# EXTRACTION OF RED GRAPE POMACE ANTIOXIDANTS WITH AQUEOUS ORGANIC ACID SOLUTIONS USING KINETIC MODELLING

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**Abstract:** Grape pomace is a food industry residual material which may contain a high load of polyphenolic antioxidants and a number of methods have been implemented for their effective recovery. Nevertheless, eco-friendly processes should embrace environmentally benign and non-toxic solvents. On this basis, this study investigated the extraction of antioxidants, by employing aqueous acetic acid and citric solutions and monitoring the reducing power ($P_R$) of the extracts. The scope was to evaluate the effect of acid concentration on the extraction yield using kinetics. The kinetic model established allowed for the credible comparison of the extraction efficiencies, achieved with acetic acid and citric acid solutions of variable concentration. The results suggested that citric acid solutions were more effective in recovering red grape pomace antioxidants. Using a 4% (w/v) citric acid solution, a maximum $P_R$ of 229.8 μM ascorbic acid equivalents per g of dry pomace could be attained. This investigation demonstrated that aqueous media used for the extraction of antioxidant compounds from food industry wastes could be significantly influenced by the acidifying agent and its concentration.

**Key words:** antioxidants, extraction, grape pomace, kinetics, organic acids, reducing power.

## Introduction

The agri-food sector is constantly under legislative pressure to reuse and/or recycle waste biomass, in an effort to minimise the associated environmental risks (Devesa-Rey et al., 2011). Industrial scale vinification produces inevitably the large volume of residues, composed of organic biomolecules (stems, seeds, skins, etc.). It is estimated that 14.5 million tonnes of grape processing residues are produced on annual basis from the viti-viniculture sector only in Europe (Pinelo et al., 2006). This waste has attracted significant attention, because of its important content in polyphenols, which may have beneficial biological properties (Yu and Ahmedna, 2013).

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The examinations on the effective polyphenol extraction from vinification wastes have focused mainly on red grape pomace (RGP), which is characterised by a significant content of polyphenols with high antioxidant potency (Makris et al., 2007). The antioxidant recovery of wine industry residues and their valorisation as food additives, cosmetic ingredients and dietary supplements are a high-value prospect (Galanakis, 2012). Numerous methodologies have been deployed to develop strategies for polyphenol extraction from RGP, with particular emphasis being given to the use of eco-friendly means, such as water/ethanol solutions (Boussetta et al., 2012; Bucić-Kojić et al., 2007; Carrera et al., 2012; Makris et al., 2008). In the examinations pertaining to extraction optimisation, the focus has been on the role of pH, a factor that may impact the extraction yield to a significant degree (Makris et al., 2008), but also it may act as a selectivity parameter, e.g. for flavanol extraction (Karvela et al., 2009a, b). The regulation of pH by the addition of acidifying agents in the extraction medium could have a profound effect on the overall efficiency of the extraction process.

Citric acid has been tested for the extraction of onion peel polyphenols (Kiassos et al., 2009; Makris, 2010) and olive leaf polyphenols (Mylonaki et al., 2008). Nonetheless, other natural organic acids have never been used for such a purpose. Both citric and acetic acids are natural organic acids occurring in foods and a more thorough study on the use of these acids in extraction processes could be of value to the cost-effective and sustainable exploitation of residues from the agri-food sector. On such grounds, this study was carried out for assessing the effect of citric and acetic acids on the efficiency with regard to the extraction of antioxidants from RGP. The extraction process was monitored using a ferric-reducing power test, which has been effectively used in previous studies on the recovery of antioxidants (Garcia-Perez et al., 2010), and the kinetic modelling was accomplished including extraction time and acid concentration as the critical variables.

## Materials and Methods

### Chemicals

Ascorbic acid (AA), 2,2′-dipyridyl and ferric chloride hexahydrate were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.). Trichloroacetic acid (TCA), citric acid and acetic acid were provided from Merck (Darmstad, Germany).

