DETERMINATION OF PHENOLIC COMPOUNDS IN APPLES AND PROCESSED APPLE PRODUCTS

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A B S T R A C T

The aim of this preliminary research was to evaluate phenolic compounds content in apples and their processed products. Four cultivars of apples: 'Jonagold', 'Sampion', 'Idared' and 'Topaz' were harvested at commercial maturity during the season of 2004 and processed into clear and cloudy juice as well as apple sauce according to typical industrial technologies.

The content of phenolics was determined by the HPLC method using a novel type of chromatographic column Phenomenex Fusion RP.

On average the total amount of the determined phenolic compounds in the investigated fresh fruits was 857 mg/kg of fresh weight. The most abundant group was flavonols (417 mg/kg), followed by phenolic acids (229 mg/kg). Significant variations were found comparing the content of different phenolic groups in the investigated cultivars. A highest content of phenolic acids was found in the 'Idared' fruits. Most abundant in flavonols was the cultivar 'Sampion' (477 mg/kg) simultaneously having the lowest amount of phenolic acids. A high content of quercetin glycosides was found in the cvs. 'Jonagold' and 'Topaz'.

Processing of apples into apple sauce caused rather small changes in phenolic content whereas during processing into cloudy juices only 53% of fruit phenolics were found in juices. Much higher losses of phenolics were found during clear juices production. A strong effect of temperature on phenolics content was found during the production of clear juices. Juices produced with Panzym MK at 50°C contained 40% of fruit phenolics but juices produced with Rohapect MA Plus at 20°C contained only 19% of initial fruit phenolics.

Key words: apple, polyphenols, juice, cloudy juice, apple sauce

INTRODUCTION

The main sources of phenolic compounds in the diet are fruits and vegetables, including apples and processed apple products. The benefits of phenolic compounds have recently been the subject of much discussion (Manach et al., 2004; Scalbert et at., 2005). Dietary intake of phenolics is estimated to be about one gram per day. This is significantly higher than that of all other dietary antioxidants, including vitamin C, vitamin E and carotenoids (Scalbert and Wiliamson, 2000).

Many different phenolic compounds have been identified in apples. The two main subtypes of polyphenols are flavonoids and phenolic acids. Some of the most important flavonoids in apples are:

- quercetins, present in glycosylated forms (flavonols); and
- catechin and epicatechin and their oligomers (proanthocyanidins), which are responsible for astringency and bitterness.

Some of the most common phenolic acids in apples are:

- caffeic acid, present in esterified form with quinic acid (chlorogenic acid); and
- p-coumaric acid, present in esterified form with quinic acid (p-coumarylquinic acid).

Other phenolics in apples include the dihydrochalcones (phloretin glycosides).

The concentration of phenolic compounds varies widely in different parts of the apple fruit. Quercetin glycosides and flavonols are present mainly in the skin (Oszmiański and Lee, 1994; Thielen et al., 2005; Awad et al., 2000). Dihydrochalcones are present in the core and seeds (Thielen et al., 2005). Phenolic acids are present mainly in the cortex (Awad et al., 2000; Russel et al., 2002).

Thee fact that different phenolics are present in different parts of the fruit affects the concentration of phenolics in juice, puree and processed apple products. Only small amounts of quercetin glycosides and dihydrochalcones are extracted during juice production because quercetin glycosides are present mainly in the skin and dihydrochalcones are present mainly in the seeds. The oxadative capacity of the phenolic group also affects the concentration of phenolics (Oszmiański and Lee, 1990). During processing, the cell wall disintegrates and the natural barrier separating enzymes and substrates disappears. Polyphenoloxidase (PPO), the main oxidizing enzyme bound to cell walls, starts to degrade phenolic compounds, ascorbic acid and other constituents of the mash (Mayer and Harel, 1979). Polyphenoloxidase catalyzes the oxidation of orthodiphenols to quinones, which are initially colourless. Quinones are very reactive and may undergo further non-enzymatic reactions with other phenolics, proteins and amino acids, condensing as brown pigments (Zimmer, 1999).

The determination of phenolics content in apples and apple products has been the subject of extensive research. There are several HPLC methods to separate and quantify polyphenolic compounds in apples and processed apple products (Spanos et al., 1990; Schieber et al., 2001; Tsao and Yang, 2003). However, the qualitative and quantitative changes in phenolic compounds during processing are not well understood.

