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# Total phenolic content and antioxidant capacity of agri-food waste and by-products

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**ABSTRACT**

Agri-food waste (AFW) and by-products represent sources of phytochemicals, such as phenols and antioxidant compounds that can be used as functional ingredients in animal feed. In this study, a selection of AFW and by-products were collected and analysed for their nutrient composition. After chemical (with methanol) and physiological (*in vitro* digestion) extraction, total phenolic content and antioxidant capacity (AOC) were determined in AFW and by-product samples using Folin–Ciocalteu and 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid)-ABTS methods, respectively. Sample digestibility was also assessed using a multi-step enzymatic technique. After chemical extraction, grape marc showed the highest total phenolic content (4480.5 ± 886.58 mg TAE/100g; $p < .05$). Fruit and vegetable waste (FVW), orange peel, strawberry, citrus pulp and *Camelina sativa* cake showed a total phenolic content ranging from 238.0 ± 4.24 to 1583.0 ± 154.35 mg TAE/100g. Grape marc also showed the highest AOC (15440.7 ± 2671.85 mg TE/100g). In all other samples, AOC ranged from 43.3 ± 3.17 to 1703.9 ± 391.07 mg TE/100g. After physiological extraction, total phenolic content values higher than 3000 mg TAE/100g were observed in FVW, grape marc and orange peel. Grape marc, *C. sativa* cake and orange peel had AOC values of over 5000 mg TE/100g. The digestibility of AFW and by-products ranged from 44.20 to 97.16%. The lowest digestibility value was observed in grape marc (44.2 ± 2.31%). In conclusion, the results obtained in this study indicate that AFW and by-products could be a source of bioaccessible phenols and antioxidant molecules as ingredients for monogastric compound feeds.

**HIGHLIGHTS**

- Agri-food waste and by-products can be reused in feed industry.
- Agri-food waste and by-products are a source of valuable compounds as phenols and antioxidant molecules.

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## Introduction

The global volume of food wastage is estimated at 1.6 billion tons of 'primary product equivalents' and its edible part is roughly 1.3 billion tons (Gustafsson et al. 2013). Food waste has considerable economic and environmental implications: not only does it represent a wasted investment, but it also has a negative environmental impact, due to the greenhouse gas emissions and inefficient use of water and land, which in turn can lead to diminished natural ecosystems (Lipinski et al. 2013).

Food waste is generated throughout the entire food life cycle: from agriculture to industrial manufacturing and pro.cessing, in retail and households (Mirabella et al. 2014). Agri-food waste (AFW) and by-products provide a high potential source of bioactive compounds, such as phenols and antioxidants, which could be exploited in the pharmaceutical, cosmetic, and food industries and used as functional ingredients in animal feeds (Fontana et al. 2013). The use of AFW or by-products, such as animal feeds, is already traditional practice in animal husbandry (Bampidis and Robinson 2006); however, the bioactive potential in feed has not been fully elucidated.

Fruit and vegetable by-products are notably rich in antioxidant phenols (Balasundram et al. 2006; Peschel et al. 2006), such as anthocyanin and flavonoids (Croft

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2016). The enrichment of animal diets with phenolic compounds may have beneficial effects on animal gut health, including anti-inflammatory and antimicrobial activity along with their antioxidant capacity (Ignat et al. 2011).

Several studies have focussed on the quantification of the total phenolic and antioxidant compounds in fruits and vegetables (Ignat et al. 2011; Pastoriza et al. 2011) using different extraction methods. Chemical extraction is widely used (Pastoriza et al. 2011; Attard 2013) which enables the extraction of a high amount of total phenolic and antioxidant compounds. From a physiological point of view, however, the bio-accessibility of phenolic compounds and antioxidants depends on their release from the food and feed matrix during the digestive process.

In this study, AFW and by-product samples were analysed in order to assess the total phenolic content and antioxidant capacity. In particular, chemical (with methanol) and physiological (*in vitro* digestion) extractions were performed in order to evaluate the potential bioaccessibility of phenols and antioxidant molecules in monogastric compound feed.

