenoori, Asl, & Hormozi, 2009), an anti-cancer compound (*damnacanthal*) from roots of Morinda (Anekpankul, Goto, Sasaki, Pavasant, & Shotipruk, 2007), and polycyclic aromatic hydrocarbons from contaminated soils (Islam, Jo, Jung, & Park, 2013). The model is an extension of the "one-site kinetic model", mostly referred as Crank's (1975) hot ball diffusion model, which is based on Fick's second law of diffusion and exploits the similarities with the diffusion of heat in a spherical hot ball cooling down in a uniform medium. It assumes that initially the solute is uniformly distributed in the solid matrix, which contains small quantities of extractable materials so that the extraction is not limited by solubility and the solute concentration in the solvent is close to zero. The two-site kinetic model considers a fast and a slow extraction period relevant to two different fractions of solute. The desorption rate of fast extracted fraction of polyphenols, F , is given by first-order rate constant k_1, and that of slowly released fraction $(1 - F)$ is given by first-order rate constant k_2 (Kubátová et al., 2002). Thus, the extraction profile is given by Eq. (2).

$$C/C_o = 1 - [Fe^{-k_1 t}] - [(1 - F)e^{-k_2 t}] \quad (2)$$

where C is the mass of TP extracted per mass of substrate and C_o is the initial mass of TP per mass of substrate. A more explicit form of Eq. (2) is given by Sovová (2012) for the extraction of solutes under the assumption of mixed flow conditions and with the existence of solute-matrix interactions.

According to Sovovà (2012), the first-order rate constants, represented as lumped parameters k_1 and k_2, are expressed by Eq.s (3) and (4).

$$k_1 = QK_m/(1 + \varepsilon Q/\gamma k_f a_0) \quad (3)$$

$$k_2 = 1/\left\{ {}^{\lambda R}/_{5D_e} + {}^{\varepsilon}/_{K_m \gamma k_f a_0} \right\} \quad (4)$$

Where Q is specific flow rate, K_m is mass partition coefficient, ε is bed void fraction, γ is solvent-to-solid mass ratio in the extractor, k_f is mass transfer coefficient in the fluid, a_0 is specific surface area, λ is characteristic particle dimension (volume-to-surface ratio), R is particle radius, and D_e is effective diffusion coefficient.

In order to reduce the number of model adjustable parameters, reference was done to the well-known representation referred as "broken and intact cells model" (Sovová, 1994) which is largely used in the extraction of solute from solid matrix. Under this assumption, the solutes are contained in cells of the plant matrix and, as a result of mechanical milling pretreatment, some cells in the solids are broken and the remaining cells in the core of the particles are intact. The solute in the broken cell is directly exposed to the particle surface and can be easily extracted (fast desorption): this solute is referred as "free solute" and the extraction rate depends on first-order rate constant k_1. Conversely, the solute in the intact cells is much more difficult to extract due to the high mass transfer resistance inside the particle itself: in this case the solute is referred as "tied solute" and the extraction rate depends on k_2. The value of F was determined following the approach of Reverchon & Marrone (2001), who assumed that the particle surface is completely covered with free solute and the thickness of this layer is equal to the radius of solute bearing cell. For grape seed oil supercritical CO_2 extraction, Fiori, Basso, & Costa (2009) found a better agreement between experimental data and model predictions by doubling the thickness of this layer under what was called "double shell hypothesis". Combining the two approaches, F is given by Eq. (5).

$$F = 6\, d_c/d_p \quad (5)$$

where d_p is the mean diameter of the particle (0.5 mm) and d_c is the solute bearing cell diameter. The solute bearing cell diameter was let equal to 20 μm, value previously measured for grape seeds

using scanning electron microscope (Fiori et al., 2009b). Accordingly, the value of $F = 0.24$ was taken for all the investigated conditions.

The model, written as a MATLAB™ code, was utilized in best-fitting the experimental data according to the least square minimization technique by using k_1 and k_2 as the model adjustable parameters. The goodness of the model fitting to experimental data was assessed considering two statistical criteria, the percent average absolute relative deviation (AARD (%)), calculated according to Eq. (6), and the root mean square error (RMSE), given by Eq. (7).

$$AARD(\%) = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{\left((C/C_o)_{exp}-(C/C_o)_{model}\right)}{(C/C_o)_{exp}}\right|_i * 100 \quad (6)$$

$$RMSE = \sqrt{\sum_{i=1}^{n}\frac{\left((C/C_o)_{exp}-(C/C_o)_{model}\right)^2}{n}} \quad (7)$$

where *n* represents the number of experimental data, and $(C/C_o)_{exp}$ and $(C/C_o)_{model}$ are the dimensionless experimental extraction yield and the extraction yield predicted by the model, respectively.

4. Statistical analysis

Influences of the TP yields were assessed by analysis of variance (ANOVA) and Tukey's post hoc test. Multiple comparison of the means was made by the least significant difference test at $p = 0.05$. The Statistica v. 6.0 software (StatSoft, Tulsa, OK, USA) was used for the analysis.

5. Results and Discussion

5.1 Total Polyphenol Yields

The TP extraction yield for both grape skins and defatted seeds at different temperatures is presented in Table 1. All the data points represent the average of at least two repeated extractions, each analyzed for TP in triplicate.

