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QUALITATIVE ANALYSIS OF THE MAIN POLYPHENOLS CONTAINED IN APPLE POMACE EXTRACT

ANALIZA JAKOŚCIOWA GŁÓWNYCH POLIFENOLI ZAWARTYCH W EKSTRAKCIE Z WYTŁOKÓW Z JABŁEK

Abstract

The paper describes chosen methods for the extraction and qualitative analysis of the main polyphenolic compounds contained in apple pomace. Apple pomace is a by-product generated during apple juice production. The extracts were obtained with the Ultrasound Assisted Micelle Mediated Extraction (UAMME) method using a 1% Rocanol B2 water solution, as well as the Ultrasound Assisted Extraction (UAE) method in water. Thin Layer Chromatography (TLC) and UV/VIS spectroscopy were applied as analytical methods in order to identify the main polyphenolic substances in the obtained extracts. The obtained results showed that both UAMME as well as UAE are effective methods for obtaining quercetin, catechin and rutin.

Keywords: apple-pomace, ultrasound assisted extraction, micelle mediated extraction, polyphenols, TLC, UV/VIS

Streszczenie

Artykuł opisuje wybrane metody pozyskiwania, a także analizę jakościową głównych związków polifenolowych zawartych w wytłokach z jabłek. Wytłoki jabłkowe stanowią produkt uboczny powstający podczas procesu produkcji soku jabłkowego. Ekstrakty uzyskano metodami ekstrakcji micelarnej wspomaganej ultradźwiękami w 1% wodnym roztworze Rokanolu B2 (UAMME) oraz ekstrakcji wspomaganej ultradźwiękami w wodzie (UAE). Do identyfikacji polifenoli w otrzymanych ekstraktach zastosowano metodę chromatografii cienkowarstwowej (TLC) oraz spektroskopię UV/VIS. Zarówno ekstrakcja micelarna wspomagana ultradźwiękami, jak i ekstrakcja w wodzie, wspomagana ultradźwiękami, okazały się skuteczne do pozyskiwania kwercetyny, katechiny oraz rutyny.

Słowa kluczowe: wytłoki z jabłek, ekstrakcja wspomagana ultradźwiękami, ekstracja micelarna, polifenole, TLC, UV/VIS

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1. Introduction

Since 90's Poland is one of World's greatest producers of concentrated apple juice. Over 70% of fruit trees in orchards are apple trees (Fig. 1).

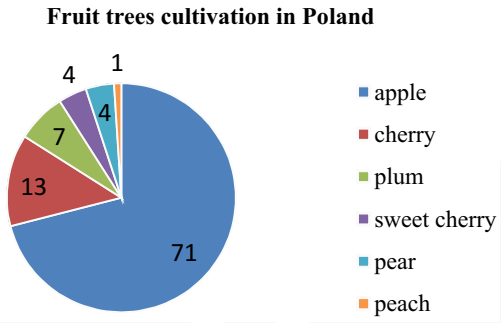

Fig. 1. The structure of fruit tree cultivation in Poland in 2014 [%] [1]

Currently, there are 47 apple processing installations, which operate in Poland, with total productivity of 32 thousand tons of apples a day. It is estimated that about 50–60% of apple fruits are processed in the food industry, mainly for juice concentrate production [1]. During the processing of apple fruits for juice production, large amounts of solid residues, such as peel, core and seeds, are generated [2]. To obtain 200 thousand tons of apple concentrate, about 1.5 million tonnes of apples have to be processed. About 0,3 million tons of residue is treated as a waste [3]. These by-products are called "apple pomace" and are mainly used as feed additives or are disposed of as waste (Fig. 2) [2, 4, 5].

Fig. 2. Dried apple pomace, a by-product of the juice production process

However, many studies show that apple pomace is a rich source of valuable nutraceuticals: carbohydrates, dietary fibres, vitamins, minerals and polyphenols (Fig. 3) [6].

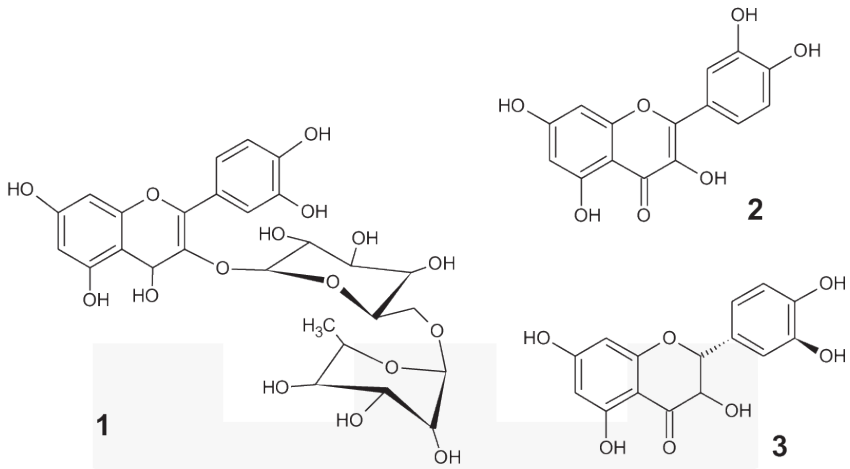

Fig. 3. The structure of the main apple pomace polyphenols: rutin (1), quercetin (2) and catechin (3)

