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APPLE POMACE AS A POTENTIAL SOURCE OF NUTRACEUTICAL PRODUCTS

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Poland with production of clear apple juice concentrates up to 200.000 tons processes yearly more than 1.5 mln t. of apples. Commercial technologies of processing are very effective but 12 to 20% of processed raw material remains as a waste product after juicing. Typically such material containing sugars, acids and traces of proteins is utilized for animal feeding or composted. Pomace after juice production still contains significant amount of valuable compounds beneficial for human health; polyphenolics and fiber. The content of dietary fiber constitutes is on the average 50% of dry weight. In this study, it was proved that pomace from modern juice production plant contained 60-64% of TDF, 1850-2550 mg/kg dm of flavan-3-ols and 7000 mg/kg dm of polyphenols (with Folin-Ciocalteau method). It was proved that such a raw material is useful for the preparation of liquid and solid phenolic concentrates containing 2.5 and 33% of polyphenols, respectively. Such extracts may be considered as an excellent components for the production of new more healthy and valuable products.

INTRODUCTION

Apple pomace is a by-product resulting from juice pressing. Although for many years regarded as a waste product, at present apple pomace should be considered as a source of DF and phenolics. The content of dietary fiber (DF) constitutes is on the average 50% of dry weight, whereas phenolics may vary from 1200 to 4000 mg/kg dry weight [Schieber *et al.,* 2003; Rodriguez *et al.,* 2006].

According to AACC [2000] definition, dietary fibre consists of edible parts of plants or non-starch carbohydrates, which are resistant to human digestive enzymes and resistant to absorption in small intestine; which undergo total or partial fermentation in large intestine. Fiber consists of polysaccharides, oligosaccharides, lignin and plant accompanying substances. Dietary fibre contributes to beneficial physiological impact, decreases cholesterol and blood glucose level [Duxbury, 2004].

DF binds the excess of hydrochloric acid in stomach and harmful substances such as metal ions and cholesterol, increases fecal bulk, improves intestinal peristalsis, and may stimulate growth of positive gut micro flora [Nawirska & Kwaśniewska, 2005]. Dietary fibre intake in Western countries is estimated at a level of 10-25 g per person per day while nutritional experts recommend *ca.* 35 g [Thebaudin *et al.*, 1997]. This undertake could be replaced by diet supplementation by dietary fibre preparations. Dietary fibre is used as component of weight control preparations and as enrichment additive for bread and bakery products [Masoodi *et al*., 2002]. Dietary fibre elongates glucose absorption flatting glucose profile by which insulin excretion is decreased. High insulin levels are connected with coronary heart diseases and diabetes [Nawirska & Kwaśniewska, 2004]. Thanks to these properties dietary fibre plays important role in prevention and treatment of obesity, arthrosclerosis, coronary heart diseases, large intestine cancer and diabetes [Jenkins *et al.*, 2004]. Dietary fibre is classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). The SDF/IDF ratio close to 1:2 indicates fibre as suitable for use as food ingredient [Figuerola *et al.*, 2005]. Fruit fibres have better quality due to higher total and soluble fibre content, water and oil holding capacity and colonic fermentability than more frequently used cereal ones, moreover fruit fibres have lower caloric value [Thebaudin *et al*., 1997]. Apples give fibre with a well balanced ratio of soluble and insoluble fibre and processed in juice production in large quantities [Gorinstein *et al.*, 2001].

Pomace produced from apples contains significant quantities of polyphenols. Polyphenol subclasses of apple pomace are: flavanols (catechin, epicatechin, procyanidins), flavonols, hydroxycinnamates and dihydrochalcones [Schieber *et al.*, 2003]. Flavonoids, are a large group of natural polyphenols commonly present in plants [Shahidi & Naczk, 2004; Oszmiański *et al.*, 2007]. Plant and plant-derived flavonoids are largely present as glycosides, where flavonoid aglycone is linked to a variable sugar moiety, most commonly by beta-glycosidic bond. Among flavonoids quercetin and its glycosides are most abundant in plants, including apples. In apple pomace Quercetin-3-O-β-D-galactopyranoside (hyperin), Quercetin-3-O-β-D-glucopyranoside (isoquercitrin), Quercetrin-3-O-β-D-xylopyranoside (reynoutrin), Quercetrin-3-O-α-L-arabinofuranoside (avicularin), Quercetin-3-O-α-L-rhamnopyranoside (quercitrin) are present in largest