Table 1

The TP yield increased with temperature for both skins and defatted seeds, while it decreased when the flow rate increased from 2 to 5 ml/min. In principle, it could be expected that, for a fixed extraction duration, higher solvent flow rate would reflect in higher extraction yield. This behavior, quite common in the literature addressing standard extraction processes, was observed for SW extraction by Khajenoori et al. (2009). The authors experienced an increase in yield at increasing solvent flow rate during the SW extraction of essential oil from *Zataria multiflora* (Khajenoori et al., 2009). Conversely, the present work testifies an opposite behavior, confirmed by some other works in the literature. Rangsriwong, Rangkadilok, Satayavivad, Goto, & Shotipruk (2009) observed a decrease in yield of corilagin from *Terminalia chebula* Retz with an increase in SW flow rate at constant temperature. According to the authors, this was probably due to the action of the higher amount of hydronium and hydroxide ions which passed through the substrate, reacting to some extent with the solute being extracted (Rangsriwong et al., 2009). Pinelo, Sineiro, & Núñez (2006) also observed a decrement in polyphenol extraction yield when studying the mass transfer during continuous solid–liquid extraction of grape pomace. The authors hypothesized that, although higher flow rates favor higher concentration gradients between the sample and the solvent, the residence time had a major weight than the concentration gradient in the mass transfer mechanisms of this process (Pinelo et al., 2006). In their work, when the flow rate was changed from 3 to 2 mL/min, the polyphenol yield increased from 16.97 to 38.13 mg_{GAE}/g, indicating a higher quantity of phenols passing from grape pomace to solvent in the second case (Pinelo et al., 2006).

Another effect can be the cause of the trend here observed. We experienced a compaction of the substrate (grape skins) due to the SW extraction process. In the experiments, as reported in Section 2.3, the milled particles to be extracted were loaded in the middle of the extractor, with bottom and top layers filled with glass beads. At the end of the extraction operations, the particles resulted in a compact cake and did not dispersed through the voids of the glass beads bed. It is possible to hypothesize that, during continuous SW extraction, the compaction degree of the substrate was directly proportional to the flow rate, thereby affecting the extraction of solute from this layer either by creating local flow inhomogeneity (channeling) or by increasing the internal mass transfer resistance. This possible explanation needs further investigation.

Given these results and considerations, the defatted seeds were extracted only with a flow rate of 2 mL/min.

5.2 Grape skins SW extraction kinetics

The TP extraction kinetics curves relevant to grape skins at the flow rate of 2 and 5 mL/min for three operating temperatures of 80, 100 and 120 °C and constant pressure of 10 MPa are presented in Figs. 2a and b.

Fig. 2

For both solvent flow rates, the TP yield increased with the increase in temperature.

At fixed temperature, the initial rate of extraction was higher at the higher solvent flow rate while, conversely, the final yield was higher at the lower solvent flow rate, as discussed in section 5.1.

Because of these opposing trends, the extraction curves at different solvent flow rates crossed each other (see also Figure 4 in section 5.4). The cross over points shifted in time to the left with the increase in temperature. At 80 °C, the two extraction kinetics curves (2 and 5 mL/min) overlapped at the end of the test (120 min); at 100 °C, they crossed at about 60 min; at 120 °C, they crossed at about 20 min.

5.3 Defatted grape seeds SW extraction kinetics

For defatted seeds, the experiments were conducted at a flow rate of 2 mL/min. In this case, the TP yields resulted higher than those of skins (see Table 1, Figs. 2 and 3). It must be stated that the TP yields reported in Table 1 are greater than the final yields presented in Figs. 2 and 3, because the values of Table 1 account also for the amount of polyphenols in the water remaining inside the extractor at the end of two hours extraction period, while in Figs. 2 and 3 only the kinetics data were plotted.

Fig. 3

Even though there is not direct comparison of TP yield from defatted grape seeds and skins in the literature relevant to SW extraction (to the best of our knowledge), some studies present interesting data for comparison. It is worth to underline that during the CO_2 defatting process the amount of polyphenols in the seeds remain unvaried as pure CO_2 is incapable of extracting such polar compounds, as demonstrated by Fiori et al. (2009a).

Casazza et al. (2010) reported a comparison between un-defatted grape seeds and skins of *Pinot Nero* extracted by different non-conventional techniques. They found out that TP in seeds is one order of magnitude higher than that in skins, and the yields of TP can vary up to 390% simply by changing the extraction technique. Aliakbarian et al. (2012) performed SW extraction of grape pomace and found a yield of 30.80±3.38 mg_{GAE}/g at operation conditions of 140 °C and 11.6 MPa when the flow rate was 1-2 mL/min. Bucić-Kojić et al. (2013) reported a TP yield from grape seeds of 130 mg_{GAE}/g when extracting at a temperature of 80 °C using an ethanol-water solution in a batch reactor. Sólyom, Solá, Cocero, & Mato (2014) studied the thermal degradation of grape marc polyphenols; they found a TP yield of 82.79±2.67 mg_{GAE}/g and hinted that grape marc may preserve at least 90% of the active compounds up to 150 °C (in their case the yield at 100 °C was higher than that at 150 °C). In fact, wide ranges of TP yields from wine industry by-products are reported in the literature due to the several factors which influence the total yield, such as the extraction temperature, time, technique, solvent type, cultivars and type of pretreatment.

