AgroLife Scientific Journal - Volume 7, Number 1, 2018
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

ACHIEVING OF FUNCTIONAL INGREDIENT FROM APPLE WASTES RESULTING FROM THE APPLE JUICE INDUSTRY

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Abstract

At industrial level, by apple processing into juice results significant amounts of waste, representing 25-40% of the mass of processed apple depending on the technology applied for juice extraction. At European level, by apple processing into juice results annual millions of tonnes of waste. Apple waste is a rich source of food fibres (cellulose, hemicellulose, lignin, pectin), minerals (calcium, magnesium, potassium, zinc, iron, etc.), phenolic compounds, mainly phenolic acids such as chlorogenic acid, protocatechuic acid and caffeic acid and flavonoids. Also, apple waste contains simple sugars (glucose, fructose and sucrose). In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from apple waste resulting in apple juice industry. For this purpose, apple waste (Jonathan, Golden Delicious and Red Delicious varieties) was subjected to a convective drying process at 50°C to protect the bioactive compounds (vitamins, phenolic compounds, etc.) to a moisture content to allow their milling and turning them into powder and their stability in terms of quality. The achieved functional ingredient was evaluated sensory, physic-chemically and microbiologically. Powder achieved from apple waste is characterized by total dietary fibre (60.62...64.75%), iron (2.31....2.73 mg/100 g), potassium (450.12... 508.45 mg/100 g), calcium (76.32 ....92.44 mg/100 g), magnesium (41.15....55.65 mg/100 g) and total polyphenol content (17.83 ...38.83 mg GAE/g). At the same time, powder achieved from apple waste has antioxidant potential. Due to its complex biochemical composition and antioxidant potential, the functional ingredient achieved from apple waste can be used to fortify bakery and pastry products.

***Key words**: apple, pomace, powder, total polyphenol, dietary fibre.*

INTRODUCTION

Apples are one of the most consumed fruits in the world and are among the main sources of phytochemicals and antioxidants in the human diet. Approximately 70 million tons of apples are produced globally per year (Massias et al., 2015). Apple fruits have a complex and balanced biochemical composition represented by vitamins, minerals, sugars, organic acids, fibres and phenolic compounds, but has a moderate energy value (Feliciano et al., 2010).

Apple is a good source of fibre with well-balanced proportion between soluble and insoluble fractions (Gorinstein et al., 2001). Also, apple fibres have better quality as compared to other dietary fibres due to presence of associated bioactive compounds (Fernendez-Gives et al., 2003; Wolfe and Liu, 2003). The prevention of various chronic diseases, especially cardiovascular diseases, was associated with apple consumption due to their content in bioactive compounds (polyphenols and fibres) (Rupasinghe et al., 2012; Serra et al., 2012). Apples contain over 60 phenolic compounds (Rupasinghe et al., 2012).

By apple processing into juice results significant amounts of waste, representing 25 - 40% of the mass of processed apples depending on the technology applied for juice extraction. In Germany, by apple processing are produced annually 200 to 250 ktons of wet apple pomace, followed by Japan, Iran, USA, Spain, and New Zealand, which reported quantities of 160, 97, 27, and 20 ktons per year, of waste resulted (Kammerer et al., 2014). In Romania, apple processing into juice is achieved both in industrial units and in small and medium capacity plants, in family business, resulting large quantities of waste. Apple waste - apple pomace - is a heterogeneous mixture of peel, seeds, calyx, stem and pulp (Paganini et al., 2005; Macagnan et al., 2015). Perussello et al. (2017) noticed that apple pomace is mainly composed of skin and flesh (95%), seeds (2% to 4%), and stems (1%). Apple pomace contains simple sugars (glucose, fructose, and sucrose), carbohydrate, pectin, crude fibre, proteins, vitamins and minerals, and as such is a good source of nutrients, worth to be recovered. Also several microorganisms can use apple residues as a substrate for growth (Kosseva, 2011; Mirabella et al., 2014).