## Materials and methods

AFW and by-products, including fruit and vegetable waste (FVW), *Citrus sinensis* L. (orange peel), *Fragaria ssp.* (strawberries), citrus pulp, *Vitis vinifera* L. (grape marc), *Camelina sativa* (L.) Crantz cake (*Camelina sativa* cake) and whey, were collected three times ($n = 21$) over a one-year period (2016/2017), according to product seasonality. *Camelina sativa* cake and whey were provided by commercial suppliers. Oranges, strawberries and grape marc were freshly collected from a local market and a winery, respectively, situated in northern Italy, and subsequently dried and ground (1mm sieve). Citrus pulp and FVW were provided by the University of Messina and their composition is reported in Chiofalo et al. (2014).

The FVW sample contained different types of vegetables and fruits including: tomato (*Solanum lycopersicum* L.), fennel (*Foeniculum vulgare* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), courgettes (*Cucurbita pepo* L.), potato (*Solanum tuberosum* L.), onion (*Allium cepa* L.), lettuce (*Lactuca sativa* L.), cauliflower (*Brassica ssp.*), mushroom (*Agaricus bisporus*, *Pleurotus ostreatus*), pear (*Pyrus communis* L.), apple (*Malus domestica*), banana (*Musa spp.*), orange (*Citrus sinensis* L.), strawberry (*Fragaria ssp.*), kiwi (*Actinidia chinensis*) and pineapple (*Ananas comosus* L.). The chemical analysis of the samples was performed following official methods (AOAC 2005; European Commission regulation 2009) and the fibre fractions were analytically determined according to Van Soest et al. (1991), using heat-stable α-amylase. Gross energy (GE) values were estimated according to Hoffman and Schiemann's equation (1980).

Each sample was weighed ($5 \pm 0.5$ g), mixed with 30 mL methanol (100%) for 48 hours at room temperature (RT) and subsequently, filtered with filter paper (Whatman 54, Florham Park, NJ). Chemical extracts were tested for total phenolic compounds and antioxidant capacity, as detailed below.

In parallel, the *in vitro* digestion was performed according to the protocol described by Regmi et al. (2009) with minor adaptations reported by Giromini et al. (2017a) (Figure 1). At the end of the *in vitro* digestion, a soluble fraction and an undigested fraction (UF) were obtained. The soluble fraction was used to quantify the phenol content and the antioxidant capacity (detailed below). The UF was then collected in a filtration unit using a porcelain filtration funnel lined with pre-weighed filter paper (Whatman no. 54). The UF, along with the filter paper, were dried overnight at 65 °C. The UF was used to calculate the *in vitro* digestibility (Equation 1):

$$\text{Digestibility } (\%\ \text{dry matter, DM}) = (\text{sample DM} - \text{UF DM})/\text{sample DM} \times 100 \quad (1)$$

In addition, total phenolic compounds were assayed according to the Folin–Ciocalteu method (Attard 2013). The total phenolic content was expressed as tannic acid equivalents (mg TAE/100g).

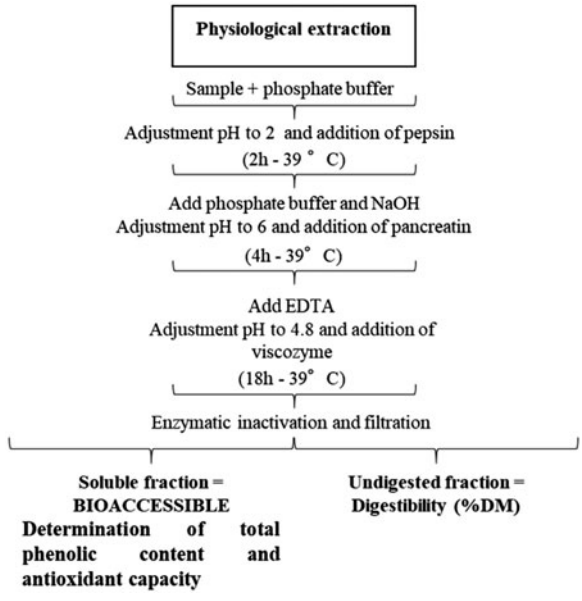


Figure 1. Graphical representation of physiological extraction.

The anthocyanin content was measured in triplicate in each chemical extract according to Theuma et al. (2015) and calculated as follows (Equation 2):

$$\begin{aligned}&\text{Anthocyanin content (mg/kg)}\\&=(1000\times\text{volume of extracted sample}\\&\times\text{absorbance value at 520})\\&/\text{extinction coefficient}\left[58.3\text{ml (mg.cm)}\right].\end{aligned}\quad(2)$$

The antioxidant capacity (AOC) was measured following Re et al. (1999). AOC results are expressed as mg Trolox equivalent (TE)/100g sample.