### Waste material

RGP was obtained from the native Greek Agiorgitiko variety (*Vitis vinifera* spp.), provided by the Department of Food Science & Human Nutrition

(Agricultural University of Athens). RGP was dried in an oven at 65 °C for 48 h and then pulverized in a domestic blender (Bosch MMB 112R). The material was stored at – 20 °C until used.

Batch extraction process

The amount of 2.4 g of pulverised RGP was transferred in a 250-mL glass vial and mixed with 120 mL of acid (citric, acetic) solution (1, 2 or 4% w/v). Extractions were performed under stirring at 80 rpm in a thermostated chamber, at 20°C. Sampling was accomplished by acquiring the volume of 1 mL of extract at regular intervals, up to 320 min.

Determination of the reducing power ($P_R$)

Prior to analysis, all samples were centrifuged in an Eppendorf centrifugator at 10,000×$g$ for 10 min. Measurement of the $P_R$ was carried out using ferric chloride as the oxidant (Psarra et al., 2002). An aliquot of 0.05 mL of extract was mixed with 0.05 mL of ferric chloride (3 mM in 5 mM citric acid) and the mixture was incubated at 50 °C, in a water bath, for 30 min. Then 0.9 mL of 2,2´-dipyridyl solution (5 g $L^{-1}$ in 1.2% TCA) was added and the absorbance was measured at 525 nm. Quantification was performed by means of a calibration curve, using ascorbic acid as standard. The $P_R$ was expressed as µM AA equivalents (AAE) per g of dry pomace weight (dpw), as follows:

$$P_R \left( \mu M \; AAE \; g^{-1} \; dpw \right) = \frac{(0.982 \times A_{515} - 0.008) \times 7}{m} \tag{1}$$

where V is the extraction medium volume (mL) and $m$ is the dry weight of grape pomace (g).

Statistics

Determinations were performed in triplicate. Values reported are means ± standard deviation. Non-linear regression correlations were performed at least at the 95% significance level ($p < 0.05$). Statistical analyses were carried out using SigmaPlot[TM] 12.0 and Microsoft Excel[TM] 2010.

**Results and Discussion**

Kinetics

Monitoring of the $P_R$ within a period up to 320 min gave a series of $P_R$/$t$ points (Figure 1) and non-linear regression between $P_R$ values and $t$ gave the best fit of a hyperbola described by the equation:
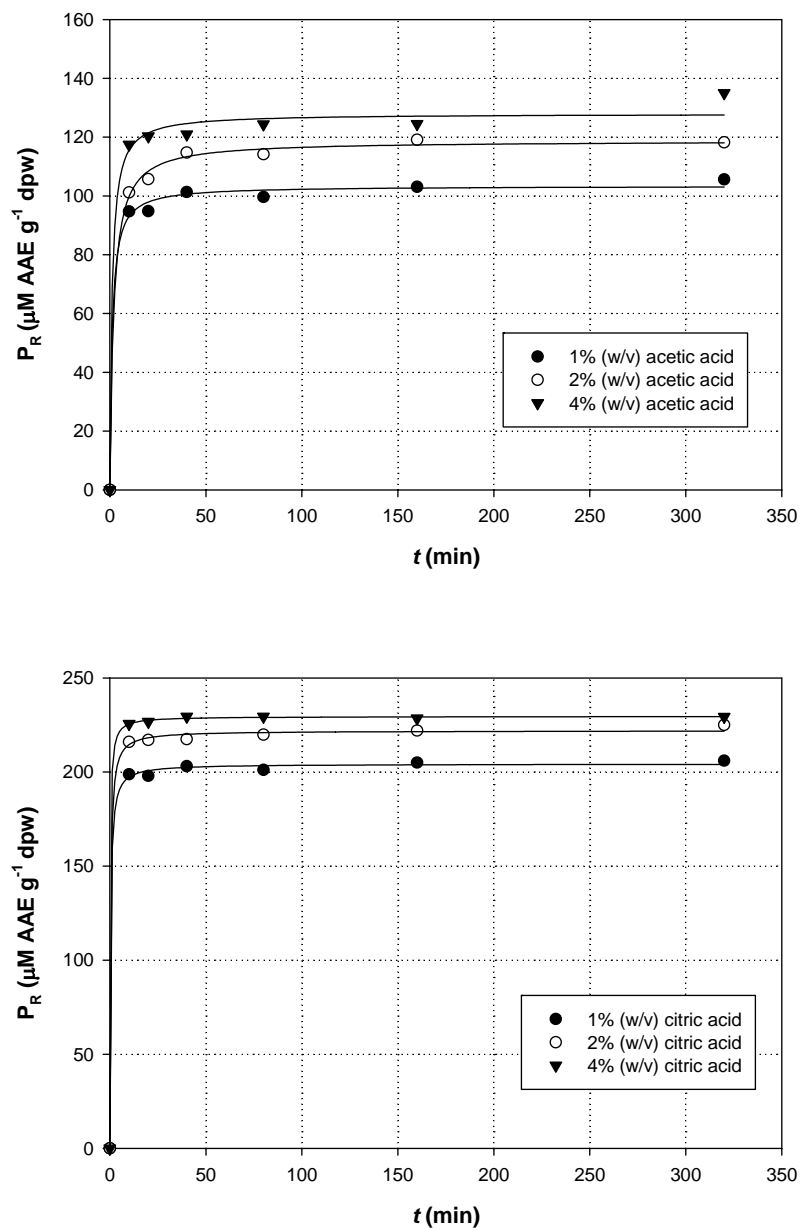
$$y = \frac{ax}{1 + bx} \tag{2}$$