Apple pomace has a complex biochemical composition: total nitrogen (6.8 g/kg DW), total carbon (6.8 g/kg DW), cellulose (127.9 g/kg DW), hemicellulose (7.2 to 43.6 g/kg DW), lignin (15.3 to 23.5), pectin (3.5% to 14.3% db), total carbohydrate (48.0% to 83.8% db), fiber (4.7% to 51.1% db), protein (2.9% to 5.1% db), lipids (1.2% to 3.9% db), reducing sugars (10.8% to 15.0% db), among which glucose (22.7%), fructose (23.6%), sucrose (1.8%), arabinose (14% to 23%), galactose (6% to 15%), and xylose (1.1%). Also, apple pomace isnoted for its mineral content, such as Ca, Fe, Mg, P (Dhillon et al., 2013). Apple pomaceis a richsource of phytochemicals such as phenolicacids (chlorogenic, protocatechuic, and caffeic acid) and flavonoids, e.g. flavanols and flavonols, presenting antioxidant potential (Cetkovic et al., 2008; Diňeiro Garcia et al., 2009; Kohajdová et al., 2014). In addition, it has been shown that polyphenols in apple peel have beneficial effects on oxidative stress and inflammation (Denis et al., 2013). At the same time, the phytochemicals which are present in apple pomace have beneficial effects on the human body such as decrease in lipid oxidation and lower cholesterol, and reducing of incidence of chronic diseases, such as heart disease, obesity and cancer (Yadav and Gupta, 2015).

Apple pomace has more pro-health compounds than processed products, like juice, nectar and other drinks which are often clarification or diluted (Kruczek et al., 2017). In this paper are presented the results of the research performed to achieve a functional ingredient (powder) from apple waste resulting in apple juice industry.

MATERIALS AND METHODS

Samples

Apple pomace resulted by apple processing into juice within the Pilot Experiments Plant for Fruits and Vegetables Processing in IBA Bucharest, using a juicer extractor (Philips). Within experiments were used three apple varieties: *Jonathan, Golden Delicious* and *Red Delicious.* Apple pomace was subjected to dehydration process in a convection dryer at temperature 50ºC to a moisture which allows their milling and conversion into flours and, at the same time, their stability in terms of quality. Milling of dried semi-finished products was performed by using Retsch mill. The achieved functional ingredients (powders) were packed in glass containers, hermetically sealed, protected by aluminum foil against light and stored in dry and cool areas (temperature of maximum 20ºC), till to the sensory, physic-chemical and microbiological analysis. In Figure 1 are presented the powders mentioned above.

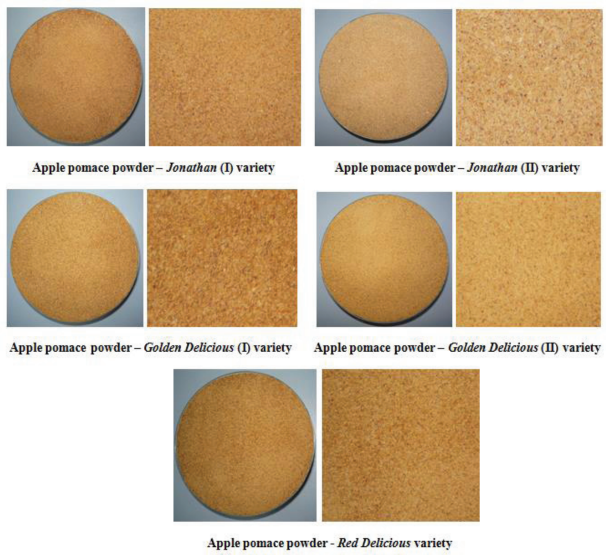

Figure 1. Apple pomace powder

Methods

Sensory analysis

Sensory analysis (appearance, taste and smell) was performed by descriptive method.

