

Phenolic profile and physicochemical characterization of quince (*Cydonia oblonga* Mill) fruits at different maturity index

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Abstract

The ripening of fruits is a determinant factor on the composition of phytochemical compounds such as phenolic compounds. In this study the phenolic profile of quince fruits was determined as a function of its maturity index. Based on the total soluble solids (TSS) and the acidity (TA) of the fruits, four maturity indexes were established (12.55, 14.56, 21.86 and 24.77), using the ratio of TSS/TA. The phenolic profile of quince fruits with different maturity indexes were obtained by a reversed-phase HPLC-DAD and HPLC-DAD/MS. A PCA loading plot was generated to explain the relationship between physicochemical parameters and the phenolic compounds. The phenolic compounds identified in the quince fruits were 3-*O*-caffeoylquinic acid, catechin, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, coumaric acid, quercetin-3-*O*-rutinoside and quercetin-3-*O*-glycosides. The maturity index increase caused in general a reduction of phenolic compounds, these compounds were also influenced by pH and acidity of fruits. Quince is a valuable source of natural phenolic antioxidants, and can be used as raw material to elaborate diverse food products, providing important functional properties.

Keywords: phenolic compounds; physicochemical parameters; quince; ripening

Introduction

Quince (*Cydonia oblonga* Miller) is a climacteric pome fruit that belongs to the Rosaceae family (Benzarti *et al.*, 2015; Hussain *et al.*, 2019). It is a round or pear-shaped fruit with yellow skin and a sour and bitter taste but at the same time is really aromatic (Rasheed *et al.*, 2018). Quince fruits have an important nutritional content as they are a rich source of organic acids, sugars, fibres and minerals. Additionally, they also contain compounds with functional and antioxidant features, being the phenolic compounds the most

important. Consumption of quince fruit have been related with health due to their hypoglycaemic action, anti-inflammatory, antimicrobial and anticancer activities (Leonel *et al.*, 2016; Rasheed *et al.*, 2018).

It is well known that the sensory, physicochemical and nutritional properties of quince fruits depend on several factors such as species, variety, crop, region, climatic conditions and maturity (Rios de Souza *et al.*, 2014). The latest factor is determinant on the quality of fruit since, the ripening involves a series of physiological and biochemical events leading to changes in colour, flavour, aroma and texture (Singal *et al.*, 2012), but also on the nutritional content and taste of products. A reliable measure of fruit quality is the maturity index, which relates sourness and sugar level (Kvikliene *et al.*, 2006). Many biochemical reactions related with flavour and taste properties during ripening are attributed to the phenolic compounds present in the fruits (Rios de Souza *et al.*, 2014). Phenolic compounds are secondary metabolites widely found in fruits, mostly represented by flavonoids and phenolic acids (Pasqualone *et al.*, 2014). These compounds are important participants also on antioxidants properties of quince fruits (Gharras, 2009; Cheynier, 2012).

The determination of phenolic compounds in quince has increasing interest in recent years. Some studies have evaluated the phenolic profile in the pulp and peel of quince (Silva *et al.*, 2002; Silvia *et al.*, 2004; Stojanović *et al.*, 2017), as well as on quince-based products (Silvia *et al.*, 2000; Ferreira *et al.*, 2004). More recently, Baroni *et al.* (2018) evaluated the content of phenolic compounds and their relationship with the antioxidant capacity of quince fruit before and after jam processing. Maghsoudlou *et al.* (2019) studied the effect of heating on the phenolic content and antioxidant capacity of quince fruit. However, there is a lack of information on the relationship of these compounds with the ripening of quince fruits. The objective of study was to determine the phenolic profile and physicochemical parameters of quince fruits as a function of maturity index.

Materials and Methods

Raw material and preparation

Quince fruits (*Cydonia oblonga* Mill) cultivar 'Gigante di Wranja' obtained from local supermarket from Italy were used for this study. Fruits were kept under refrigeration conditions (4 °C) until analysis (less than 1 week). The samples were washed and cut into pieces, removing cores and seeds. The quince puree was obtained using a Microtron MB 550 Laboratory mixer (Kinematica, Russia) and was obtained by mixing the quince pulp and peel.