Figure 1. Time course of $P_R$ during antioxidant extraction from RGP using acetic acid (upper plot) and citric acid (lower plot) solutions. Extractions were performed at 20 °C, 80 rpm and 1:50 solid-to-liquid ratio.

Model fitting was significantly high (Table 1), suggesting that prediction of the extraction yield as a function of $t$ can be performed using the equation (2). This corresponds to the 2nd-order extraction model, in line with previous findings (Ho et al., 2005; Rakotondramasy-Rabesiaka et al., 2007), considering the boundary conditions $t = 0$ to $t$ and $P_{R(t)} = 0$ to $P_{R(t)}$. The outcome indicated that there were two phases involved in the antioxidant extraction, an initial washing stage and a slow, rate-determining stage, governed by internal diffusion. These assumptions were made by admitting that (i) leaching of antioxidants occurred through diffusion, and (ii) at the equilibrium (saturation), the $P_R$ remained constant. Thus, extraction kinetics could be described as follows:

$$P_{R(t)} = \frac{P_{R(s)}^2 kt}{1 + P_{R(t)} kt} \tag{3}$$

Table 1. Statistical parameters determined after correlating $P_R$ and $t$, using non-linear regression.

| | Statistical parameters | |
|---|---|---|
| $C_{acid}$ (% w/v) | $R^2$ | $p$ |
| *Acetic acid* | | |
| 1 | 0.997 | < 0.0001 |
| 2 | 0.998 | < 0.0001 |
| 4 | 0.994 | < 0.0001 |
| *Citric acid* | | |
| 1 | 0.999 | < 0.0001 |
| 2 | 0.999 | < 0.0001 |
| 4 | 1.000 | < 0.0001 |