5.4 Extraction kinetics: modeling results

The extraction kinetics of both grape skins and defatted seeds was modeled with the two-site kinetic model described in Section 3. The model curves are reported together with the experimental data in Fig.s 4 and 5. The model adjustable parameters from best fitting are presented in Table 2 along with the deviation of model predictions from experimental data. There are clear trends for both fast and slow desorption rate constants k_1 and k_2, for both skins and defatted seeds. The desorption rate of fast extracted fraction of polyphenols, expressed as first order rate constant k_1, increases both with temperature and flow rate. Generally, an increase in temperature enhances the solvent power of water (an increase in temperature makes the polarity of SW to decrease and therefore the solubility of less polar organic solute to increase), while an increase in flow rate increases the concentration gradient. As the characteristic particle dimensions are similar for all the tests, the increase in k_1 with temperature can be explained in terms of the mass partition coefficient of the solute (which is defined as the ratio of equilibrium concentration of the solute in the fluid phase at the particle surface to the solute concentration in the solid phase). Looking at Eq. (3), the first order rate constant k_1 is directly proportional to the partition coefficient. So, with the increase in temperature the solute partition coefficient will increase, and hence the desorption rate constant k_1 will also increase. This can be also observed from Fig. 4 where the initial rate of extraction increases with both temperature and flow, while in the following the flow makes an inversion of the trends (see the discussion on crosses over at Section 5.2). For grape skins, except at the lowest temperature of 80

°C, the desorption rate constant of slowly released fraction k_2 decreases when flow rate increases. Consequently, the decrease in the TP yield when the flow rate increases incurred in second part of the extraction (Fig. 4). k_2 reflects the characteristics of the matrix and should largely depend on effective diffusivity, Eq. (4). Accordingly, the structure of the bulk material must have changed with flow rate as hypothesized in Section 5.1.

Table 2

Fig. 4

When we compare the model parameters for defatted seeds and skins at 2 mL/min, since in both cases the experiments were conducted at constant specific flow rate and bed void volume, the external mass transfer coefficients are largely expected to be similar. This hypothesis is also confirmed by the value of adjustable parameters in Table 2, with small variations which can be attributed to the structural difference between skins and defatted seeds.

Fig. 5

The deviation between model predictions and experimental data are quantified and compared using RMSE and AARD (%), as shown in Table 2. Remarkable agreement between model predictions and experimental data was achieved. The values of model adjustable parameters are also consistent with the values reported elsewhere in the literature (Kubátová et al., 2002; Anekpankul et al., 2007; Khajenoori et al., 2009).

The conventional two-site kinetic model, supplemented with Eqs. 3 and 4 for the definition of fast and slow extracted fractions rate constant k_1 and k_2, can persuasively describe the underlining physical phenomena during the extraction processes. The model is reasonably simple and information generated thereof has a vast practical importance especially in scale up and process design.

6. Conclusions

Subcritical water extractions of polyphenols from grape skins and defatted grape seeds were conducted in semi-continuous extractor. Relatively high yields of total polyphenols were obtained for both skins and seeds. Increasing the extraction temperature, the total polyphenols yields increased. Increasing the solvent flow rate resulted beneficial only in the initial extraction phase, while in the following the extraction rate decreased substantially: the final total polyphenols yields were higher for the lower solvent flow rate.

The kinetics of extraction was modeled by the two-site kinetic model; remarkable agreement between model and experimental data was observed with root mean square error in the range of 10^{-2}-10^{-1} and percent average absolute relative deviation of 0.5-4%. The model adjustable parameters were also in satisfactory agreement with values reported elsewhere in the literature.

Acknowledgement

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Nomenclature

Symbol	Caption	Unit
a_o	Specific surface area	L^2L^{-3}
C	Mass of TP extracted per mass of substrate	MM^{-1}
C_o	Initial mass of TP per mass of substrate	MM^{-1}
d_c	Solute bearing cell diameter	L
d_p	Diameter of the particle	L
D_e	Effective diffusion coefficient	L^2T^{-1}
F	Fast extracted fraction of solute	
i	Integer number	

k_1	Desorption rate constant of fast extracted fraction	T^{-1}
k_2	Desorption rate constant of slowly released fraction	T^{-1}
k_f	External mass transfer coefficient	LT^{-1}
K_m	Mass partition coefficient	MM^{-1}
n	Number of expermental points	
Q	Specific solvent flow rate	T^{-1}
R	Particle radius	L
t	Extraction time	T
Greek Letters		
γ	Solvent-to-solid mass ratio in the extractor	MM^{-1}
ε	Bed void fraction	
λ	characteristic particle dimension (volume-to-surface ratio)	L

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Figure Captions

Figure captions

Fig. 1. P&ID of the extraction equipment.

Fig. 2. TP yield (mg_{GAE}/g) relevant to SW extraction from grape skins at different temperatures. (a) solvent flow rate equal to 2 mL/min; (b) solvent flow rate equal to 5 mL/min. Experimental data.

Fig. 3. TP yield (mg_{GAE}/g) relevant to SW extraction from defatted grape seeds at different temperatures and at a solvent flow rate equal to 2 mL/min. Experimental data.

Fig. 4. TP yield (dimensionless) relevant to SW extraction from grape skins at different temperatures and solvent flow rates. Experimental data and model curves.

Fig. 5. TP yield (dimensionless) relevant to SW extraction from defatted grape seeds at different temperatures and at a solvent flow rate equal to 2 mL/min. Experimental data and model curves.