Physicochemical determinations

Measurement of total soluble solids (TSS) and pH were carried out at 25 °C using a SMART-1 Digital Benchtop refractometer (Atago, USA) and a HI 5521 pH meter (Hanna, Instruments, USA), respectively. Titratable acidity (TA) was measured through the titration of fruit juice with 0.1 N NaOH, until achieving the neutralization by phenolphthalein indicator (Mkhathini *et al.*, 2017). The results were expressed as a percentage of citric acid equivalents. Maturity index was calculated as the TSS/TA ratio (Navarro Acosta *et al.*, 2010; Mkhathini *et al.*, 2017). Dry matter was determined according to the method described by Behboudi-Jobbehdar *et al.* (2013) in a convection oven (SMO1E, Shel-Lab, USA) at 105 °C during 24 hours until a constant mass was reached. All physicochemical parameters were analysed in triplicate.

Phenolic compounds extraction

Extraction of phenols compounds was performed following the methodology reported by Silva *et al.* (2002) with some modifications. Briefly, a portion (30 g) of quince puree was added in an aqueous solution containing 0.3 M of NaCl and ascorbic acid (0.5%) (ratio 1:1, w/w) and homogenized with an Ultra Turrax (IKA-Werke mod. T 25 basic, Staufen, Germany) at 17500 rpm for 1 min. The blend was let to stand for 2 hours and then 10 g of sample was taken and mixed with 10 mL of the solution of NaCl and ascorbic acid, the

blend was homogenized at 11,000 rpm for 1 min, and centrifuged at 24,000 rpm at 10 °C for 10 min. The supernatant liquid was recovered and placed on an SPE C18 column previously activated with 5 mL of methanol, 5 mL of distilled water and 5 mL of solution formic acid solution (3.0%). The retained phenolic fraction was then eluted with methanol (2 mL), filtered and placed in the freezer at -47 °C. The determination was made in triplicate.

HPLC analysis and phenolic compounds determination

The polyphenolic extracts were analysed in the 1100 series HPLC equipped with a binary pump, a degasser, automatic sampler, UV-vis Diode array detector and a mass spectrometer detector (Agilent Technologies, Palo Alto, CA). A Gemini 3M C18 column (100 x 2 mm x 3 mm, Phenomenex, Torrance, CA) was used. The mobile phase was: acidified water (2.5% v/v formic acid) (solvent A) and acid methanol (2.5% v/v formic acid) (solvent B). The HPLC system was conditioned with the mobile phase at least for an hour or until a stable baseline was obtained. The following linear elution gradient was employed: at 0 min 95% solvent A, at 10 min 88% A was reached and held constant (81%) from 25 and 35 min, at 40 min the solvent A decreased to 76% and finally at 56 min 30% solvent A was reached. Total execution time was 70 min. An injection volume of 5 µl and a flow rate of 0.25 mL/min were used (Comandini *et al.*, 2008; Blanda *et al.*, 2009). Chromatograms were recorded at 280, 320 and 350 nm.

The different phenolic compounds were identified by their UV spectra recorded with the diode array detector, chromatographic comparisons (retention times) and fragmentation of molecular ions. Phenolic quantification was achieved by the absorbance recorded in the chromatograms and was expressed as a relative area percent.

Statistical analysis

The one-way analysis of variance (ANOVA) and the Tukey-test were used to determine statistically significant differences between the variables studied. A multivariate analysis was also applied using PCA and the geometric representation for the main factors plotted. Statistical analysis was carried out using Minitab 16 software (Statistical Software, USA).

Results

Physicochemical properties of quince fruits

The quince samples were classified in four groups basing on their maturity index (MI). The MI values obtained were 12.55 ± 0.62 , 14.56 ± 0.37 , 21.86 ± 0.59 and 24.77 ± 0.51 . Figure 1 shows the physicochemical properties of quince fruits at different maturity index.

