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KINETIC MODELLING FOR POLYPHENOL EXTRACTION FROM ONION *(ALLIUM CEPA)* SOLID WASTES USING ACIDIFIED WATER/ETHANOL MIXTURE

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Previous reports highlighted the onion solid wastes as abundant, residual material that might contain a significant load of antioxidant polyphenols. Although there have been studies pertaining to polyphenol recovery from onion wastes, the effect of temperature has not been adequately addressed. In this line, this study was undertaken with the aim of establishing a correlation between the extraction yield in total polyphenols and the extraction temperature, using acidified aqueous ethanol as the solvent system. Extraction of polyphenols from onion solid wastes was found to obey 2nd-order kinetics. On such a basis, the yield in total polyphenols at saturation could be very effectively determined and correlated with temperature using non-linear regression. The results indicated that the extraction yield at saturation is highly correlated with temperature, following a quadratic function. The extract obtained at optimal temperature (40 °C) had a total polyphenol yield of 21.10 mg gallic acid equivalents per gram of dry weight, and it was further analysed by liquid chromatography-mass spectroscopy to characterise its major constituents. The polyphenols detected were quercetin glucosides, as well as quercetin oxidation derivatives, including certain degradation products and dimers. The outcome of this study outlined that temperatures above 40 °C are rather not favourable for polyphenol extraction from onion solid wastes, as suggested by the model established through kinetics. The extract obtained under optimal conditions contained peculiar polyphenolic composition, not encountered in any other food processing residue.

Keywords: extraction kinetics, liquid chromatography-mass spectrometry, onion solid wastes, polyphenols

Dumping of food processing by-products and wastes is an issue of high significance for the agri-food sector, because these wastes usually bear a significant load of organic bio-molecules. Thus, their direct disposal to the environment might pose severe pollution risks. In this regard, there is a need for the development of processes aiming at diminishing food waste polluting load, by removing added-value phytochemicals (Galanakis, 2012).

It is estimated that about 450 000 tonnes of onion solid waste (OSW) are produced on an annual basis in Europe (Moure et al., 2001). The rejected parts of the onion bulb (outer dry layers and the apical parts) have been shown to contain a wide spectrum of polyphenolic components (Ly et al., 2005; Ramos et al., 2006). Most of these substances do not occur in the edible part of the onion bulb, but they represent oxidation products of quercetin and its glucosides. Some of these phenolics have been shown to possess antiplatelet properties (Furusawa et al., 2003).

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Optimisation of polyphenols from onion solid wastes has been previously reported with regard to solvent composition (Kiassos et al., 2009). However, the effect of temperature, which is a critical factor in extraction processes, has not been addressed, although it has been evidenced that increasing temperature might not favour polyphenol extraction from onion solid wastes (Khiari et al., 2009). This is in contrast with investigations on the extraction of other food processing residues, which demonstrated a positive impact of increased temperatures on the extraction yield of polyphenols (Aliakbarian et al., 2012; Radojković et al., 2012; Lataoui et al., 2014).

On such a background, the study presented herein was an approach in evaluating the effect of extraction temperature on the recovery of polyphenols from onion solid wastes, within a range varying from 20 to 60 °C, using an acidified hydroalcoholic medium as the extracting solvent. Ethanol is a bio-solvent, produced via fermentation of various carbohydrate-containing raw materials. Unlike other solvents, such as methanol or acetone, ethanol is non-toxic and can be reused following its recovery after removal from the extract through distillation, thus generating practically zero wastes. Therefore, ethanol can be considered as an environmentally benign solvent. Kinetics and non-linear regression analysis were employed as a first step to defining a set of conditions that may be used for further engineering the extraction process. Some principal polyphenols, detected in the richest extract obtained, were tentatively identified using liquid chromatography-mass spectroscopy.

1. Materials and methods

1.1. Chemicals

All solvents used for chromatographic analyses were of HPLC grade. Folin-Ciocalteu phenol reagent and absolute ethanol were from Fluka (Steinheim, Germany). Gallic acid was from Sigma Chemical Co (St. Louis, MO, U.S.A.).

