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Screening of Four Different Agro-Food By-Products for the Recovery of Antioxidants and Cellulose

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Carried out under the European Project SusFoFlex, this study aimed the investigation and selection of different agro-food by-products for the recovery of antioxidants and cellulose to be further exploited for application in the development of innovative and sustainable food packaging materials. Based on literature data and partners' countries availability, four by-products were considered: brewers' spent grains, wheat straw, hazelnut shells and orange peels. A common process for the production of antioxidant extracts (a two-step hydro alcoholic solvent extraction) and another for the fractionation of cellulose (an acid hydrolysis step followed by an alkali hydrolysis and, then, an oxidative bleaching step) was applied to all the materials with the objective of selecting ideally a unique by-product for the production of both antioxidants and cellulose. The extracts were characterised in terms of total phenols content (based on Folin assay and expressed as gallic acid equivalents) and antioxidant activity (evaluated with both the radical ABTS test and the Ferric Reducing/Antioxidant Power assay).The highest total phenols recovery was obtained for orange peels (9.50±0.66 mg per g of dried by-product), while the yields for the other by-products were not statistically different and < 2 mg/g. Regarding the antioxidant activity, all the extracts showed very similar results with the ABTS assay, whereas orange peels extract exhibited the highest level of FRAP activity. The obtained cellulose residues were analysed for the total cellulose content and the level of lignin and hemicellulose impurities. In this case, the best by-product resulted wheat straw, with the recovery of 45 % of the original cellulose, a cellulose content (purity) of 84 % and residual impurities of hemicelluloses and lignin of 2 % and 12 %, respectively.

1. Introduction

Agro-food by-products are promising sources of natural antioxidant compounds to be used for different applications, including antioxidant food packaging. Also, because of environmental pollution of plastic based food packaging materials, researchers are looking for bio-degradable polymers prepared from or reinforced with natural fibres like cellulose. Thus, in search of natural and economic sources of phenolics and fibres, the agro-food by-products receive increasing attention, since they are easily available and their recycling would be beneficial to the human society. In this context, the European commission has sanctioned recently the research project "Susfoflex (Smart and sustainable food packaging utilizing flexible printed intelligence and materials technologies)" under the 7th framework programme. Within the project, the present study focused on antioxidants and cellulose recovery from four selected by-products: brewer's spent grain (BSG), hazelnut shells (HS), orange peels (OP) and wheat straw (WS).

BSG is a residue generated in large volumes in the production of beer after separation of the wort during the brewing process. It is a lignocellulosic material that could be further exploited since it is rich in oligo- and polysaccharides as well as polyphenols with ferulic and p-coumaric acids being the most abundant identified phenolic acids (Moreira et al., 2013). Its use on a biorefinery concept has been analysed and shown economically feasible (Mussatto et al., 2013).

Hard nutshells are one of the major by-products from hazelnut industrial processing and represent a huge amount of discarded material available at very low cost. Nowadays, HS is mostly used as fuel for burning, mulching and as raw material for production for furfural in the dye industry. Recent researches on crude

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extracts obtained from hazelnut by-products, hypothesised that hazelnut wastes, especially skin (Nazzaro et al., 2012) and hard shell (Stevigny et al., 2007), could be a reliable source of new and efficient natural antioxidants. HS have also been investigated for exploitation of the cellulose fraction (Di Blasi et al., 2010).
As concerns OP, during citrus fruit processing to make juice, peels are the primary by-products. Extraction of phenolic compounds from fruit wastes (Peschel et al., 2006), such as citrus peels (Lagha-Benamrouche and Madani, 2013), has attracted considerable scientific interest to its use as natural antioxidants in food and other industrial applications. This by-product has also revealed a good source of cellulose due to the low lignin content (Bicu and Mustata, 2013).
Finally, WS is an agricultural residue with many interesting characteristics that facilitate its biotechnological upgrade in a bio-refinery framework. Namely, as an herbaceous crop, its soft material can be transported in relatively high density form and typically has a low water content that enables its easy storage. It has been mainly investigated for lignocellulosic fractionation processes, focusing on the recovery of phenolic compounds from the acid hydrolysed liquors (Akpinar et al., 2012) or by alkaline hydrolysis (Tilay, 2008), such as on cellulose fractionation, mainly for bioethanol production (Asgher et al., 2013).
The aim of this study was to select the best by-products among the raw materials presented above, for the isolation of both phenolic compounds (based on total yield and antioxidant activity) and cellulose (based on total yield and purity of the final residues).

