

Apple pomace from eleven cultivars: an approach to identify sources of bioactive compounds

Mariana Fátima Sato, Renato Giovanetti Vieira, Danianni Marinho Zardo, Leila Denise Falcão, Alessandro Nogueira and Gilvan Wosiacki*

*Departamento de Engenharia de Alimentos, Setor de Ciências Agrárias e de Tecnologia, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900, Ponta Grossa, Paraná, Brazil. *Author for correspondence. E-mail: gilvan.wosiacki@pesquisador.cnpq.br*

ABSTRACT. The dried apple pomace composition of eleven cultivars was assessed in this work. The drying process of apple pomace spread in a thin layer in the trays of an oven with circulating heated air at 60°C showed a 3rd order polynomial tendency and after 10 hours the product, with an equilibrium moisture of 10%, showed an homogeneous appearance according to colorimetric parameters. There are significant differences in the content of lipids, proteins, total titratable acids, total reducing sugars, dietetic fibers total phenol compounds and also in an oxidant activity. Total dietary fibers include pectin, 35%, and insoluble fibers (65%). The content of total phenolic compound, determined with the Folin Ciocalteu reagent and expressed as catechin, goes from 2.29 to 7.15 g kg⁻¹ of dried apple pomace and the antioxidant capacity, expressed as total equivalent (TEAC), from 17.41 to 77.48 mMol g⁻¹. A correlation of 82% between both these quality factors was found. The principal component analysis established the efficiency of total phenol compound, antioxidative capacity, total fiber and total reducing sugars to identify the best cultivar set as source of bioactive compound. Cv. M-2/00 shows high content of total phenol compound and antioxidant capacity, cv. Catarina, of pectin while cv. MRC 11/95, M-12/00, M-8/00, M6/00 e M-11/00, of malic acid and of total reducing sugars. The other cultivars show high content of fibers, ashes and lipids.

Key words: dried apple pomace, inverted sugar, dietary fiber, total phenolic compound, antioxidant capacity.

RESUMO. Bagaço de maçã de 11 cultivares: uma abordagem identificando fontes de compostos bioativos. A composição do bagaço seco de maçã de 11 novas cultivares foi determinada neste trabalho. A secagem do bagaço de maçã disposto em camada fina em estufa de secagem por convecção com ar aquecido a 60°C apresentou uma tendência polinomial cúbica e após 10 horas o produto continha uma umidade de equilíbrio de 10% com uma aparência homogênea, conforme parâmetros colorimétricos, sem evidências de superaquecimento. Houve diferenças significativas entre os teores de lipídeos, de ácido málico, de compostos fenólicos totais, de açúcares redutores totais e de fibras alimentares das amostras analisadas. O total de fibras dietéticas se constituiu de 35% de pectina e 65% de fibras insolúveis. O teor de compostos fenólicos totais (CFT), determinado com o reagente de Folin-Ciocalteu e expresso como catequina, variou de 2,29 a 7,15 g kg⁻¹ de bagaço de maçã e a capacidade antioxidante, expressa como valores de total de equivalentes (TEAC), variou de 17,41 a 77,48 mMol g⁻¹. Foi observada uma correlação de 82% entre estes dois atributos de qualidade. A análise dos componentes principais identificou a significância dos compostos fenólicos totais, capacidade antioxidante, fibras totais e açúcares redutores totais na qualificação das amostras de bagaço de maçã como fonte de compostos bioativos. A cultivar M-2/00 apresentou maior teor de compostos fenólicos e capacidade antioxidante e a variedade Catarina está mais relacionada aos teores de pectinas enquanto as cultivares MRC 11/95, M-12/00, M-8/00, M6/00 e M-11/00 apresentam teores mais elevados de ácido málico e açúcares redutores totais. As demais cultivares apresentaram teores elevados de fibras, cinzas e lipídios.

Palavras-chave: bagaço seco de maçã, açúcar invertido, fibras dietéticas, compostos fenólicos totais, capacidade antioxidante.