Physic-chemical analysis

Measurement of the color parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), *L**, *a** and *b**: *L** - color luminance (0 = black, 100 = white); *a** - red-green coordinate (-a = green, +a = red); *b** - yellow-blue coordinate (-b = blue, +b = yellow).

Moisture determination was performed with Ohaus Moisture Analyzer MB45 at temperature 105°C.

Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according to AOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).

In order to determine minerals samples were mineralized by calcination, with the addition of hydrochloric acid and hydrogen peroxide. The minerals sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and zinc (Zn) were determined by Atomic Absorption Spectrophotometer (type *AAnalyst* 400, Perkin–Elmer). Iron (Fe) was determined by Graphite Furnace Atomic Absorption Spectrophotometer (type *AAnalyst* 600, Perkin–Elmer).

Total sugar content was determined according to Schoorl method.

Total dietary fibre (TDF) was determined by enzymatic method using the assay kits: K-TDFR ''Total dietary fibre'' (AOAC Method 991.43).

Total polyphenol content

Total polyphenol content was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extraction media, time and mode of extraction, extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in three extraction media (methanol: water = 50:50; ethanol: water = 50:50; acetone: water = 70:30) and the absorbance of the extracts were determined at a wave lenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents (GAE) per g apple pomace powder.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald and Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g apple pomace powder.

Microbiological analysis

The water activity (*A*w) was determined by an instrument Aquaspector AQS-2-TC, Nagy. The measurements were performed at 25ºC. Yeasts and molds were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* were determined according to the SR ISO 21528-2:2008 method and *Escherichia coli* by SR ISO 16649-2:2007 method. *Salmonella* was determined by the method SR EN ISO 6579:2003/AC: 2006.

RESULTS AND DISCUSSIONS

Sensory analysis

After sensory analysis it was found that the obtained powders have specific characteristics. Powders obtained from the apple waste arein the form of dark beige-to-light brown powder, reddish tint, with characteristic pleasant taste and smell.

Following instrumental colour analysis (Figure 2), it was found that apple pomace powder - *Jonathan* (I) variety is the darkest, recording the minimum luminance value (L* = 55.80), while apple pomace powder - *Red Delicious* variety is the lightest (L* = 65.56). Apple pomace powder - *Golden Delicious* variety recorded luminance values close to that of apple pomace powder - *Red Delicious* variety (L* = 61.31, respectively, L* = 63.30).

Also, the minimum positive values of parameter a* (red colour coordinate) and parameter b* (yellow colour coordinate) were recorded in case of apple pomace powder - *Red Delicious* variety.

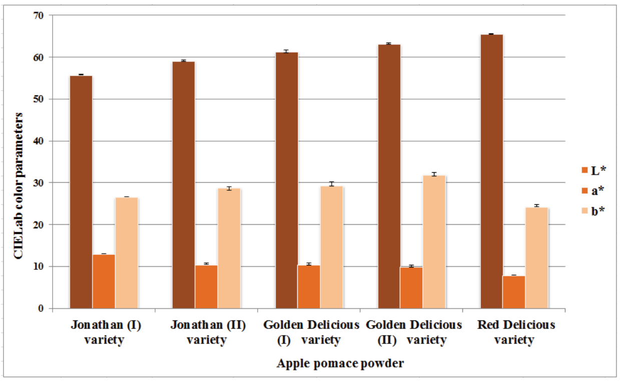

Figure 2. Colour parameters of the powders achieved from apple pomace

Physic-chemical analysis

Composition of the powders achieved from apple waste is presented in Table 1. Water content of apple pomace powders resulted by processing of apple varieties studied into juice *(Jonathan, Golden Delicious* and *Red Delicious)* is comparable to that reported by Younas et al. (2015) (7.82±0.2%, in case of *Kala Kullu* variety).

