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Optimization of the key parameters for extraction of polyphenol compounds from tomato fruits (*Solanum lycopersicum* L.). Kinetics of the process.

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Dedicated to Acad. Dimiter Ivanov on the occasion of his 120th birth anniversary

The main parameters that affect extraction process of polyphenolic compounds from tomato were investigated. The most suitable solvent for reaching maximum yield of polyphenols was acetone-water mixture 80:20 (v:v). Using this extragent the concentration of extracted polyphenols was approximately 17% higher than using ethanol and methanol. The optimal extraction time, temperature and solid to solvent ratio were 45 min, 60ºC and 1:40, respectively. Using these extraction parameters the yield of total polyphenols in tomato variety Desperado was 27.80 GAE/100 g fresh weight. The kinetics of extraction process was investigated and theoretical model describing extraction process was proposed. This mathematical model provides the theoretical initial amount of polyphenols in tomato fruits which could be useful for the breeding programs of varieties with high amount of polyphenol compounds.

Key words: tomatoes, polyphenols, extraction, optimization, kinetics

INTRODUCTION

Polyphenols are secondary metabolites that are synthesized mainly in plants [1,2]. It is known that these components protect plants from pathogens, UV-B light and play role as signal molecules in the interaction between plants and environment [3]. In the recent years polyphenol compounds gained a lot of attention because they act as antioxidants and protect human body from oxidative stress which is the main reason for different degenerative processes. Because of the polyphenol components, the consumption of fruits and vegetables is reversely correlated with the development of chronic diseases [4,5]. Therefore, contemporary breeding programs are directed to the selection of cultivars with increased content of polyphenols and other antioxidant components.

The accurate determination of polyphenols depends on the methods of extraction and analysis. Since the extraction is the main part of sample preparation, there are many methods for isolation of phenolic compounds from different plant matrices. The yield of analytes is influenced by many factors such as: chemical structure, solvent, pH, temperature, etc. Optimization of extraction parameters is critical for precise and reproducible analysis and there is no protocol, suitable for all classes of phenolic compounds [6]. Therefore, optimization of extraction parameters for different plant matrices is necessary [2].

Tomato is among the most consumed vegetables in the world. Generally, tomato fruits are not very rich of polyphenols, but high consumption of tomato and tomato products make them an important source of these compounds [7]. The main part of the hydrophilic antioxidant capacity of tomatoes is due to the presence of polyphenols [8]. For example, Grozeva *et al.* (2013) compared the polyphenol content and antioxidant activity of cherry and small sized tomatoes and observed that cherry tomato line $1620/_{10}$ is distinctive with the highest polyphenol content and the highest antioxidant activity, measured by ORAC (11.54 μmol TE/g) and HORAC (6.69 μmol GAE/g) methods [9]. Similar results were obtained by Toor *et al.* (2005) who investigated antioxidant properties of tomatoes grown in New Zealand [8].

Predominate phenolic compounds in tomatoes are chlorogenic acid, rutin, naringenin, naringenin chalcone, quercetin [10]. All plant phenolics are conjugated with sugars, rather than free aglycones, which make them more soluble in water. Therefore, for extraction of these compounds mixtures of

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ethanol, methanol and acetone with water are most commonly used [11-13]. For obtaining better yield of these compounds optimization of extraction parameters such as temperature, extraction time, solid to solvent ratio is also necessary. These parameters were already optimized in other plant materials but to our knowledge the optimization of these key factors for tomato polyphenols extraction has not been addressed [14,15]. Therefore, the aim of this study was to find the optimal parameters for extraction (temperature, extraction time, solid to solvent ratio) of phenolic compounds from tomatoes and to develop mathematical models describing the process.

EXPERIMENTAL

Reagents, Solvents and Apparatus

Ethanol, methanol and acetone used in experimental work were pure for analysis. Folin-Ciocauteu reagent was from Merck, gallic acid was from Sigma Aldrich. UV/VIS Spectrophotometer used in this work was from Camspec Ltd., Cambridge, UK.

Plant Material

Optimization of extraction parameters was carried out by using freeze-dried tomato samples, variety Desperado (Enza Zaden, the Netherlands). The plants were grown under greenhouse conditions during the period March-July 2012. Randomized samples of tomato fruits were harvested at red ripening state.

Sample Preparation

About 700-800 g of tomatoes were washed, packed in plastic bag and frozen in refrigerator at -20˚C. After that, samples were freeze-dried, subsequently crushed to powder using laboratory blender and stored at -20˚C prior analysis.

