ASSESSING COMPARATIVELY THE BIOACTIVE COMPOUNDS COMPOSITION OF APPLE POMACE OBTAINED FROM THREE APPLE CULTIVARS AFTER JUICE EXTRACTION

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Abstract

The nutrients composition of dried apple pomace derived from three different apple cultivars (Granny Smith, Golden and Red delicious) were compared in the present study. The pomace was obtained by lyophilisation from fresh apples after juice extraction. Weende method was used to determine the proximate composition. Total polyphenols were determined by Folin-Ciocalteu method, individual polyphenols, carbohydrates and organic acids by HPLC while minerals were measured by atomic absorption spectrometry. The cultivar had no effect on protein and lipid percentage, but influences the concentration of different active molecules. Thus, Granny Smith pomace contained higher amount of dietary fiber (23.46%) compared to Golden (22.42%) and Red delicious (19.85%) as well as a higher level of natrium and iron. By contrast, Red delicious pomace had a larger amount of total polyphenols and epicatechin and a higher concentration of magnesium, potassium and zinc. No influence of apple cultivar on vitamin C level was notice. The introduction of this by-product into the feed chain have taken into account the variations in the chemical composition due to the apple variety.

Key words: *apple cultivar, apple pomace, composition, bioactive compounds*

INTRODUCTION

Apple is one of the most important fruit species registering the second worldwide production after banana [1], due to the nutritional qualities derived from its high content of micro- and macronutrients such as polyphenols, fiber, carbohydrates, organic acids, vitamins, minerals etc and last but not least due to some facilities for cultivation, transport, price storage, etc [2].

The bioactive compounds it contains give it beneficial properties for health including antioxidant, anti-inflammatory, anti-microbial, anti-cancer activity [3]. Thus, apples are rich in polyphenols, a class of powerful antioxidants with recognized benefits for human and animal health. Accumulating findings showed that polyphenols alleviated inflammation and oxidative stress, two process involved in intestinal inflammatory diseases like Crohn's disease and ulcerative colitis, rheumatoid arthritis or other degenerative diseases (cardiovascular disease, diabetes mellitus etc [4]. Resveratrol, for example, a polyphenol with important biologic activity downregulated the MAPK pathway leading to a reduction of intestinal pro-inflammatory cytokines which include tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1 (IL-1β) in weaned pigs and reduced also the level of TNF-α in plasma of patients with ulcerative colitis [5,6]. Study of [7] demonstrated that polyphenols extract from apple is able to decreased *Clostridioides difficile*-induced

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intestinal infections in mice by modulating intestinal microbiota, enhancing anti-inflammatory effects and colon barrier integrity. Catechins polyphenols and phytosterols (e.g., β-sitosterol), found in different fruits, apples included has the capacity to reduce the oxidative stress generated in the liver by preventing the lipid oxidation and reduction of fat in the body of experimental obese mouse C57BL/6 J [8] as well as the levels of low-density lipid cholesterol (LDL cholesterol), pro-inflammatory cytokines, triglycerides.

Large quantities of apples are consumed fresh (~200g/capita/day), but also as juice and many other culinary preparations. About 30% of apple production are used to obtain apple juice, cider, wine, distilled spirit and dried products. About 25-30% of the total processed fruits remain as solid residue/waste [9,10]. A large and significant amount of apple pomace containing active phytoconstituents (e. g. dietary fibre, phenolic, terpenic compounds, minerals, vitamins etc) with potential beneficial for health (increased lipid metabolism, hypoglycaemic, hypocholesterolaemic and anti-cancerogenic activity, anti-inflammatory, antiviral, antibacterial, antiallergic and anticoagulant effects [9,11,12] remains available. The pomace has higher content of nutrients than the apple itself [11] and could be also added safely in the feed for farm animals (e.g., pigs, [13]. It was shown that dietary inclusion of apple pomace in weaning pigs improved blood parameters, intestinal bacteria and gene expression of immune parameters [14]. Diet with apple pomace increased the antioxidant capacity and activity of antioxidant enzymes (superoxide dismutase) in blood and serum of rats and reduced blood glucose and [15]. The effect of polyphenol from apple peel, another by-product derived from apple on intestinal ecology in Balb/c mice revealed an increase in diversity of intestinal flora especially of beneficial bacteria as well as of the content of short-chain fatty acids. From the immunological point of view an upregulation of tight junction proteins and the downregulation of the TLR4 and NF-κB protein and mRNA expression was also observed [16]. Gastroprotective effects against indomethacin-induced ulcer either by the inhibition or stimulation of two key genes involved in inflammation and oxidative stress, cyclooxygenase (COX-2) and heat shock protein 70 (HSP-70) and subsequent reduction of inflammatory cytokines (TNF-alpha, IL-6) and of oxidative stress markers (MDA) was reported by [3] after feeding Wistar rats with 100 and 200 mg of apple leaves extract.