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quantities [Lu & Foo, 1997]. Much attention has been paid to potentially beneficial effect of quercetin and its glycosides for prevention of many diseases [Boyer & Liu, 2004; Hollman *et al.*, 1995]. Those compounds express antioxidant properties and play an important role as dietary agents in human antioxidant defense [Van Der Sluis *et al.*, 2002; Wolfe *et al.*, 2003]. Oxidative stress caused by free radicals can damage lipids, proteins, enzymes, carbohydrates and DNA, which can lead to membrane damage, fragmentation or random cross linking of molecules like DNA, damage of structural proteins, enzymes and eventually cause cell death [Ratman *et al.*, 2006; Grajek 2007]. Polyphenols inhibit LDL oxidation, which is responsible for atherogenesis, and reduce coronary heart disease [Meyer *et al.*, 1997]. Studies have shown that a high level of polyphenols in diet can reduce the incidence cancer and heart disease [Hertog *et al.*, 1993]. Flavonoids possess a number of biological activities such as anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, anti-carcinogenic, anti-allergic effects, which have been shown both *in vitro* and *in vivo* [Middleton, 1996]. Until now the use of pomace as a source of nutraceuticals was limited to the production of dietary fibre preparations and seed oil [Schieber *et al.*, 2003; Lu & Foo, 1997].

Pomace contains 5% of seeds, which contain 15% of fat and are a good source of oil. Apple seeds oil can be either cold-pressed or hot extracted. The main component of apple seeds oil is linoleic acid – about 50% [Lu & Foo, 1997].

Phenolics from apple pomace although known for their health-beneficial properties seem to be not reasonably used until now. The process of combined recovery of pectin and phenolics was described [Schieber *et al*., 2003], the technology led to pectins of lighter colour and lower astringency and lyophilized extract containing 11.8% of phenolics.

The aim of experimental study was to asses available Polish industrial pomace focusing on their fibre and phenolics content and to obtain and characterize phenolics preparation.

MATERIAL AND METHODS

Material. Industrial apple pomaces from apple cultivars mixture and Antonówka cultivar from the harvest season 2006 were obtained from juice producing factory ALPEX (Łęczeszyce, Poland). The pomace was air-dried in a large laboratory dryer at below 70°C.

Industrial apple pomace. Industrial apple pomace were ground in a laboratory mill (Lab-Mill-1, Labor-Mim, Hungary) to particle size below 0.5 mm and stored in a closed container before analyzed.

Extraction of apple pomace and dietary fibre preparates. Extraction was performed as follows: 0.5 g of sample was mixed with 4 mL of solvent (70% methanol) and sonificated for 15 min. After centrifugation, the supernatant was collected, and the sample was re-extracted two times with solvent (3 mL). Pooled extracts were refilled up to 10 mL. Extraction of the insoluble plant material was repeated with a second solvent (70% acetone) in the same way. All extractions were made in duplicate.

Sample preparation for sugars determination with HPLC. A sample (2 g), 20 mL of water and 3 g of $CaCO_3$ were mixed together, cooked for 5 min, chilled, fill up to 50 mL and filtrated. The extract was desalted on ionite (2 parts of anionite to 1 part of cationite). After centrifugation the solution was ready to be analyzed by HPLC.

Polyphenol concentrates were dissolved in methanol, sonificated, centrifuged if necessary and analyzed by means of HPLC.

Analytical methods. The methanolic extract containing most soluble polyphenols was analysed with HPLC and spectrophotometrically by Folin-Ciocalteau method [De Pascual-Teresa & Santos-Buelga, 2000] and Vanillin test [Nakamura *et al.*, 2003]. The acetone extract was analyszed spectrophotometrically by Folin-Ciocalteau method and Vanillin test. Sum of polyphenols was calculated as a total of results of assays for methanolic and acetone extracts. Sum of procyanidins was calculated as a total of results of assays for methanolic and acetone extracts.

HPLC analysis of phenolic compounds. Analysis was carried out with Dionex (Germering, Germany) HPLC system with a DAD detector equipped with 150 x 2.00 mm Phenomenex Synergy 4μm Fusion-RP 80A column (Torrance, CA, USA).