Introduction

Traditional apple harvesting technology treats pomace as waste because its disposal creates expensi-

ve environmental problems. However, apple pomace is an interesting raw material and has attracted considerable attention as a potential sugar,

dietary fiber, pectin, and phenolic source. These products can be then be used for many purposes in the pharmaceutical, cosmetic and food industries.

Commercial apple production in Brazil, based on only two cultivars, was driven to supply the highly demanding national retailers and, more recently, the apple juice and wine industry. Seventy percent of the production is commercialized for *in natura* consumption, while 30% is considered industrial fruit. A third of this fraction is comprised of inferior quality fruit, which is discarded or used for vinegar fermentation and distilled beverage production, and the other 2/3 are fruit that can be used for apple juice production (WOSIACKI et al., 2002). From the latter fraction, 75% of the product becomes juice or must and 25% is moistened pomace although nowadays there are developed technology to change these numbers to 91% and 9%, respectively, employing new generation enzymes (ISSENHUTH; SCHNEIDER, 2008).

Industrial apple pomace is composed of press residue from cider apples, wines, brandies, distilled or spirits and vinegars (SMOCK; NEUBERT, 1950) as well as components from the residual epidermis and endocarp obtained in the semi-industrial processes of freezing, canning, dehydration, and other processing (VIRK; SOGI, 2004). Drying apple pomace seems to be the most economically viable approach to stabilization because it drastically reduces the volume and makes for lower transportation costs. The drying yield at 60°C is around 50.0 g kg⁻¹ in 10 hours, or 5% from raw material.

The appearance of dried pomace is dependent on the drying temperature. From 50 to 60°C the enzymatic browning reactions are stimulated (WOSIACKI; SATAQUE, 1987), while from 90 to 100°C, Maillard reactions occur, with products appearing darker than those obtained in range from 70 to 80°C. However, if the criterion to stop the process is the time when the pomace temperature begins to rise, such temperature will never be higher than 52°C and the final product appearance tend to be homogeneous.

The instability of apple pomace is related to its physicochemical composition and to the presence of enzymes activated after plant tissue disintegration (ENDREB, 2000; KENNEDY et al., 1999; SMOCK; NEUBERT, 1950). Apple pomace is composed of water (76.3%) and dry solids (23.7%), and is generated from pulp and epidermis (95.5%), seeds (4.1%), and stems (1.1%). It contains an average moisture of 80% and 14% of its total soluble

solids include glucose, fructose, and sucrose. Its composition is related to the apple cultivar and to the processing (KENNEDY et al., 1999). The fiber content varies from 11.6 to 44.5%, and includes cellulose (12.0 to 23.2%), lignin (6.4 to 19.0%), pectin (3.5% to 18.0%), and hemicellulose (5.0 to 6.2%). The average dietary fibers (35.8%) and residual sugars (54.4%) comprise 91.2% of the pomace, and the remaining components are proteins, lipids, and ashes (CARSON et al., 1994). The chromatic characteristics of L=51.8, a=5.4 and b=18.2 have been determined in an apple pomace sample (SHUDA et AL., 2007).

The use of apple pomace as a potential source of nutrients for the production of glucosidase by *Aspergillus foetidus* was suggested by Hang and Woodams (1994). Ten years later, Schieber et al. (2004) proposed its utilization for other technological purposes like polyphenolic compound recovery. Pomace was also recommended for biotechnological applications like ethanol production (PAGANINI et al., 2005), scents, citric acid, pectin, enzymes, and molds after the extraction of dietary fibers and vegetal coal (TSURUMI et al., 2001).

Fuji and Gala are the most cultivated varieties in Brazil, but they do not fit the industrial apple standard of quality due to their low acidic content and total phenolic compounds levels. The industrial orchard practice is only now beginning in Brazil (WOSIACKI et al., 2007) and information about potential new cultivars, such as their usefulness for juice or wine processing and their pomace, are necessary. The aim of this work was to characterize the physicochemical composition and antioxidant capacity of pomace from eleven apple cultivars still under agricultural studies and to identify the best source for the bioactive compound remaining in this important by-product of apple juice processing.