The composition and quality of by-products derived from apples are influenced by different factors, for example, the cultivars, climate and soil condition, the enzymes used for juice extraction, the method used for pressing and the drying process, [13,17,18]. That is why the aim of the present study was to compare the proximate chemical composition and the content in several phyto-active constituents of three apple pomaces derived from three different cultivars in order to include the best one in the diet of pigs after weaning. Chemical characterization is important for feed producers and nutritionists to establish the optimal level of inclusion in the animal diet.

MATERIAL AND METHOD

Obtention of apple pomace

Pomace was obtained by lyophilisation (LyoQest-55 freeze dryer) in 45 hours and 4 phases from three commercial cultivars apple, Granny Smith, Golden and Red delicious apples. The biomass was grinding to obtain a fine powder.

Measurement of apple pomace cultivar proximate chemical composition

Weende method (Commission Regulation (EC) No 152/2009) was used to determine the proximate chemical composition. According with EC regulation (EC/152/2009, SR ISO 6496:2001), the dry matter substance was determined by the gravimetric method meaning the drying samples at 103^{0}C until constant weight using ECOCELL (Neuremberg, Germany) oven for drying and a Gridomix GM 200 mill for grinding. Crude protein was determined by the measurement of nitrogen concentration following the Kjeldahl principle (EC/152/2009 SR EN ISO 5983- 2:2009 AOAC 2001.11) using a FOSS Tecator1030—FOSS Tecator AB (Höganäs, Sweden). Crude fat was measured by using Soxhlet method, extraction with organic solvents (EC/ 152/2009 SR ISO 6492:2001) and a FOSS Tecator SOXTEC-HT 1043 (Höganäs, Sweden). Crude cellulose was determined by the intermediate filtration method (EC/152/2009 SR EN ISO 6865:2002) using a FOSS Tecator FIBERTEC 2010 (Höganäs, Sweden). Neutral and acid detergent fibre fractions was measured with a Van Soest method (SR EN ISO 16472:2006) and a FOSS Tecator FIBERTEC 2010 (Höganäs, Sweden). Ash was determined based on gravimetric method (EC/152/2009SR EN ISO 2171:2010) and a Nabertherm calcination furnace (Nabertherm GmbH, Lilienthal, Germany).

Measurement of total polyphenol from dried apple pomace cultivars

The total polyphenol concentration was measured by the Folin-Ciocalteu method after the extraction in acetone 80% p.a., (1:7, w/v). A mix of apple extracts, water and Folin-Ciocalteau reagent plus Na2CO2 (20%) was vortexed and left for 2 hours in the dark. The absorbance of each apple mix was read at 750 nm, using UV-Vis Spectrophotometer Specord 205 (Analytik Jena, GMbH, Germany) and the concentration of total polyphenols was calculated from a calibration curve obtained by using gallic acid as a standard. The results were expressed in mg gallic acid equivalent per 100 g dry weight (mg GAE/100 g).

Measurement of antioxidant activity of dried apple pomace cultivars

The antioxidant activity of apple meal extracts was determined as described by Taranu et al., (2018) consisting in the measurement of their radical scavenging ability by using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, an aliquot from a mixture of diluted apple extract and DPPH solution (60µM in methanol) was vortexed and the absorbance was read at 515 nm and two times (0 and 30 minutes) with a Specord 250 (Analytik Jena, Jena, Germany) spectrophotometer. The results were expressed as µM of Trolox equivalent (TRE).