1.2. Onion solid wastes (OSW)

The material used was obtained from a catering facility (Chania, Crete) after processing of brown-skin onion bulbs. The OSW consisted of the apical trimmings of the bulbs, as well as the outer dry and semi-dry layers. The material was transferred to the laboratory immediately after processing and stored at –20 °C until analysed.

1.3. Extraction procedure

OSW was ground in a domestic blender and approximately 3 g of the ground material was placed in a 100 ml conical flask with 30 ml of solvent (solvent-to-solid ratio 10), composed of 0.1% HCl in 60% (v/v) aqueous ethanol. The OSW was extracted under stirring at 1200 r.p.m. using a magnetic stirrer, and samples were withdrawn at predetermined time intervals for total polyphenol (TP) determination. For the extractions carried out at 20 °C, stirring was performed in a temperature-controlled chamber. Extractions at 40 and 60 °C were performed in a temperature-controlled water bath. All samples were filtered through 0.45 μm syringe filters prior to analyses.

1.4. Determination of the extraction yield in total polyphenols (Y_{TP})

Analysis was carried out employing the Folin-Ciocalteu methodology (Arnous et al., 2002). Yield in total polyphenols (Y_{TP}) was expressed as mg gallic acid equivalents (GAE) per g of solid waste, using the following equation:

$$Y_{TP}\ (mg\ GAE\ g^{-1}\ dw) = \frac{(951 \times A_{750} - 1.49) \times V}{m} \quad (1)$$

where A_{750} is the absorbance at 750 nm, V is the volume of the extraction medium (ml) and m the weight of onion solid wastes (g).

1.5. Liquid chromatography-mass spectroscopy (LC-MS)

A Finnigan MAT Spectra System P4000 pump was used coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. Analyses were carried out on a Superspher RP-18, 125 × 2 mm, 4 µm column (Macherey-Nagel, Germany), protected by a guard column packed with the same material, and maintained at 40 °C. Analyses were carried out employing electrospray ionization (ESI) at the positive ion mode, with acquisition set at collision energies of 12 and 80 eV, capillary voltage 4 kV, source voltage 45 V, detector voltage 650 V, and probe temperature 400 °C. Eluent (A) and eluent (B) were 2.5% acetic acid and MeOH, respectively. The flow rate was 0.33 ml min^{-1}, and the elution programme used was as follows: 0–5 min., 20% B; 5–25 min., 80% B; 25–30 min., 80% B.

1.6. Statistical analysis

All determinations were carried out at least in triplicate and values were averaged and given along the standard deviation (± S. D.). Kinetics was performed by carrying out non-linear regression between Y_{TP} and t values. Kinetics and linear and non-linear correlations were established at least at a 95% significance level ($P<0.05$). For all statistics, SigmaPlot™ 12.0 and Microsoft Excel™ 2010 were used.

2. Results and discussion

2.1. Kinetic model for TP extraction

The model fitted to the extraction kinetics using non-linear regression between extraction yield in TP (Y_{TP}) values and t (Fig. 1) was a hyperbola described by the equation:

$$y = \frac{ax}{1+bx} \quad (2)$$

For all temperatures tested, fitting was statistically significant (Table 1), suggesting that extraction yield as a function of t can be adequately predicted by the eq. (2). This equation actually describes a 2nd-order extraction rate, as previously reported (Ho et al., 2005; Rakotondramasy-Rabesiaka et al., 2007; Pan et al., 2012), when the boundary conditions t=0 to t and $Y_{TP(t)}$=0 to $Y_{TP(t)}$ are considered. The 2nd-order extraction rate might indicate that there are two phases implicated in the leaching of polyphenols from the solid particles; first, there is a high rate of extraction, possibly owed to the most polar polyphenols; afterwards, the rate is significantly slowed down, suggesting extraction of less polar substances.

