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Total phenolic content and antioxidant capacity of former food products intended as alternative feed ingredients

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ABSTRACT

The application of Former Food Products (FFPs) as feed ingredients is already documented in swine, as well as their beneficial nutritional value. To date, FFPs extra-nutritional bioactive effect in feed has not been elucidated. Therefore, the aim of the present study was to investigate and compare the total phenolic content and antioxidant capacity (AOC) in six samples of FFPs extracted by different solvent systems. After methanol and acetone extraction, total phenolic content and AOC were determined in FFPs and wheat sample (CTR) using Folin–Ciocalteu and 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonic acid)-ABTS assay, respectively. Results demonstrated that FFPs samples were characterised by an average amount of total phenolic content of 129.3 ± 15.1 mg tannic acid equivalents (TAE)/100g in methanol extracts and 156.4 ± 25.8 mg TAE/100g in acetone extracts. Whereas, the ABTS assay revealed that FFPs showed also anAOC of 138.0 ± 14.3 mg Trolox Equivalent (TE)/100 g in methanol extracts and 173.3 ± 18.8 mg TE/100 g in acetone extracts. Former Food Products represent relevant sources of phenols and antioxidant compounds, which can be beneficial for animal health.

HIGHLIGHTS

- Former Food Products (FFPs) reprocessing is a way to convert food losses into ingredients for animal diets;
- Valuable content of total phenolic compounds and notable antioxidant capacity were observed in FFPs after extraction with methanol and acetone;
- FFPs demonstrated to be a source of bioactive compounds with extra-nutritional activities which can be beneficial for animal health.

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KEYWORDS
Ex-food; circular economy; phenols; antioxidant capacity

Introduction

For a long time, our economy has been 'linear'. This means that raw materials have been used to make a product, and after its use, any waste has been thrown away. In a circular economy almost all materials are reused. The case of reuse of ex-food also termed former food products (FFPs) in animal nutrition perfectly fit with this concept. Indeed, FFPs are represented by foodstuffs, which were manufactured for human consumption in full compliance with the food law and standards but which are no longer intended for human consumption for practical or logistical reasons or defects, which however do not present any health risks when used as feed. As consequence, they represent a way by which maintaining ex-food in the feed/food chain (Lipinski et al. 2013; Pinotti et al. 2014; Featherstone 2016; Pinotti et al. 2019). Food leftovers and waste have considerable economic and environmental implications: not only they represent a wasted investment, but they also have a negative environmental impact, due to the greenhouse gas emissions and inefficient use of water and land, which in turn can lead to negatively affect the natural ecosystems (Lipinski et al. 2013). Industrial by-products, former foods, co-products, insect and seaweed ingredients have been largely proposed as alternative feed ingredients (Pinotti et al. 2014). Among these, Food leftovers (FFPs) have been proposed as one of the categories with great potential as alternative ingredients

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for the animal diet which can represent a sustainable way of converting losses from the food industry into ingredients for feed, thereby reducing overall food losses (Featherstone 2016; Pinotti et al. 2019). Moreover, FFPs are regarded as interesting alternative energy sources for animal feed (Giromini et al. 2017; Tretola et al. 2017) and are expected to be increasingly used in the near future as a replacement for conventional feedstuffs due also to the recently published European Union guidelines on the use of food no longer intended for human consumption in animal feed (European Commission 2018).

Even if the application of FFPs is already documented in swine, as well as their valuable nutritional value (Tretola et al. 2019), FFPs functional effect in feed has not been elucidated yet. Phenols present in feed play an important role in animal health because of their highly antioxidant capacity. Phenolic compounds, a specific group of secondary metabolites, can control oxidative stress in the organism by maintaining a balance between oxidants and antioxidants (Van Hung 2016). Antioxidants molecules, including phenols, vitamins and carotenoids, are proven to be effective in the prevention of oxidative stress-related diseases in animals and humans. Food waste and industrial by-products previously demonstrated to be not only a source of nutrients for feeding animals, but also valuable source of bioactive compounds (Castrica et al. 2019). However, the potential of FFPs has not yet been fully exploited with regard to their functional properties. More studies are needed to determine the functional characteristics of FFPs to improve their application in feed. Therefore, the aim of the present study was to investigate and compare the total phenolic content and antioxidant activity of FFPs samples.

Materials and methods

Samples composition and extraction

FFPs samples composition was detailed in a previous study (Giromini et al. 2017) and in Table 1. Briefly, 5 ± 0.5 g of each FFP sample were ground and extracted for 15 hours in 20 mL acetone (50%*v/v*) or methanol (80%*v/v*) at room temperature according to a previous study (Zieliński and Kozłowska 2000). Subsequently, samples were filtered with filter paper (Whatman 54, Florham Park, NJ, USA). Filtered sample extracts were tested for total phenolic content and antioxidant capacity, as detailed below. Wheat sample was included as control (CTR).