$P_{R(s)}$ and $k$ correspond to the $P_R$ at saturation and the extraction rate constant. Modification of the equation (3) provides its linearized form:

$$\frac{t}{P_{R(t)}} = \frac{t}{k P_{R(s)}^2} + \frac{t}{P_{R(s)}} \tag{4}$$

When $t$ approaches 0, the initial extraction rate, $h$, can be determined as:

$$h = k P_{R(s)}^2 \tag{5}$$

Plotting $t/P_{R(t)}$ as a function of $t$ gives a straight line in the form of y = ax + b is (Figure 2), where a = $1/P_{R(s)}$ and b = $1/h$. Thus, in every case, $P_{R(s)}$, $k$ and $h$ could be determined graphically. The correlation between $t/P_{R(t)}$ and $t$ ($R^2 > 0.99$, $p < 0.0001$) enabled the estimation of the representative kinetic parameters (Table 2).
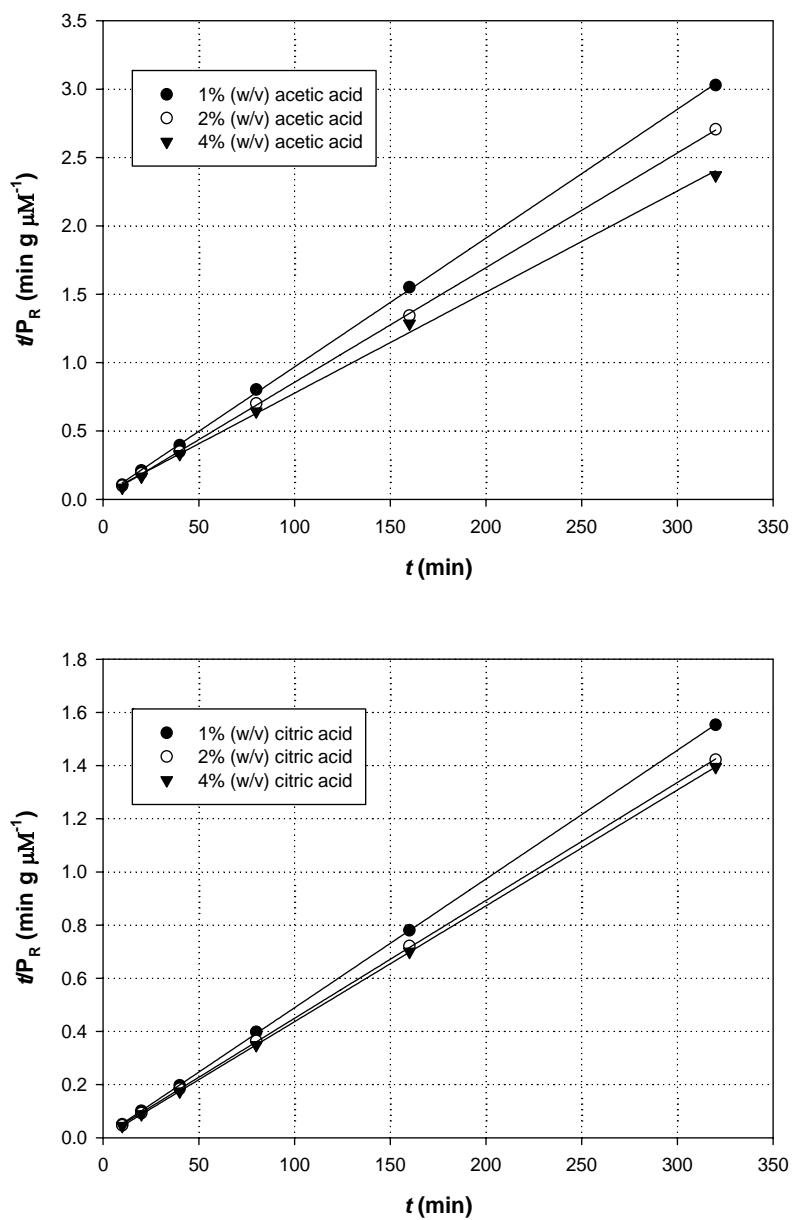
Figure 2. The second-order kinetics of antioxidant extraction from RGP with
aqueous acetic acid (upper plot) and citric acid (lower plot) solutions.
Extractions were performed at 20 °C, 80 rpm and 1:50 solid-to-liquid ratio.