An inverse relationship between titratable acidity and maturity index was observed, resulting in a TA reduction from 1.44 to 0.58% when MI increased; while that the pH values raised when the maturity index of fruit increased (Figure 1). Total soluble solids (TSS) and dry matter (DM) of quince fruits varied from 13.96 to 18.14 °Bx and from 16.84 to 22.5%, respectively by effect of MI (Figure 1).

The equations obtained can be used to estimate the physicochemical parameters of quince fruits as a function of maturity index. The correlation coefficient values (R^2) were equal or greater than 0.91, indicating an adequate adjustment of the experimental data.

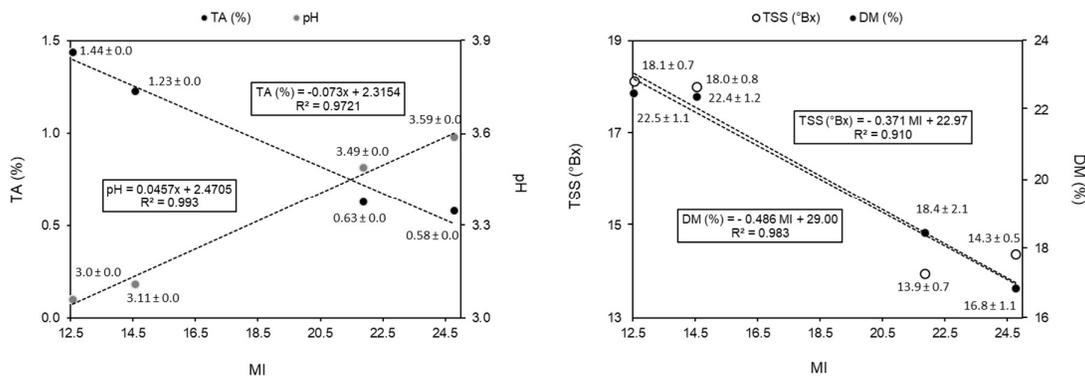


Figure 1. Correlation between physicochemical characteristics of quince fruit and its maturity index (MI). TA-titratable acidity; TSS- total soluble solids; DM-dry matter

Phenolic profile of quince fruits

The phenolic profile of quince fruits was obtained by HPLC (Figure 2). Phenolic compounds were identified on the basis their UV-VIS spectra, retention times and fragmentation of molecular ions according to data from the literature (Table 1).

The chromatogram presented in Figure 2 shows the peaks of the 8 compounds identified. The caffeoylquinic acids: 3-*O*-caffeoylquinic acid its isomers 4-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid were found in the quince fruits analysed (Figure 2). Absorbance (325 nm: 290 sh:245 nm), retention times from 14.36 to 27.37 and fragmentation of ions characteristic *m/z* of these caffeoylquinic acids, allowed their identification based on literature data (Table 1) (Plazonić *et al.*, 2014; Chen *et al.*, 2014; Baroni *et al.*, 2018). 5-*O*-caffeoylquinic acid was the predominant compound in the quince (largest peak). Quince fruits were also source of p-coumaric acid, which is a hydroxycinnamic acid derivative (Figure 2 and Table 1).