Material and methods

Materials

Samples (10 kg) of selected apple cultivars were given by the *Empresa de Pesquisa e Extensão Agropecuária de Santa Catarina – Estações Experimentais de Caçador e de São Joaquim*, codified cv. 1 (Catarina), cv. 2 (Joaquina), cv. 3 (M-11/00), cv. 4 (M-11/01), cv. 5 (M-11/00 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). Chemical products were of 'pro analysis' (p.a.) quality.

Methods

Process

After juice extraction in a vertical press, the apple pomace was rinsed once with tap water (1:1:w:v) and centrifuged at 860 x g in a small scale domestic equipment until total drainage. The rinsed apple pomace was then spread as a thin layer in circular bamboo support in each of the six trays of a laboratory oven, and was left to dry under circulating air at 60°C. The temperature of the apple pomace as well its weight was monitored hourly to determine the end of the drying process either by increase in temperature or weight stabilization. The dried product was milled in a Waring blender, sieved to separate fragments of skin, seeds and stems from the 60 MESH fraction, which was then stored at 22°C±3°C in hermetically sealed containers for further analysis.

The extraction of pectin was made in accordance with the procedures previously described by Fertoni et al. (2006). A mixture of raw material (10 g) with 400 mL aqueous HCl (100 mM) was boiled during 10 min and the reaction was stopped in an ice bath; the slurry was filtered through cheese cloth and the pectin was precipitated from the clear extract using alcohol (1:2::v:v). After filtration through cheese cloth and drying in an oven with circulating dry heated air at 50 °C, pectin was triturated in a Waring blender and stored at 22°C±3°C in plastic bags containing silica gel for further analysis.

Analysis

The appearance was evaluated by looking at relative color attributes measured by the CIELAB method, which measures luminosity (L*) and chromatic coordinates (a* and b*) using a Sony Cyber-shot 4.1Mpixels camera to acquire the images and Corel® Photo Paint 12.0 software to treat them (CAMELO; GÓMEZ, 2004). pH was measured with a digital pH meter (Tecnal TEC3MP, São Paulo, Brazil), which was calibrated with standard solutions of pH 7.0 and 4.0. Total soluble solids were determined using a refractometer at 20°C. Moisture and mineral contents were determined by weight loss at 105°C (until constant value) and 550°C, respectively (AOAC, 1998). Lipid content was calculated as the gravimetric difference in the sample after 4 hours of extraction with hexane in Soxhlet, and the protein content was calculated considering the nitrogen content and the factor 6.25 (AOAC, 1998). Reducing sugars and total reducing sugars, after mild hydrolysis with HCl, were determined by the classic methodology of Somogyi

(1945) modified by Nelson (1944), and expressed as glucose in g 100g⁻¹. Sucrose was calculated as the difference between total reducing sugar and reducing sugar. Glucose content was determined by oxidation to gluconic acid with the GOD kit (AOAC, 1998) and fructose content was calculated as the difference between reducing sugar and glucose. Total acidity, determined by titrimetry with 0.1 N NaOH, was expressed as malic acid in g 100 g⁻¹ using 0.64 as conversion factor (AOAC, 1998). Dietary fiber was gravimetrically determined after amylolysis and proteolysis with commercial enzymes (AOAC, 1998). Total phenolic compound was determined with the Folin-Ciocalteu reagent according to Singleton and Rossi (1965) and expressed as mg of catechin equivalent per kg of apple pomace. Antioxidant activity was determined by the Ferric Reducing Ability of Plasma (FRAP) assay carried out as described by Benzie and Strain (1996) with the modifications of Pulido et al. (2000).

Results and discussion

Dehydration of pomace

The drying kinetics of apple pomace fits a cubic or 3rd order model as follows:

$$Y = -a \cdot x^3 + b \cdot x^2 - c \cdot x + d$$

where:

y = value of total mass (in kilograms) and x = time (in hours)

Processing under standard conditions in the laboratory convective dryer with heated circulating air at 60°C allowed us to observe 50% weight loss in 4 hours, although the weight was considered constant only after 10 hours since the curve is asymptotic to the time axis, reaching equilibrium moisture of around 10%. The dehydration in this case is composed of three distinct phases: [1] heating the pomace until it reaches the equilibrium temperature, around 42°C; [2] drying the pomace by evaporation at a constant temperature, which results in loss of weight; and [3] warming of the pomace until it reaches the temperature of circulating air, while maintaining a constant weight. The last step should be omitted to avoid the spoilage of the temperature-sensitive compounds or even to prevent oxidative reactions resulting in a clear product. The product of the drying process, after milling in a Waring blender, is a powder which can be sieved through 60 MESH, and is stable if stored at 22°C±3°C in a closed container.