The effect of acid concentration

The increased acetic acid concentration yielded decreased $k$ values (Table 2), but $P_{R(s)}$ values exhibited an increasing tendency in response to $C_{acid}$ and the highest $P_{R(s)}$ (128 µM AAE g$^{-1}$ dpw) was achieved with 4% (w/v) acetic acid. Irrespective of the $t/C_{acid}$ combinations used, $h$ and $P_{R(s)}$ values were constantly higher in citric acid solutions, but $k$ values were in all cases lower. The extract with the highest $P_{R(s)}$ value (229.8 µM AAE g$^{-1}$ dpw) was obtained using 4% (w/v) citric acid.

Table 2. Parameters of the 2$^{nd}$-order kinetics for the extractions performed with acetic acid and citric acid solutions.

| $C_{acid}$ (% w/v) | Kinetic parameters | | |
|---|---|---|---|
| | $k$ (g µM$^{-1}$ min$^{-1}$) × 10$^{-3}$ | $h$ (µM g$^{-1}$ min$^{-1}$) | $P_{R(s)}$ (µM AAE g$^{-1}$ dpw) |
| *Acetic acid* | | | |
| 1 | 6.98 | 92.6 | 103.5 |
| 2 | 2.60 | 62.6 | 118.8 |
| 4 | 1.53 | 119.2 | 128.0 |
| *Citric acid* | | | |
| 1 | 1.90 | 579.0 | 204.3 |
| 2 | 2.28 | 647.5 | 222.0 |
| 4 | 3.06 | 1272.9 | 229.8 |

For the extractions carried out using acetic acid solutions, non-linear regression between $P_{R(s)}$ and $C_{acid}$ obeyed a single rectangular hyperbola correlation, as follows:

$$P_{R(s)} = -3.57 C_{acid}^2 + 26 C_{acid} + 81.07 \qquad (R^2 = 1.000, p < 0.0001) \qquad (6)$$

Similarly, a correlation between $h$ and $C_{acid}$ obeyed a quadratic function:

$$h = 19.43 C_{acid}^2 - 88.3 C_{acid} + 161.47 \qquad (R^2 = 1.000, p < 0.0001) \qquad (7)$$

In a similar manner, the equations obtained for the extraction with citric acid solutions were:

$$P_{R(s)} = -4.6 C_{acid}^2 + 31.5 C_{acid} + 177.4 \qquad (R^2 = 1.000, p < 0.0001) \qquad (8)$$

$$h = 81.4 C_{acid}^2 - 175.7 C_{acid} + 673.3 \qquad (R^2 = 1.000, p < 0.0001) \qquad (9)$$

Rearrangement of the equation (3) would enable calculation of $Y_{TF}$ at any time, $t$:

$$P_{R(t)} = \frac{t}{\frac{1}{h} + \frac{t}{P_{R(s)}}} \qquad (10)$$

For the extraction with acetic acid solutions, combination of equations (6), (7) and (10), would give:

$$P_R(t, c_{acid}) = \cfrac{t}{\cfrac{1}{19.43c_{acid}^2 - 88.36c_{acid} + 161.47} + \cfrac{t}{-3.37c_{acid}^2 + 36c_{acid} + 81.07}} \qquad (11)$$

Likewise, the combination equations (8), (9) and (10) would give the corresponding function for citric acid solutions:

$$P_R(t, c_{acid}) = \cfrac{t}{\cfrac{1}{21.41c_{acid}^2 - 176.61c_{acid} + 543.63} + \cfrac{t}{-4.61c_{acid}^2 + 61.61c_{acid} + 177.4}} \qquad (12)$$

These equations describe an empirical model for antioxidant extraction from RGP with aqueous acetic and citric acids and provide the values for $P_R$ at any time $t$ and any concentration $C_{acid}$ within the limits set by the experimental design.

Model validation

Several combinations of $C_{acid}$ and $t$ were used to assure model validity (Table 3) and for this purpose linear regression was performed between the observed and the predicted values to ascertain the degree of correlation (Figure 3).

