Journal of the Serbian Chemical Society

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J. Serb. Chem. Soc. 77 (10) 1381–1389 (2012)
JSCS–4359

UDC 544.4.004.12+541.515:635.64+633.879.6

Original scientific paper

Kinetic behaviour of the DPPH radical-scavenging activity of tomato waste extracts

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(Received 10 April, revised 16 June 2012)

Abstract: The kinetic behaviour of tomato waste extracts (obtained from six genotypes) and standard antioxidant compounds (ascorbic and caffeic acid) were investigated using the 2,2-diphenyl-1-picrylhydrazyl radical test. Based on the time required for the reaction to reach steady state, the investigated extracts showed very slow (steady state ≥ 180 min) antiradical behaviour, ascorbic acid acted as a rapid antioxidant (steady state < 5min) while caffeic acid is a rapid–intermediate antioxidant (5 min < steady state < 20 min). The efficient concentrations at different kinetic times EC_{50},t were determined for all extracts, as well as for ascorbic and caffeic acid. The $EC_{50,t}$ was used as a parameter to screen and compare antiradical activites of food extracts with slow kinetic action. Irrespective of the time considered, a comparison of the $EC_{50,t}$ values for extracts of tomato waste obtained from different tomato genotypes showed that their DPPH radicals-scavenging activity decreased in the order O_2 > Knjaz > Bačka > Saint Pierre > Rutgers > Novosadski niski. The tomato waste extracts showed very slow kinetic action, which is probably the result of the different kinetic bevaviour of the phenolic compounds present in tomato waste, as well as other antioxidants (vitamins, carotenoids, *etc*.).

Keywords: tomato waste; DPPH radicals; free radical scavenger; kinetic behaviour.

INTRODUCTION

By-products of fruits and vegetables processing represent a major disposal problem for the industry concerned, but they are also promising sources of antioxidant compounds, which may be used for various purposes in the food, pharmaceutical and cosmetic industries.[1] The most abundant vegetable-processing waste is tomato pomace, produced in tomato juice and paste factories. It consists

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doi: 10.2298/JSC120410065S

of tomato peel, seeds and a part of the pulp, and contains valuable nutritional compounds (on a dry weight basis): mainly fibres (59.03 % d.w.), total sugars (25.73 % d.w.), proteins (19.27 % d.w.), pectins (7.55 % d.w.), total fats (5.85 % d.w.), minerals (3.92 % d.w.) and antioxidants.[2–4]

Several methods have been proposed to measure the antioxidant activity of pure compounds and plant extracts, such as FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity), ESR (Electron Spin Resonance), ABTS (2,2-azinobis(3-ethyl-benzothiazoline-6-sulphonate) and DPPH (2,2-diphenyl-1-picrylhydrazyl).[5] Usually, the antioxidant activity is examined at a fixed endpoint, which may not consider the kinetic characteristics of the antioxidant. However, an investigation of the kinetic behaviour could provide more complete information about the antioxidant properties and could be more important than the total antioxidant capacites determined at a fixed endpoint.[6]

The DPPH test is one of the oldest and the most frequently used methods for the determination of the antioxidant activity of food extracts.[7–9] Some authors used different initial DPPH radical concentrations and reaction times in order to define the kinetic model for understanding the antioxidant behaviour. In the case of food extracts with very slow kinetic behaviour, the treatment of DPPH test data could be simplified to easily screen food extracts.[10]

The main objective of this study was to evaluate the kinetic behaviour of radical scavenging activity of tomato waste (from juice processing, obtained from different tomato genotypes – Bačka, Knjaz, Novosadski niski, O_2, Rutgers and Saint Pierre).

MATERIALS AND METHODS

Chemicals and materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH), caffeic acid and L-ascorbic acid were obtained from Sigma (St. Louis, USA). All other chemicals and reagents were of the highest analytical grade.

Tomato genotypes (Bačka, Knjaz, Novosadski niski, O_2, Rutgers and Saint Pierre) grown in the fields of the Institute of Field and Vegetable Crops, Novi Sad, Serbia were taken for the experiments. The materials include new (Bačka, Knjaz, Novosadski niski and O_2) and traditional (Rutgers and Saint Pierre) genotypes.

