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# THE CONTENT OF TOTAL POLYPHENOLS, ASCORBIC ACID AND ANTIOXIDANT ACTIVITY IN SELECTED VARIETIES OF QUINCE (*CYDONIA OBLONGA* MILL.)

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## ABSTRACT

Quince fruit (*Cydonia oblonga* Miller) is an important source of bioactive compounds, especially of polyphenolic compounds, phenolic acids, flavonoids also of minerals and vitamins. This compounds exhibit health promoting properties including antibacterial, anti-inflammatory, anticancer, antidiabetic and cardioprotective properties. Quine fruit have a high therapeutic value, can be used as good sources of antioxidants. This study provides some knowledge about content of total polyphenols, ascorbic acid and antioxidant activity in selected varieties of quince fruit samples. Four quince fruit cultivars (Semenáč, Konstantinopler Apfelquitte, Cydora Robusta, Mammut) were analysed. The content of the total polyphenols (TPC) was determined by the Folin-Ciocalteu reagent (FCR) at 765 nm using spectrophotometer. Ascorbic acid (AsA) content was determined using standard HPLC gradient method. Antioxidant activity (AA) was measures using a compound DPPH$^{\cdot}$(2.2-diphenyl-1-picrylhydrazyl). The content of (TPC) in fresh samples of quince fruit ranged from 661 ±11.60 $mg.kg^{-1}$ to 1044 ±11.03 $mg.kg^{-1}$ and content of AsA were in interval from 151 ±0.58 $mg.kg^{-1}$ to 215 ±0.75 $mg.kg^{-1}$. The values of antioxidant activity in quince fruit samples were in range from 26.90 ±0.61% to 49.14 ±0.38%. Statistically significant highest content TPC, AsA and AA was recorded in cultivar Konstaninopler Apfelquitte and statistically lowest content was recorded in cultivar Semenáč. The content of TPC, AsA and AA beside the variety may be affected by many factors also climatic conditions and the agrochemical composition of the soil.

**Keywords:** quince fruit; total polyphenol; ascorbic acid; variety

## INTRODUCTION

Quince fruit (*Cydonia oblonga* Miller) is a fruit of the *Rosaceae* family. It is one of the oldest crops, originates in the warmer areas of Sout-west Asia and has spread to Europe. It was a popular fruit species in Ancient Roma as well. The main production areas are Iraq, France and Portugal.

Quince fruit (*Cydonia oblonga* Miller) is mostly consumed in processed form. Because of its astringency, bitterness and firmness, it belongs to less popular species of the core fruit. However, when ripe, it is very demanded fruit for processing of marmalades, cakes and aromatic distillates as well.

The scientific investigations showed that quince fruit contains high quantities of bioactive substances, which are effective in prevention of different types of cancer and heart diseases and has beneficial effects on the human health **(Trigueros et al., 2011; Pacifico et al., 2012; Benzarti et al., 2015)**. Quince fruit is rich in useful secondary metabolites such as phenolics, steroids, flavonoids, terpenoids, tannins, sugars, organic acids, and glycosides **(Ashraf et al., 2016)**. Quince healthy properties have been attributed to the high level of phenolic compounds which provide interesting antioxidant properties in quince fruits. Polyphenols are able to act as antioxidants in a number of ways. Quince polyphenols include mainly flavonoids (flavonols, flavanols), quercetin and kaempferol derivatives and proanthocyanidins **(Wojdylo et al., 2013; Benzarti et al., 2015)**. **Fattouch et al. (2007)** and **Costa et al. (2009)** concluded that chlorogenic acid (*5-O-caffeoylquinic acid)* is the major phenolic compound in quince. Organic acids, identified in quince fruit, are citric, ascorbic, malic, shikimic and fumaric **(Silva et al., 2002)**. Monosaccharides, situated in quince fruit, include: rhamnose, mannose, glucose and galactose **(Hopur et al., 2011)**. The nutritional value of quince fruit is high. It is also considered as a good source of vitamins and minerals such as K, Ca, Mg, Fe, Cu, Zn, and Mn **(Al-Snafi, 2016)**.

**Wani et al. (2013)** also considers quince to be a good source of bioactive substances like vitamin (A, C, E, riboflavin, folic acid), carotenoids, and flavonoids (isorhamnetin, quercetin, myricetin) and their glucoside compounds.

The aim of the present study was to determine the content of polyphenols, ascorbic acid and antioxidant activity in selected varieties of quince fruit.