Measurement of individual phenolic compounds from dried apple pomace cultivars

Individual phenolic compounds (flavonoids and phenolic acids) were measured by HPLC gradient methods as described by [19] and using a Jasco Chromatograph (Jasco Corporation, Tokyo, Japan) equipped with UV/Vis detector, Refractive Index detector and an injection valve. Apple samples extracted in methanol 80% (Vlassa et al., 2022) were separated by gradient on Lichrosorb RP-C18 column using as mobile phase a mix of methanol (A, HPLC grade) and 0.1% formic acid solution (ultrapure water). Chromatographic data collection and processing was carried out with ChromPass software.

Measurement of carbohydrates from dried apple pomace cultivars

The samples of dried apple meal were subjected to an aqueous extraction (Millipore ultrapure water) and the carbohydrates (fructose, glucose, sucrose, maltose) were separated on Kromasil-NH2 column as described by [19]. The elution of extract samples was performed with acetonitrile–water, 70:30 (v/v) as the mobile phase, a flow rate of 1 mL/min and column temperature 25^0 C and RI detection.

Measurement of organic acids from dried apple pomace cultivars

The organic acids (oxalic, citric, tartric, succinic, malic and fumaric) were extracted with water (Millipore ultrapure water), separated on a CarboSep Coregel 87H3 column with sulfuric acid (5µM) eluent solution and detected by HPLC as described by [19]. The flow rate was 1 mL/min and the detection was by UV at 214 nm.

Measurement of minerals from dried apple pomace cultivars

Macro-elements Calcium (Ca), Natrium (Na), Potassium (K) and Magnesium (Mg) concentration was determined by atomic absorption spectrometry according to the standard SR ISO 6490-2: 1983 for Ca and SR ISO 7485: 2000 for Na, K, Mg using an atomic absorption spectrophotometer Thermo Electron (SOLAAR M6 Dual Zeeman Comfort, Cambridge, UK). The concentration of micro-minerals Copper (Cu), Iron (Fe), Zinc (Zn) and Manganese (Mn) was measured by atomic absorption spectrometry (SOLAAR M6 Dual Zeeman Comfort, Cambridge, UK) after microwave digestion and mineralization with nitric acid (EC/152/2009 STAS 9597 / 16-86STAS 9597 / 17-86).

Statistical analysis

The effect of apple cultivar on the different components were evaluated using one-way ANOVA and Student-t test followed by Fisher's least square difference procedure in order to differentiate between all determined parameters. *P*-value between 0.05 and 0.1 or equal to 0.1 was considered a trend. Results represent the means ± standard error (SEM) from two independent determinations.

RESULTS AND DISCUSSION

Effects of the cultivar on the proximate chemical composition of the apple pomace

Table 1 showed that the three apple pomace cultivars analysed had a low content of protein and fat, but similar to the results described by [11]. Nevertheless, these authors reported that the pomace contains a higher amount of protein and fat than those in the whole apple probably due to the content of the seed (2.7-5.3 protein in pomace *versus* 0.24-0.28 in whole apple and 1.1-3.6 fat in pomace *versus* 0.16-0.18 in whole apple). Even higher values of protein and fat was determined by [20] in apple pomace (6.91% and 3.30 respectively). The protein level did not differ significantly among the three apple pomace cultivars analysed in this study (Table 1). However, the crude fat and ash registered significant higher level in Red delicious pomace compared to Granny Smith or Golden while cellulose was higher in Golden than in Granny Smith and Red delicious. According to [21] and [22] the apple cultivars can influence the physico-chemical composition of apple residue. Differences in the content of protein, lipid and especially of total polyphenol content was reported by [21] who investigated the physicochemical composition and antioxidant capacity of pomace from eleven apple cultivars (cv. 1-Catarina, cv. 2-Joaquina, cv. 3-M-ll/00, cv. 4-M-ll/01, cv. 5-M-ll/00 AGR, cv. 6-M-12/00, cv. 7-M-13/(00), cv. 8-M-2/00, cv. 9-M-6/00, cv. 10-M-8/01 and cv. 11-MRC-11/95). Differences in basic composition were found also by [23] in an apple pomace derived from 17 varieties of Irish apple used for cider production (protein, 2.4%DM, fat 2.7%DM and ash 1.7%DM). By contrast [9] which reported a higher concentration of crude protein and fat from a mixture of various varieties of apples grown in eastern Poland (3.8g/100g DM for protein, 3.8g/ 100g DM for fat and 1.8g 100g DM for ash) affirmed that the differences in chemical composition due to the composition of the soil, climate and apple varieties are few. There are also authors who determined very low concentrations of protein, fat and ash in apple pomace such as 1.2 for protein, 0.6 for fat 2.5 for ash respectively [24].