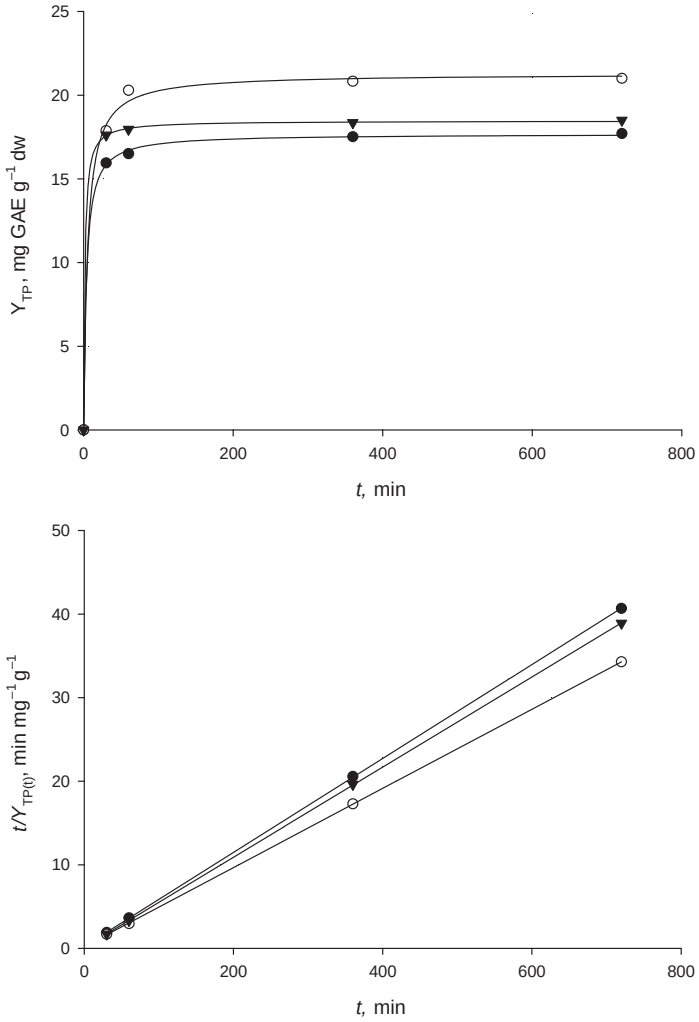

Fig. 1. Time course of Y_{TP} during the extraction of OSW using 0.1% HCl in ethanol/water 6/4 (v/v) (upper graph) and second-order extraction kinetics of TP from OSW at various temperatures, using 0.1% HCl in ethanol/water 6/4 (v/v) (lower graph). Extractions were carried out at (●): 20 °C; (○): 40 °C and (▼): 60 °C

Table 1. Parameters of 2nd-order kinetics determined for the extraction of TP from OSW

T (°C)	Kinetic parameters		
	k (min g mg^{-1})	h (mg g^{-1} min^{-1})	$Y_{TP(s)}$ (mg GAE g^{-1} dw)
20	0.016	5.04	17.79
40	0.009	4.23	21.10
60	0.036	12.23	18.55

These considerations were made on the concession that (i) polyphenols leached from the solid parts into the solution through diffusion, and (ii) at saturation conditions Y_{TP} remained constant. Based on such a simplification, the integrated law for the extraction kinetics would be:

$$Y_{TP(t)} = \frac{Y_{TP(s)}^2 kt}{1 + Y_{TP(s)} kt} \quad (3)$$

where $Y_{TP(s)}$ and k represent the TP yield at saturation and the extraction rate constant, respectively. Transformation of eq. (3) yields the following linearized form:

$$\frac{t}{Y_{TP(t)}} = \frac{1}{Y_{TP(s)}^2} + \frac{t}{Y_{TP(s)}} \quad (4)$$

When t approaches 0, the initial extraction rate, h, given as $Y_{TP(t)}/t$, is defined as:

$$h = kY_{TP(s)}^2 \quad (5)$$

Plotting $t/Y_{TP(t)}$ versus t, would give a straight line in the form of y=ax+b (Fig. 1), where $a=1/Y_{TP(s)}$. Thus, for each temperature tested, $Y_{TP(s)}$, h, and k could be determined graphically.