Mobile phase consisted of 0.05% phosphoric acid (solvent A) and 0.05% phosphoric acid in acetonitrile (solvent B). The gradient applied at a flow rate of 0.25 mL/min was: stabilization for 10 min with 4% B, 0-33 min 4-50% B, 33-34 min 50% B, 34-35 min 4% B. Column temperature was 25°C.

HPLC analysis of sugars. Analysis was carried out with Knauer (Berlin, Germany) system with an RI detector equipped with 300 x 7.6 mm Bio-Rad Aminex HPX-87C column (Hercules. CA, USA). Mobile phase was water, isocratic flow rate was 0.5 mL/min, column temperature was 70°C.

Total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) were determined according to AOAC Official Method 993.42 [1995].

Titratable acidity was assayed according to the Polish Standard [PN-90 A-75101/04, 1990].

Total pectins were determined according to the International Federation of Fruit Juice Producers method IFU 26 [IFU, 2001].

Preparation of dietary fibre, phytocomponent concentrates and post extraction dietary fibre. Dietary fibre preparation was obtained by grinding 0.5 kg of apple pomace or apple pomace without seeds and stalks to particle size below 0.5 mm in a laboratory mill (Lab-Mill-1, Labor-Mim, Hungary).

Liquid polyphenol concentrates were obtained by previously published method [Król *et al.*, 2007]. A portion of 1.5 kg of dried and ground pomace was extracted in three batches with 7 L of 15%, 5 L of 70% and 3.5 L of 50% ethanol, re-

spectively. Extract from the first batch was rejected. Extracts from the second and the third batch were joined, ethanol was evaporated, water extract was concentrated to 54.3°Bx.

Solid polyphenol concentrates were obtained by previously published method [Król *et al.*, 2007]. Dried and ground pomace was extracted as above. Ethanol from extracts was evaporated, resulting water solution was adsorbed on ion exchange resin, then desorbed with 60% ethanol. Ethanol from eluate was evaporated, solution was concentrated and cooled down to 5°C, crystallized quercetin was removed, solution was dried by lyophilization resulting in solid polyphenol concentrate.

Post-extraction dietary fibre was obtained from pomace extracted as above by drying in a laboratory hot air drier at 70°C, followed by grinding in a laboratory mill (Lab-Mill-1, Labor-Mim, Hungary) to particle size below 0.5 mm.

Statistical analysis. The determinations were performed in duplicates or triplicates, standard deviations were calculated.

RESULTS AND DISCUSSION

Apple pomace was characterised by a high content of total dietary fibre. The material contained significant amount of mono- and disaccharides as well (above 30%). The content of polyphenols in the examined pomace was 0.3 g/100 g (by HPLC). The composition of apple pomace is illustrated in Figure 1. The composition of phenolics is shown in Table 1.

Most of apple pomace was consisted with pulp, more then one third was peel (Figure 2). After the removal of seeds and stalks, pulp and peel were a predominant part of raw material for preparation of dietary fibre. Commercial preparation of dietary fibre contains above 50% of total dietary fibre, moisture lower than 9%, low content of lipids and is characterized by low caloric value, neutral flavor and neutral taste [Larrauri, 1999]. In our experiments, the fibre preparation of higher TDF content was obtained. A disadvantage of apple fibre when it is produced from pomace including seeds is its bitter taste [Carson *et al.*, 1994]. Dietary fibre can be produced by separation of seeds and hard parts, followed by grinding and micronization to suitable particle size [Masoodi *et al*., 2002]. Such prepared dietary fibre contains phenolics responsible for darkening and bitter taste of the preparation, which is not desired in such a product. In the presented work, it was proved that the pomace from 'Antonówka' cultivar contained less polyphenols, especially phloridzin, procyanidins and flavan-3-ols comparing to pomace from a mixture of red apples cultivars. It indicates the usefulness of that pomace for the production of dietary fibre preparations, characterized by acceptable colour and taste. On the other hand, the pomace from red apples cultivars can be considered as better raw material for the preparation of phytocomponents, especially polyphenol concentrates.