Waste preparation and extraction procedure

Tomatoes (1 kg) of each genotype were washed and cut into four pieces and tomato juice was prepared using a juice processor Neo, SK-400. Fresh tomato waste was dried (25 °C, 1.03 mbar, 15 h, and 30 °C, 0.001 mbar, 4.5 h) in a vacuum-dryer (Alpha 2-4 LSC Martin Christ, Osterode, Germany). The weights of the dry tomato wastes were measured in triplicate.

Samples of dried tomato waste (5 g) were treated with *n*-hexane to remove non-polar compounds, then extracted with ethanol at room temperature, using a high performance homogenizer, Heidolph DIAX 900 (Heidolph Instruments, Kelheim, Germany). The extraction was performed three times with different amounts of 80 % ethanol: 80 ml in 30 min, 40 ml in 30 min and 40 ml in 15 min at room temperature. The obtained three extracts were combined and

evaporated to dryness under reduced pressure at 40 °C on a water bath. The weights of the extracts, *i.e.*, ethanol extractive values, were taken as the average of triplicate analyses.

DPPH Radical scavenging activity

The DPPH radicals scavenging activity of the tomato waste extracts was determined spectrophotometrically using the DPPH method of Espin *et al.*,[11] modified for this assay. Briefly, 0.5 ml of a solution containing from 0.05 to 2 mg of extract in distilled water or 0.5 ml of distilled water (control) were mixed with 1.5 ml of a 90 μM DPPH radical solution and 3 ml of methanol. The mixture was shaken vigorously and incubated at room temperature for 120 min. The absorbance at 515 nm was measured at different intervals (after 10, 20, 30, 60, 120, 150 and 180 min) using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) against a blank that had been prepared in a similar manner as the control, by replacing the DPPH radicals solution with methanol. The level of remaining $DPPH^{\bullet}$ in the reaction medium was calculated using the following equation:

$$\text{Remaining DPPH}^{\bullet}\ (\%) = 100 \times A_{\text{Sample}}/A_{\text{Control}}$$

where A_{Control} is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the extract measured at different time intervals.

The efficient concentration at different times $EC_{50,t}$ (mg extract mg^{-1} $DPPH^{\bullet}$) was the amount of the extracts in relation to the amount of initial $DPPH^{\bullet}$, which was calculated using the following equation:

$$EC_{50,t} = IC_{50,t}/[\text{DPPH}^{\bullet}]_{t=0}$$

where $IC_{50,t}$ is the inhibitory concentration at different times, defined as the concentration of extract (mg mL^{-1}) required to scavenge 50 % of $DPPH^{\bullet}$ and $[DPPH^{\bullet}]_{t=0}$ is the initial concentration of $DPPH^{\bullet}$ (mg mL^{-1}).

Ascorbic and caffeic acid were used as reference radical scavengers.

Statistical analysis

All measurements were performed in triplicate and the results are presented as mean ± *SD*. The IC_{50} values were calculated using Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

Waste from six tomato genotypes were obtained as by-products in juice processing. In a previous study,[12] it was shown that tomato waste contained significant amounts of hydrophillic antioxidants, polyphenolics and ascorbic acid, which were identified as being responsible for antiradical activities. Based on the significant antioxidant activity of the selected tomato waste at a fixed end-point, as well as the well known health benefits of polyphenolics and ascorbic acid, this by-product has potential as value-added ingredients for functional foods. The results of the antioxidant activity based on measurments at a fixed end-point together with those based on kinetic data provide comprehensive information on the total antioxidant property of sample.[13] In this work, kinetic parametars were evaluted to clarify the antioxidant activity of tomato waste extracts.

The scavenging stable DPPH radicals is a widely used method to evaluate antioxidant activities due to its simple, rapid, sensitive and reproducible procedure.[14,15] A freshly prepared DPPH solution exhibits a deep purple colour with