Figure 1

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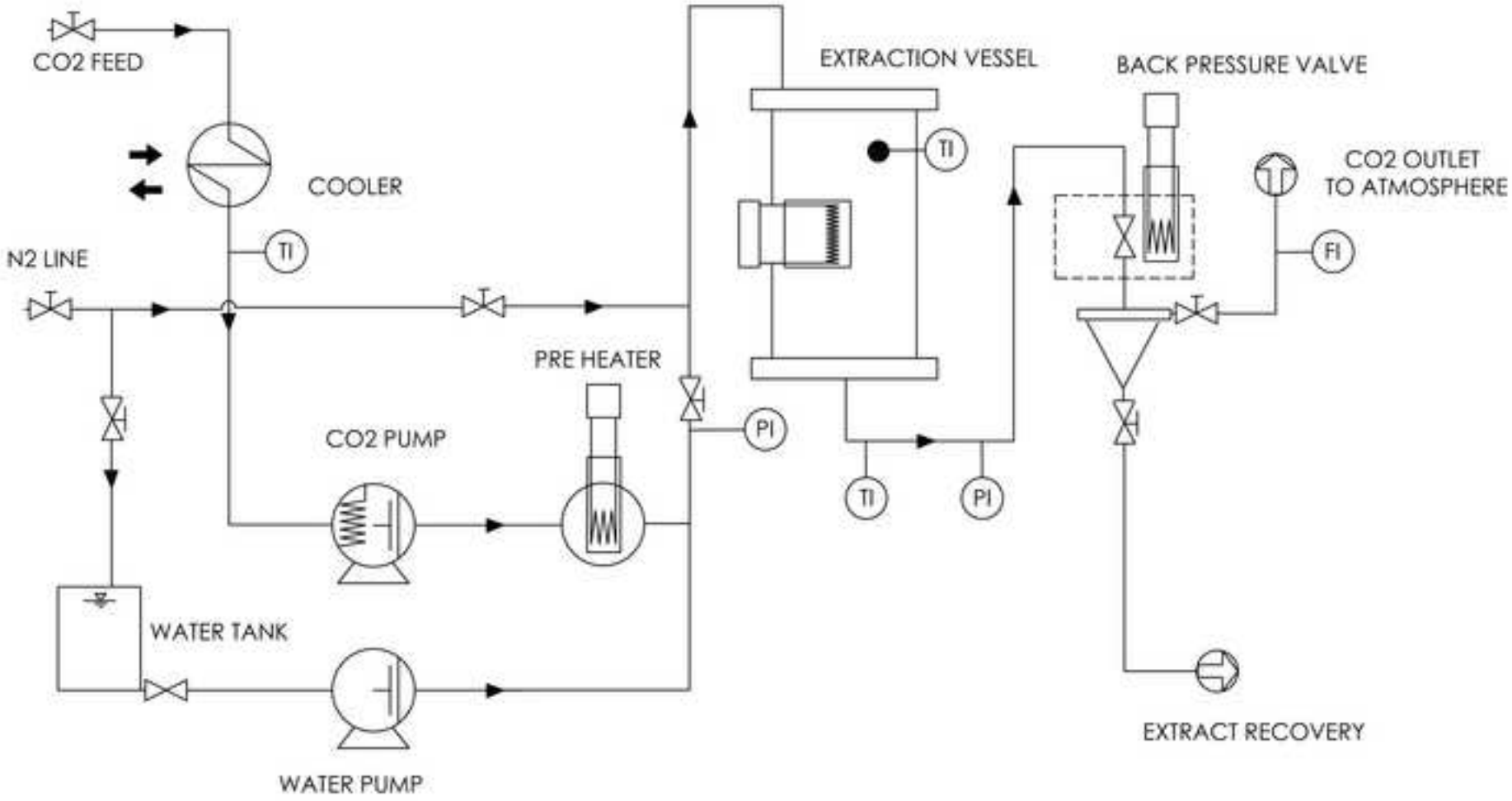

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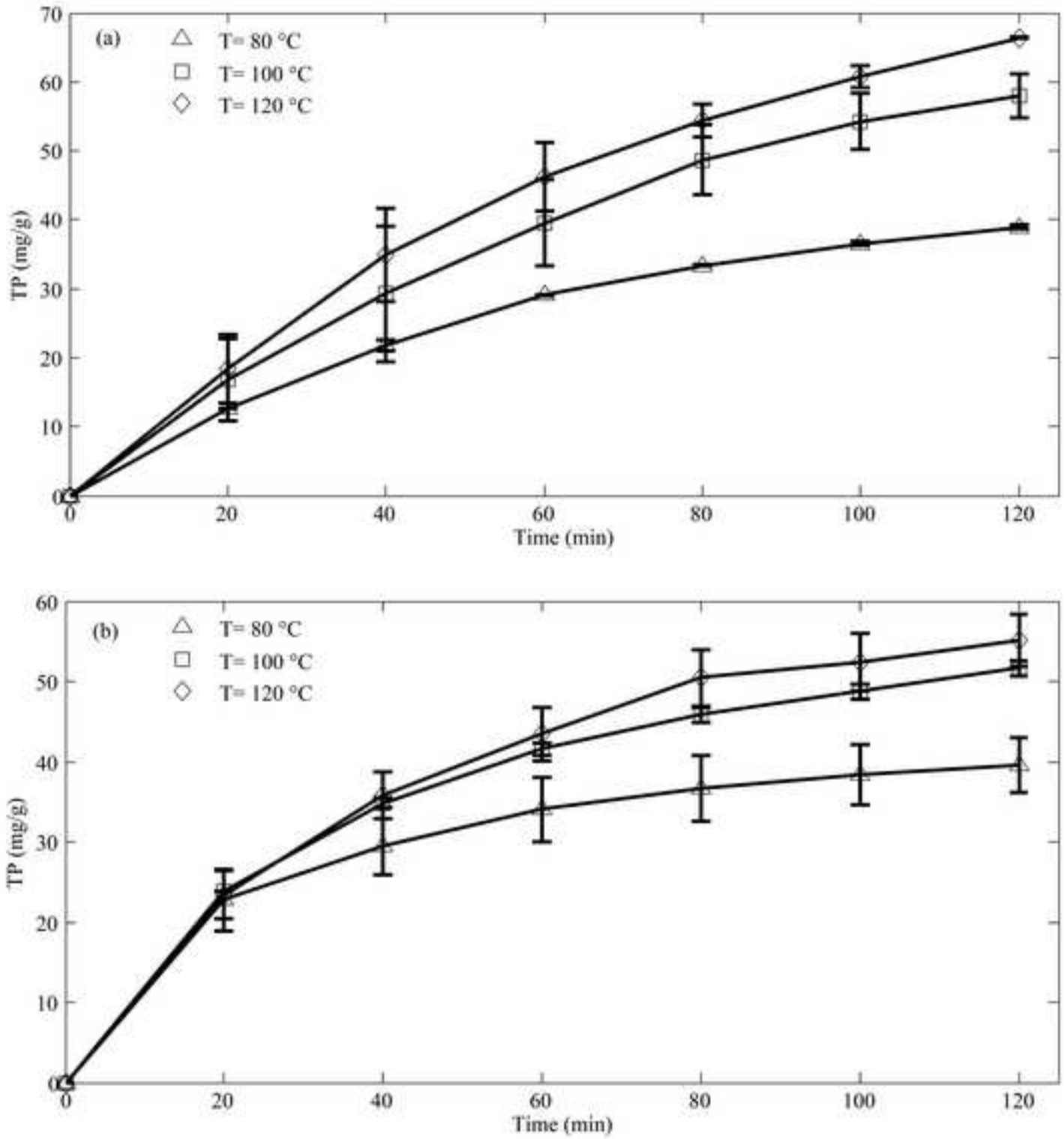