In our experiments after seeds removal (and suitable grinding) the preparation of dietary fibre characterized by the increased content of TDF comparing to apple pomace was obtained. Soluble fibre ratio in the preparation remained similar (12.9% *versus* 12.6%). After extraction of phenolics, TDF content in DF preparation increased to 72.2%. Masoodi *et al*. [2002] found that undesired sensory properties of dietary fibre can be limited or eliminated by the extraction of phenolics. TDF and its SDF fraction content in raw pomace and fibre preparations are summarized in Table 2.

Both liquid and solid preparations contained significant amounts of phenolics (2.5% and 37% respectively), most of all quercetin glycosides (Tables 3 and 4). For a comparison,

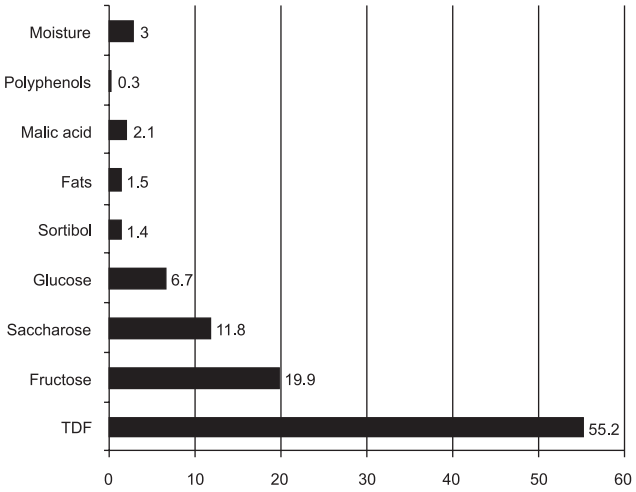

FIGURE 1. Chemical composition of apple pomace (g/100 g).

TABLE 1. Characteristics of polyphenols from apple pomace (mg/kg dm) (mean value of 2 determinations).

	Red apples cultivars pomace (ALPEX)	'Antonówka' apple pomace (ALPEX)
Hydroxycinnamic acids	122.2±2.8	166.2±3.3
Flavan-3-ols by HPLC including:	728.0±12.0	269.5±1.9
Procyanidin B2	102.2±1.6	71.0±1.5
Epicatechin	114.6±3.6	54.9±0.6
Procyanidin C1	98.9±7.4	88.3±1.0
Quercetin glycosides	973.6±28.8	855.4±18.1
Quercetin	4.8±0.12	5.8±0.12
Phloridzin	879.8±14.0	654.6±14.3
Sum of flavan-3-ols VT	2550±100	1850±60
Sum of polyphenols FC	6900±120	7000±130

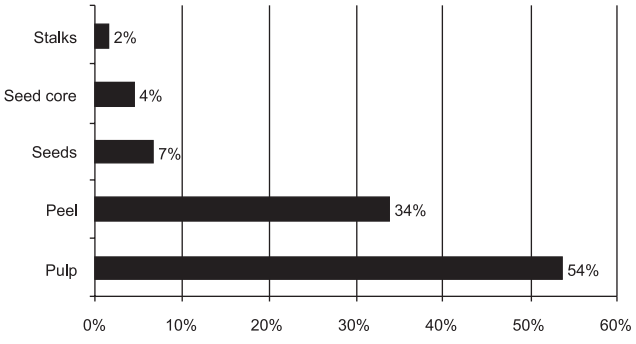

FIGURE 2. Morphological composition of apple pomace.

TABLE 2. Composition of pomace and dietary fibre preparations (mean value of 2 determinations).

	TDF (g/100g)	SDF (g/100)	SDF/TDF (%)
Apple pomace	60.9±0.89	7.7±0.25	12.6
Seedless DF preparation	64.1±0.75	8.3±0.35	12.9
Post extraction dietary fibre preparation	72.2±0.95	10.4±0.16	14.4

TABLE 4. Composition of solid glycosides preparations (mean value of 3 preparations).