2.2. Effect of temperature

As shown in Table 1, both k and h displayed a decreasing tendency upon an increase in T from 20 to 40 °C. By contrast, further increase at 60 °C was accompanied by a concomitant rise in both kinetic parameters. On the other hand, the maximum $Y_{TP(s)}$=21.10 mg GAE g^{-1} dw was obtained at 40 °C, while either at 20 or 60 °C, $Y_{TP(s)}$ values were lower.

The non-linear regression between $Y_{TP(s)}$ and T values (Fig. 2) was shown to obey a quadratic function. This function was described by the following equation:

$$Y_{TP(s)} = -0.0074T^2 + 0.609T + 8.57 \quad (R^2=1.000, P<0.0001) \quad (6)$$

The same was observed after applying non-linear correlation between h and T values (Fig. 2), and the equation extracted was:

$$h = 0.011T^2 - 0.701T + 4.66 \quad (R^2=1.000, P<0.0001) \quad (7)$$

After rearrangement of eq. (4), Y_{TP} at any time, t, can be calculated:

$$Y_{TP(t)} = \frac{t}{\frac{1}{h} + \frac{t}{Y_{TP(s)}}} \quad (8)$$

Thus, combining eqs (6), (7), and (8), the following equation is obtained:

$$Y_{TP(t,T)} = \frac{t}{\frac{1}{0.011T^2 - 0.701T + 14.66} + \frac{t}{-0.0074T^2 + 0.609T + 8.57}} \quad (9)$$

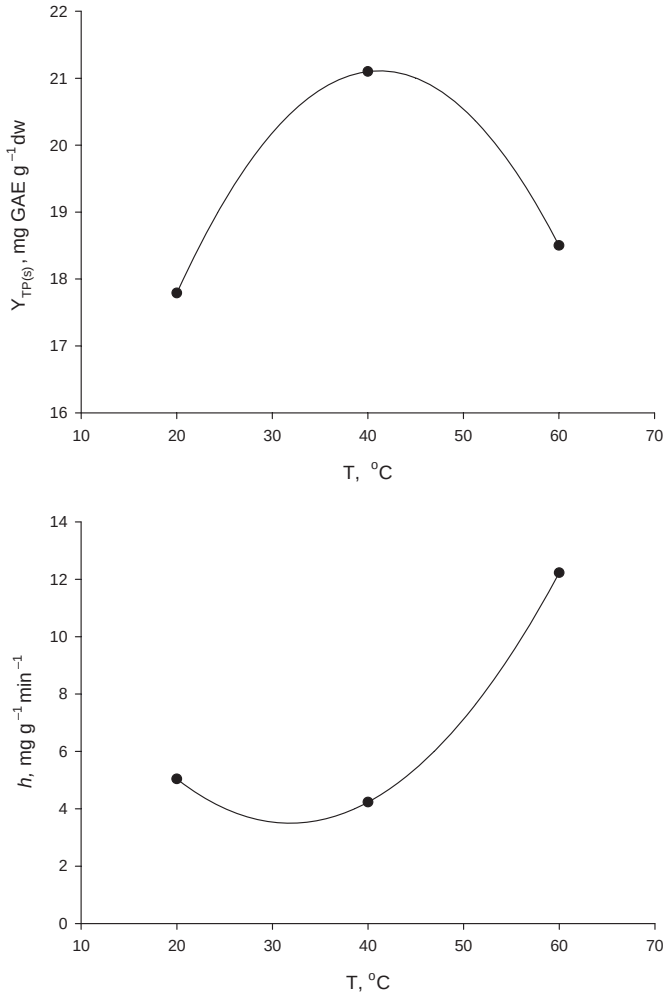

Fig. 2. The dependence of $Y_{TP(s)}$ as a function of temperature (upper plot) and the dependence of *h* as a function of temperature (lower plot) (●): measured values; (---): fitted model

This empirical equation represents the evolution model of Y_{TP} during extraction of OSW and provides the values for Y_{TP} at any time *t* and any temperature T ranging between 30 and 720 min and 20 and 60 °C, respectively.