MATERIAL AND METHODS

The study was carried out in 2004 and 2005 at the Department of Storage and Processing of the Research Institute of Pomology and Floriculture in Skierniewice, Poland. Phenolics content was measured in four cultivars: 'Jonagold', 'Sampion', 'Idared' and 'Topaz'. Apples were harvested at commercial maturity. Replicate batches were processed into clear juice, cloudy juice, and applesauce in accordance with industrial procedures. Clear apple juices were digested with Panzym MK at 50°C or Rohapect MA Plus at 20°C. Cloudy juices were produced without mash enzymation. 200 mg/kg ascorbic acid was added to the cloud juice.

Reagent grade sodium acetate, chlorogenic acid, p-coumaric acid, catechin, epicatechin, phloridzin and quercetin were purchased from Sigma-Aldrich. Water used for HPLC analysis was first purified using the Elix 3 system and then using the Simplicity™ system (Millipore). HPLC grade acetonitrile was purchased from Baker.

To determine phenolic compounds in fresh fruits, thirty apples were divided into octants with a ceramic knife. Opposite parts from each apple were frozen to -25°C before being ground up.

For HPLC, 10 g of ground up apples were homogenized for one minute with 70% aqueous methanol (Ultra Turrax® T 25 Basic IKA®-WERKE). The slurry was transferred to a 50 ml volumetric flask, which was then filled to the mark with 70% methanol. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was stored at −18°C prior to analysis.

Samples of clear juices were diluted before injection. Samples of sauces and cloudy juices were filtered, diluted and extracted with 70% methanol in an ultrasonic water bath for ten minutes before injection. Before HPLC, all samples were diluted 1:3 (v/v) with sodium acetate buffer (solvent A).

HPLC was carried out using an Agilent 1100 Series HPLC system equipped with a DAD detector. Polyphenolics were separated using a Phenomenex® Fusion RP column (250 mm × 4.6 mm; 3 μm) with a guard column. The mobile phase consisted of 10.2% acetic acid in 2 mM sodium acetate (solvent A) and acetonitrile (solvent B). The flow rate was kept constant at 0.5 ml/min for a total run time of 72 min at 25°C. The system was run with a gradient program: 3% B (0-20 min); 3-35% B (45 min); 35-90% B (3 min); 90-90% B (4 min); 90-0% B (1 min). The column was equilibrated for ten minutes at initial conditions. The injection volume was 20 μl. A representative separation profile is presented in Table 1 and Figure 1.

Data were statistically elaborated by analysis of variance, followed by means separation with Duncan's multiple-range t-test at $P \leq 0.05$.

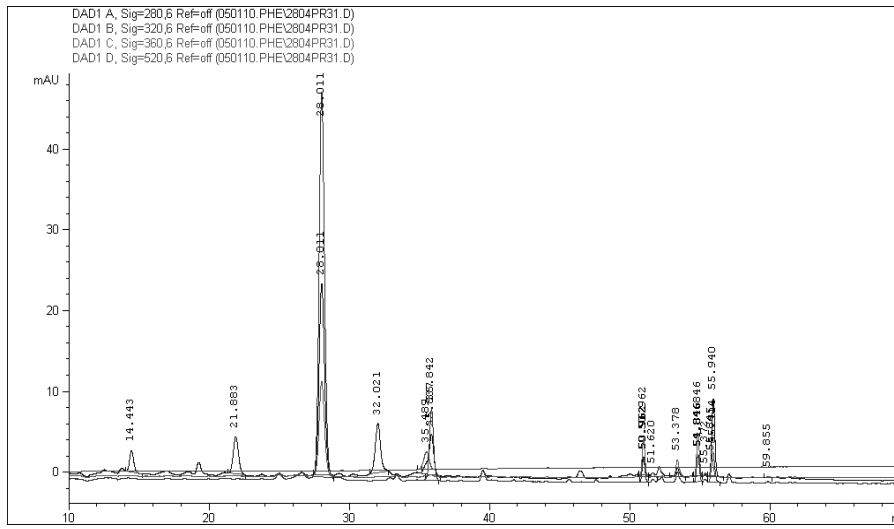

Figure 1. Example of phenolics separation: 1 – flavonols; 2 – phenolic acids; 3 – dihydrochalcones; 4 – quercetin glycosides

RESULTS AND DISCUSSION

The concentration of phenolic compounds in the cultivars evaluated was 857 mg/kg of fresh weight (Tab. 1). The concentration of different groups of phenolic compounds varied widely from cultivar to cultivar. The cultivar with the highest level of flavonols was 'Sampion' (477 mg/kg). The cultivar with the highest level of phenolic acids was 'Idared', and the cultivar with the lowest level of phenolic acids was 'Sampion'. The cultivars with the highest levels of quercetin glycosides were 'Topaz' and 'Jonagold'.