Table 3. Predicted and observed $P_R$ values obtained using the extraction models.

| Run | Time (min) | $C_{acid}$ (% w/v) | $P_R$ (µM AAE g$^{-1}$ dpw) | | | |
| | | | Acetic acid | | Citric acid | |
| | | | Observed | Predicted | Observed | Predicted |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | 10 | 1 | 83.7 | 93.1 | 198.8 | 197.3 |
| 2 | 160 | 1 | 103.1 | 102.8 | 205.0 | 203.9 |
| 3 | 320 | 1 | 105.6 | 103.1 | 206.0 | 204.1 |
| 4 | 10 | 2 | 101.2 | 99.8 | 216.0 | 214.6 |
| 5 | 160 | 2 | 119.1 | 117.4 | 222.0 | 221.5 |
| 6 | 320 | 2 | 118.2 | 118.1 | 225.0 | 221.8 |
| 7 | 10 | 4 | 117.5 | 115.5 | 225.7 | 225.7 |
| 8 | 160 | 4 | 124.5 | 127.1 | 228.6 | 229.5 |
| 9 | 320 | 4 | 135.0 | 127.5 | 229.4 | 229.7 |

The values showed a high correlation, suggesting that, within the limits set by the experimental design, a credible prediction of $P_R$ as a function of $C_{acid}$ and $t$ can be done using the equations (11) and (12). The trends in $P_R$ recorded were given as 3D plots (Figure 4).
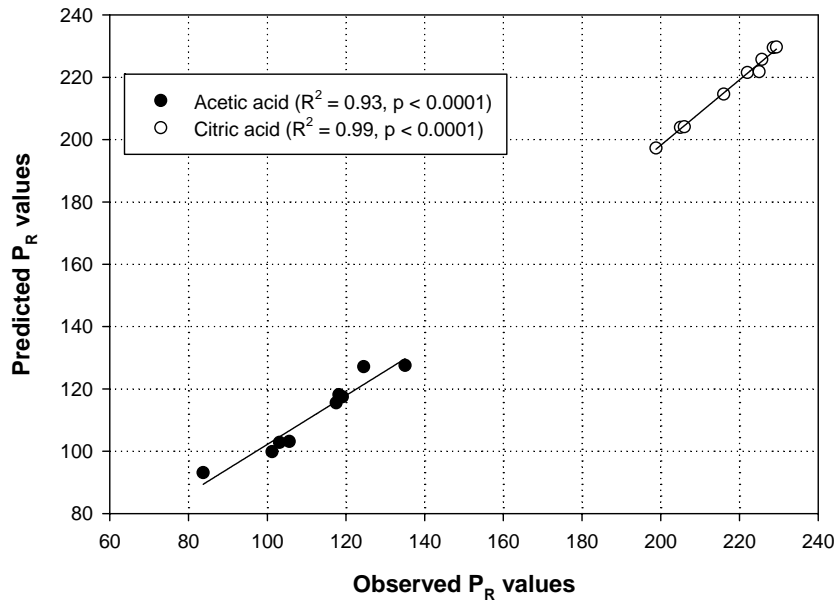
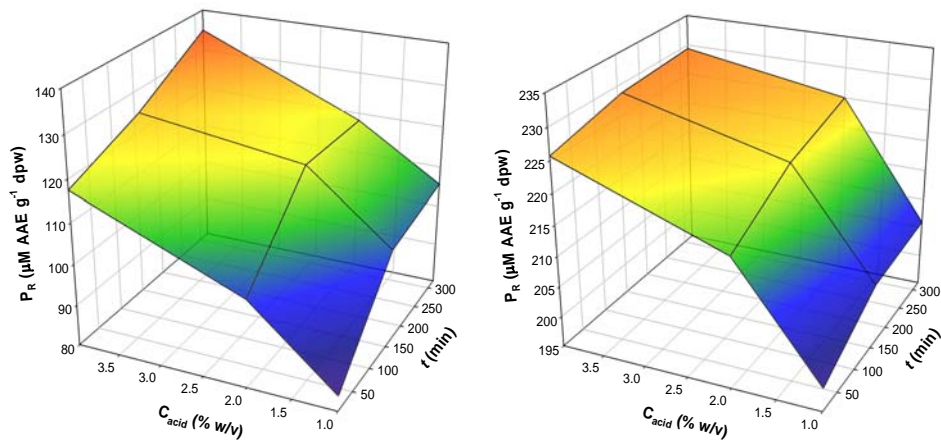Figure 3. Linear regression between observed and predicted $P_R$ values.