Table 1. Physic-chemical composition of powders achieved from apple waste

Apple pomace powder variety	Water (%)	Ash (%)	Protein (%)	Fat (%)	Total sugar (%)	Total fibre (%)
Jonathan (I)	7.67±0.19	1.69±0.02	4.57±0.04	2.08±0.023	18.56±0.053	64.57±1.20
Jonathan (II)	7,49±0.18	1.73±0.02	4.62±0.04	2.27±0.025	18.69±0.053	64.75±1.20
Golden Delicious (I)	7.35±0.18	1.87±0.03	4.75±0.04	2.75±0.030	19.22±0.055	60.85±1.13
Golden Delicious (II)	7.22±0.18	1.92±0.03	4.84±0.04	2.84±0.031	19.48±0.056	60.62±1.12
Red Delicious	7.43±0.18	1.75±0.03	4.55±0.04	2.21±0.024	19.25±0.055	63.75±1.18

Ash content of apple pomace powders varied in the range 1.69 - 1.92% (the minimum value was recorded in case of apple pomace powder - *Jonathan* (I) variety, and the maximum one in case of apple pomace powder - *Golden Delicious* (II) variety). Ash content of apple pomace powder is comparable with that reported by Younas et al. (2015) (1.66±0.10%, respectively, 1.88±0.13%), but higher than that presented by O'Shea et al. (2015) (1.60±0.02%). The protein content of apple pomace powders of three varieties is higher than that reported by Younas et al. (2015) (4.53±0.37%, respectively, 3.43±0.03%) and by O'Shea et al. (2015) (2.37±0.12%).

Powders achieved from apple waste have the lipid content in the range 2.08-2.84%, comparable to that reported by O'Shea et al. (2015) (2.27±0.23%), but higher than that presented by Yadav and Gupta (2015). It is noted the high content of total sugar (expressed as % invert sugar) of apple pomace powders, obtained from experiments. Values obtained for this chemical parameter varied within the small range (18.56 - 19.48%).

Apple pomace powders are important sources of dietary fibre. The total fibre content varied in the range 60.62- 64.75% (the minimum value was recorded in case of apple pomace powder - *Golden Delicious* (II) variety, and the maximum one in case of apple pomace powder - *Jonathan* (II) variety).The fibre content of apple pomace powders obtained in experiments is higher compared to that reported by Yadav and Gupta (2015), respectively 30.86%. Dietary fibre intake in Western countries is of 18 g per person per day, but, according World Health Organization, population's fibre intake should increase to 30 g a day (British nutrition fundation, 2015). A proper intake of dietary fibre in diet has beneficial effects on health, such as regulation of intestinal transit, prevention of diabetes, cardiovascular diseases and colon cancer. Food fibres reduce the risk of hyperlipidaemia, hypercholesterolemia and hyperglycaemia, through specific mechanisms that modulate and influence the digestion, absorption and metabolism of nutrients (Macagnan et al., 2015). Apple pomace is a good source not only of total dietary fibre, but also contains a large amount of soluble dietary fibre, represented by pectin (Shah and Masoodi, 1994; Issar et al., 2016). Apple pectin is distinguished by a high degree of esterification, has a prebiotic effect and is fermented by the microflora in the large intestine. Researches in the field have shown a relationship between pectin consumption and maintenance of normal blood cholesterol levels or a reduction of post-prandial glycaemic responses (Ferretti et al., 2014).

The powders achieved from apple waste are an important source of minerals (K, Ca, Mg, Fe, Zn). Their content in minerals is presented in Figures 3 and 4.

Some mineral elements, such as sodium and potassium, have mainly electrolyte function;

others such as magnesium and calcium serve a structural function in addition to acting as electrolytes.