Apple pomace is composed of apple peels, pulp as well as seeds. It is a rich source of valuable biological active compounds, especially with antioxidant activity, e.g. polyphenols, procyanidins or glycosides [7]. After the apple juice extrusion process, only 5% of the pectines, vitamin C and polyphenols contained in a fresh apple remains in the apple juice. Procyanidins are compounds, which remain in the juice at the lowest concentration level. All of the mentioned active substances are bound with apple tissue cells and stays within pomace after the pressing process [8, 9]. Polyphenols are known for their wide range of biological activity [10]. Flavonoids and polyphenolic acids can inhibit the proliferation of cancer cells, which has been proven in many *in vitro* tests. An ethanolic extract from apple pomace, as a rich source of polyphenols, exhibits a very high antioxidant activity, which can be compared to ascorbic acid activity [7]. Because of the presence of triterpene compounds, it can also act as an anti-inflammatory, immune-stimulatory, antiviral, antibacterial and antifungal agent [11]. There are many *in vitro* and *in vivo* experiments confirming the biological and therapeutic activity of apple pomace compounds and the possibility of their application as a valuable source for the pharmaceutical, cosmetic and food industries. In cosmetic products, the apple pomace extracts are used as additives in order to neutralise free radicals and prevent many skin diseases and skin damage [9]. Importantly, in order to obtain a maximum concentration of polyphenols, the apple pomace has to be extracted directly after the extrusion process. The storage of fresh pomace causes changes of its composition and decreases its usability. In order to prevent the fermentation process, apple pomace should be dried-up directly after it is obtained. It can be prepared in the closed technological cycle [12].

2. Methodology

Champion apple-pomace was used as the raw material. It was supplied by the Maurer Juice Extraction Plant, Poland. Before extraction, the pomace was dried at 105°C for 90 min. The water content in the pomace was measured using the OHAUS MB25 moisture analyser.

The completely dried apple pomace was ground using a mortar in order to obtain a grounded material with particle sizes below 1 mm.

2.1. Extraction process

The extraction processes of dried pomace were conducted according to the following two methods: ultrasound assisted extraction (UAE) and ultrasound assisted micelle-mediated extraction (UAMME). The solvents applied for the extraction processes were: deionised water in the case of UAE method and a 1% water solution of Rokanol B2 (alkoksylated alcohols, C16-18, PCC Exol) in UAMME. The water used was deionised with the Merck Simplicity Milipore water purification system. Dried apple pomaces (10 g each) were extracted in an ultrasonic bath (InterSonic IS-3, frequency 50 Hz, power 300 W) at room temperature for 30 min, using 200 cm^3 of proper solvent.

2.2. Qualitative analysis

The identification of polyphenols in the obtained extracts was performed with the TLC analysis. Two different mobile phases, containing chloroform and ethyl acetate (10:1 v/v) – *solution 1* or ethyl acetate, water, formic acid (85:15:0.5 v/v) – *solution 2,* were applied to separate some of the flavonoids occurring in the extracts, i.e. rutin, quercetin and catechin. At the beginning, TLC plates (Polygram Sil G/UV 254, Macherey Nagel) were sprayed with a solution containing anisaldehyde (98%, Sigma Aldrich), acetic acid (99%, POCH), methanol (99.8%, POCH), sulphuric acid (38%, POCH) (0.5:10:85:5 v/v). The extracted ingredients, as well as standards of the flavonoids, triterpenes and polyphenols, become visible after heating the plates at 100°C for 2–3 min. Retention times of the extracted ingredients and standard compounds were compared in order to identify particular spots on the TLC plate. Moreover, UV/VIS spectra were measured for all of the extracts as well as for three standard compounds (catechin, rutin and quercetin). The spectra were prepared in the wavelength range of 200–800 nm, using the Macherey Nagel Nanocolor UV/VIS Spectrophotometer. The obtained absorption spectra for the analysed extracts and standard compounds were compared in order to determine the presence of the polyphenolic compound groups in apple pomace extracts.