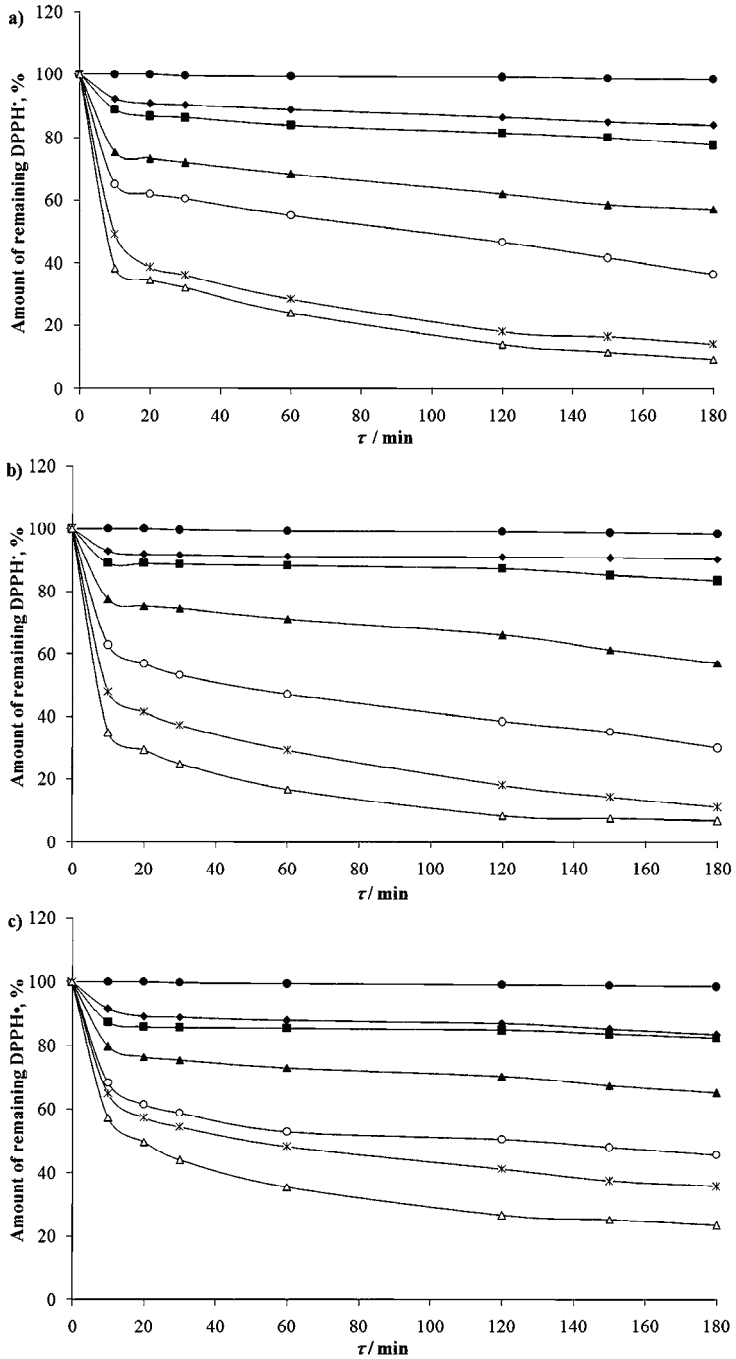
an absorption maximum at 515 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium.[16] Antioxidant molecules can quench DPPH free radicals (*i.e.*, by providing hydrogen atoms or by electron donation, conceivably *via* a free-radical attack on the DPPH molecule) and convert them to a colourless/bleached product (*i.e.*, 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 515 nm.[17,18]

Kinetic studies of the DPPH–extract reaction were performed to estimate the scavenging activity of the extracts as a function of time. The free DPPH radical is quite stable for more than 180 min at 20 °C in the reaction medium. Hence, it allows the evaluation of the radical scavenging activity of the extracts within that time. The kinetics of DPPH annihilation by different tomato waste extracts are shown in Figs. 1a–1f. Immediately after the addition of the tomato waste extracts to the reaction medium, the absorbance of DPPH at 515 nm dropped, due to the decrease of DPPH concentration in the medium. Clearly, the highest rate of DPPH decay occurs within the first 10 min of reaction. Although, the extract solutions maintained their antioxidant effect until the end of the experiment (180 min).

Based on the time required for the reaction to reach steady state, four reaction kinetic types (rapid, intermediate, slow and very slow) were found.[8,10] For all tomato waste extracts, a steady state was not attained even after 180 min of reaction. Thus, the investigated extracts are classified as showing very slow behaviour. At each time, it was possible to compare the antioxidant activity of extracts.[10] For example, in the presence of 37.04 mg of Novosadski niski extract per mg DPPH radicals, after 180 min, when the steady state was almost reached, about 23.39 % of the initial DPPH radicals remained in the medium. In the presence of the same concentration of O_2 extract, 4.40 % of initial DPPH radicals remained in the medium after the same time.