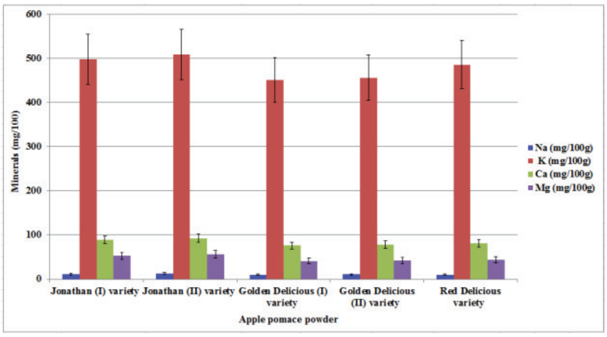

Figure 3. Mineral content (Na, K, Ca, Mg) of the powders achieved from apple pomace

Also, many minerals interfere for the production and function of a wide variety of enzymes necessary for normal metabolic reactions. Other minerals have an important role in hormones synthesis, antioxidant activity, cell signalling, and energy production (Mahan et al., 2012; Mueller, 2012; NIH, 2016).

Apple pomace powders have high potassium content in the range 450.12-508.45 mg/100 g, the maximum value being recorded by apple pomace powder - *Jonathan* (II) variety. Their potassium content is higher than that reported by O'Shea et al. (2015) (253.1±28.4 mg/100 g). Potassium is important in lowering blood pressure. A high-potassium diet reduces cardiovascular disease or renal disease and decrease the risk of osteoporosis (He and MacGregor, 2008). Also, Sousa et al. (2014) mention that this may lead to a balance of minerals, which favours hypertension control.

The calcium content of the powders achieved from apple pomace varied in a relatively small range (76.32 - 92.44 mg/100 g), the maximum value being recorded by apple pomace powder - *Jonathan* (II) variety. The results obtained for the calcium content are consistent with those reported by Dhillon et al. (2013) (60 – 100 mg/100 g), but smaller than those presented by O'Shea et al. (2015) (126.5±14.2 mg/100 g). Also, the powders achieved from apple pomace are noted by magnesium content (41.15 - 55.65 mg/100 g), which is 3.27 - 4.42 times higher than that reported by O'Shea et al. (2015) (12.6±1.42 mg/100 g).

Iron content of the powders achieved from apple pomace in this study varied in the range 2.31 - 2.73 mg/100 g.

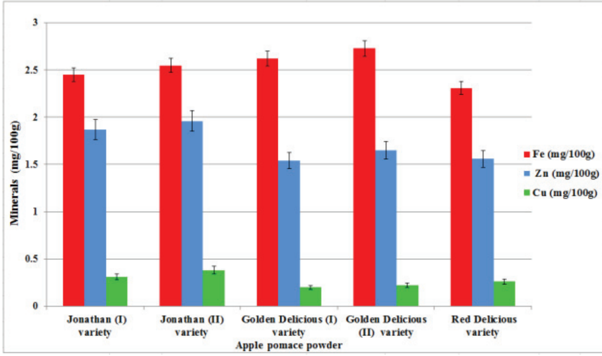

Figure 4. Mineral content (Fe, Zn, and Cu) of the powders achieved from apple pomace

Minimum value was registered for apple pomace powder - *Red Delicious* variety and the maximum one was registered for apple pomace powder - *Golden Delicious* (II) variety. Iron content of the apple pomace powders is 2.75 - 3.25 times higher than that reported by O'Shea et al. (2015) (0.84±0.12 mg/100 g). Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014).

Apple pomace powders have a zinc content in the range 1.54-1.96 mg/100 g, in conformity with the one presented by Dhillon et al. (2013) (1.65 mg/100 g). Zn is an important element of the immune system. Also, Bashandy et al. (2016) showed that the protective effect of zinc can be attributed to its antioxidant and anti-inflammatory properties.

Copper content of apple pomace powders achieved within this study (0.20-0.38 mg/100 g) is comparable to that reported by Bhushan et al. (2008) and Kruczek et al. (2017) (0.11 mg/100 g).

Total polyphenol content

Apple pomace powders are important sources of phenolic compounds. Their total polyphenol content is presented in Figure 5.

Total polyphenol content of apple pomace powders varied in the range 17.83 ...38.83 mg GAE/g (the minimum value was recorded in case of apple pomace powder - *Golden*

Delicious (II) variety and the maximum one in case of apple pomace powder - *Jonathan* (I) variety).