Figure 4. 3D graphs illustrating the effect of $C_{acid}$ and $t$ on $P_R$, using acetic acid solutions (upper plot) and citric acid solutions (lower plot).

## Conclusion

Antioxidant extraction from RGP with acetic acid and citric acid solutions was shown to depend on both the acids and their concentration. Extractions were described by the determination of basic kinetic parameters, which permitted the establishment of a reliable model. This model enabled the reliable prediction of the $P_R$ of the extracts as a function of both $t$ and $C_{acid}$, under the experimental conditions employed. This investigation illustrated that aqueous solvents used for antioxidant extraction from RGP can be profoundly affected by the acidifying agent. This is of particular importance for the development of the efficient extraction process. Similar examinations might be used for engineering extraction procedures involving eco-friendly and food compatible solvent systems.

Nomenclature: AA, ascorbic acid; AAE, ascorbic equivalents; Cacid, acid concentration (% w/v); dpm, dry pomace weight; $h$, initial extraction rate (μM g-1 min-1); $k$, extraction rate constant (min g μM-1); PR, reducing power (μM AAE g-1 dpw); $t$, time (min); PR(s), reducing power at saturation (μM AAE g-1 dpw); PR(Cacid,$t$), reducing power as a function of Cacid and $t;$ TCA, trichloroacetic acid.

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# EKSTRAKCIJA ANTIOKSIDANASA IZ KOMINE CRVENOG GROŽĐA VODENIM RASTVORIMA ORGANSKIH KISELINA KORIŠĆENJEM KINETIČKOG MODELOVANJA

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R e z i m e

Komina grožđa je rezidualni materijal prehrambene industrije koji može sadržati veliku količinu polifenolnih antioksidanasa i poznate su brojne metode za njihovo efikasno dobijanje iz navedenog materijala. Međutim, ekološki procesi bi trebalo da obuhvate i ekološki neškodljive i netoksične rastvarače. Na osnovu toga, u ovom istraživanju ispitana je ekstrakcija antioksidanasa, upotrebom vodenih rastvora sirćetne i limunske kiseline, kao i praćenje redukcione moći (engl. *reducing power* − PR) ekstrakta. Cilj je bio da se pomoću kinetike ispita uticaj koncentracije kiseline na prinos ekstrakcije. Uspostavljeni kinetički model omogućio je verodostojno poređenje efikasnosti ekstrakcije, postignute korišćenjem rastvora sirćetne i limunske kiseline različitih koncentracija. Rezultati ukazuju da su rastvori limunske kiseline efikasniji u dobijanju antioksidanasa iz komine crvenog grožđa. Korišćenjem 4% (w/v) rastvora limunske kiseline, mogla bi se postići maksimalna redukciona moć, PR, od 229,8 μM ekvivalenata askorbinske kiseline po g suve komine. Ovo istraživanje je pokazalo da sredstvo za zakišeljavanje i njegova koncentracija mogu značajno uticati na vodenu sredinu koja se koristi za ekstrakciju jedinjenja sa antioksidativnim osobinama iz otpada u prehrambenoj industriji.

**Ključne reči:** antioksidanti, ekstrakcija, komina grožđa, kinetika, organske kiseline, redukciona moć.

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