Optimization of Extraction Parameters

The main factors that affect the extraction such as type of solvents (methanol, ethanol and acetone) and their mixtures with water (20%, 40%, 60%, 80% (v:v)), extraction time (15, 30, 45, 60, 75 and 90 min), temperature (room, 40˚C and 60˚C) and plant material to solvent ratio (1:20, 1:40 and 1:80 (w:v)) were studied. For the extraction two grams of dry powdered sample were weighted accurately and mixed with 40 ml of extragent in extraction tubes with caps. Samples were extracted in shaking water bath for 1 hour.

Extraction solvent and temperature: Three solvents (acetone, methanol and ethanol) and their mixtures with water (20%, 40%, 60%, 80% (v:v)), as well as three different temperatures of extraction (room temperature, 40˚C and 60˚C) were studied.

Time of extraction: The influence of time on extraction process was examined in optimal conditions, found for type of extragent and temperature. The tested time intervals were 15, 30, 45, 60, 75 and 90 min.

Sample to solvent ratio: The optimal solvent, temperature and extraction time were used to determine the optimal sample to solvent ratio (v:v) of the extraction process. For that aim 1 gram of the sample were weighted accurately and mixed with 20, 40 or 80 ml of the extragent (80% acetone) to obtain 1:20, 1:40 and 1:80 sample to solvent ratio, respectively. All samples were put in shaking water bath for 45 min at 60˚C. After that, samples were filtrated and supernatants were collected for determination of total polyphenol contents.

Determination of Total Polyphenols

The influence of different extraction parameters on the yield of polyphenols was estimated according to the method of Singleton & Rossi (1965) with Folin-Ciocalteu's reagent [16]. Briefly, 100 µl of extract was mixed with 3100 µl water, 200 µl Folin-Ciocalteu's reagent and 600 µl 20% Na_2CO_3. The mixture (final volume 4 ml) was vortexed, incubated for 5 min at 50˚C, cooled in ice bath for 5 min and then absorbance was measured spectrophotometrically at 750 nm. The amount of polyphenols was determined by standard curve of seven different concentrations of Gallic acid (0.04; 0.06; 0.08; 0.10; 0.20; 0.40 and 0.60 mg/ml) and results were expressed as mg GAE/100 g fresh weight (FW).

Statistics

Data were subjected to Duncan's Multiple Range Test to evaluate the statistical significance among means. Each sample was measured in triplicates (or quintuplicates) and variations between these technical triplicates in each analysis were less than 1% (data not shown). Method of least squares was also used.

Kinetics of Extraction Process

Based on data obtained from the experiments the rate constants (k_1, k_2) of the process and the amount

of polyphenols in matrices (A_0) were determined (equation 1). The principal of Nuton-Rafson For was used for determination of nonlinear regression correlation and implementation was performed using NONLIN program compiled on FORTRAN IV, with adapted sub program FUNCTN for the specific mathematical correlation.

RESULTS AND DISCUSSION

We investigated the influence of the main factors that affect the extraction of polyphenols from tomato matrix, namely type of solvent, temperature, solid to liquid ratio (w:v) and extraction time. In Table 1 the results for the influence of temperature and type of solvent on yield of total polyphenols from tomato fruits are presented.

Type of Solvent

From the results in Table 1 it is evident that ethanol-water mixtures have a better extraction efficiency compared to methanol-water mixtures at room temperature and at 40ºC. With increasing of the temperature, the yield of total polyphenols for these two solvents has also increased, but differences between obtained values are very low and statistically insignificant (22.79 mg GAE/100 g for methanol-water and 22.97 mg GAE/100 g for ethanol-water). Similar results were reported by Mukhopdhyay *et al.* in 2006 in different plant matrices [6]. In these cases ethanol is preferable solvent because of it's lower toxicity compared to methanol. Among the three solvents used in this investigation acetone exhibited extraction efficiency resulting in up to 17% higher yield of total polyphenols in all studied temperatures. Our results are in line with other studies pointing out acetone as suitable extragent for polyphenol compounds [14]. Acetone has polar index 5.1 which makes it a solvent with intermediate polarity. Phenols, which are present in the tomatoes are compounds with different polarity. Therefore, such types of solvents are preferable for their extraction from this matrix. The higher yield of total polyphenols from tomatoes using acetone extract is probably due to the presence of high molecular polyphenols as tannins that are also extracted with acetone [17].