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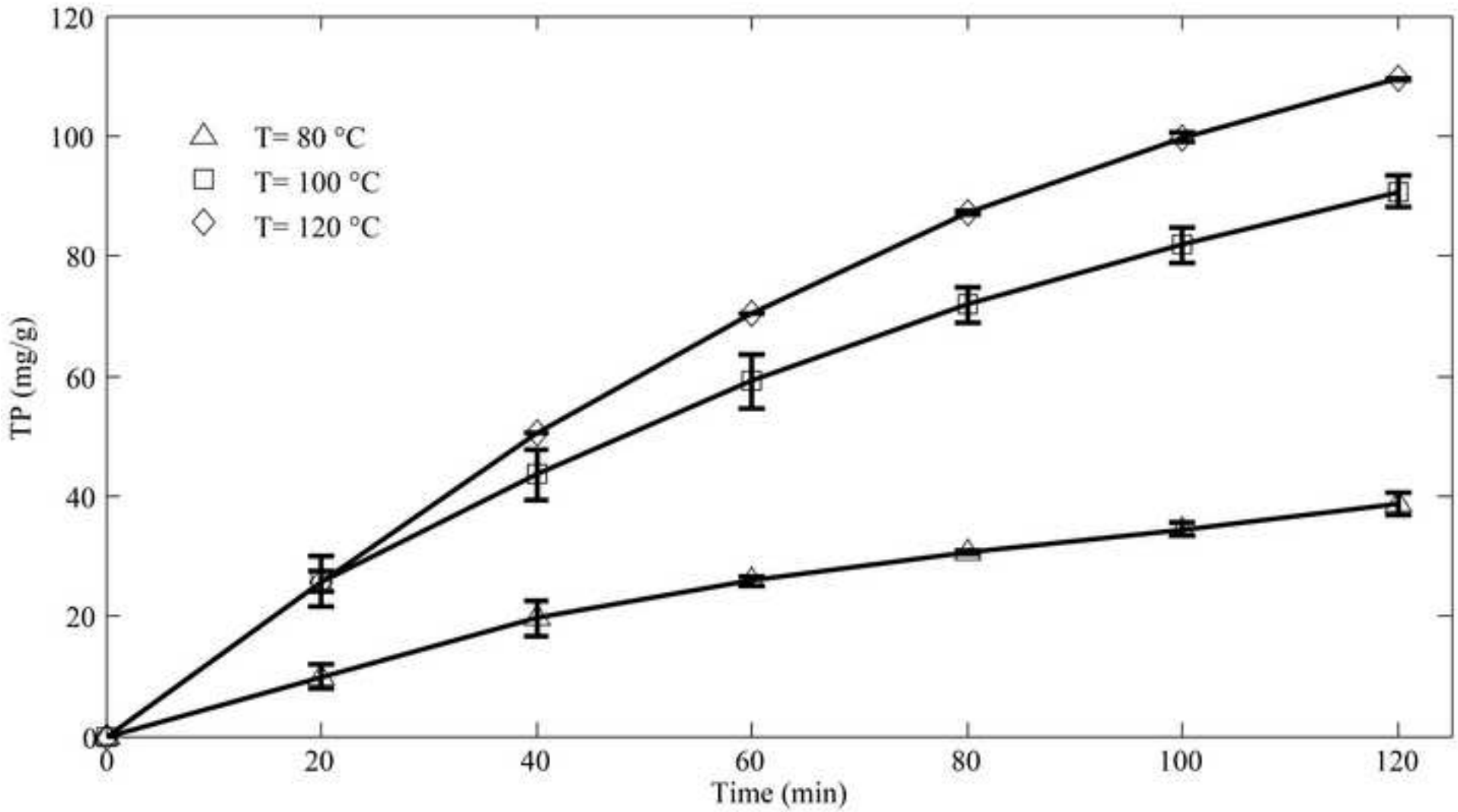