All samples were prepared and analysed in triplicate. The data from total phenolic content and AOC were analysed through one-way analysis of variance (ANOVA) using GLM procedure of SAS (SAS, 9.3 version, Cary, NC). Results are expressed as mean ± standard deviation. Data were analysed using Shapiro–Wilk test to evaluate the normality of sample distribution. The Pearson correlation coefficient ($r$) and probability-value ($p$) were used to show correlation and their significance by using SPSS software, Version 24. Chicago, IL: SPSS Inc; 2002. A probability value of $p < .05$ was considered statistically significant.

## Results and discussion

The total phenolic content and AOC of the various AFW and by-product matrixes were analysed in order to assess their use in the animal feed sector. Exploiting AFW and by-products plays an important role in the production of high value functional feed ingredients along with socio-economic and environmental sustainability, according to the circular economy strategy (Mirabella et al. 2014) and according to the 'biorefinery' approach where value-added molecules are recovered from waste biomass (Di Donato et al. 2017; Rodríguez-González et al. 2018). The nutrient composition and gross energy content of AFW and by-products are reported in Table 1.

Our results confirm that *C. sativa* cake and whey represent valuable protein sources. As for the lipid content, grape marc and *C. sativa* cake had the highest values in terms of ether extract (EE) compared to the other samples. Overall, our results confirm notable amounts of nutrients in the AFW and by-products with a high potential for feeding animals.

Table 2 shows the total phenolic content, AOC and anthocyanin content of AFW and by-products after chemical extraction. In particular, the highest total phenolic content was found in grape marc (4480.5 ± 886.58 mg TAE/100g; $p < .05$). The total phenolic content of FVW, orange peel, strawberry, citrus pulp and *C. sativa* cake ranged from 238.0 ± 4.24 (*C. sativa* cake) to 1583.0 ± 154.35 mg TAE/100g (orange peel). Grape marc showed the highest AOC value (15440.7 ± 2671.85 mg TE/100g; $p < .05$) and a notable amount of anthocyanin compared to all other samples. In all other samples, AOC ranged from 427.2 ± 109.91 (citrus pulp) to 1703.9 ± 391.07 mg TE/100g (orange peel). In the whey sample, the total

**Table 1.** Chemical composition of AFW and by-products (% w/w on DM basis).

| | DM | CP | EE | NDF | ADF | ADL | Ash | GE, MJ/kg |
|---|---|---|---|---|---|---|---|---|
| FVW | 12.3 ± 2.20 | 9.9 ± 1.11 | 0.8 ± 0.50 | 22.2 ± 0.41 | 16.6 ± 0.52 | 10.2 ± 0.28 | 6.1 ± 0.22 | 16.4 |
| Orange peel | 26.6 ± 1.24 | 3.5 ± 2.02 | 1.7 ± 0.52 | 10.0 ± 1.30 | 7.6 ± 1.71 | 1.8 ± 0.01 | 3.8 ± 0.24 | 15.92 |
| Strawberries | 7.0 ± 1.21 | 7.7 ± 1.40 | 1.5 ± 0.30 | 12.8 ± 0.54 | 9.7 ± 1.58 | 5.7 ± 0.81 | 4.9 ± 0.58 | 16.99 |
| Citrus pulp | 93.7 ± 1.22 | 5.0 ± 1.14 | 2.6 ± 0.32 | 23.0 ± 0.60 | 16.2 ± 0.76 | 3.8 ± 1.03 | 9.0 ± 0.88 | 16.04 |
| Grape marc | 26.4 ± 1.30 | 8.0 ± 0.30 | 8.5 ± 0.22 | 20.8 ± 0.70 | 18.9 ± 1.49 | 12.9 ± 0.42 | 1.3 ± 1.26 | 16.86 |
| *Camelina sativa* cake | 92.3 ± 1.40 | 32.2 ± 1.40 | 7.7 ± 0.82 | 46.1 ± 1.03 | 22.0 ± 0.91 | 5.1 ± 0.39 | 5.9 ± 1.60 | 19.3 |
| Whey | 92.1 ± 0.22 | 13.0 ± 1.22 | n.d. | n.d. | n.d. | n.d. | 8.7 ± 0.56 | 18.54 |

Data are presented as mean ± standard deviation, ($n = 3$).
AFW: Agri-food waste; FVW: fruit and vegetable waste; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; GE: gross energy; n.d.: not detected.