The concentrations required to decrease twofold the DPPH concentration at the chosen reaction time ($EC_{50,t}$) were calculated from the reaction kinetics obtained with different concentrations of the tomato waste extract. First, the remaining DPPH radicals percentage was plotted as function of extracts concentration at applied reaction time (10, 20, 30, 60, 120, 150 and 180 min). The $EC_{50,t}$ values were determined at all the kinetic times. The EC_{50},t values of tomato waste extracts and their kinetic classification are presented in Table I.

A lower $EC_{50,t}$ value indicates a higher DPPH radicals scavenging activity. Ascorbic and caffeic acid were used as reference radical scavengers. A comparison of the $EC_{50,t}$ values for the extracts showed that the DPPH radical scavenging activity of the extracts decreased in the order of O_2 > Knjaz > Bačka > Saint Pierre > Rutgers > Novosadski niski. The $EC_{50,t}$ value for ascorbic acid, was 0.14 mg mg^{-1} $DPPH^{\bullet}$ at 5 min (steady state < 5 min; rapid antioxidant), while the $EC_{50,t}$ value for caffeic acid was lower, 0.09 mg mg^{-1} $DPPH^{\bullet}$ at 20 min (5 min < steady state < 20 min; rapid–intermediate antioxidant). It was ob-



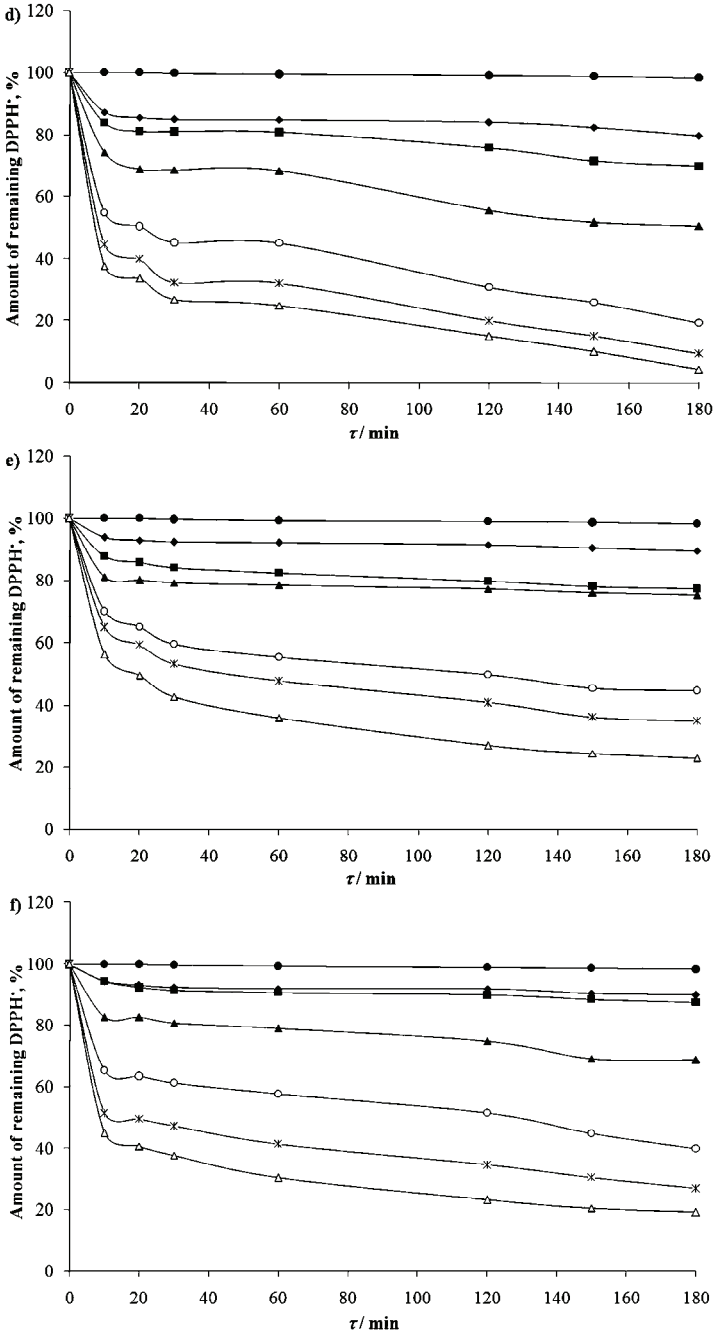


Fig. 1. Kinetic behaviour of: a) Bačka, b) Knjaz, c) Novosadski niski, d) O_2, e) Rutgers and f) Saint Pierre tomato waste extract. Concentrations of extracts in the medium are: ● 0; ♦ 0.93; ■ 1.85; ▲ 9.26; ○ 18.52; ✳ 27.50 and Δ 37.04 mg extracts mg^{-1} DPPH.