The average total phenolics content in the four cultivars was flavonols (857 mg/kg), consisting mainly of flavonols (417 mg/kg) and phenolic acids (229 mg/kg).

Table 1. Content of phenolic compounds in fruits [mg/kg]

Phenolics group [mg/kg]	Cultivar				Average
	Jonagold	Sampion	Idared	Topaz	
Flavonols and their dimers	371	477	374	446	417
Dihydrochalcones	119	118	97	85	119
Phenolic acids	235	145	346	192	229
Quercetin glycosides	87	65	41	83	69
Total phenolics	**839**	**840**	**910**	**840**	**857**

During the production of applesauce, phenolics content essentially did not change. During the production of cloudy juices, phenolics content dropped by 47%. During the production of clear juices with Panzym MK, phenolics content dropped by 65%. During the production of clear juices with Rohapect MA Plus, phenolics content dropped by 81% (Tab. 6).

The results of polyphenols determination are presented in Tables 2-6.

Table 2. Content of flavonols in fruits and processed products

Product	Cultivar			
	Jonagold	Sampion	Idared	Topaz
Fruit [mg/kg]	371.1 hi	477.4 k	373.6 hi	446.3 j
Sauce [mg/kg]	387.8 i	462.3 jk	359.7 h	452.5 j
Cloudy juice [mg/l]	209.4 e	284.9 f	82.7 b	329.8 g
Clear juice at 50°C [mg/l]	62.5 b	267.5 f	70.8 b	219.8 e
Clear juice at 20°C [mg/l]	15.3 a	158.3 d	12.9 a	108.1 c

Remark: Averages marked by the same letter do not differ significantly at P=0.05

Table 3. Content of dihydrochalcones in fruits and processed products

Product	Cultivar			
	Jonagold	Sampion	Idared	Topaz
Fruit [mg/kg]	119.4 h	117.8 h	97.0 g	85.0 f
Sauce [mg/kg]	88.8 fg	71.5 e	86.8 fg	52.6 d
Cloudy juice [mg/l]	37.6 bc	40.2 c	42.6 c	23.2 a
Clear juice at 50°C [mg/l]	42.3 c	58.8 d	55.8 d	41.8 c
Clear juice at 20°C [mg/l]	19.3 a	28.6 ab	24.1 a	19.9 a

Remark: See Table 2

Table 4. Content of phenolic acids in fruits and processed products

Product	Cultivar			
	Jonagold	Sampion	Idared	Topaz
Fruit [mg/kg]	235.1 j	145.1 g	346.3 l	191.7 h
Sauce [mg/kg]	262.5 k	120.6 f	353.2 l	156.1 g
Cloudy juice [mg/l]	213.4 i	106.2 e	225.0 ij	150.1 g
Clear juice at 50°C [mg/l]	69.5 c	88.1 d	115.6 ef	71.5 c
Clear juice at 20°C [mg/l]	51.9 ab	46.1 a	51.0 ab	62.5 bc

Remark: See Table 2

Table 5. Content of quercetin glycosides in fruits and processed products

Product	Cultivar			
	Jonagold	Sampion	Idared	Topaz
Fruit [mg/kg]	87.5 fg	65.2 de	41.2 c	82.9 f
Sauce [mg/kg]	92.6 g	74.0 e	65.6 de	112.1 h
Cloudy juice [mg/l]	9.6 a	5.1 a	5.0 a	7.1 a
Clear juice at 50°C [mg/l]	35.2 c	21.3 b	20.9 b	57.9 d
Clear juice at 20°C [mg/l]	5.9 a	3.1 a	2.4 a	7.3 a

Remark: See Table 2

Table 6. Content of total phenolics in fruits and processed products

Product	Cultivar			
	Jonagold	Sampion	Idared	Topaz
Fruit [mg/kg]	839.4 hi	840.0 hi	909.6 j	840.1 hi
Sauce [mg/kg]	853.1 i	762.5 g	912.2 j	806.6 h
Cloudy juice [mg/l]	469.9 e	462.2 e	377.3 d	539.4 f
Clear juice at 50°C [mg/l]	215.5 b	460.2 e	274.7 c	412.0 d
Clear juice at 20°C [mg/l]	92.4 a	251.2 bc	90.2 a	207.7 b