2.3. Experimental fitting (model validation)

A series of nine combinations of T and *t* were used to test the validity of the model in predicting Y_{TP} values (Table 2). The observed and the predicted values were then analysed by linear regression to ascertain the degree of correlation (Fig. 3). It was found that the observed and the predicted values were highly correlated (R^2=0.997, P<0.0001), suggesting that, under the given experimental conditions, Y_{TP} can be calculated with high reliability as a function of T and *t*, using the eq. (9). The tendency in Y_{TP} recorded was given in the form of a three-dimensional plot (Fig. 4).

Table 2. Observed and predicted values of Y_{TP} for a number of runs performed to assess the validity of the extraction model established

Run	*t* (min)	T (°C)	Y_{TP} (mg GAE g^{-1} dw)	
			Observed	Predicted
1	30	20	15.95	15.91
2	360	20	17.52	17.62
3	720	20	17.70	17.70
4	30	40	17.88	18.08
5	360	40	20.83	20.80
6	720	40	21.00	20.94
7	30	60	17.62	17.58
8	360	60	18.36	18.39
9	720	60	18.50	18.43

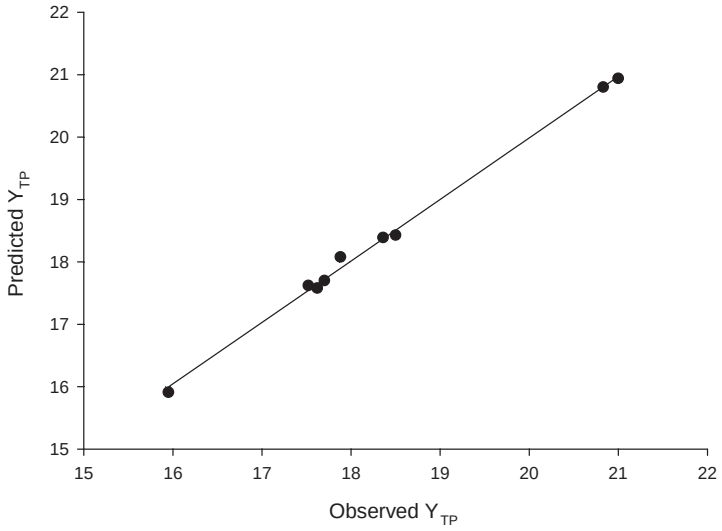

Fig. 3. Correlation between the observed Y_{TP} values and those predicted by the kinetic model established

2.4. Polyphenolic composition

The outer dry layers of onion bulbs contain a peculiar variety of polyphenols, many of them considered to derive from quercetin and quercetin glucosides, upon the action of peroxidase (Ly et al., 2005; Ramos et al., 2006; Khiari & Makris, 2012). The chromatogram of the extract obtained at 40 °C, which was monitored at 290 nm, revealed the presence of 5 major peaks accompanied by several minor substances. These minor constituents were eluted at longer retention times and thus they appeared less polar in a reversed-phase HPLC system.

Compound (1) gave a pseudo-molecular ion at *m/z*=155 and based on its UV-Vis spectrum was tentatively identified as protocatechuic acid (Fig. 5). Compound (2) showed a pseudo-molecular ion at *m/z*=319, but also fragments with *m/z*=337 and 341, presumed to

correspond to adducts with water and Na^+, respectively. Taking into consideration the UV-Vis spectral characteristics, this compound was assigned to 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one (GÜLŞEN et al., 2007a) (Table 3).