TABLE I. $EC_{50,t}$ (mg extract mg^{-1} DPPH) values of tomato waste extracts and their kinetic classification; the results are mean values of three determinations ± standard deviation. Range of time to reach steady state: ≥ 180 min; kinetic classification: very slow

t / min	Genotype					
	Bačka	Knjaz	Novosadski niski	O_2	Rutgers	Saint Pierre
10	27.3±1.21	26.6±1.24	47.1±2.15	22.9±1.06	46.8±2.15	30.0±1.35
20	23.3±1.08	22.7±1.04	36.9±1.64	18.9±0.87	36.6±1.71	27.6±1.26
30	22.5±1.05	20.4±0.98	31.8±1.35	16.6±0.71	30.7±1.36	26.0±1.02
60	20.3±0.96	17.4±0.76	25.4±1.20	16.6±0.65	25.0±1.05	23.0±1.00
120	16.5±0.72	14.6±0.65	19.1±0.90	11.3±0.50	18.4±0.86	17.3±0.60
150	13.9±0.54	13.2±0.52	17.6±0.85	9.9±0.42	17.1±0.64	16.6±0.63
180	12.5±0.52	11.7±0.51	16.4±0.74	9.4±0.38	16.9±0.70	15.3±0.59

served that the $EC_{50,}t$ value of the tomato waste extracts were higher than those of the individual antioxidant compounds.

According to literature data, phenolic compounds, vitamins, carotenoids, *etc*., show different antiradical kinetic action. For example, ascorbic acid is a rapid antioxidant, caffeic acid was classified as a rapid–intermediate antioxidant, tocopherol and gallic acid show intermediate kinetic behaviour, while ferulic acid, quercetin and rutin are slow antioxidants.[6] Furthermore, carotenoids act as intermediate or slow antioxidant.[19,20] It can be proposed that the different kinetic behaviour of the phenolic compounds, present in tomato waste, as well as other constituents (vitamins, carotenoids *etc*.), determined the antiradical activity of this natural source of antioxidants.

CONCLUSIONS

In this study, DPPH radicals scavenging activity of tomato waste extracts (obtained from six genotypes) was applied to determine their kinetic behaviour. Expression of the results in terms of the kinetic approach not only takes into account the activity of an antioxidant, but also provides information on how quickly the antioxidant acts. All extracts showed a very slow kinetic action, which is probably result of the different kinetic behaviour of the antioxidants present in tomato waste. The chemical characteristics of the antioxidant compounds in tomato waste will be further investigated and more research is required to establish bioavailability and real benefits *in vivo*.

Acknowledgement. This research is part of the Project TR 31044 that is financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ИЗВОД

DPPH АНТИРАДИКАЛСКО КИНЕТИЧКО ПОНАШАЊЕ ЕКСТРАКАТА ОТПАДА ПАРАДАЈЗА

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Кинетичко понашање екстраката отпада парадајза (добијених од шест генотипова) и антиоксидативних једињења (аскорбинска и кафена киселина) испитано је 2,2-дифенил-1-пикрилхидразил радикал тестом. На основу времена потребног за успостављање динамичке равнотеже реакције, испитивани екстракти су показали веома споро (≥ 180 min), аскорбинска киселина брзо (< 5 min), а кофеинска киселина умерено–брзо антирадикалско понашање (5 до 20 min). Ефективне концентрације у различитим кинетичким временима, $EC_{50,t}$, се користе као параметар за поређење антирадикалске активности антиоксиданата и екстраката прехрамбрених производа различитог кинетичког деловања и одређене су за све екстракте, аскорбинску и кофеинску киселину. Без обзира на разматрано време, поређењем $EC_{50,t}$ испитиваних екстраката утврђен је следећи редослед антирадикалске активности екстракта: O_2 > Књаз > Бачка > Сент Пјер > Rutgers > Новосадски ниски. Екстракти отпада парадајза су показали веома споро антирадикалско понашање, што је вероватно последица различитих кинетичких особина како фенолних једињења присутних у отпаду парадајза, тако и других присутних антиоксиданата (витамини, каротеноиди, итд.).

(Примљено 10. априла, ревидирано 16. јуна 2012)

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