2. Materials and Methods

2.1 Materials

BSG were kindly provided by a small brewing plant close to Piacenza (Northern Italy). The material was further pressed (by a laboratory hand press) to remove excess water and oven-dried at 50 °C for 1 day.
HS and WS were kindly provided by a Company and a farmer in Piemonte (Northern Italy), respectively, and simply stored without additional drying.
OP were kindly provided by Anecoop S.Coop (Spain) after a drying treatment at 60 °C of almost 5 days. Lighter material and without moulds contamination was manually selected for the trials.
All raw materials were milled through a 2 mm sieve obtaining a powder with medium particle size ≤ 2 mm and used for the following trials.
Moisture content of the dried materials (and also of fresh raw materials in the case of OP and BSG) was assessed by constant weight measurement in oven at 105 °C. Fiber content and composition (cellulose, hemicelluloses and lignin) were evaluated according to TAPPI standards (Amendola et al., 2013).

2.2 Solvent extraction

The raw materials (10 g) were extracted twice with ethanol 80 %, for 2 h at room temperature, in darkness, under stirring at 150 rpm in an orbital shaker (HT Infors AG CH-4103, Switzerland) and with a solid/liquid ratio of 1/10 (g/mL) for BSG, HS, OP or 1/20 (g/mL) for WS (only in the first extraction, due to a high imbibition ratio of the solids). Solids were then separated with a paper filter (Whatman 595 1/2) and the liquids (extracts) analysed for total phenols content (TPC) and antioxidant activity.

2.3 Cellulose isolation

A common fractionation process was adopted for all the by-products combining the methods reported by Asgher et al. (2013) and Spigno et al. (2008). Briefly, 20 g of raw material were mixed with 200 mL of 4.7 % sulphuric acid and autoclaved at 121 °C for 45 min. The solid was separated by filtration using Whatman filter paper (No. 595 ½, 185 mm), washed several times with distilled water until neutral pH and oven-dried at 50 °C for 24 h. Then, the dried solid was taken in a 250 mL screw-capped flask, 200 mL of 4 M NaOH were added and the flask was kept on the orbital shaker at 180 rpm for 24 h at room temperature. The final solid residue was separated again with filter paper, oven-dried at 50 °C for 24, bleached using 200 mL 5 % H_2O_2 (pH 11.5) in the orbital shaker at 180 rpm at 45 °C for 8 h and let stand for 24 h at room temperature. The final solid residue was recovered through filter paper, washed with 50 mL of 50 % glacial acetic acid followed by washing with distilled water until neutral pH, dried at 50 °C for 24 h and weighed to calculate the cellulose residue yield. The residue was analysed for the cellulose, hemicelluloses and acid insoluble lignin (AIL) content.

2.4 Analytical methods

- ✓ Total phenols content was evaluated by Folin-Ciocalteau analysis (Folin Index), expressed as gallic acid equivalents (GAE) based on a calibration curve with standard gallic acid (García et al., 2011).
- ✓ Antioxidant activity was evaluated both according to the radical ABTS test and expressed as the percentage inhibition of radical oxidation (AOP%) (García et al., 2011); and the Ferric Reducing

/antioxidant power (FRAP) test according to the procedure described by Pulido et al. (2000). FRAP results were expressed as $mmol_{FeII}/g_{GAE}$.