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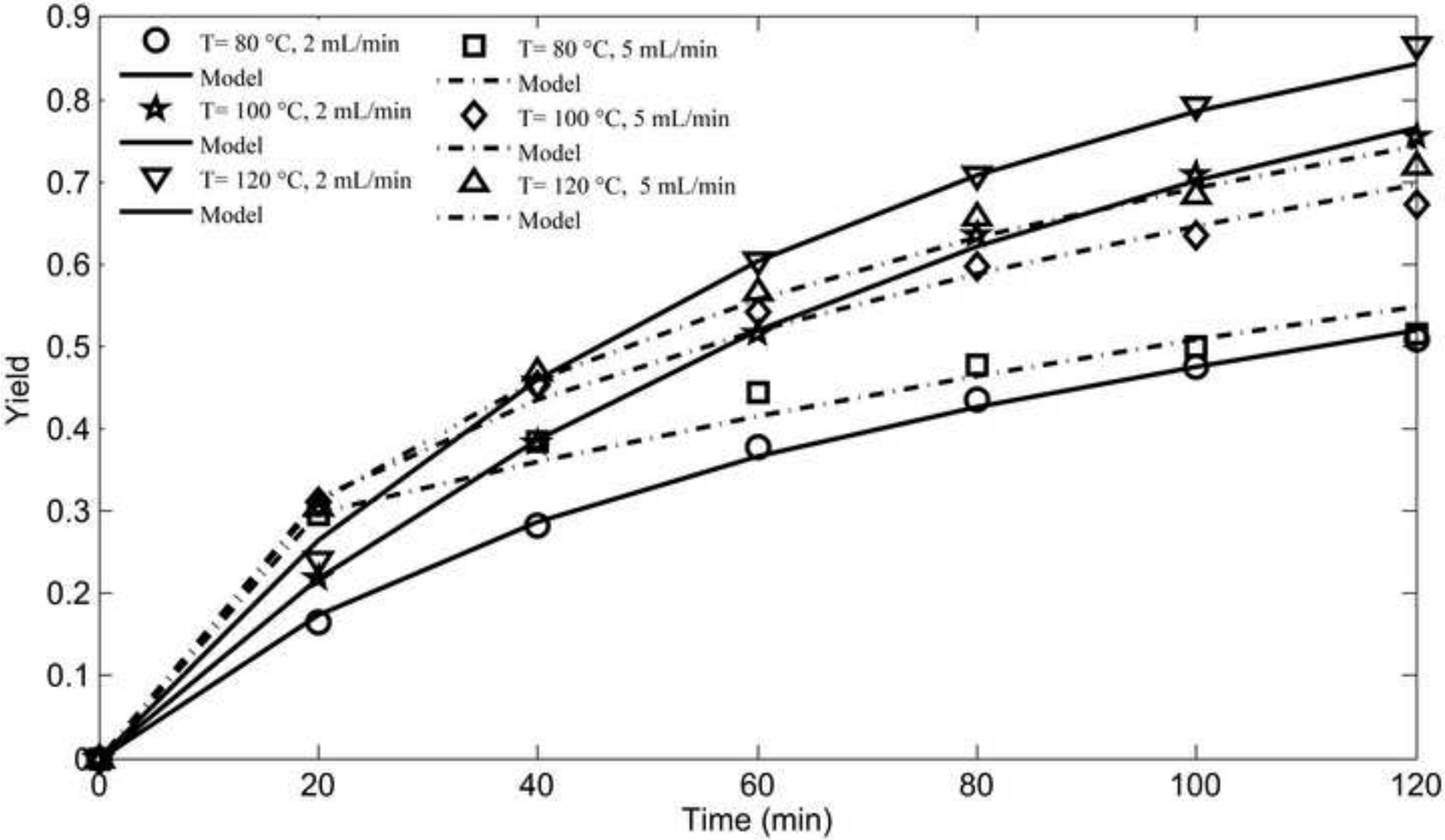

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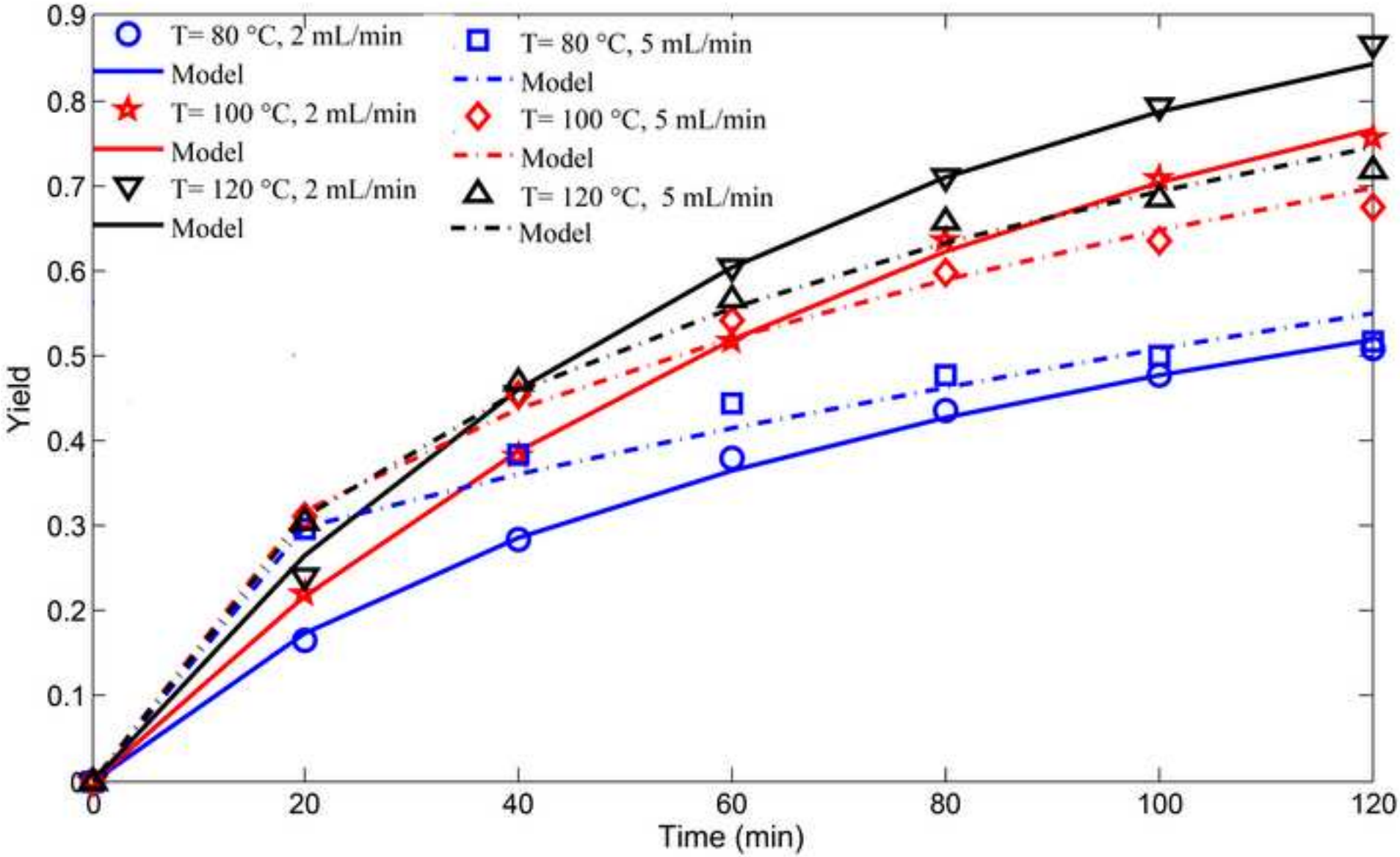