Figure 1A shows apple pomace drying as a 3rd order polynomial model, as seen in the 60 °C isotherm asymptotic to the time axis. Although 50% of the weight is lost in the first 4 hours, the entire process theoretically demands 15 hours, but in 10h the equilibrium moisture of 12% is acquired and can be interrupted, thus avoiding overheating. The apple pomace temperature never reached 45°C during the entire drying process. Figure 1B shows the first derivative of the previous equation, which represents the speed of weight loss by water evaporation, making the equation negative, and Figure 1C shows the linear deceleration crossing the time axis indicating the end of the process more precisely, somewhat longer than 15h. Wang et al. (2002), looking for a mathematical model on hot air drying of thin layer apple pomace, studied the process at 75, 85, 97 and 105°C in a convective air dryer as thin layer thickness of 10 mm. As increasing the temperature speeds up the drying process thus shorting the drying time, the authors determined the entire length of process, which is similar to that reported here.

Figure 2 shows the results from dried apple pomace samples related to the color parameters, where homogeneity of all the products can be easily seen. This colorimetric analysis was done in order to determine the appearance characteristics of the product when the drying process was conducted aiming to avoid any spoilage due to temperature stress. The air and the pomace temperature were 60°C and 42.5°C, respectively, and under such conditions the luminosity values of the product ranged from 56 to 63 on a scale of 0-100, with the average being of $59.7 \pm 2.93\%$, which points to a homogeneous group of dried apple pomace. The low coefficient of variation for all colorimetric parameters state that all samples indeed have a similar appearance due to the same drying procedures and suggesting some homogeneity of composition. With different figures, Shuda et al. (2007) found more difference among the cultivars

they studied. It must be stressed that there are at least two factors that affect the final appearance: the cultivar itself and the drying process employed.

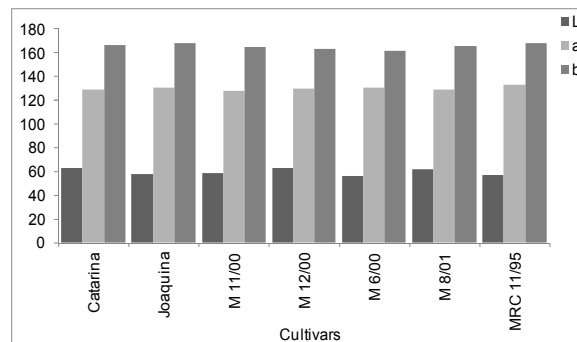


Figure 2. Colorimetric parameters of seven samples of dried apple pomace.

Physico-chemical composition and antioxidant activity

Table 1 shows the composition of the minor components found in dried apple pomace vs. moistened pomace, with the significant differences between the two in humidity, lipid content, and malic acid calculated using ANOVA ($F_{cal}/F_{tab} = 21.50, 1.68$ and 90.36 , respectively).

The moisture (11.43% on average) is low enough to maintain microbiological stability. After one year of storage at $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$, the microbiological load was the same as at the start of the experiment, and lower than the limits imposed by federal laws. Smock and Neubert (1950) cited the range of 11.00 to 12.50 g 100 g⁻¹ as the humidity usually found in the United States. Shuda et al. (2007) described the features of commercial dried apple pomace in India, which showed humidity values of 10.80 ± 0.03 g 100 g⁻¹.

The ash fraction appears at an average concentration of 1.84 g 100 g⁻¹ in our study. Smock and Neubert (1950) reported similar results ranging from 2.11 to 3.50 g 100 g⁻¹, Cho and Hwang (2000) of 0.56 g 100 g⁻¹, and Teixeira et al. (2007) of 0.56 g 100 g⁻¹.