- ✓ Cellulose, hemicelluloses and acid insoluble lignin content was evaluated according to the structural carbohydrate analysis (Spigno et al., 2008). After complete hydrolysis by 72 % sulfuric acid, the glucose content was measured by a Megazyme kit and used to estimate the cellulose content using the 0.88 conversion factor from monomer to polymer. The xylose content (measured by a Megazyme kit) was used to estimate the hemicelluloses content using the 0.90 conversion factor. The solid residue was washed with 50 mL of distilled water and dried in a pre-weighed crucible at 105 ºC for 24 h. Then, the sample was cooled to room temperature in a desiccator and weight was recorded. It was ignited in muffle furnace at 550 ºC for 3 h and the final weight was. The AIL content was calculated based on the difference between weight of oven dried and ignited residue.

2.5 Statistics

All the trials and analyses were carried out in three replicates and the values are reported as means ± SD. The significance of the influence of the by-product type on the fiber composition of raw material, total phenols yield and FRAP value of antioxidant extracts, was assessed by ANOVA and Tukey's post-hoc test for means discrimination at a confidence level of 99 % (IBM SPSS Statistics 19).

3. Results and Discussion

3.1 Raw materials

Table 1 shows the chemical characterization of the investigated by-products. BSG and OP have high moisture content before drying. Therefore, the drying yield is very low and the drying process increases the costs of the antioxidants and cellulose production, compared to WS or HS which do not require any drying. However, HS are very hard and their milling step would require more energy compared to the other materials. The hemicelluloses content was generally low, with the highest value found for WS which showed also the highest cellulose content. As expected, HS had high lignin content, while OP a very low content that might favour the cellulose isolation compensating its quite low level.

Table 1: Composition of by-products. Same letter under each parameter indicates means not statistically different.

Component	Brewer's spent grains (BSG)	Wheat straw (WS)	Hazelnut shells (HS)	Orange peels (OP)
Moisture content of fresh material	69.80 ± 0.13^{a}	5.85 ± 0.73^{c}	10.51 ± 0.12^{b}	75.64 ± 2.32^{d}
Moisture content of dried material	2.47 ± 0.19^{c}	5.85 ± 0.73^{b}	10.51 ± 0.12^{a}	6.47 ± 0.19^{b}
Drying yield ($g_{dry\ material}$ / g $_{fresh\ material}$)	0.31	1.00	1.00	0.26
Hemicelluloses (%)	11.76 ± 1.17^{b}	19.47 ± 0.22^{a}	13.32 ± 0.72^{b}	6.66 ± 0.39^{c}
Cellulose (%)	10.07 ± 1.06^{c}	33.78 ± 0.2^{a}	29.23 ± 2.18^{a}	16.37 ± 0.77^{b}
Lignin (%)	13.87 ± 0.13^{b}	21.53 ± 0.73^{b}	39.45 ± 2.67^{a}	5.00 ± 0.41^{c}

3.2 Antioxidants extraction

Table 2 reports the results of total phenols recovery from the four used by-products.

In general, second step of extraction led to an extract less concentrated in terms of total phenols in all raw materials. The extract from OP, compared with the other by-products, showed the highest phenols concentration both in the first and in the second extraction. Considering the phenols yields on dry weight, no significant differences were found between HS, WS and BSG, while OP yield was about six times higher. Total phenols yield on fresh weight considerably decreased in OP and BSG due to the high water content of the original by-products (Table 1), even though OP still remained the material with the highest yield.

Table 2: Comparison of total phenols recovery for the solvent extraction trials. Same letter under each parameter indicates means not statistically different. I, II: first, second extraction step.