Temperature

Values obtained for total polyphenol content of tomato extract obtained by methanol-water mixtures at room temperature and 40˚C were very close (Table 1). Increasing the temperature inten-sified the extraction process, yielding higher amounts of extractible polyphenols. The highest yield of polyphenolic compounds was achieved at temperature 60˚C. It is known that high temperature promotes high analite solubility and increases mass transfer. Viscosity and solvent surface tension are decreased by high temperature and solvent could easily reach sample matrices which leads to increased extraction rate [13].

Table 1. Influence of type solvent, percentage of solvent with water (v:v), and temperature on yield of total polyphenols from freeze-dried tomato.

Room temperature		**Temperature 40˚C**		**Temperature 60˚C**	
Solvent, %	**Polyphenols, mgGAE/100g**	**Solvent, %**	**Polyphenols, mgGAE/100g**	**Solvent, %**	**Polyphenols, mgGAE/100g**
Methanol	18.36±0.36 ^{ef}	Methanol	18.23±0.15 ^{fg}	Methanol	20.91±0.40 ^{f}
20	18.07±0.38 ^{g}	20	18.20±0.05 ^{fg}	20	20.67±0.58 ^{f}
40	19.36±0.26 ^{ef}	40	18.29±0.47 ^{fg}	40	22.79±0.28 ^{de}
60	19.18±0.39 $^{e-g}$	60	18.79±0.39 ^{f}	60	22.07±0.06 ^{e}
80	19.54±0.36 ^{de}	80	19.58±0.45 ^{e}	80	22.34±0.33 ^{e}
Ethanol	15.87±0.49 ^{h}	Ethanol	17.39±0.23 ^{h}	Ethanol	18.39±0.58 ^{g}
20	18.56±0.71 $^{e-g}$	20	19.73±0.40 ^{e}	20	22.78±0.75 ^{cd}
40	19.08±0.47 $^{e-g}$	40	21.19±0.11 ^{d}	40	22.78±0.06 ^{de}
60	22.07±0.80 ^{c}	60	22.76±0.66 ^{c}	60	22.57±0.40 ^{e}
80	22.62±1.06 ^{bc}	80	20.95±0.46 ^{d}	80	22.97±0.61 ^{de}
Acetone	18.13±0.45 ^{i}	Acetone	17.54±0.31 ^{h}	Acetone	12.55±0.24 ^{h}
20	20.40±0.30 ^{d}	20	21.38±0.20 ^{d}	20	23.15±0.54 ^{de}
40	23.07±0.54 ^{b}	40	22.68±0.35 ^{c}	40	24.34±0.46 ^{c}
60	26.05±0.53 ^{a}	60	23.88±0.56 ^{b}	60	26.21±0.59 ^{b}
80	25.82±0.42 ^{a}	80	26.14±0.11 ^{a}	**80**	**27.80±0.45** ^{a}

Results are expressed as mean value ± standard deviation from three measurements. Differences between values marked with different superscript letters are statistically significant at $P<0.05$ based on Duncan's Multiple Range Test ($n = 3$).

Solvent Concentration

Obtained results about the influence of different concentrations of organic solvents on the extraction efficiency of polyphenols indicate that pure solvents have the lowest extraction ability in comparison to solvent-water mixtures. Mixtures with water are preferable because water facilitates the penetration of organic solvent into the plant cells and increase polyphenols extraction [6]. In our study, the highest yields were obtained with 60% and 80% water solution of the used organic solvents (Table 1). It has been observed that 80% methanol was better extragent for extraction of some flavonoids from vegetables compared to 50% and 90% methanol [14]. Gyenai *et al.* in 2012 also reported 80% acetone as better solvent for extraction of polyphenols from tomato than methanol and acetonitrile [15].

Extraction Time

The influence of extraction time on polyphenols extraction is shown on Fig. 1.

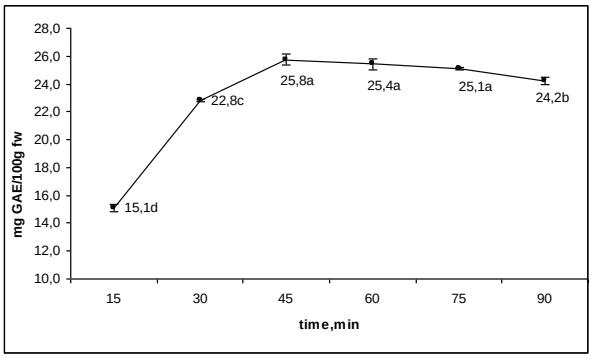

Fig. 1. Influence of extraction time on the yield of total polyphenols in freeze-dried sample tomato.