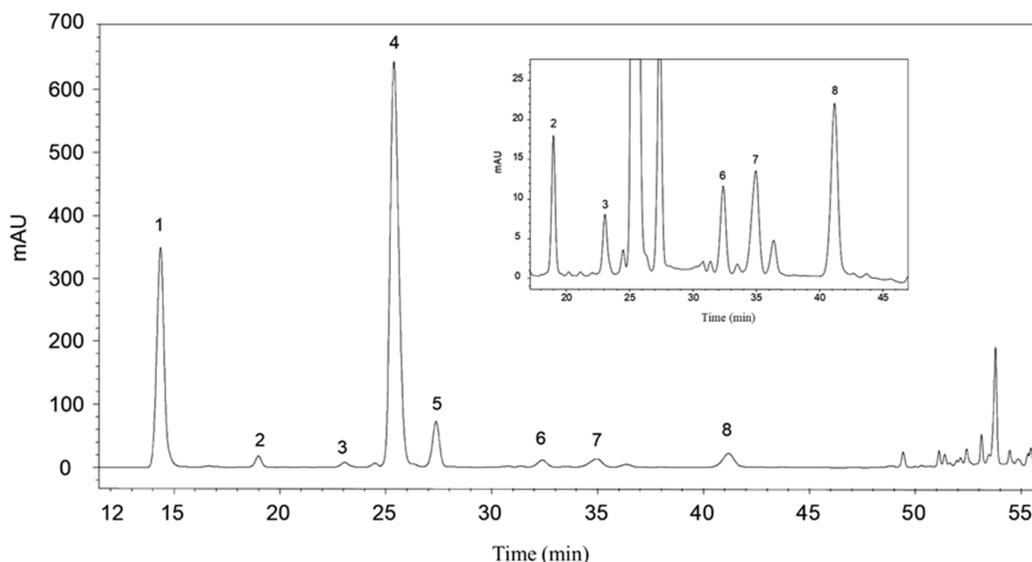


Figure 2. HPLC phenolic profile of quince fruits. Detection at 350 nm. Peaks: (1) 3-*O*-caffeoylquinic acid, (2) catechin, (3) 4-*O*-caffeoylquinic acid, (4) 5-*O*-caffeoylquinic acid, (5) 3,5-dicaffeoylquinic acid, (6) coumaric acid, (7) quercetin-3-*O*-rutinoside and (8) quercetin-3-*O*-glycosides

Table 1. Identification of compounds found in quince fruits, according to their retention time, maximum

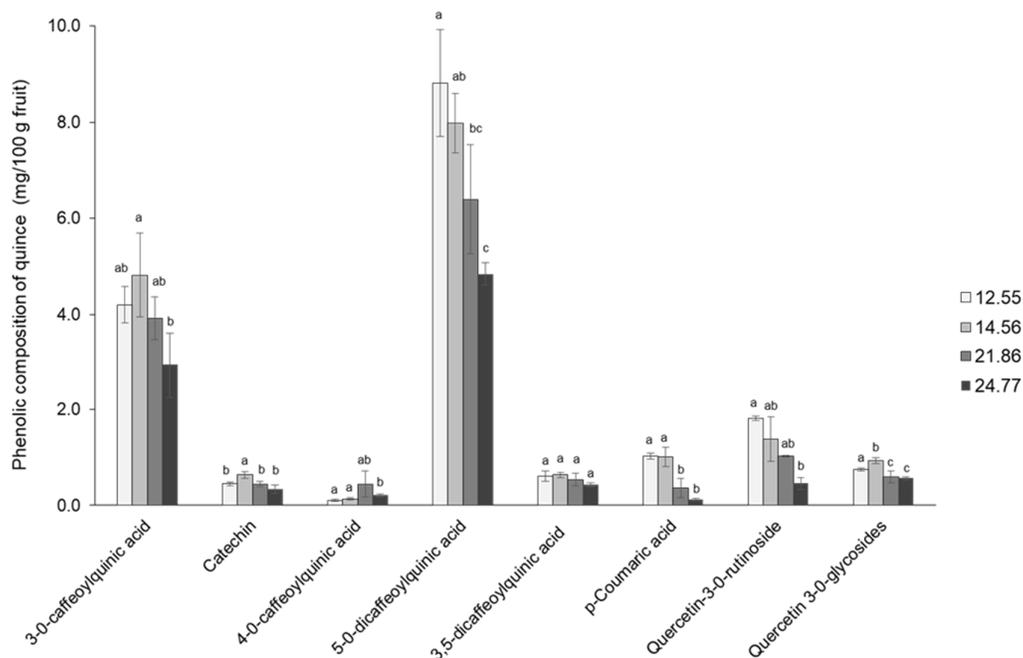
Peak	Compound	RT (min)	λ_{\max} (nm)	Characteristic ions (m/z)
1	3- <i>O</i> -caffeoylquinic acid	14.36	325; 290; 245	353; 354
2	Catechin	18.98	310; 240	-
3	4- <i>O</i> -caffeoylquinic acid	23.06	325; 285; 242	181; 223; 399; 471; 580
4	5- <i>O</i> -dicaffeoylquinic acid	25.38	325; 295; 245	191; 353; 354
5	3,5-dicaffeoylquinic acid	27.37	325; 295; 245	289; 431; 432
6	<i>p</i> -Coumaric acid	32.38	305; 240	433; 547; 593
7	Quercetin-3- <i>O</i> -rutinoside	34.96	315; 240	289; 461
8	Quercetin-3- <i>O</i> -glycosides	41.17	330; 295; 245	180; 435; 345; 391