Table 1 Proximate chemical composition of dried apple pomace cultivars

Chemical composition (%)	Apple cultivars			SEM	*p*- value
	Granny Smith	Golden	Red delicious		
Dry mater	94.55a	95.04a	89.96b	0.823	0.005
Crude Protein	2.96	2.43	2.45	0.067	0.112
Crude fat	1.08a	0.76c	1.16a	0.062	0.001
Cellulose	15.09a	14.48a	13.09b	0.203	0.001
Ash	2.16a	1.48c	1.75b	0.043	0.035

SEM=standard error of the mean

Effects of the cultivar on the dietary fiber of apple pomace

Our chemical analysis showed that the apple pomace is very rich in dietary fiber, the values ranging from 13.09% in Red delicious to 14.48% in Golden ($p \leq 0.05$) for cellulose with difference between cultivars (Table 1). As for detergent fiber fractions, the NDF was higher than ADF, the highest level been obtained in both cases for Granny Smith cultivar and the lowest for Red delicious (Table 2). As in other studies fiber are the most important compounds with the highest concentration in apple pomace [9,21]. These authors highlighted the important role of dietary fiber within the physiological processes by their contribution to the formation of short chain fatty acids in the large intestine, in the reduction of hyperglycaemic level and the maintenance of normal cholesterol [9,21]. For example, in a feeding study on rats with induced obesity, the results showed that rats receiving the diet supplemented with 10% apple pomace had reduced body fat percentage, lower level of LDL-cholesterol and increased HDL-cholesterol in comparison to the rats fed the high fat diet [11].

Table 2 Fiber composition of dried apple meal cultivars

Fiber (%)	Apple cultivars			SEM	*p*- value
	Granny Smith	Golden	Red delicious		
Neutral detergent fiber (NDF)	23.46a	22.42a	19.85b	0.110	0.002
Acid detergent fiber (ADF)	14.91a	13.89b	14.90a	0.014	0.547

SEM=standard error of the mean

Effects of the cultivar on the carbohydrates content of apple pomace

Studies analysing the chemical composition of apple and apple pomace have indicated that carbohydrates are the constituents with high concentration in apple and apple pomace both of them being important sources of fructose, glucose etc [1,7], valuable substances for their positive health effects. Moreover, the pomace which contained seeds has even a higher content of fructose and glucose than apple. It is well known that carbohydrates are fermented in the large intestine by bacteria and other microorganism resulting in short-chain fatty acids that are particularly important in various metabolic processes in the intestine and distant sites, such as the muscle, liver, and brain [25]. Especially, butiric acid has an important role on colon epithelial cells by reducing the inflammation and improving the barrier function [1]. Like as in the case of other constituents, it seems that the apple variety influences the carbohydrate content. For example, [1] found that apple pomace from Belgian apple cultivar used for juice extraction had a significant higher content of fructose

(55g/100g dw) than Spanish apple cultivar (46g/100g dw) while pomace derived from Spanish cultivar presented a higher concentration of sucrose (7.4g/100 dw) than Belgian one (1.8g /100 dw). In our study we also observed differences between different level of carbohydrates, fructose and glucose being higher in Golden cultivar and Red delicious and Granny Smith (Table 3). Sucrose and maltose are in lower concentration in all the three cultivars.