Research reported is in full compliance with all relevant codes of experimentation and legislation

Table 1. Samples ingredients (Giromini et al., 2017).

ID	Ingredients in descending order of inclusion
FFP1	Leftover of food industry (confectionery products, bakery products (cookies, biscuits), products of pastry); wheat by-products (e.g. bran); wheat flour
FFP2	Leftover of food industry (bakery products, pasta, of pastry products industry, confectionery products); wheat by-products (e.g. bran); wheat flour
FFP3	Leftover of food industry (bakery products, pasta, pastry products industry, confectionery products); wheat by-products (e.g. bran); wheat flour
FFP4	Extruded and puffed rice cakes and corn extruded
FFP5	Leftover of food industry (pasta, bakery products, confectionery products, products of pastry products industry)
FFP6	Bakery products (e.g. biscuits)

FFP: former food products

Total phenolic content

The total phenolic content in acetone and methanol extracts was estimated using Folin–Ciocalteu reagent (Yu et al. 2002). In brief, the reaction mixture contained 500 µL of extract, 2.5 mL of freshly prepared Folin–Ciocalteu reagent, 2 mL of 20% sodium carbonate. After 20 min of reaction at room temperature, the absorbance at 765 nm was measured and used to calculate the total phenolic content. Tannic acid was included as a standard. The total phenolic content was expressed as mg tannic acid equivalents (TAE)/100 g. Results are the mean values (Lsmean ± SEM) of three replicates.

Antioxidant capacity (AOC): ABTS assay

Measurement of antioxidant activity was performed using the method of Re et al. (Re et al. 1999). Results are expressed as mg Trolox equivalent (TE)/100 g sample. Results are the mean values (Lsmean ± SEM) of three replicates.

Statistical analysis

Statistical analysis was performed using GraphPad-Prism version 8 software package. Total phenolic content and AOC were analysed by one-way ANOVA. Pearson's correlation method was used to study the association between the AOC and total phenolic content in both acetone and methanol extracted FFP samples. In this case, FFP samples altogether were considered the experimental unit for Pearson's correlation analyses. Pearson's correlation, *r*, was considered to determine the degree to which a relationship is linear and values $<.05$ were considered significant.

Results

Food leftovers were characterised by a mean value of total phenolic content of 129.3 ± 15.1 mg TAE/100 g in methanol extracts and 156.4 ± 25.8 mg TAE/100 g in acetone extracts. Although acetone extraction generally produced higher total phenolic content in all FFPs samples compared with methanol, the extraction solvents did not significantly influence ($p>.05$) the total phenolic content estimation in the FFPs samples tested. The highest total phenolic content was observed in FFP3 (250.0 ± 4.0 mg TAE/100 g acetone extract versus 186.1 ± 2.0 mg TAE/100 g methanol extract), compared to all other samples. Moreover, also FFP1 and FFP2 showed significant higher total phenolic content (+190% and + 288% in acetone extracts, +129% and +136% in methanol extracts, respectively; $p<.05$), compared to CTR (Figures 1 and 2). The lowest total phenolic content was observed in FFP4, FFP5 and FFP6.

The ABTS assay revealed that FFPs showed a mean value of AOC of 138.0 ± 14.3 mg TE/100 g in methanol extracts and 173.3 ± 18.8 mg TE/100 g in acetone extracts. In acetone extracted samples, FFP1, FFP2 and FFP3 showed a significantly higher AOC, compared with CTR (Figure 3). While in methanol-extracted samples, FFP2, FFP3 and FFP4 showed significantly higher AOC values ($p<.05$) compared with CTR (Figure 4).

Pearson's correlation method was applied to study the association between the total phenolic content and AOC in FFP samples. A positive correlation was observed between the total phenolic content and AOC in both acetone ($r = 0.88$; $p<.009$) and methanol ($r = 0.89$; $p<.007$) extracted samples.

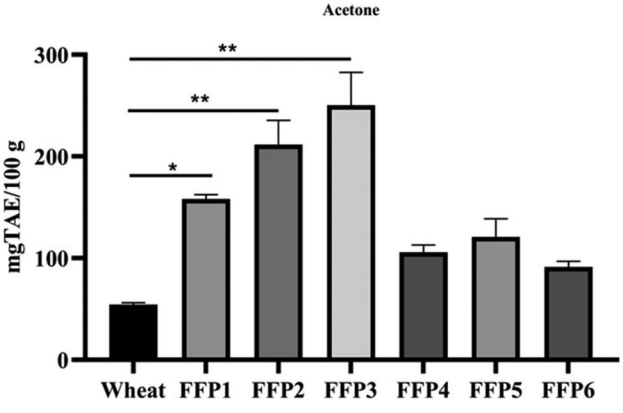

Figure 1. The total phenolic content in six FFPs samples extracted in acetone (50 *w/w*). Total phenolic content was determined by Folin-Ciocalteu colorimetric method. Each sample was extracted and analysed in triplicate ($n = 3$). **($p<.01$) and *($p<.05$) denote significant differences between FFPs samples and wheat. FFP: former food products