**Table 2.** Total phenolic content, antioxidant capacity (AOC) and anthocyanin content of chemical extracts ($n = 3$) (methanol extraction) from AFW and by-products (mean ± standard deviation).

| Chemical | Total phenolic content, mg TAE/100g | AOC, mg TE/100g | Anthocyanin content, mg/100g |
|---|---|---|---|
| FVW | 1307.0 ± 182.85$^{b}$ | 1112.8 ± 340.62$^{c}$ | 18.8 ± 1.92$^{b}$ |
| Orange peel | 1583.0 ± 154.35$^{b}$ | 1703.9 ± 391.07$^{b}$ | 5.2 ± 0.53$^{c}$ |
| Strawberries | 1253.6 ± 98.57$^{b}$ | 1163.7 ± 276.90$^{cb}$ | 14.7 ± 2.13$^{b}$ |
| Citrus pulp | 565.6 ± 106.80$^{c}$ | 427.2 ± 109.91$^{d}$ | 3.4 ± 0.41$^{c}$ |
| Grape marc | 4480.5 ± 886.58$^{a}$ | 15440.7 ± 2671.85$^{a}$ | 29.6 ± 4.21$^{a}$ |
| *Camelina sativa* cake | 238.0 ± 4.24$^{c}$ | 730.5 ± 16.90$^{cd}$ | 1.9 ± 0.48$^{c}$ |
| Whey | 89.5 ± 18.41$^{c}$ | 43.3 ± 3.17$^{d}$ | 0.4 ± 0.02$^{d}$ |

AFW: Agri-food waste; FVW: fruit and vegetable waste; TAE: tannic acid equivalents; TE: trolox equivalents.
Different superscript letters in columns indicate significant different data ($p < .05$).

**Table 3.** Total phenolic content, antioxidant capacity (AOC) and anthocyanin content of physiological extracts ($n = 3$) (*in vitro* digestion) from AFW and by-products (mean ± standard deviation).

| Physiological | Total phenolic content, mg TAE/100g | AOC, mg TE/100g | *In vitro* digestibility (% of DM) |
|---|---|---|---|
| FVW | 3230.7 ± 122.26[a] | 3783.4 ± 604.43[b] | 78.7 ± 2.84[b] |
| Orange peel | 3596.0 ± 420.37[a] | 5233.9 ± 1518.18[ac] | 88.7 ± 3.44[a] |
| Strawberries | 2335.5 ± 462.26[c] | 4346.5 ± 1065.86[bc] | 86.8 ± 2.61[a] |
| Citrus pulp | 836.5 ± 18.43[b] | 4059.8 ± 55.84[b] | 78.9 ± 1.01[b] |
| Grape marc | 3552.2 ± 446.17[a] | 5511.4 ± 938.07[a] | 44.2 ± 2.31[d] |
| *Camelina sativa* cake | 879.7 ± 74.87[b] | 5262.3 ± 449.76[a] | 66.8 ± 0.82[c] |
| Whey | 219.2 ± 8.05[d] | 3258.3 ± 215.44[b] | 97.2 ± 1.61[a] |

AFW: Agri-food waste; FVW: fruit and vegetable waste; TAE: tannic acid equivalents; TE: trolox equivalents.
Different superscript letters in columns indicate significant different data ($p < .05$).

phenolic content, AOC and anthocyanin content were the lowest detected. The high content of phenols and anthocyanin in grape marc samples are in accordance with those reported by Larrauri et al. (1997). The AOC of grape marc was higher in our study than in data reported by Heng et al. (2017). The total phenolic content of orange peel and citrus pulp was similar to values reported for orange peel extract by Attard (2013) and Tzanakis et al. (2006). Strawberry AOC was at least two-fold higher (1163.8 ± 276.90 mg TE equivalent/100g) than values reported by Özşen and Erge (2013) (568–642 mg TE equivalent/100g) and Gössinger et al. (2009) (530–805 mg TE/100g). The total phenolic content, AOC and anthocyanin content obtained after chemical extraction show a large variability among different studies due to the lack of assay standardisation (Pellegrini et al. 2003). As demonstrated by Thomas et al. (2018), the variations in the total phenol content might be due to several factors such as genetic variability, environmental pressure, cultivation techniques, age and maturity of the plants and postharvest treatments.