Table 3 Carbohydrates composition of dried apple meal cultivars

Carbohydrates (g/100g)	Apple cultivars			SEM	*p*- value
	Granny Smith	Golden	Red delicious		
Fructose	8.96b	31.62a	28.18a	7.05	0.05
Glucose	9.31	11.22	12.94	1.05	0.04
Sucrose	0.023	0.076	0.027	0.02	0.05
Maltose	0.012	0.00	0.00	0.04	0.00

SEM=standard error of the mean

Effects of the cultivar on the polyphenols content of apple pomace

According to [11] apple pomace of all apple cultivars contains important concentration of polyphenols located mainly in the skin representing 95%. They are valuable active compounds with a lot of health benefits for both animal and human. In piglets for example, they improve the digestive and the fermentative process [13], increase the diversity of intestinal flora and decrease the intestinal inflammation [26,27]. There are evidences showing that the apple cultivar can influence the amount of polyphenols in the apple and apple pomace. Thus, by comparing six single-cultivars (Limon Montes, Meana, Durona de tresali, De la Riga, Perezosa and Carrio) used from cider production, [28] reported that the phenolic content and the antioxidant capacity differ significantly. Similarly, the analysis of the chemical composition of apple pomace derived from eleven apple cultivars highlighted a variation of total phenolic compounds from 2.29 to 7.15g/kg and of antioxidant capacity from 17.41 to 77.48 mMol/g [21]. Also, an even wide range of variation (66.2 to 211.9mg/100g) in the polyphenolic content was reported by [29] after investigation of the total polyphenols from 40 apple varieties. Significant differences concerning the total polyphenol level were also observed between the three apple cultivar pomaces from our study (Table 4), Red delicious cultivar containing the highest amount (+32.50% versus Golden and +6.38% versus Granny Smith) of total polyphenol and by consequence the highest DPPH activity which increased with 44.77% than Golden and with 22.38% than Granny Smith).

Table 4 Total polyphenol composition and antioxidant activity (DPPH) of dried apple meal cultivars

Item	Apple cultivars			SEM	p- value
	Granny Smith	Golden	Red delicious		
Total polyphenols (mg GAE/100 g)	711.20a	512.80b	759.70a	0.523	0.002
DPPH activity (mM Trolox Equivalent)	0.520b	0.370c	0.670a	0.357	0.003

SEM=standard error of the mean

As for the classes of polyphenols the pomace derived from Red delicious cultivar are also characterized by the highest content in catechin and epicatechin two of the most important flavonoids which act as powerful antioxidants in comparison with Golden and Granny Smith pomace (Table 5). Pomace of Golden cultivar contained however a larger number of polyphenols even in a lower concentration. The results showed clearly the influence of apple variety on polyphenols level (table 5).

Table 5 Classes of polyphenols of dried apple meal cultivars

Polyphenols (mg/g)	Apple cultivars			SEM	*p*- value
	Granny Smith	Golden	Red delicious		
Catechin	9.32b	3.74c	11.42a	2.29	0.005
Epicatechin	11.56c	29.33b	47.65a	9.42	0.050
Vanilic acid	0.19b	1.74a	-	0.55	0.001
Cafeic acid	1.56b	-	4.03a	1.17	0.045
p-Cumaric acid	0.16b	-	1.52a	0.48	0.003
Ferulic acid	-	16.70	-	-	-
Rutin	11.68a	3.93c	6.77b	2.65	0.012
Quercitin	1.11a	0.12b	-	0.35	0.056
Luteolin	-	0.47	-	-	-

SEM=standard error of the mean

Effects of the cultivar on the mineral and vitamin C content of apple pomace

Apple pomace are also a valuable source of minerals for both macro- and micro-elements. Significant concentration (p<0/05) of macroelements such as Calcium, Potassium and Magnesium was found in the Red delicious dried pomace compared with Golden and Granny Smith. Similar concentration was reported by [10] Bhushan et al., (2009), but higher minerals concentration was found by Pieska et al.,[20] An appreciable content in zinc was also recorded in Red delicious pomace (+185.86% against that in Granny Smith and +449.42% in Golden pomace), a very important result taken into consideration the essential role of this micro-element in the function of over 300 enzymes and different metabolic processes (e. g. metabolism of RNA and DNA, signal transduction and gene expression, transport of albumin and transferrin etc). Zinc is cofactor for many enzyme classes [30], being involved in the immune response (e. g. regulation of apoptosis, [31]. By contrast, the analysed pomaces showed a grater increase ($p<0.05$) in iron in the dried pomace derived from Granny Smith cultivar compared with Red delicious and Golden pomaces. Like zinc, iron is a very important compound due to its exceptional biological properties of which the most known is the presence in heme proteins (haemoglobin, myoglobin, cytochrome P450) with their essential and various physiological implication.