Discussion

The application of FFPs in animal feed is growing and this represents a valid opportunity for food industries to align themselves with the principles of circular economy, through the implementation of innovative approaches and techniques to recycle food wastage with also direct benefits for the environment. The reprocessing of FFPs is a procedure used to convert human food losses into suitable ingredients for animal feed. These materials are usually rich in heat-treated carbohydrates that make them not only rich in energy but also highly digestible (Luciano et al. 2020). In spite of that, there has not been conducted much research

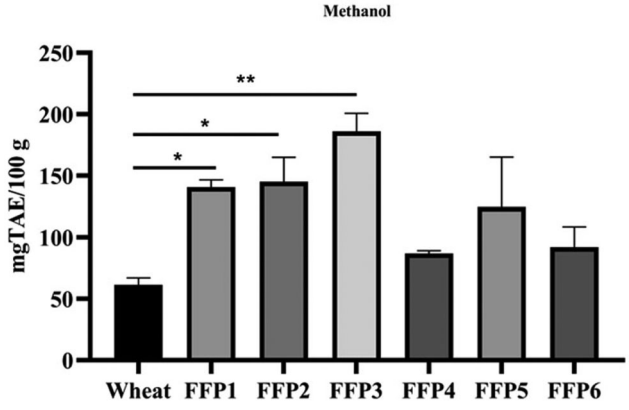

Figure 2. The total phenolic content in six FFPs samples extracted in methanol (80 *w/w*). Total phenolic content was determined by Folin-Ciocalteu colorimetric method. Each sample was extracted and analysed in triplicate ($n = 3$). **($p<.01$) and *($p<.05$) denote significant differences between FFPs samples and wheat. FFP: former food products.

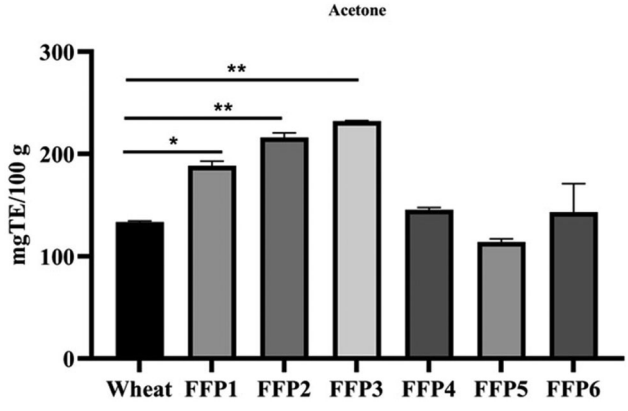

Figure 3. The antioxidant capacity (AOC) of six FFPs samples extracted in acetone (50 *w/w*). AOC was determined by ABTS + colorimetric method. Each sample was extracted and analysed in triplicate ($n = 3$). **($p<.01$) and *($p<.05$) denote significant differences between FFPs samples and wheat CTR. FFP: former food products; AOC: antioxidant capacity; CTR: control.

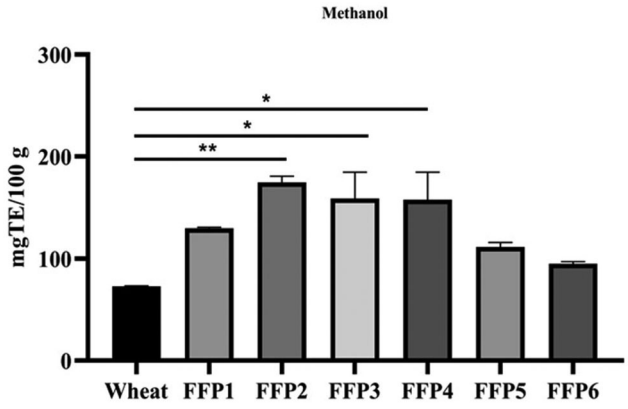

Figure 4. The antioxidant capacity (AOC) of six FFPs samples extracted in methanol (80 *w/w*). AOC was determined by ABTS + colorimetric method. Each sample was extracted and analysed in triplicate ($n = 3$). **($p < .01$) and *($p < .05$) denote significant differences between FFPs samples and wheat CTR. FFP: former food products; AOC: antioxidant capacity; CTR: control.