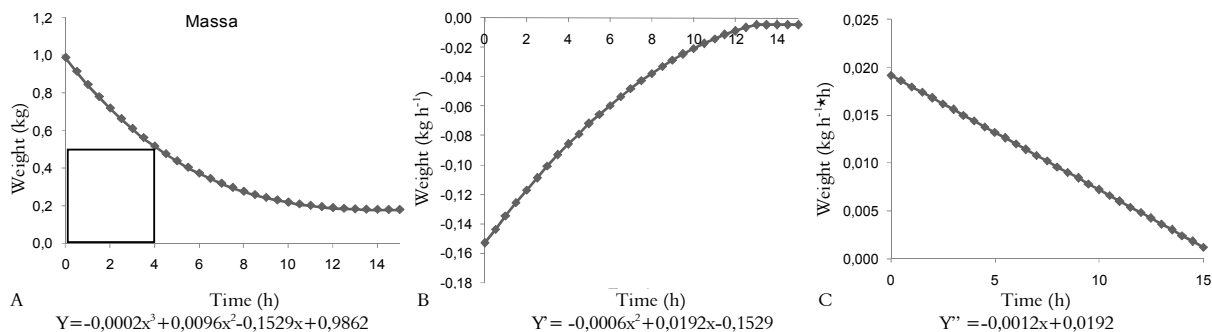


Figure 1. The theoretical trend of the drying model at 60 °C showing the lost of water (A), the fall of the rate (B) and the deceleration (C) of the process.

The lipid content was 1.72 g 100 g⁻¹ on average, lower than the results reported by other authors, from 3.01 to 4.70 g 100 g⁻¹ (SMOCK; NEUBERT, 1950; CHO; HWANG, 2000; SHUDA et al., 2007). The most probable source of variation in the lipid fraction is the seed composition, which can range from 2.20 to 4.40 g 100 g⁻¹ (CARSON et al., 1994; KENNEDY et al., 1999).

With regards to the protein content, our samples ranged from 3.75 to 4.65 g 100 g⁻¹, which was higher than the average 2.06 g 100 g⁻¹ found by Shuda et al., (2007), but lower than the 4.45 to 5.67 g 100 g⁻¹ range reported by Smock and Neubert (1950) and the 11.40 g 100 g⁻¹ reported by Cho and Hwang (2000). The protein content in apple pomace leads to the potential for its use as an ingredient for fermentation by *Saccharomyces cerevisiae* to obtain stable products or even to mature distilled alcohol in oak barrels (PAGANINI et al., 2005).

Malic acid is a component that is present in pomace in varied amounts and this variation is amplified by the accuracy in detection methodology. Malic acid is a functional compound that plays a role in peristaltic movements in the human intestine. The amount found in pomace was, on average, 1.08 g 100 g⁻¹, which is higher than that found in apple juice. Malic acid is also a quality indicator that differentiates sweet apple juice from tart apple juice or commercial and industrial fruits, with the reference 4.5 g L⁻¹, generally acting as the cutoff, with some influence on concentrated apple juice prices (HALBWARE-PREISNOTIERUNG, 2007).

The average total polyphenol content detected in our study was 4620 mg kg⁻¹ and the average antioxidant activity was 36.69 mMol g⁻¹. For these data, the R² = 0.82 reveals that remaining polyphenolic compounds in the apple pomace have a high correlation with the antioxidant activity. An even higher correlation was supposed to be found if the total phenol compound profile were more

homogeneous. In the apple epidermis, many polyphenolic compounds like antocianins can be found, and when the fruit are peeled (SMOCK; NEUBERT, 1950), these bioactive compounds are lost. It is well known that these epidermal compounds show higher bioactivity than the pulp (WOLFE et al., 2003).