3. Results

Figure 4 presents the visible spots on TLC plate obtained for two different eluents. Table 1 shows the retention times (R_f) of the identified polyphenols. Three of the obtained visible spots on TLC plate: 1, 2 and 3 (Fig. 4), corresponded to the applied standard compounds: rutin, quercetin and catechin. The identified flavonoids are the major polyphenols contained in apple pomace extracts [6, 13].

Figure 5 shows the spectra of the analysed extracts and standard polyphenols. The absorbance maximum, corresponding to the extracts, rutin, quercetin and catechin, is presented in Table 1. The results showed that the extraction procedure influences the concentration of

the analysed polyphenolics. The ultrasound assisted micelle mediated extraction (UAMME) method allowed us to obtain a higher concentration of polyphenols than in the case of the ultrasound-assisted extraction (UAE-E). In case of the extract obtained by UAMME, a higher value of absorption is visible at a wavelength of 210 nm (Fig. 5). The micelles are capable of dissolving organic compounds that are less soluble in the water, such as catechin comparing with rutin, by positioning them inside the micelle structure. Most likely, ingredients that have a slightly lipophilic character are dissolved in water by surface active substance like Rokanol B2 (alkoksylated alcohols C16-18).

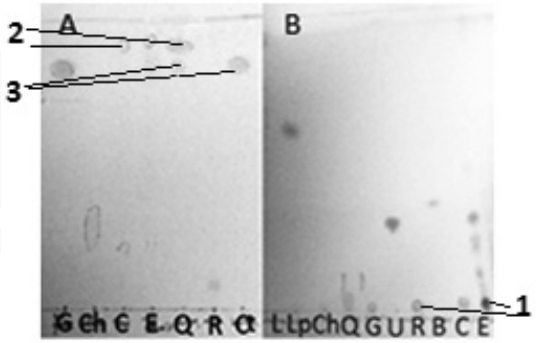

Fig. 4. TLC plates with apple pomace extracts and polyphenolic standard compounds (C, E,-apple pomace extracts, R-rutin, Q-quercetin, G-gallic acid, Ch-chlorogenic acid, L-luteolin, Lp-lupeol, U-ursolic acid, Ct-catechin, B-betulin) – eluent A chloroform : ethyl acetate (10:1 v/v), eluent B – ethyl acetate, water, formic acid (85:15:0,5 v/v)

Table 1

Polyphenol compounds contained in apple pomace extracts, UAE – Ultrasound Assisted Extraction, UAMME – Ultrasound Micelle Mediated Extraction

Compound	R_t (*solution 1*)	R_t (*solution 2*)	Absorbance max. [nm]
UAE	0.03; 0.06; 0.05	0.08; 0.82; 0.87	212, 250, 260, 360, 370
UAMME	0.03; 0.06; 0.05	0.08; 0,82; 0.90	211, 251, 262, 290, 368
Rutin standard	0.03	0.07	260, 365
Quercetin standard	0.06	0.83	250, 370
Catechin standard	0.05	0.92	210, 290

The obtained results confirmed that both of the applied extraction processes are effective in isolating rutin, quercetin and catechin, but ultrasound-assisted micelle mediated extraction is more efficient for obtaining less hydrophilic substances.

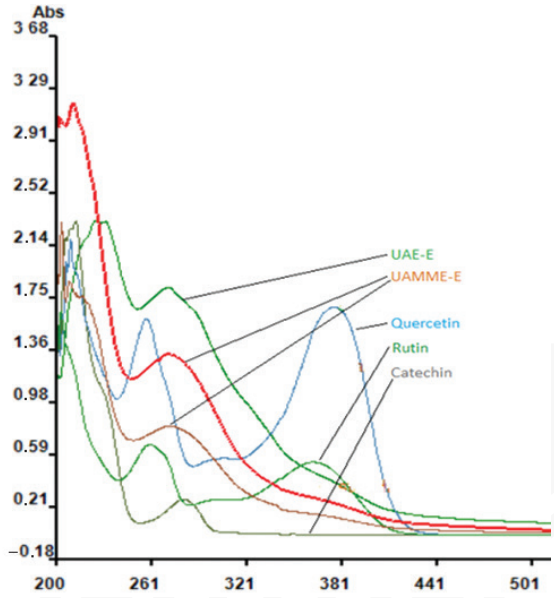

Fig. 5. UV-VIS spectra of apple pomace extracts and polyphenols standards (quercetin, rutin and catechin)

4. Conclusions

The flavonoids (rutin, quercetin and catechin) identified in the obtained extracts are one of the major polyphenols in apple pomace. Both of the applied extraction methods are effective ways for obtaining extracts that are rich in antioxidant polyphenolic compounds. TLC and UV/VIS spectrophotometric methods are useful tools for a qualitative analysis of the extracted polyphenols. Due to presence of antioxidants, the obtained apple pomace extracts can be applied directly as cosmetic raw materials, which are rich in biological active substances.

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