Remark: See Table 2

During the production of applesauce, flavonols content essentially did not change, dihydrochalcones content decreased significantly, phenolic acids content decreased by only a small amount, and quercetin glycosides content even increased, perhaps due to evaporation (Tab. 2, 3, 4 and 5). The decrease in dihydrochalcones content is probably a result of the processing technology. Before applesauce production, the cores were removed. The core and seeds contain the highest levels of flavonols (Awad et al., 2000; Thielen et al., 2005).

During the production of clear juices, levels of every class of phenolic substances significantly decreased.

Phenolics content was highest in cloudy juices (Tab. 4). Quercetin glycosides content in cloudy juices was on average 7 mg/l (Tab. 5). This may be due to different concentrations of phenolic compounds in the fruit tissue. Due to short processing time and low temperature during clear juice production, quercetin glycosides remained with the skin in the pomace. Phenolic acids are more evenly distributed in the cortex and were better extracted in juices.

On the basis of the content of individual groups of phenolic compounds in cloudy juices, the cultivars can be divided into two groups. The first consists of two traditional cultivars suitable for processing, 'Idared' and 'Jonagold', which had the highest content of phenolic acids (Tab. 4). The second consists of the cultivars 'Sampion' and the scab resistant cultivar 'Topaz', which had the highest content of flavonols (Tab. 2). This may be due to a difference in polyphenol oxidase (PPO) level (Kuczyński, 1995; Podsędek et al., 2000). PPO activity is lower in 'Sampion' than in all popular cultivars. Unfortunately, there are no data available for Topaz.

Phenolics levels in cloudy juices are consistent with the literature (Markowski, 1998; Will and Dietrich, 2005). However the degree of phenolic preservation depends significantly on the addition of ascorbic acid during juice production (Markowski, 1998). Adding more ascorbic acid prevents oxidation of phenolics because of their higher content in cloudy juices.

Clear juices produced from 'Sampion' with Panzym MK at 50°C had about the same phenolics content as cloudy juices (Tab. 5). This may be due to extremely low PPO activity in 'Sampion' and a low rate of enzymatic browning (Kuczyński, 1995). In the other cultivars, phenolics level decreased. The content of individual phenolic groups in clear juices produced with Panzym MK at 50 ^{o}C differed significantly from cloudy juices. The dominant group was phenolic acids , and the content of flavonols was lower than in cloudy juices (Tab. 2 and 4) with the exception of 'Sampion' and 'Idared', in which the difference was statistically insignificant.

Dihydrochalcones and quercetin glycosides contents were higher in clear juices produced with Panzym MK at 50^{o}C than in cloudy juices (Tab. 3 and 5). This may be due to better extraction of these substances from the skin and seeds because of the increased temperature and enzymatic action.

Using the new enzyme Rohapect MA Plus, which has an optimum activity at 20^{o}C, caused a significant decrease in phenolic content (160 mg/l as opposed to 341 mg/l for clear juices produced with Panzym MK at 50°C). The lowest decrease was found for phenolic acids (Tab. 4).

The content of quercetin glycosides was very low and comparable to cloudy juices (Tab. 5). There were no significant differences in quercetin glycosides content in cloudy juices and clear juices produced with Rohapect MA Plus at 20°C, even though the enzymatic treatment lasted one hour. This proves that temperature has a significant effect on the extraction of quercetin glycosides. During enzymatic treatment with Panzym MK at 50°C, the content of quercetin glycosides was on average almost eight times higher than in the other juices. This cannot be explained by the enzyme dose and efficiency because the pressing yield was about the same for both clear juices (82%).

Cloudy juices made from 'Sampion' and 'Topaz' had the highest flavonols content. This seems not to depend on the technique of juice

production or the temperature during enzyme treatment. Juices made from 'Jonagold' and 'Idared' distribution of phenolic compounds that is typical for commercial juices and contained the highest levels of phenolic acids (mainly chlorogenic acid), Juices made from ''Sampion' and 'Topaz' contained high levels of flavonols (primarily epicatechin). Further study is need on the role of PPO activity.