## MATERIAL AND METHODOLOGY

### Characteristic of varieties

Semenáč – the oldest and unbred cultivar. The fruits are similar to apples or pears, golden-yellow in colour.

Konstantinopler Apfelquitte – bred cultivar, is among the best tasting and oldest cultivars. Fruits are very aromatic.

Cydora Robusta – is among the most recent bred cultivars. The fruits are big, similar to the pears, bright yellow in colour.

Mammut – It is very profitable cultivar. Fruits are big and pear shaped, bright yellow in colour.

### Chemical and instruments

Total polyphenol content (TPC) and antioxidant activity (AA) were analysed by colorimetric methods (Shimadzu UV/VIS-1240 spectrophoto-meter; Shimadzu, Kyoto, Japan).

The chemicals used for all analyses were as follows: Monohydrate of gallic acid, p. a., Folin-Ciocalteu reagent, anhydrous sodium carbonate, p. a., methanol, p. a., ethanol, p. a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical, p.a., Trolox (97%).

### Plant samples

Four quince fruit cultivars (Semenáč, Konstantinopler Apfelquitte, Cydora Robusta, Mammut) were obtained from a local producer in area Banka, Slovak Republic. All cultivars were cultivated conventionally under the same condition.

Samples of quince fruit were homogenized (50 g) in 100 mL 80% ethanol 12 h at 250 rpm. Extracts were then filtered through filter paper (130 $g.m^{-2}$; Filtrak Brandt GmbH, Thermalbad Wiesenbad, Germany) and kept at 8 °C for further analysis. The experiment was based on four replications.

### Total polyphenol content (TPC) determination

Total polyphenol content was determined by the method of **Lachman et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content because a wide spectrum of phenolic compounds. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 mL of extracts. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

### Antioxidant activity (AA) determination

Antioxidant activity was measured by the **Brand-Williams et al. (1995)** method-using a compound $DPPH^{\cdot}$ (2.2-diphenyl-1-pikrylhydrazyl).

The compound 2.2-diphenyl-1- pikrylhydrazyl ($DPPH^{\cdot}$) was pipetted to cuvette (3.9 mL) then the value of absorbance which corresponded to the initial concentration of $DPPH^{\cdot}$ solution in time Ao was written. Then 0.1 mL of the followed solution was added and then the dependence A = f (t) was immediately started to measure. The absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer Shimadzu UV/VIS – 1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove $DPPH^{\cdot}$ radical at the given time.

Inhibition (%) = (Ao – At/Ao) x 100

### Ascorbic acid (AsA) determination

Determination of ascorbic acid (AsA) using standard HPLC gradient method (Waters Separation module 2696 with DAD detector Waters 2996). The aliquots of the extract (extraction of samples using meta-Phosphoric acid, homogenization, filtration) were taken for HPLC analysis using syringe filter (PTFE 0.45 μm, Teknokroma). Chromatographic conditions: HPLC column NovaPak C18 (4 μm), 150 x 3.4 mm (Waters, USA), column temperature 25 °C, flow rate 1.0 $mL.min^{-1}$, DAD detector set to wavelength λ = 251 nm, mobile phase MetOH:water – 5:95 (v/v), injection aliquot 5 μL, retention time Rt = 1.4 min.

### Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA).

## RESULTS AND DISCUSSION

Quince (*Cydonia oblonga* Miller) is regarded as an important source of beneficial effective bioactive compounds, which have a positive effect on human health.

**Table 1** Total polyphenol content (TPC) in mg $GAE.kg^{-1}$, ascorbic acid content (AsA) in $mg.kg^{-1}$ and antioxidant activity (AA) in % inhibition.

| **Cultivars** | **Quince (*Cydonia oblonga* Miller)** | | |
|---|---|---|---|
| | TPC | AsA | AA |
| Semenáč | 661 ±11.60$^{a}$ | 187 ±0.75$^{c}$ | 26.90 ±0.61$^{a}$ |
| Konstantinopler Apfelquitte | 1044 ±11.03$^{d}$ | 215 ±0.75$^{d}$ | 49.14 ±0.38$^{d}$ |
| Cydoria Robusta | 799 ±13.55$^{b}$ | 183 ±0.65$^{b}$ | 30.17 ±0.64$^{b}$ |
| Mammut | 819 ±7.14$^{c}$ | 151 ±0.58$^{a}$ | 38.03 ±0.52$^{c}$ |

Note: $^{a-d}$ values with different letters mean significant differences ($p$ <0.05) among selected varieties, values TPC, AsA and AA are expressed as arithmetic mean.