absorbance and negative ionisation mass spectra

RT, retention time; MS2 (*m/z*), daughter ions produced from [M-H] – fragmentation; λ_{\max} , maximum absorbance for compound identification by UV-VIS spectra

Phenolic composition of quince fruits

Figure 3 shows the phenolic composition of quince fruits at different maturity index. Statistically significant differences ($p < 0.05$) were obtained in the composition of phenolic compounds of fruits with different maturity index (Figure 3). The concentration of the 3-*O*-caffeoylquinic and 5-*O*-dicaffeoylquinic acids in the quince fruits varied from 2.93 to 4.20 and from 4.84 to 8.81 mg/100 g fruit, respectively. In general, the phenolic composition decreased by the rise of fruits MI, obtaining a decrease up 39% and 45% on 3-*O*-caffeoylquinic and 5-*O*-dicaffeoylquinic acids, respectively (Figure 3).

**Figure 3.** Phenolic Composition of quince fruits with different maturity index (values obtained at 320 nm)

Correlation between phenolic compounds and physicochemical properties of quince fruits

The relationship between physicochemical characteristics and phenolic compounds of quince fruits is shown in the loading plot (Figure 4). To explain the graph, it is important to know that when two vectors are close, forming a small angle, the two variables they represent are positively correlated (Eriksson *et al.*, 2013). The ripeness degree inversely affected the phenolic content of quince fruits, therefore, when the MI increased,

a reduction of phenolic compounds was obtained (Figure 4). Likewise, a direct relationship between the pH and the maturity stage of the quince's fruits can be observed.

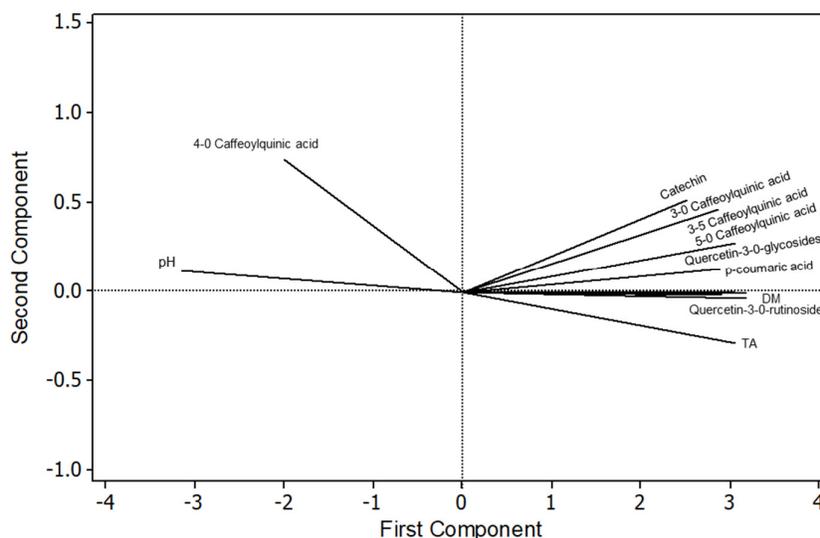


Figure 4. Principal component analysis (PCA) loading plot of physicochemical characteristics and phenolic compounds of quince fruits

Discussion

Physicochemical properties of quince fruits

The acidity of quince fruits decreases when the MI increased. This parameter is generally attributed to proton release from organic acids, which are metabolized in the respiration process as that fruit ripens (Akhtar and Rab, 2015; Famiani *et al.*, 2015). Rasheed *et al.* (2018) studied also quince and reported a similar trend in the acidity of fruits.