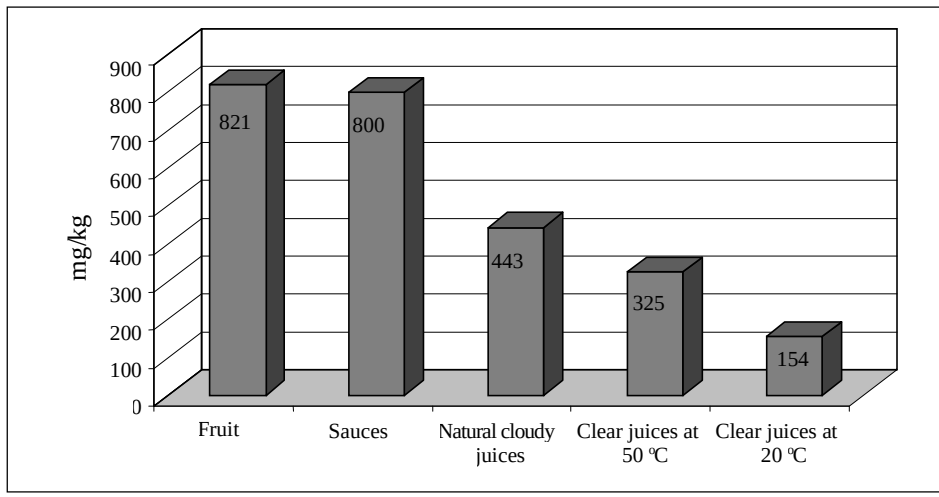

Figure 2. Total phenolics content in fruits and processed products

CONCLUSIONS

1. Even though the cultivars differed significantly in terms of morphology, they all contained about the same amount of phenolics, the most abundant of which were flavonols.
2. Apple sauces contained more phenolics than juices.
3. Natural cloudy juices contained more phenolic compounds than clear juices.
4. In the production of clear juices, phenolic compounds are more effectively extracted when the temperature during enzyme treatment is higher.

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OZNACZANIE ZWIĄZKÓW FENOLOWYCH W JABŁKACH I PRZETWORACH Z JABŁEK

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S T R E S Z C Z E N I E

Celem prezentowanych wstępnych badań była ocena zawartości związków fenolowych w jabłkach i przetworach z jabłek. Cztery odmiany jabłek: 'Jonagold', 'Szampion', 'Idared' i 'Topaz' zebrano w stanie dojrzałości zbiorczej w sezonie 2004 i przetworzono na soki klarowne, mętne oraz na przeciery stosując typowe technologie przemysłowe. Zawartość związków fenolowych określono metodą HPLC stosując nowy typ kolumny chromatograficznej Phenomenex Fusion RP.

Średnia całkowita zawartość oznaczanych związków fenolowych w badanych owocach wyniosła 857 mg/kg świeżej masy. W owocach w największych ilościach występowały flawonole (417 mg/kg), następnie kwasy fenolowe (229 mg/kg). Istotne różnice stwierdzono porównując zawartość poszczególnych grup związków fenolowych w owocach badanych odmian jabłek. Najwyższą zawartość kwasów fenolowych stwierdzono w owocach odmiany 'Idared'. Z kolei najbogatsze w katechiny (477 mg/kg) były owoce odmiany 'Szampion', które jednocześnie były najuboższe w kwasy fenolowe. Wysoką zawartość glikozydów kwercetyny stwierdzono w owocach odmian 'Jonagold' i 'Topaz'. Niemniej jednak zawartość związków fenolowych w badanych odmianach jabłek była stosunkowo mało zróżnicowana.

Produkcja przecierów z jabłek powodowała stosunkowo małe straty związków fenolowych, podczas gdy przy przetwarzaniu owoców na soki mętne stwierdzono w nich tylko 53% początkowej ilości związków fenolowych. Znacznie wyższe straty związków fenolowych wystąpiły podczas produkcji soku klarownego. Stwierdzono silny wpływ temperatury na zawartość związków fenolowych. Soki produkowane z użyciem enzymu Panzym MK w temperaturze 50°C zawierały 40% związków fenolowych obecnych w owocach, a soki produkowane z użyciem enzymu Rohapect MA Plus w temperaturze 20°C zawierały jedynie 19% początkowej ilości związków fenolowych.

Słowa kluczowe: jabłka, związki fenolowe, sok, mętny sok, przecier