Table 2 shows the sugar and fiber content in apple pomace. The sugar content in pomace was 40 g 100 g⁻¹, on average. To measure sugar content, the pomace must first be rinsed with tap water to avoid the formation of a layer that can prevent water evaporation, thus avoiding dried pomace with a high degree of moisture. Rinsing pomace promotes the drying process, leading to a stable pomace. There was a difference in sugar content among the cultivars. The simple sugars, known as ‘inverted sugars’, are usually present in apples juice with the glucose:fructose:sucrose ratio of 1.00:3.51:1.64 (WOSIACKI et al., 2007), but in these pomace samples, the ratios were different. Fructose is still the prevalent sugar, but the average ratio of sugars was glucose:fructose:sucrose 1.00:1.43:0.56. The amount of total ‘reducing sugar’ or ‘inverted sugar’ in the apple pomace and the ease of extracting that sugar makes it possible to use this raw material to obtain natural sweeteners.

The dietary fiber fraction, containing both soluble and insoluble fiber, was considered heterogeneous, with values from 33.40 g 100 g⁻¹ to 51.85 g 100 g⁻¹, and significant differences between varieties (F_{cal}/F_{tab} of 3.2340). Shuda et al. (2007), reported 51.10 g 100 g⁻¹ dietary fiber in their study, with 36.50 g 100 g⁻¹ as insoluble fiber, and 14.60 g 100 g⁻¹ soluble.

The appeal of foods rich in dietary fiber is based on the physiological observation that they may play a role in the enterohepatic cycle of cholesterol, contributing to the reduction of the blood cholesterol levels.

Table 1. Physicochemical characterization and antioxidant activity of dried apple pomace.

Cultivars	Physicochemical characteristics of apple pomace						Antioxidant activity TEAC (mMol g ⁻¹)
	Moisture*	Ash*	Lipids*	Protein (N Kjeldhal)*	Malic Acid*	Total Polyphenols (g kg ⁻¹)	
cv. 1	8.93±0.64	1.73±0.07	1.06±0.13	2.69±0.38	1.14±0.02	7.15±0.05	44.46±0.02
cv. 2	11.36±0.03	1.72±0.08	1.56±0.19	2.85±0.02	0.75±0.01	4.87±0.14	38.78±0.06
cv. 3	13.72±1.22	1.62±0.12	1.20±0.13	2.84±0.01	0.78±0.02	3.20±0.18	31.52±1.12
cv. 4	11.10±0.18	1.82±0.11	1.37±0.15	2.90±0.07	1.07±0.01	2.71±0.05	27.62±0.48
cv. 5	12.40±0.35	2.00±0.13	1.12±0.22	2.45±0.01	1.09±0.04	n.a.**	n.a.**
cv. 6	10.16±0.12	1.93±0.09	1.44±0.06	2.72±0.08	1.18±0.03	3.59±0.13	26.88±0.13
cv. 7	9.45±0.36	1.86±0.25	2.14±0.84	2.84±0.01	1.10±0.02	2.62±0.81	17.41±0.56
cv. 8	10.99±0.18	1.82±0.07	1.43±0.06	3.01±0.09	1.02±0.02	8.56±0.28	77.48±0.30
cv. 9	13.20±0.60	1.84±0.14	1.87±0.40	2.76±0.03	1.11±0.03	2.29±0.025	24.14±0.81
cv. 10	11.64±0.83	1.77±0.19	1.86±0.15	2.72±0.01	0.73±0.03	4.37±0.08	46.32±0.51
cv. 11	11.95±0.90	1.66±0.11	1.76±0.18	2.42±1.86	1.32±0.02	3.19±0.11	32.24±0.64
Mean	11.43	1.80	1.53	2.74	1.09	4.61	36.69

*results in mg 100 g⁻¹; **n.a. not analyzed.

Table 2. Sugars and fibers fractions of dried apple pomace.