Contrarily to data reported for other fruits, the total soluble solids (TSS) decreased as that MI increased, this result was verified with the starch-iodine test, being consistent. This can be due to quince composition, which includes high content of pectin (1.83%) (Acikgoz, 2011; Borazan and Acikgoz, 2017). TSS assessment by refractometry applied include sugars but also acids and small amounts of dissolved vitamins, proteins, pigments, phenolics, and minerals (Magwaza and Opara, 2015). The trend obtained for TSS is also supported by the reduction in dry matter obtained (Figure 1).

Phenolic profile of quince fruits

The phenolic profile obtained to quince fruits is similar to reported in previous studies (Silva *et al.*, 2000; Silva *et al.*, 2004; Baroni *et al.*, 2018).

As well as in other fruits like apples and pears (Zampelas and Micha, 2015), the 5-*O*-caffeoylquinic acid was the major the phenolic acid of the quince fruits. This phenolic compound is the main substrate of polyphenol oxidase enzyme, which explains the susceptibility of these fruits to enzymatic browning (Sunil, 2016). The range obtained of 5-*O*-caffeoylquinic acid (4.84-8.81 mg/g) is higher than reported for pulp and peel of pear (0.08-0.66 and 0.32-3.33 mg/g, respectively) (Brahem *et al.*, 2017) and less than range reported in other studies for quince fruits (10.7 to 15.7 mg/g) (Carvalho *et al.*, 2010; Costa *et al.*, 2009). This last can be

attributed to factors, such as variety, cultivar, soil and climate related factors, fertilization and others (Klepcka *et al.*, 2011).

Caffeic acid has been found to be the most effective agent to diseases resistance response found in many fleshy fruits during ripening (Singh *et al.*, 2010). The biosynthesis of caffeic acids involves in a broad range of stress responses, due to mechanisms underlying their biosynthesis and protective action. Like other phenolics, they are accumulated inside vacuoles or in the apoplast during leaf ageing, and their biosynthesis apparently occur within chloroplasts since the last enzyme that catalysis their biosynthesis is described as chloroplastic (Mondolot *et al.*, 2006). The monomeric flavonols as the quercetin-3-*O*-rutinoside and quercetin-3-*O*-glycosides were also detected in the quince in a range to 0.46-1.81 and 0.57-0.93 mg/g, respectively. These values are much higher than those reported for pears fruits (Brahem *et al.*, 2017). Both compounds are considered potent antioxidants due to their ability to scavenge the free radicals (Gharras, 2009). Additionally, it has been reported that they also contribute to the quality characteristics of fruits including astringency, texture, taste and colour (Arena *et al.*, 2012).

Relationship between phenolic profile and physicochemical properties of quince fruits

The reduction of phenolic compounds during the maturation of fruits explains the decline astringency and bitterness in fruits (Butkhup and Samappito, 2011). A decline in the phenolic acids content during ripening was also reported in fruits such as blackberries, strawberries (Häkkinen *et al.*, 2000), white grapes, mango, banana (Wang and Lin, 2000) and tomato (Gougoulis *et al.*, 2018) and apple (Silva *et al.*, 2019). This pattern suggests the degradation of phenolic compounds and their utilization in the biosynthesis of other compounds and/or association with other cellular compounds by stable covalent links (Arena *et al.*, 2012).

Conclusions

In this study determined the phenolic profile and physicochemical parameters of quince fruits as a function of maturity index. Eight phenolic compounds were identified in quince fruits, being the major the 3-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acids. Significant differences on phenolic composition by effect of maturity index were obtained. The phenolic compounds were correlated with the maturity index and the physicochemical factors of the quince fruits, obtaining in general, an inverse relation between the phenolic compounds and maturity index. The quince fruits are an important source of phenolic compounds especially when they have a low maturity index.

Authors' Contributions

GB (Methodology); PC (Methodology); MJR-R (Methodology, Review and Editing); MAF-C (Methodology, Review and Editing); NAS-S (Methodology, Review and Editing); OC-A (Review and Editing); MCS-C (Methodology, Supervision and Writing)

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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