	Total Phenols Content mg_{GAE}/L		Total Phenols Yield $mg_{GAE}/g_{dry\ weight}$		Total Phenols Yield $mg_{GAE}/g_{fresh\ weight}$		FRAP $mmol_{FeII}/g_{GAE}$	
Sample	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.	Mean	± s.d
Hazelnut shells I	181.17^{bc}	9.08	1.670^{c}	0.087	1.495^{b}	0.078	20.84^{a}	1.36
Hazelnut shells II	75.92^{d}	1.57	0.749^{d}	0.006	0.670^{d}	0.006	23.22^{a}	1.81
Wheat straw I	168.46^{bc}	7.91	1.189^{c}	0.048	1.120^{c}	0.045	11.80^{bc}	1.20
Wheat straw II	101.04^{d}	2.31	0.594^{d}	0.023	0.560^{d}	0.012	11.76^{bc}	1.21
Brewer's spent grains I	167.07^{b}	20.45	1.451^{c}	0.182	0.368^{e}	0.046	10.22^{bc}	1.34
Brewer's spent grains II	66.75^{d}	4.41	0.652^{d}	0.042	0.165^{f}	0.011	18.00^{b}	6.12
Orange peels I	1013.59^{a}	70.20	9.501^{a}	0.664	2.310^{a}	0.161	9.79^{c}	1.71
Orange peels II	225.26^{b}	7.31	2.405^{b}	0.080	0.585^{c}	0.019	9.14^{c}	0.23

The obtained results are generally in agreement with literature data, although different extraction methods might have been applied. Stèvigny et al. (2007) recovered from 3 to 9 mg_{GAE}/g from HS of different cultivars (extraction with 55.7 % ethanol in water, at pH 4 for 109 min at 20-22 °C, solvent-to-solid ratio 50 and particle size 0.5 mm) and Nazzaro et al. (2012) extracted up to 2.30 mg_{GAE}/g from HS using 70 % methanol in water for 5 days at room temperature. Logha-Benam and Madani (2013) obtained values between 9.61 and 31.62 mg_{GAE}/g from different cultivars of OP with 80 % methanol in water, solvent-to-solid ratio 10, particle size 0.5 mm, for 22 h at room temperature. Alkali extraction can allow for higher extraction yield in case of cereal residues, as reported by Moreira et al. (2013) who obtained 16-20 mg_{GAE}/g from BSG using 0.75 % NaOH for 15 min at 100 °C.
The ABTS scavenging test was selected because is one of the most widely used and simplest techniques, and has been proposed as one of the methods considered for standardization of antioxidant activity in food (Prior et al., 2005). Our results showed similar trend for the extracts of all the by-products expressing the results as a function of total phenols content (Figure 1). In general, HS first extract had higher antioxidant power than other samples, while for the same by-product the specific activity was almost not influenced by the extraction step. Due to the low initial concentration of some of the extracts, it was not always possible to evaluate the complete 0-100 % range for the AOP.

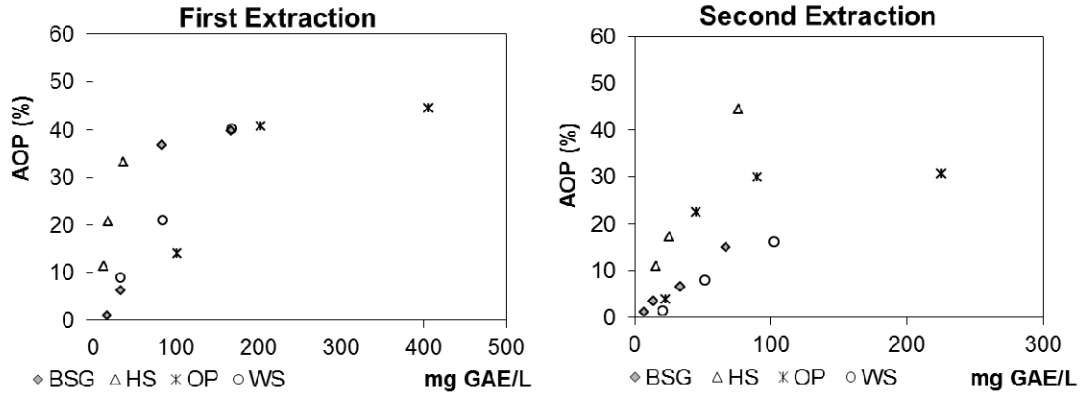

Figure 1: Antioxidant activity (AOP%) of extracts obtained by solvent extraction for the different by-products as a function of total phenols concentration (GAE).