The lowest yield of total polyphenols was obtained after 15 minutes of extraction at optimum condition (80% acetone at 60ºC). Accumulation of polyphenols increased gradually and reached maximum value at the 45th min. After that the yield slightly decreased. Phenolic compounds are not uniformly distributed in plants. Some of them are strongly linked with cellular walls, while others like hydroxycinamic acid are linked with various cell components and more time is required for their penetration into the solvent. This could explain the low extraction of polyphenols in the first 15 minutes and their gradual increment [1]. On the other hand, polyphenols are prone to degradation if exposed to harsh conditions and long extraction times, which could lead to reduction of their yield [17]. The values for total polyphenols yield in the 3 time points between 45 and 75 min were very close and mathematical modeling was necessary for prediction of the most effective extraction time.

The influence of solid to solvent ratio (w:v) is shown in Fig. 2. Among the ratios studied, the lowest yield of polyphenols was obtained with 1:20 (w:v). The highest concentration of investigated compounds was achieved with 1:80 (w:v) but the difference between values obtained with 1:80 and 1:40 was not statistically significant. The extraction process is characterized with mass transfer process, where the main driving force is concentration gradient between the solid and the solvent. The transition (moving) of components of solid matrix to the solvents continues until equilibrium of the system is reached. The equilibrium constant of the mass transfer process can be affected by increasing the solid to solvent ratio because the concentration gradient also increases. On the other hand increasing the solid to solvent ratio affects the active coefficient of components and their solubility in the solvent, and thus the yield of analytes is increased [18].

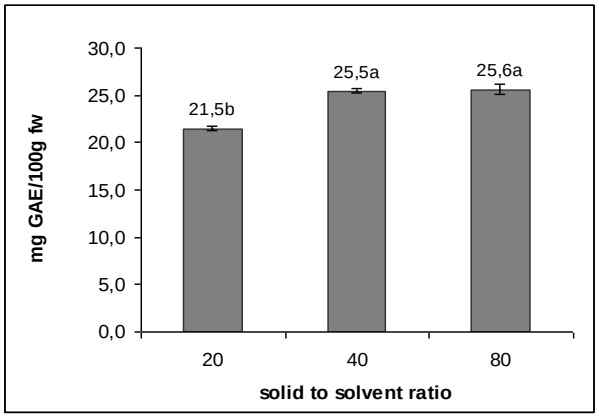

Fig. 2. Influence of ratio sample:solvent (w:v) on the yield of total polyphenols in freeze-dried tomato sample.

Kinetics of Extraction Process in Case of Solid-Liquid Extraction

The extraction processes are dynamic and therefore characterized with their own kinetics. The kinetics of the process is influenced by many factors such as plant matrices, shape and size of participle and especially by temperature and extraction time [19]. The extraction process starts with the crossing of the plant phenolic compounds of the matrix to the extragent. This process is characteri-

zed by rate constant k_1, which includes a diffusion coefficient. In this case, the extraction of polyphenols for a certain time t can be described by the differential equation 1.

$$\frac{dz(t)}{dt} = -k_1 z(t) \quad (1)$$

where $z(t)$ is the amount of polyphenol remaining in the plant tissue, k_1 is the rate constant and t is time.

The extraction of the polyphenols can also be described as an accumulation in the extractant, and the degradation of the components for a given time t. Degradation is a process which is also characterized by a rate constant k_2. These processes can be described by a differential equation 2.

$$\frac{dy(t)}{dt} = k_1 z(t) - k_2 y(t) \quad (2)$$

where $y(t)$ is the accumulation and at the same time the decomposition of polyphenols.