A positive correlation ($r = 0.95$, $p = .01$) was observed between the total phenolic content and AOC in the chemical extracted samples. The anthocyanin content was correlated with the total phenolic content ($r = 0.87$, $p = .01$) and with AOC ($r = 0.80$, $p = .01$). The positive linear relationships between the total phenolic content and AOC values are in accordance with the results of other authors (Ehlenfeldt and Prior 2001; Connor et al. 2002) confirming that total phenolic compounds largely contribute to the AOC of AFW and by-products (Dudonné et al. 2009).

*In vitro* digestion (physiological extraction) was performed to evaluate the bioaccessibility of total phenolic and antioxidant compounds in AFW and by-products. The soluble fraction of the digestion was used to measure the phenol content and AOC, and the results are reported in Table 3. We found that the total phenolic content was significantly high (3000 mg TAE/100g; $p < .05$) in FVW, grape marc and orange peel, compared with the other samples. The AOC was higher than 5000 mg TE/100g in grape marc, *C. sativa* cake and orange peel. A high AOC value was also observed in the whey sample (3258.3 ± 215.44 mg TE/100g), compared with the value obtained after chemical extraction, thus suggesting the liberation of antioxidant compounds encrypted in whey proteins. Thus, the AOC of whey mainly depends on the high biological value of bioactive peptides (Giromini et al. 2017b) and on the high oligosaccharides and B-vitamin content.

In addition, the undigested fraction obtained from the physiological extraction (Figure 1) was used to calculate the *in vitro* digestibility. Notably, the digestibility of AFW and by-products showed a mean value of 77.33%. The highest digestibility was observed in whey (97.2 ± 1.60% DM), while the lowest (44.2 ± 2.30% DM) was reported for grape marc (Table 3).

The *in vitro* digestion protocol exploited in the present study had previously been used to test the monogastric digestibility of feeds, showing a great correlation with the *in vivo* digestibility values (Regmi et al. 2009). The feed bioaccessibility corresponds to the feed portion effectively released from the matrix and available for intestinal absorption. The application of *in vitro* digestion to AFW and by-products enables the physiological bio-accessible phenols and antioxidant compounds to be studied in more depth in soluble fractions. However, the UF obtained may still contain bioactive components (Chen et al. 2014) which may play an essential role at the gut level (e.g. intestinal epithelial cells, microbiota).

In our study, total phenolic content and AOC values were higher in the physiological than in the chemical extracts. This suggests that digestion can enhance their bioaccessibility, except for grape marc in which the phenols and antioxidant molecules were lower in the physiological than the chemical extracts. The latter aspect is related to the low digestibility observed in grape marc (44% DM, Table 3) which may have

negatively affected the liberation of phenols and the AOC, although no overall correlation was observed ($p > .05$). This aspect, however, needs further investigation, using an improved protocol to simulate the *in vitro* digestion and assess the digestibility of the grape marc, also taking into consideration the relatively high lipid content of the sample. From an application point of view, dietary supplementation with enzyme-based additives could be a valid technique to improve the bioaccessibility of phenols and antioxidant molecules of AFW and by-products and to implement their use in animal nutrition (Chamorro et al. 2015).

Other aspects related to data application in animal nutrition should also be considered, i.e. palatability, stability, storage conditions and food safety issues such as the risk of mycotoxin contamination in AFW and by-products.

## Conclusions

This study contributes to the current knowledge on the functional role of agri-food waste and by-products in the diet of monogastric animals. The results indicate that agri-food waste and by-products are a good source of phenols and antioxidant molecules. Further issues, however, need to be considered when using agri-food waste and by-products in feed formulations. The highly variable chemical composition, along with the storage and processing conditions need to be correctly addressed in order to guarantee the stability of the bioactive components in agri-food waste and by-products.

Overall, the reuse of agri-food waste and by-products as functional ingredients in animal feed is crucial, not only because it reduces the costs of disposal costs and the amount of food waste, but also because of the promising potential as functional feed ingredients.

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No potential conflict of interest was reported by the authors.

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