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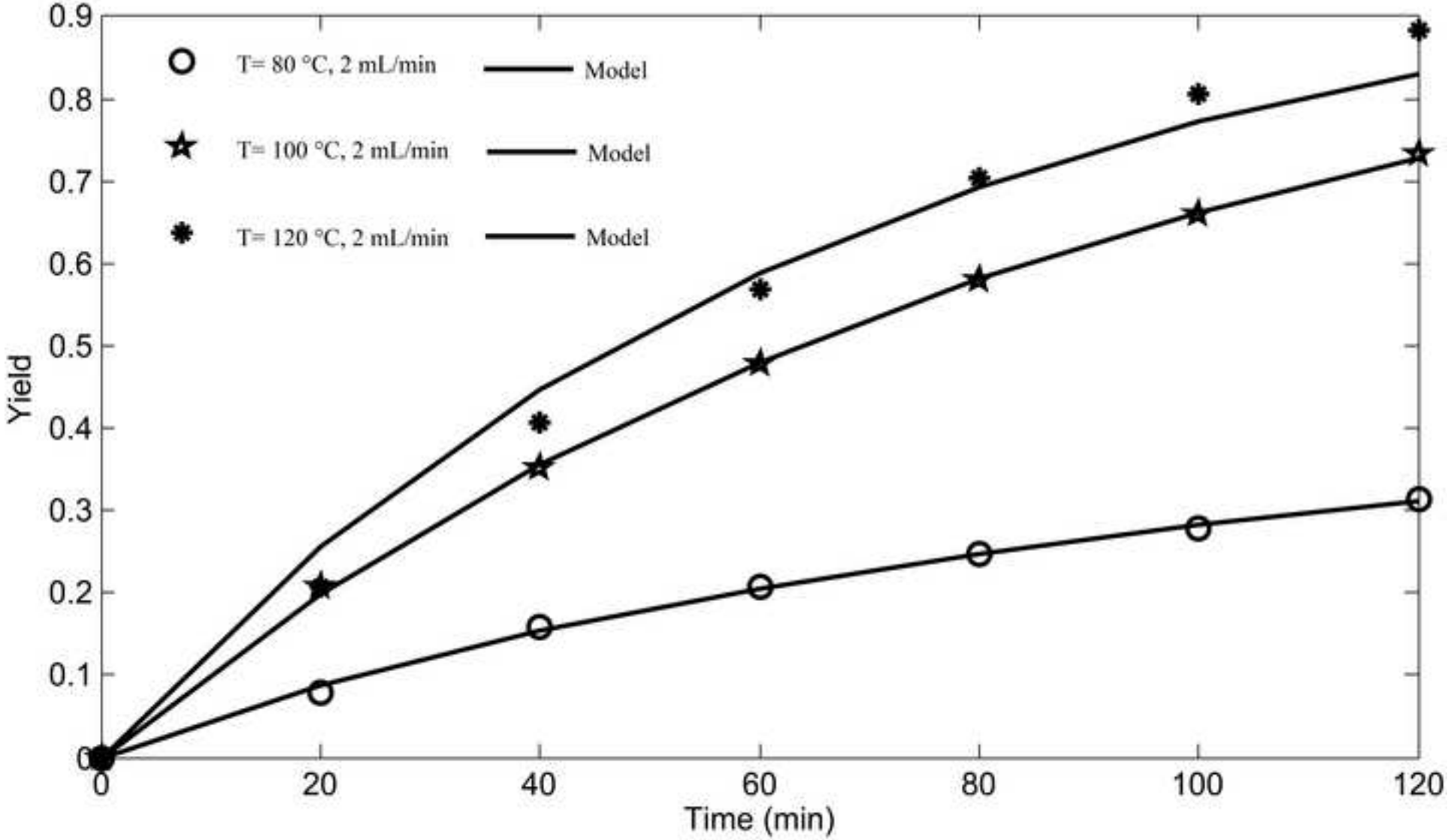