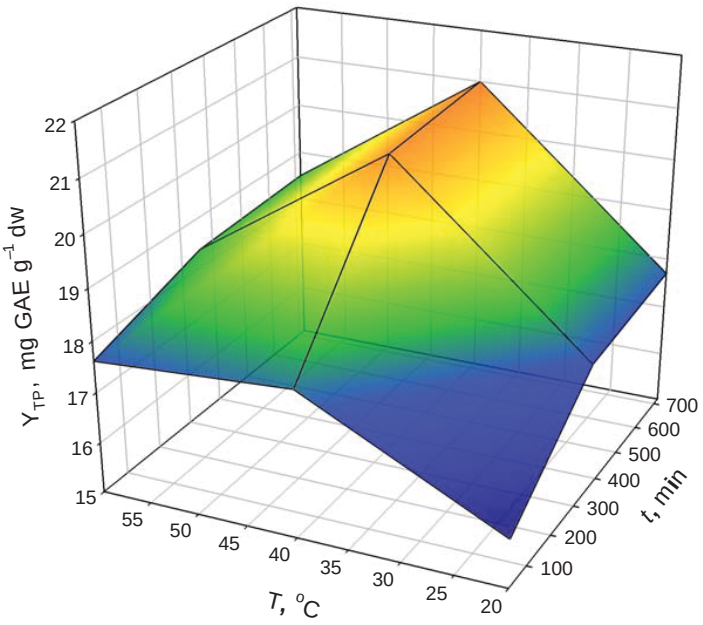

Fig. 4. Three-dimensional plot illustrating the Y_{TP} tendency as a function of *t* and T

Table 3. Spectral characteristics of the polyphenol tentatively identified in the OSW extract obtained at 40 °C

Peak #	Rt (min)	UV-Vis	$[M+H]^+$	Other ions	Tentative identity
1	2.45	258, 294	155	–	Protocatechuic acid
2	5.40	294	319	341, 337	2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one
3	9.06	252, 364	627	649 $[M+Na]^+$, 465, 303	Quercetin 7,4′-*O*,*O*-diglucoside
4	10.61	264, 318, 344	627	649 $[M+Na]^+$, 465, 303	Quercetin 3,4′-*O*,*O*-diglucoside
5	14.98	252, 316, 368	465	303	Quercetin 4′-*O*-glucoside
6	18.13	254, 370	303	–	Quercetin
7	21.57	240, 254, 274(s), 304, 370	765	787 $[M+Na]^+$, 603, 303	Quercetin 4′-*O*-glucoside/quercetin dehydrate adduct
8	23.50	240, 270, 304, 362	765	787 $[M+Na]^+$, 603, 303, 273	Quercetin 4′-*O*-glucoside/quercetin adduct
9	24.89	240, 250, 312, 364	603	303, 273	Quercetin dimer

Compound (3) exhibited typical flavonol UV-Vis spectral characteristics and a pseudo-molecular ion at *m/z*=627, a diagnostic fragment at *m/z*=465 (loss of a glucosyl moiety), and a fragment at *m/z*=303 corresponding to the aglycone. This compound was identified as quercetin 7,4′-*O*,*O*-diglucoside (Lee et al., 2012). Likewise, compound (4) gave an identical fragmentation pattern and it was assigned to quercetin 3,4′-*O*,*O*-diglucoside (Kiassos et al., 2009; Lee et al., 2012). Compound (5) with pseudo-molecular ion at m/z=465 and a fragment at *m/z*=303, as well as compound (6) with pseudo-molecular ion at *m/z*=303 were identified as quercetin 4′-*O*-glucoside and quercetin, respectively.

Compounds (7) and (8) showed identical pseudo-molecular ions at *m/z*=765, Na^+ adducts at *m/z*=787, loss of a glucosyl moiety (*m/z*=603) and a fragment *m/z*=303, corresponding to quercetin. These compounds were assigned to adducts of quercetin with quercetin 4′-O-glucoside (Fig. 5) in accordance with previously reported data (Ly et al., 2005). In a similar fashion, compound (9) was identified as a quercetin dimer (Ly et al., 2005; Gülşen et al., 2007a).