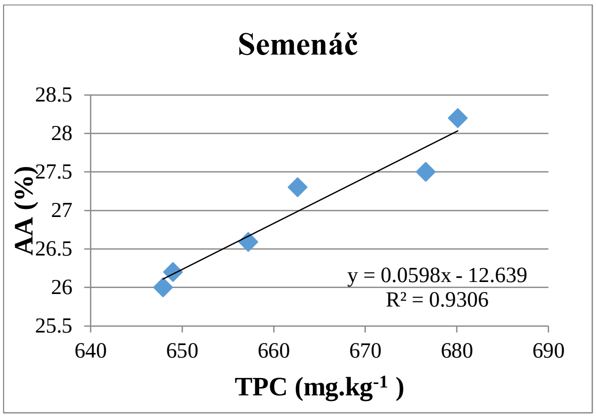


**Figure 1** Relationship between TPC ($mg.kg^{-1}$) and AA (%) cultivar Semenáč.

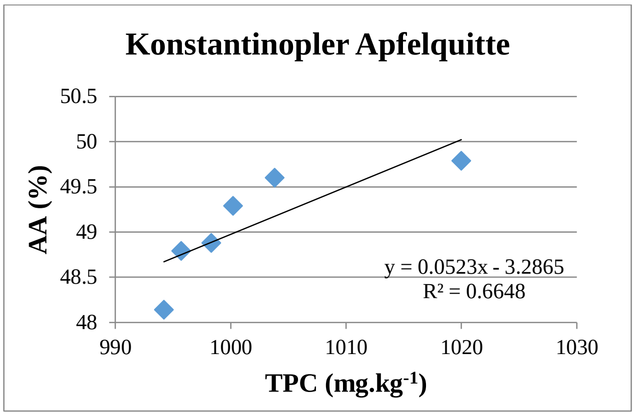


**Figure 3** Relationship between TPC ($mg.kg^{-1}$) and AA (%) cultivar Apfelquitte.

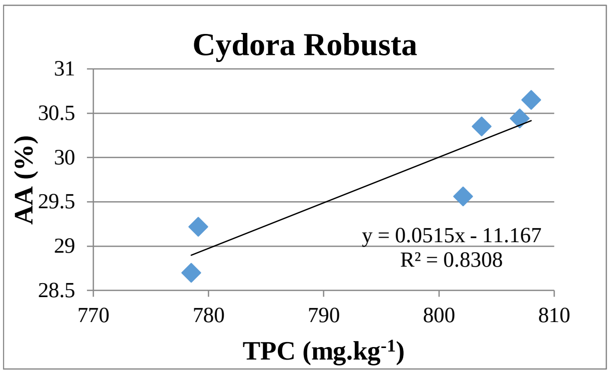


**Figure 2** Relationship between TPC ($mg.kg^{-1}$) and AA (%) cultivar Cydora Robusta.

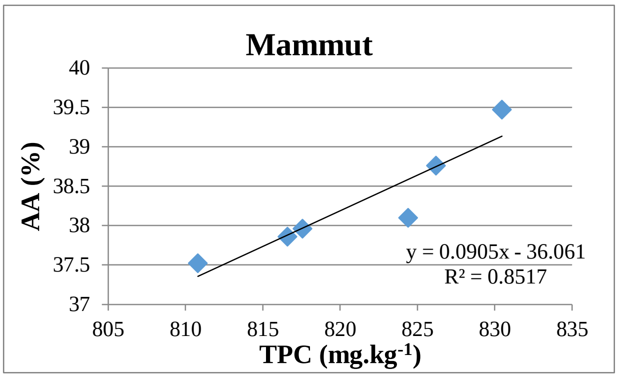


**Figure 4** Relationship between TPC ($mg.kg^{-1}$) and AA (%) cultivar Mammut.

**Figure 5** Cultivar Semenáč (URL 1).

**Figure 6** Cultivar Konstantinopler Apfelquitte (URL 2).

**Figure 7** Cultivar Mammut (URL 3).

**Figure 8** Cultivar Cydora Robusta (URL 4).

These health-promoting substances have antimicrobial, antioxidant, anticancer, cardiovascular and anti-inflammatory effects **(Al-Snafi, 2016; Sadeghpour et al., 2016)**. The results of the phytochemical analysis of the individual cultivars are presented in Table 1.