Sum of polyphenols by HPLC (mg/g)	Sum of quercetin glycosides by HPLC (mg/g)
516±32	337±44

hawthorn leaf and flower liquid extract quantified according to European Pharmacopoeia contains from 0.8 to 3.0% of flavonoids expressed as hyperoside [European Pharmacopoeia 5.3, 2006]. Schieber *et al.* [2003] obtained a preparation containing 11.8% of phenolics. Commercial preparation of apple phenolics "Pomactiv HFV®" contains *ca*. 40% of phenolics in dry weight, including 15-20% of quercetin [Val de Vire, 2005]. Liquid preparation obtained in our experiments was less concentrated in phenolics, but contained 22 g/100 g pectins (which are very valuable as soluble dietary fiber), about 20 g/100 g sugars and 5% of acids. Such a preparation could possibly be used directly as an additive to drinks of special nutrition design. The admixture of solid quercetin glycosides preparations to apple juices could possibly increase the concentration of glycosides to 50 mg/L without negative taste effect (data not published). For now the addition of glycosides to commercial juices is not possible due to strict regulations concerning juices, but the preparations could be added to nectars and drinks in order to increase their health-promoting properties. Potential applications of phenolic solid extracts from apples are quite broad. They can be used in anti-radical, anti-aging preparations, in glycemia regulation products, hypocholesterolic preparations, skin and care products, skin pigmentation control, anti-cariogenic preparations or slimming products [Sanoner, 2005]. Besides nutraceutical applications, phenolic extracts can be used in cosmetics for hair and skin care, anti-decay and anti cellulite products. Broad application possibilities of apple polyphenols together with their not very broad applicability prompt a growing need for reasonable use of apple pomace as a source of valuable phenolic compounds and for new technologies of phenolic preparation production.

Poland with production of clear apple juice concentrates up to 200.000 tons processes yearly more than 1.5 mln t of apples. Commercial technologies of processing are very effective but 12 to 20% of processed raw material remains as a waste product after juicing.

Calculations lead to the conclusion that in Poland yearly 20.000 tons of dietary fibre and up to 250 tons of valuable phenolic compounds are simply wasted. According to our investigation dry weight of the pomace depending on juice technology production (application of enzymes, enzymation time) or pomace washing and repeated pressing may vary from 20 to 30%. Phenolics content in the pomace depends, to a large extent, on cultivar and processing technology. The use of apple pomace as a raw material should be extended to the production of nutraceutical products.

CONCLUSIONS

Industrial apple pomace resulting from a modern apple juice production plant can be considered as a raw material for direct preparation of dietary fibre, since it contains above 50% of TDF. Selection of raw material can result in higher quality dietary fibre preparations; raw material less useful for direct dietary fibre preparation due to its high polyphenol content can be used for production of phytocomponents concentrates. The use of such products as an admixture to fruit drinks is a very promising perspective for designing new food products.

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TABLE 3. Composition of liquid polyphenol extracts (mean value of 3 extracts).

Extract (%)	Pectins	Fructose	Other sugars	Sorbitol	Titratable acidity	Sum of polyphenols (mg/g DM)
	(g/100 g)					
54.3±2.8	22±4.4	14.8±0.8	5.3±2.5	2.4±0.3	5.1±0.1	25.7±3.8

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WYTŁOKI JABŁKOWE JAKO POTENCJALNE ŹRÓDŁO NUTRACEUTYKÓW

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Polska z produkcją koncentratu jabłkowego na poziomie do 200 000 ton przerabia corocznie ponad 1,5 mln ton jabłek. Przemysłowe technologie przerobu są bardzo wydajne, jednak 12 do 20% przerabianego surowca pozostaje po produkcji soku jako produkt odpadowy. Zwykle taki zawierający cukry, kwasy i śladowe ilości białka produkt jest wykorzystywany do pasz lub kompostowany. Wytłoki po produkcji soku zawierają znaczące ilości cennych prozdrowotnych składników takich jak polifenole i błonnik pokarmowy. Zawartość błonnika pokarmowego stanowi przeciętnie 50% suchej substancji. W prezentowanej pracy wykazano, że wytłoki z nowoczesnego zakładu produkującego soki zawierają 60-64% całkowitego błonnika pokarmowego, 1850-2250 mg/kg s.s. flawan-3-oli i 7000 mg/kg s.s. polifenoli (oznaczonych metodą Folin-Ciocalteau). Wykazano, że taki surowiec jest użyteczny do produkcji ciekłych i stałych ekstraktów polifenolowych, zawierających odpowiednio 2,5 i 33% polifenoli. Ekstrakty takie można uważać za znakomity składnik do otrzymywania nowych cennych produktów prozdrowotnych.