Results of the FRAP assay test are reported in Table 2. Since only the phenolic compounds present in the samples are supposed to have the ferric reducing power, data was expressed based on the total phenols content. Confirming the ABTS assay, except for HS extracts, all the other extracts showed a very similar activity, and almost the same specific activity was observed in the second extracts. In this case, the less powerful extracts resulted from OP, even though it must be underlined that OP had been dried at 60 °C for 5 days and this might have led to partial degradation of the phenolic compounds.

3.3 Cellulose isolation

Table 3 reports the cellulose recovery after the applied acid / alkali fractionation process. Cellulose content of fiber residue indicates that WS residue has the highest level, followed by BSG, OP and HS, and slightly higher recovery for HS. Both WS and HS showed higher levels of residual hemicelluloses than OP and BSG. In all the investigated materials, a certain amount of acid insoluble lignin was still present as impurity, mainly in the HS cellulose, confirming a more lignified structure for this by-product. The highest recovery of the initial cellulose was reached for HS, followed by WS. However, the residue from WS showed the highest purity in terms of cellulose content. The hemicelluloses impurity was low in all the residues, even though these values could be underestimated since they were calculated based only on the xylose content, while other sugars can be released from hemicelluloses hydrolysis. Also the visual observation of the residues (Figure 2) clearly indicates an incomplete fractionation and a lignin contamination of the fiber residues from HS, OP and BSG. The cellulose residue from WS was relatively white indicating a higher purity.

Generally, the adopted fractionation process revealed to be too severe since a significant degradation of cellulose occurred. In particular for OP and BSG, which are the less lignified materials, up to 75 and 65 %, respectively, of the initial cellulose was lost.

Table 3: Characterization of the final cellulose residues obtained after lignocellulosic fractionation of the different agro-food by-products.

Component	Wheat Straw	Hazelnut Shells	Orange Peels	Brewers' Spent Grains
Cellulose residue yield (g/100 g_{dm})	16.91 ± 2.72	41.17 ± 0.23	6.78 ± 0.11	5.66 ± 0.37
Cellulose content (%)	84.07 ± 1.80	37.01 ± 1.15	56.11 ± 0.43	61.34 ± 1.63
Cellulose recovery (%)	44.70 ± 0.92	58.68 ± 7.99	24.91 ± 1.84	35.84 ± 6.28
Hemicellulose content (%)	2.10 ± 0.12	2.58 ± 0.09	1.28 ± 0.09	1.57 ± 0.08
Acid insoluble lignin (%)	12.43 ± 0.24	27.99 ± 2.72	13.46 ± 1.17	16.48 ± 2.25

Figure 2: Cellulose residues from hazelnut shells (A), wheat straw (B), orange peels (C) and brewer's spent grain (D) after lignocellulosic fractionation.

4. Conclusions

The obtained results showed that it would not be possible to exploit only one of the four investigated by-products for the recovery of both antioxidants and cellulose, based on the applied common experimental protocols. Regarding the extraction of antioxidants, orange peels gave the highest total phenols yield and the extracts showed antioxidant activity comparable to the extracts from the other materials. Thus, orange peels should be selected for this purpose, compared to wheat straw and hazelnut shells, that require a

drying-pretreatment. However, other factors should be taken into account, such as their production in large amounts by one of the partners and the possibility of recovering also pectins that can be used always in the project for the production of food edible films. As for the cellulose isolation, only wheat straw allowed to obtain good recovery of an almost pure cellulose residue.

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