on the functional effect of FFPs with regard to animal nutrition. In this direction, data obtained in the present study demonstrate a valuable content of total phenolic content and notable AOC in FFP samples obtained with acetone and methanol extraction methods compared to wheat control. FFP1, FFP2, and FFP3 generally showed the highest total phenolic content and antioxidant capacity while the lowest total phenolic content and AOC was observed in FFP4, FFP5 and FFP6. The difference observed among sample is probably due to the inner composition. In fact, FFP1, FFP2 and FFP3 have a similar ingredient composition, which account leftovers of food industries, wheat by-products and wheat flour, while FFP4, FFP5 and FFP6 are mainly composed by leftover of food industry and bakery products but without bran or wheat by-products (Giromini et al. 2017). Consequently, FFP1, FFP2 and FFP3 were characterised by a higher dietary fibre content compared to the others FFPs which can explain the higher AOC and total phenolic content. These results can also be explained by the processing to which FFPs are subjected. It is well known that thermal processing can greatly affect the total phenolic content and antioxidant capacity of nutrients (Gunathilake et al. 2018).

One of the mechanisms that can explain those differences between raw and processed food is that thermal treatment can induce the oxidation of total phenolic content, leading to an intermediate oxidation state characterised by a higher radical scavenging activity (Nicoli et al. 1999). FFPs are products originally produced for human consumption and, consequently, subjected to mechanical/thermal processes such as heating (cooking), rolling, pelleting, flaking, extrusion, and expander processing before consumption (Singh et al. 2010). Therefore, beside an increased food digestibility, these processing techniques could lead also to an increased antioxidant capacity of FFPs compared to unprocessed counterparts. Pearson's correlation coefficient demonstrated a positive correlation between the total phenolic content and AOC in FFPs extracted with acetone or methanol. This positive linear correlation is in line with previous results (Ehlenfeldt and Prior 2001; Connor et al. 2002) and confirms that total phenolic content strongly impacts AOC of FFPs. Folin-Ciocalteu test determines the reducing capacity and thus, it quantifies all reducing compounds (such as ascorbic acid/reducing sugars). As the ABTS assay determines the electron transfer capacity of compounds/solutions, there is often a good correlation between Folin and ABTS assays.

Several solvent systems have been used to extract phenols and antioxidant compounds from cereal products (Zhou and Yu 2004) such as ethanol, methanol and acetone-based systems. These organic compounds are commonly used to extract free and soluble conjugate phenolic acids and antioxidant molecules, whereas the extraction of other molecules such as bound phenols can be obtained through a prior alkali, acid or enzymatic treatment of samples that allows their release. Several organic solvents such as water, ethanol, methanol and an aqueous ethanol solution were used with wheat samples and this allowed to obtain antioxidant-rich extracts (Zieliński and Kozłowska 2000; Vaher et al. 2010). Although the comparison of cereals antioxidant and total phenolic content results among different laboratories is difficult due to different solvent systems and extracting conditions applied. In our study, we have employed methanol:water and acetone:water extraction systems as they showed to be efficient in total phenolic and antioxidant molecules extraction from wheat samples which showed similar nutrient composition to FFPs (Giromini et al. 2017).

The nature of phenolic components is important in evaluating the antioxidant activity of a sample. The antioxidant activity of FFP samples may be due to other non-phenolic components. In addition, phenolic compounds can occur covalently bound to sugar molecules or cell wall components. Therefore, further studies are needed for a deeper characterisation of different phenols classes and on the presence of bound phenols and antioxidants in FFPs.These investigations will be useful in defining and quantifying especially bound phenols, that are known to have health-promoting effects. The reason is that that these

compounds may escape from digestion in the upper gastrointestinal tract along with cell wall materials and may be absorbed into blood plasma during digestion by intestinal microflora (Andreasen et al. 2001).

Moreover, a further step to be performed is related to the characterisation and quantification of phenolic and antioxidant compounds in FFPs to determine if AOC depends on the presence of natural or synthetic antioxidants, such as butylated hydroxyanisol and butylated hydroxyltoluene (Ratnam et al. 2006), which are seldom added to processed food to avoid oxidation. The latter points remain to be addressed in further studies.

Conclusion

In conclusion, the reprocessing of FFPs represents a valuable opportunity to meet circular economy strategy FFPs demonstrated to be a source of phenols and antioxidant compounds, which can be beneficial for animal health. The nature of these polyphenolic components is important in evaluating the antioxidant activity of the analysed samples. The antioxidant activity of these samples may be due to other non-phenolic components. Further studies should be performed to identify the specific classes of phenols and antioxidant compounds in FFPs.

Ethical approval

All procedures performed were in accordance with the 1964 Helsinki declaration.

Disclosure statement

No potential conflict of interest was reported by the authors.

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