Table 6 Minerals composition of dried apple meal cultivars

	Apple cultivars			SEM	*p*- value
	Granny Smith	Golden	Red delicious		
Calcium (Ca, %)	0.040b	0.040b	0.380a	0.113	0.642
Phosphorus (P, %)	0.110	0.110	0.110	0.000	0.100
Magnesium (Mg, %)	0.011c	0.060b	0.105a	0.030	0.045
Natrium (Na, %)	0.010a	0.008a	0.006b	0.001	0.040
Potassium (K, %)	0.947b	0.746c	1.216a	0.136	0.012
Iron (Fe, ppm)	50.22a	26.09	34.91b	7.055	0.031
Manganese (Mn, ppm)	4.00c	6.07a	5.06b	0.598	0.021
Zinc (Zn, ppm)	6.65b	3.46c	19.01a	4.742	0.001

SEM=standard error of the mean

Effects of the cultivar on the organic acids of apple pomace

Among other nutrients contained by apple pomace are counted the organic acids that include hydroxyl or carboxyl group. They are very important for their effect on bacteria, especially those bacteria called "*pH-sensitive*" which cannot tolerate a large pH gradient such as *Escherichia coli, Salmonella spp., Clostridium perfringens, Listeria etc.* That is why, apple pomace can be used in food and feed preservation. Organic acids are also known for their beneficial effect on animal performance [19]. The growth rate increased in piglets after weaning fed diet containing organic acids which reduced gastric pH preventing the pathogen multiplication and increased apparent total tract digestibility [32]. The level of organic acids differed from one cultivar to another. However, similarities between the concentrations of organic acids (citric, malic, oxalic) between Granny Smith and Golden pomace were recorded. Fumaric acid was lower and tartric acid was higher in Granny Smith than in Golden and Red delicious. The available literature showed also higher or similar level of organic acids in dried pomace derived from Romanian Johnatan cultivar [33] apart malic acid. Comparing the concentration of organic acids from crude apple and dried apple pomace these authors found that drying process enriched the concentration of acids, higher amount being recovered in pomace than in crude apple [33].

Table 7 Organic acid composition of dried apple meal cultivars

Acids (mg /100g)	**Apple cultivars**			**SEM**	***p*- value**
	Granny Smith	**Golden**	**Red delicious**		
Oxalic acid	0.0	0.0	0.0	-	-
Citric acid	30.90a	27.40a	16.90c	1.55	0.023
Tartric acid	2.80	0.0	0.0	-	-
Succinic acid	548.45b	803.40a	869.80a	97.94	0.049
Malic acid	2912.04a	2448.30a	1981.40b	13.20	0.048
Fumaric acid	0.66b	1.20a	1.20a	0.18	0.05

CONCLUSIONS

In this study we assessed comparatively the influence of cultivar on content and the concentration of several bioactive compounds of apple pomace derived from three apple cultivars (Granny Smith, Golden and Red delicious). Similarly with other literature data, our results showed that irrespective of the cultivar, apple pomace contains active biomolecules with important and beneficial role for animal and human health as dietary fiber, polyphenols, etc). The cultivar did not influence the protein and lipid percentage of apple pomace, but influence the level of several bioactive constituents. Thus, apple pomace is rich in dietary fiber and from the three cultivars, Granny Smith contained the higher level as well as the higher level of natrium and iron. By contrast, Red delicious cultivar had a larger amount of total polyphenols and epicatechin and a higher concentration of magnesium, potassium and zinc. These results show that the introduction of this by-product into the feed chain have take into account the variations in the chemical composition due to the apple variety.

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