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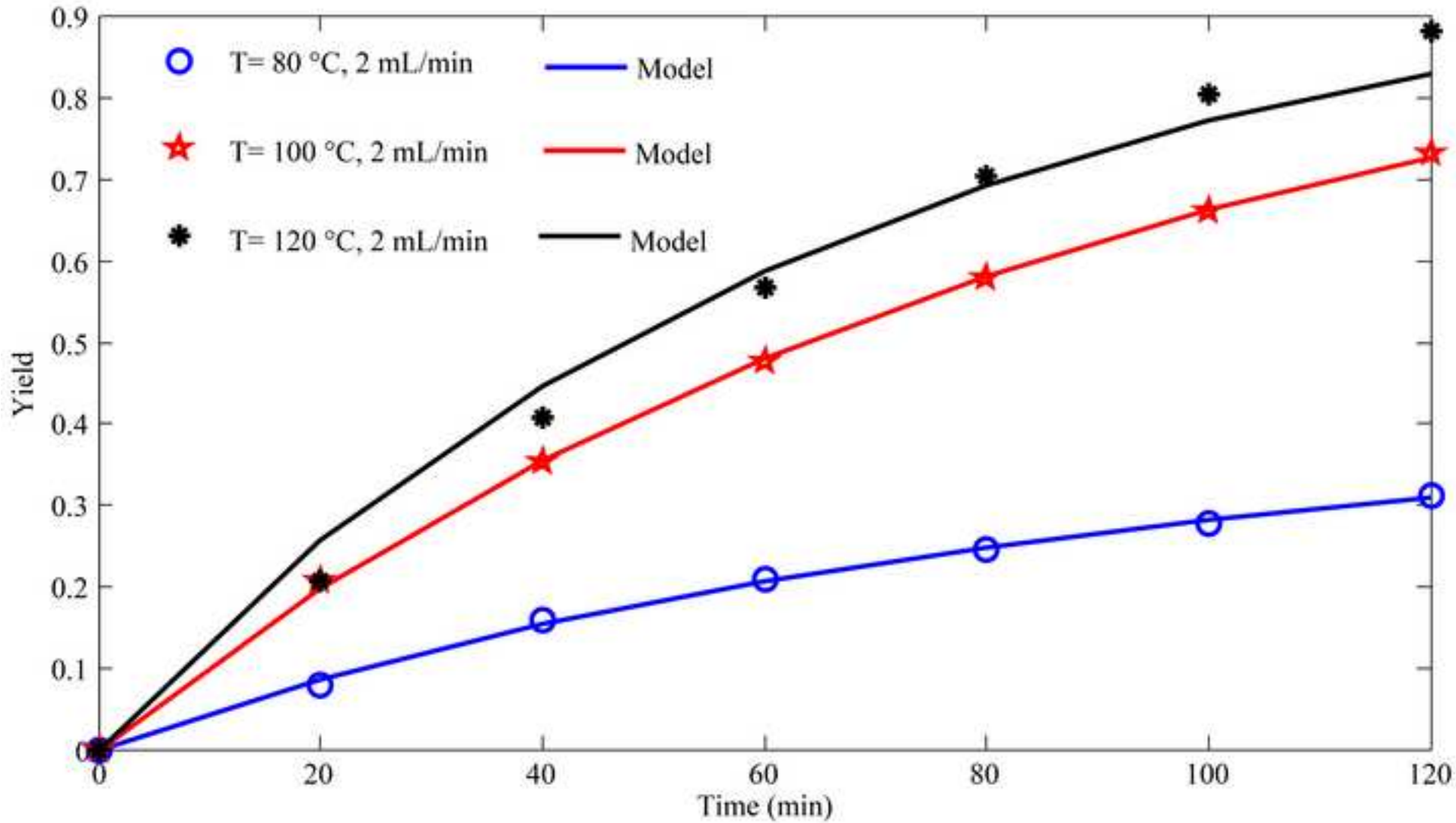

Table 1

Extraction yield of TP for *Pinot Nero* grape skins and defatted seeds.

	Skins TP (mg_{GAE}/g)		**Defatted seeds TP (mg_{GAE}/g)**
Temp.(^oC)	2 mL/min	5 mL/min	2 mL/min
80	44.3±0.4[a]	40.7±1.8[a]	44.2±2.4[a]
100	66.3±4.2[b]	54.7 ±1.0[b]	101.6±1.6[b]
120	76.7±2.8[c]	58.0±3.3[b]	123.9±0.7[c]

Different letters (a-c) within columns show significant differences at $p < 0.05$

Table 2

Model adjustable parameters for SW extraction of grape skins and defatted seeds.

$\boldsymbol{T}$ (°$\boldsymbol{C}$)	Skins								Defatted seeds			
	2 mL/min				5 mL/min				2 mL/min			
	k_1 (min^{-1})	k_2 (min^{-1})	$RMSE$ $*10^2$	$AARD$ (%)	k_1 (min^{-1})	k_2 (min^{-1})	$RMSE$ $*10^2$	$AARD$ (%)	k_1 (min^{-1})	k_2 (min^{-1})	$RMSE$ $*10^2$	$AARD$ (%)
80	0.0154	0.0039	2.16	1.19	0.0739	0.0044	5.20	1.99	0.0146	0.0012	0.99	1.27
100	0.0163	0.0111	1.84	0.57	0.1019	0.0077	3.98	1.38	0.0148	0.0099	1.28	0.66
120	0.0334	0.0155	3.42	1.22	0.1865	0.0091	4.04	1.21	0.0168	0.0148	9.11	3.78