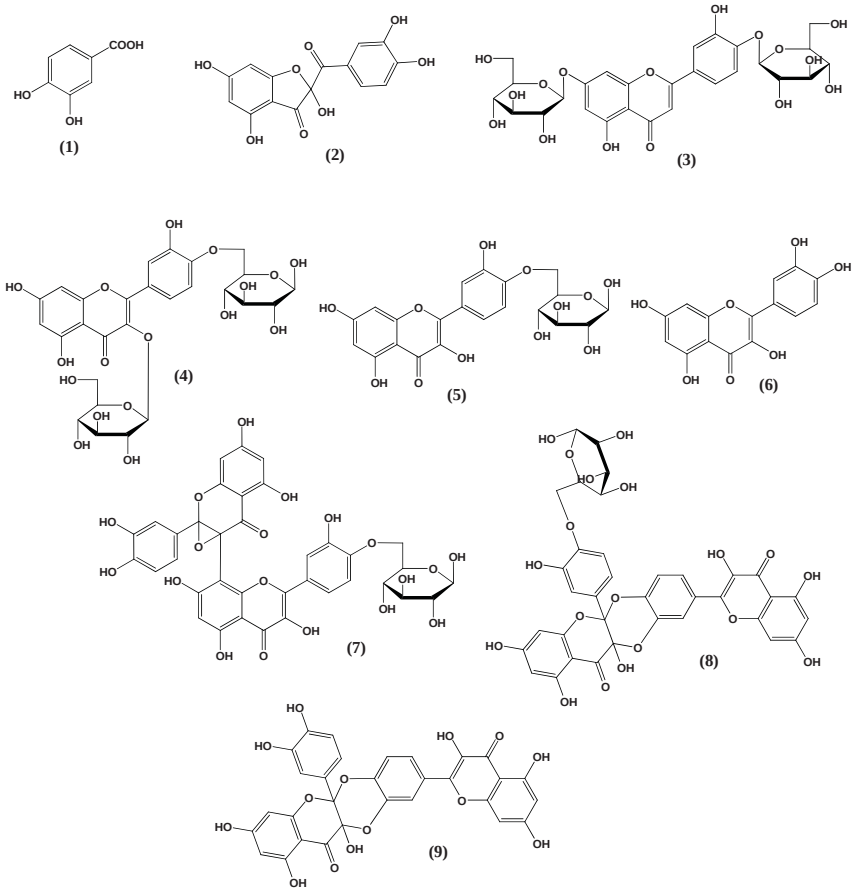

Fig. 5. Chemical structures of the polyphenols tentatively identified using liquid chromatography-diode array-mass spectroscopy. Assignments: (1): protocatechuic acid; (2): 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2H)-one; (3): quercetin 7,4′-*O*,*O*-diglucoside; (4): quercetin 3,4′-*O*,*O*-diglucoside; (5): quercetin 4′-*O*-diglucoside; (6): quercetin; (7): quercetin 4′-*O*-glucoside/quercetin dehydrate adduct; (8): quercetin 4′-*O*-glucoside/quercetin adduct; (9): quercetin dimer

Compounds (1) and (9) have been demonstrated to derive from quercetin through oxidative cleavage by onion peroxidase (Osman et al., 2008). Further, compounds (2) and (9) have been generated using bio-mimetic, metal-catalysed oxidation reactions (Gülşen et al., 2007a, b).

3. Conclusions

This study demonstrated that T beyond 60 °C do not favour increased polyphenol recovery. Non-linear regression between Y_{TP} and *t* values revealed that polyphenol extraction obeyed 2nd-order kinetics. On such a basis, for each temperature tested, the saturation Y_{TP} values ($Y_{TP(s)}$) could be determined graphically. Likewise, non-linear regression between $Y_{TP(s)}$ and T showed that the correlation followed a quadratic function. The non-linear regressions performed were used to establish a model, which enabled the reliable prediction of Y_{TP} values as a function of both *t* and T, within determined ranges. The major polyphenols detected in the optimally obtained extract were tentatively identified as quercetin glucosides, as well as quercetin oxidation and degradation products.

Abbreviations:
dw: dry weight (g)
GAE: gallic acid equivalents
h: initial extraction rate (mg g^{-1} min^{-1})
k: extraction rate constant (g mg^{-1} min^{-1})
OSW: onion solid wastes
T: temperature (°C)
t: time (h)
TP: total polyphenols
Y_{TP}: extraction yield in total polyphenols (mg GAE g^{-1} dw)
$Y_{TP(s)}$: extraction yield in total polyphenols at saturation (mg GAE g^{-1} dw)

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