Since degradation and accumulation of phenolic compounds are simultaneous processes, they are described by the two differential equations (1 and 2). After mathematical processing, the following equations describing the whole process of extraction are derived:

$$z(t) = A_0 e^{-k_1 t} \quad (3)$$

$$y(t) = A_0 \frac{k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \quad (4)$$

$$q(t) = A_0\left(1 + \frac{k_2}{k_1 - k_2} e^{-k_1 t} - \frac{k_2}{k_2 - k_1} e^{-k_2 t}\right) \quad (5)$$

where $q(t)$ is the amount of degraded polyphenols time t.

$$t_{\max} = \frac{\ln \frac{k_1}{k_2}}{k_1 - k_2} \quad (6)$$

$$y_{\max} = A_0\left(\frac{k_1}{k_2}\right)^{\frac{\frac{k_2}{k_1}}{1-\frac{k_2}{k_1}}} \quad (7)$$

The proposed theoretical model describing the extraction and degradation of polyphenols is practically verified by the experimental data shown on Fig. 1. Accumulated experimental data approximated with equation (4) and equations (6) and (7) are provided for maximum yields during extraction. Data are presented on Fig. 3.

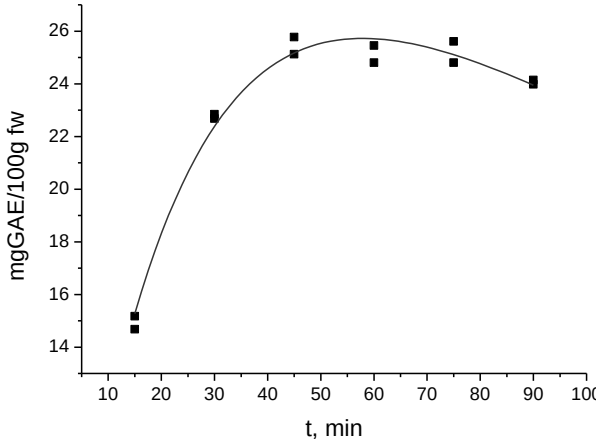

Fig. 3. Theoretical model of the influence of the extraction time on the yield of total polyphenols from tomato: R^2= 0.98599; A_0 = 34.60136 ± 2.68647; K_1 = 0.04112 ± 0.00466; K_2 = 0.00512 ± 0.0013; t_{max} = 57.87 min; y_{max} = 25.73 mg GAE/100g.

From Fig. 3 it is evident that the established theoretical model describes well the experimental data. Statistical processing were performed by the method of least squares and the correlation coefficient is R^2 = 0.98599, which shows that more than 98% of the experimental data are described by the model. The optimal extraction time t_{max} is 57.87 min (6) and the optimal extraction yield of polyphenols y_{max} is 25.73 mgGAE/100g fresh weight at the selected primary extraction conditions (temperature, solid to liquid/solvent ratio and type of extragent). The coefficient A_0, known after solving equation (1) is a very important indicator of the model, which theoretically calculates the initial amount of total polyphenols in the matrix. The application of the model could provide valuable information about the theoretical contents of polyphenols in different varieties of tomatoes, and thus allow the choice of polyphenol-rich genotypes for the purposes of tomato breeding.

CONCLUSION

The main extraction parameters that affect extraction process of polyphenolic compounds in tomato were investigated. Maximum amounts of these components were obtained with 80% acetone at 60˚C and 1:40 solid to solvent ratio. The kinetics of extraction was also studied. An equation showing what was the theoretical initial quantity of polyphenols in raw material was elaborated, which could be useful for the breeding programs of

varieties with high amount of polyphenol compounds.

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ОПТИМИЗАЦИЯ НА КЛЮЧОВИ ПАРАМЕТРИ НА ЕКСТРАКЦИЯ НА ФЕНОЛНИ КОМПОНЕНТИ ОТ ДОМАТЕНИ ПЛОДОВЕ (*Solanum lycopersicum* L.). КИНЕТИКА НА ПРОЦЕСА.

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(Резюме)

Проучени бяха основните параметри, оказващи влияние върху процеса на екстракция на полифеноли от доматени плодове. Най-подходящ разтворител, с който се достигна максимален добив на полифеноли от плодовете, беше смес от ацетон и вода в съотношение 80:20 (v:v). Използвайки този разтворител, концентрацията на търсените компоненти е приблизително 17% по-висока в сравнение с етанол и метанол. Оптималните време на екстракция, температурата и хидромодул (съотношение проба-екстрагент) бяха съответно 45 мин, 60ºC и 1:40. Използвайки оптималните параметри на екстракция, добивът на полифенолни вещества от доматени плодове сорт Desperado достигна 27.80 GAE/100g свежо тегло. Кинетиката на екстракционния процес също беше проучена и бе предложен теоретичен модел, описващ екстракционния процес. Този математически модел дава сведения за първоначалното съдържание (теоретично изчислено) на търсените компоненти в доматените плодове, което може да бъде използвано успешно за нуждите на селекцията.