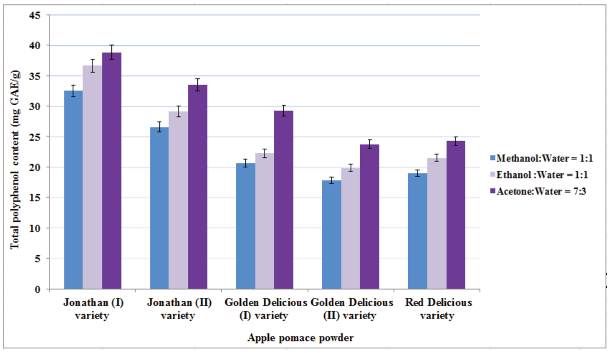

Figure 5. Total polyphenol content of the powders achieved from apple pomace

Values of polyphenol content obtained within this study are higher compared to that reportedfor apple pomace powder: Diňeiro Garcia et al. (2009) (5.5 ...10.9 mg GAE/g), Rana et al. (2015) (3.98 … 5.78 mg GAE/g) and Younas et al. (2015) (4.93±0.39 mg GAE/g, respectively, 6.11±0.15 mg GAE/g). The results obtained for total polyphenol content of apple pomace powders achieved within experiments are due to the apple varieties used in the experiments, but also to the fact that apple waste was subjected to dehydration to a low temperature (50°C), immediately after juice extraction, for inactivation of enzymes favoring the degradation of phenolic compounds. Lavelli and Corti (2011) showed that enzymes mediate the degradation of polyphenols and therefore for polyphenol preservation in apple pomace, it should be subjected to the dehydration process immediately after apple processing, which should inactivate the degrading enzymes.

The most effective solvent for the extraction of total polyphenols was acetone:water = 70:30, the results obtained being in conformity with those obtained by Rana et al. (2015).

The combination of apple pectin and a polyphenol-rich apple concentrate significantly decreased plasma cholesterol and triglyceride levels, as well as intestinal cholesterol absorption (Aprikian et al., 2003). Also, polyphenol-rich fractions from an apple pomace ethanol extract have anti-inflammatory activity (Yue et al. 2012). In a recent study, Sharma et al. (2016) showed the protective effect of apple pomace aqueous extract against CCl4 damage-induced hepatic injury. Treatment with apple pomace aqueous extract reduced the liver damage caused by CCl4, indicated by improved serum level of liver marker enzymes, antioxidative capacity, apoptosis inhibition, and Nrf2 induction.

Antioxidant capacity

Due to their content in phenolic compounds apple pomace powders have antioxidant capacity. Antioxidant capacity of the powders achieved from apple pomace is presented in Figure 6.

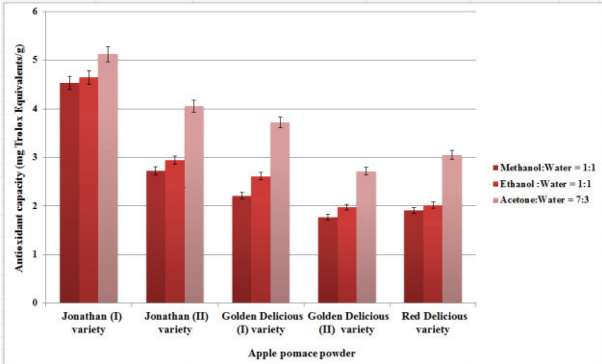

Figure 6. Antioxidant capacity of the powders achieved from apple pomace

Antioxidant capacity of apple pomace powders varied in the range 1.77 ...... 5.12 mg Trolox Equivalents/g, taking into account those three extraction media used (the minimum value was recorded in case of apple pomace powder - *Golden Delicious* (II) variety and the maximum one in case of apple pomace powder - *Jonathan* (I) variety). When using the acetone:water = 70:30 mixture as an extraction medium, higher values of antioxidant capacity were obtained, ranging from 2.71 .... 5.12 mg Trolox Equivalents/g. The values obtained in this case for antioxidant capacity are higher than those reported by Rana et al. (2015) (2.09...3.74 mg Trolox Equivalents/g).