Cultivars	Compounds in g 100 g ⁻¹						
	Glucose	Fructose	Sucrose	Total sugar	Dietary Fiber	Soluble fiber	Insoluble fiber
cv. 1	14.07	18.00	4.86	36.93	44.50	17.65	26.85
cv. 2	n.a.	n.a.	n.a.	53.63	43.77	12.26	31.51
cv. 3	9.77	19.30	3.11	32.17	45.95	16.09	29.86
cv. 4	13.78	15.37	n.d.	28.83	48.48	13.26	35.22
cv. 5	17.35	18.23	5.16	40.74	46.05	17.11	28.94
cv. 6	13.78	21.16	11.78	46.72	40.89	14.48	26.41
cv. 7	9.92	12.02	7.28	29.21	46.52	15.07	31.45
cv. 8	6.15	22.31	3.13	31.59	33.40	14.79	18.61
cv. 9	11.85	16.72	16.13	44.71	51.85	14.9	36.95
cv. 10	18.62	15.90	5.84	41.79	34.19	14.66	19.53
cv. 11	10.36	20.31	13.41	44.08	45.24	15.22	30.02
Average	12.57	17.93	7.04	39.35	43.63	14.78	28.85
Standard deviation	3.74	3.06	5.17	8.45	5.91	1.34	5.96
Variation's coefficient (%)	29.79	17.07	73.43	21.00	0.14	9.00	21.00

n. a. = not analyzed; n. d. = not detected.

Apple pomace is therefore even more appealing than apples, as the fiber is more concentrated.

Minor compounds such as minerals, lipids, and proteins are relatively homogenous among the various cultivars ($p < 0.05$). The major compounds, even without precise quantification of the F_{ca}/F_{tab} ratio, were present at different levels in the various cultivars. These differences were seen in total sugar (glucose, fructose and sucrose), and alimentary fibers, such as pectin, but not starches and proteins.

Figure 3 shows the results of principal component analysis (PCA) of the physicochemical profile of ten different apple pomaces. The PCA was performed on a correlation matrix. Factor 1 x Factor 2 axes explain 57.00% of the total variance amongst the data; the first represents 32.40% and the second 24.60% of the total dispersion.

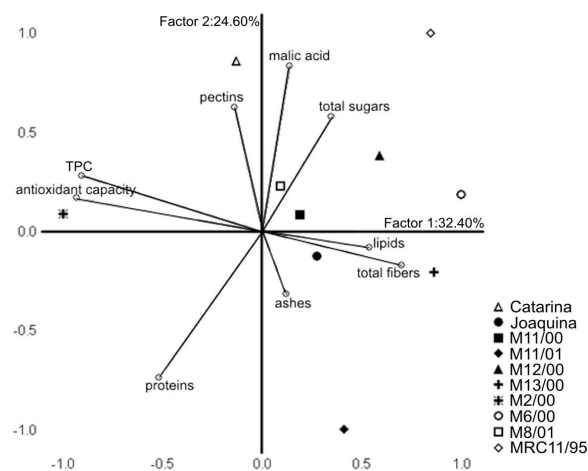


Figure 3. PCA of the physicochemical profile of ten different apple cultivars (2005 vintage) using Factor 1 x Factor 2 analysis.

The scores of the apple cultivars are based on these first two components and overlay loadings (the location in the PC space of the original variables). The factorial coordinate shows that lipids and total fibers

were strongly positively correlated with Factor 1, while antioxidant activity, TPC, and proteins were strongly negatively correlated with Factor 1. Factor 2 presents the overall opposition between the malic acid and protein variables, which are strongly positively and negatively correlated, respectively. Factor 2 was strongly positively correlated with the total sugar and pectin variables. Projection of these cases onto the two axes showed that the M-2/00 cultivar appears to have higher values for TPC and antioxidant properties. cv.1 was more correlated to pectin, and the cv.11, cv.6, cv.10, cv.9, cv.3 cultivars appear to have higher values for malic acid and total sugar variables, while Joaquina, M-11/01 and M-13/00 showed high values for total fiber, ashes, and lipids.

Conclusion

Apple pomace dried at 60°C has an equilibrium moisture of 10%. The minor (mineral, lipids, proteins, and total polyphenols) and major (malic acid, inverted sugars, and dietary fibers) components were quantified with significant differences among samples in relation to malic acid, inverted sugars, and dietary fiber contents ($p < 0.05$). The polyphenolic compounds have a high correlation with antioxidant activity. Apple pomace is a source of compounds that are potentially interesting to the functional food industry. PCA results showed that the apple pomace from different cultivars can be differentiated by their physico-chemical composition and antioxidant activities.

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