The content of total polyphenols in quince fruit samples ranged from 661 ±11.60 $mg.kg^{-1}$ to 1044 ±11.03 $mg.kg^{-1}$. The highest level of total polyphenols content was found in cv. Konstantinopler Apfelquitte and the statistically significant lowest content of total polyphenols was recorded in cv. Semenáč. Based on the measured values of total polyphenols content, cultivars of quince can be classified as follows: Konstantinopler Apfelquitte (1044 $mg.kg^{-1}$) >Mammut (819 $mg.kg^{-1}$) >Cydoria Robusta (799 $mg.kg^{-1}$) >Semenáč (661 $mg.kg^{-1}$). Many studies have dealt with the content of polyphenols in the parts of quince. The research results vary depending on the cultivars and also climatic and agrochemical conditions. Our results are in correspondence with the results of **Silva et al. (2005)**, who indicated the content of polyphenols within the range of 200 $mg.kg^{-1}$ – 1700 $mg.kg^{-1}$. **Fattouch et al. (2007)** determined a lower content of polyphenols in quince fruit, in comparison with our results. Their value ranged from 370 to 470 $mg.kg^{-1}$. **Mir et al. (2015)** referred, that total polyphenol content in fresh quince was in interval from 412.3 to 975.10 $mg.kg^{-1}$. It follows from the above, that the quince ranks among the significant and easily available sources of the polyphenols substances.

Another significant parameter, that was monitored, was the content of ascorbic acid in selected varieties of quince. The highest level of ascorbic acid content was found in cv. Konstantinopler Apfelquitte, (215 ±0.75 $mg.kg^{-1}$) and the statistically significant lowest content of ascorbic acid was recorded in cv. Mammut (151 ±0.58 $mg.kg^{-1}$). **Sharma et al. (2011)** determine similar results; their average value presents 168 $mg.kg^{-1}$. **Souci et al. (2008)** determines slightly lower value; it presents 130 $mg.kg^{-1}$. On the other hand, **Rop et al. (2011)** determined in Czech cultivars several times higher values, in comparison with our results. Their value ranged from 500 to 800 $mg.kg^{-1}$ and cultivar Muškatová contained the highest amount, 793.1 ±2.01 $mg.kg^{-1}$. This determination proves that the cultivars of the quince show great genetic variability. In this study, we evaluated the value of antioxidant activity in selected cultivars of quince fruit. Our values were in interval from 26.90 ±0.61% to 49.14 ±0.38 (Table 1). Based on the measured values of antioxidant activity in quince fruit, cultivars can be classified as follows: Konstantinopler Apfelquitte (49.14%) >Mammut (38.03%) >Cydoria Robusta (30.17%) >Semenáč (26.90%). **Mir et al. (2015)** determines in his study slightly higher values, in comparison with our results. Their values were in interval from 43.20 to 69.40%. **Gheisari** and **Abhari (2014)** compared antioxidant activity in peel, flesh extracts and oven-dried fruits and determine following values: 87%, 23.20% and 83.35%, respectively. In this study we have found positive correlation between the content of total polyphenols and antioxidant activity ($r = 0.967$, $r = 0.815$, $r = 0.911$, $r = 0.922$). Results are shown in Figure 1, Figure 2, Figure 3 and Figure 4. These results are in good accordance with **Drogoudi et al. (2008)**, who reported a positive correlation between total antioxidant activity and total phenolic content in apple flesh and peel ($r = 0.914$ and $r = 0.977$, respectively). **Silva et al. (2008)** also observed a positive relationship between the content of polyphenolic coumpounds and antioxidant activity.

## CONCLUSION

Despite the fact, that Quince is among the less popular fruit, this study indicates, that it is a rich source of compounds with interesting biological effects. The Quince is mainly valued for its high content of polyphenols (flavonols, flavanols, proanthocyanidins and many more), which protect the body against many diseases of civilization. The highest content of polyphenols was determined in breeding cultivar Konstantinopler Apfelquitte. The content of bioactive compounds may be affected by many factors, e.g. cultivars, agrochemical composition of the soil and climatic conditions. In the future, the information determined in this study, should be used for development of new functional foods with the addition of quince.

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URL 4 Available at: http://gyumolcspedia.hu/cydora-robusta-birs

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