For the powders achieved from apple pomace between the total polyphenol content and antioxidant capacity it is a linear correlation, regression coefficient R^2 being 0.9355 in case of use as extraction medium of methanol:water = 50:50, 0.9323 in case of ethanol:water = 50:50, and respectively, 0.9755 in case of acetone:water = 70:30. In Figure 7 is presented the correlation between the total polyphenol content and antioxidant capacity in case of the powders achieved from apple pomace (acetone:water = 70:30, as extraction medium). The results presented are consistent with those reported by Rana et al. (2015), which also obtained a linear correlation between the total polyphenol content and the antioxidant capacity values.

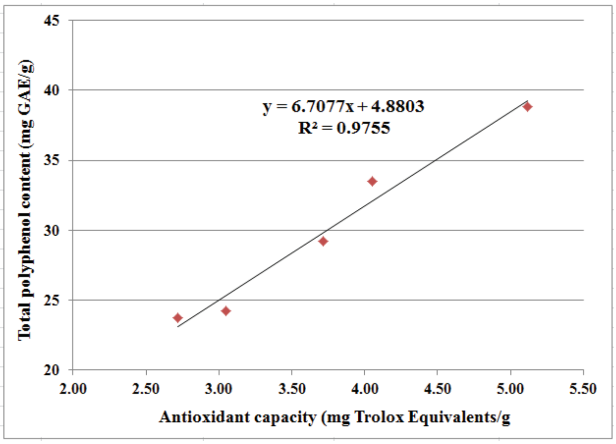

Figure 7. Correlation between the total polyphenol content and antioxidant capacity in case of powders achieved from apple pomace (extraction medium acetone:water = 70:30)

Microbiological analysis

Results of the microbiological analysis of the powders achieved from apple pomace are presented in the Table 2.

Table 2. Microbiological analysis of powders achieved from apple waste

Apple pomace powder variety	Yeast and mold (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)	*Salmonella* (in 25 g)	Water Activity (Aw)
Jonathan (I)	< 10	< 10	< 10	absent	0.341
Jonathan (II)	< 10	< 10	< 10	absent	0.334
Golden Delicious (I)	< 10	< 10	< 10	absent	0.330
Golden Delicious (II)	< 10	< 10	< 10	absent	0.324
Red Delicious	< 10	< 10	< 10	absent	0.332

Microbiological analysis shown that the achieved powders are in the frame of the provisions of the legislation into force. These powders show low values of water activity (0.324-0.341), which give them microbiological stability.

CONCLUSIONS

Powders achieved from apple pomace are important sources of minerals (K, Fe, Mg, Ca, Zn), dietary fibres and bioactive compounds.
Thus, apple pomace powders are important sources of polyphenols (17.83 ...38.83 mg GAE/g). Also these powders have antioxidant potential being beneficial in ahealthy diet for prevention of diseases caused by free radicals.
On the other hand, powders achieved in this study are characterized by high dietary fibre content (60.62...64.75%) being important sources to increase the fibre content of foods (bakery products, pastry products etc.). Increase of the fibre content in case of the sweet flour products is very important because it reduces their glycemic impact on the human body, thus preventing the development ofdiabetes mellitus and obesity. Also, dietary fibre have an important role in promoting feeling of satiety and detoxification of the human body.
Powders achieved from apple waste can be regarded as functional ingredients and can be used to fortify food products (bakery and pastry products, especially) in order to increase the nutritional and their antioxidant potential.

ACKNOWLEDGEMENTS

This study was achieved through Core Programme (PN 18 02), supported by the Ministry of Research and Innovation, contract 25N/2018, project no. PN 18 02 02 02.

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