Chapter N 302

**Green technologies and LCA for the analysis of bioactive compounds in wheat husk: an integrated study for sustainability assessment.**

**Abstract.** Wheat is the third most cultivated cereal in the world and is one of the most important cereals for production in the food industry. FAO has estimated that its production worldwide is about 760 million tons, of which Europe produces about 33%. Wheat husk, which is the external leathery part of the grain, is one of the main by-products of the wheat supply chain. This low-cost by-product is unstudied and underutilized, although it is generated in abundance in wheat production (about 17-20% of grain yield). Therefore, it is important to extract residues of high nutritional value (e.g., polyphenols), thus valorizing agri-food by-products.

For this purpose, the study will examine the possibility of recovering bioactive compounds (BCs) (e.g., polyphenols) from wheat by-products using green technologies (such as ultrasound-assisted extraction, Natural Deep Eutectic Solvents or NADES, water, etc.), which have been proposed to be the *greener* replacement for conventional solvents (e.g., Methanol, n-Hexane, Ethyl Acetate, etc.) In particular, NADES will be applied for the extraction of BCs, as they are natural solvents compatible with food, pharmaceuticals, and cosmetics. Furthermore, the application of the Life Cycle Assessment (LCA) methodology would allow a sustainability assessment for the recovery of bioactive components in comparison to wheat by-products.

**Keywords.** Green solvents, NADES, bioactive compounds, LCA, environmental sustainability.

# Introduction

The agri-food industry generates significant amounts of by-products that are normally discarded and can be a serious environmental problem. Wheat is the most common type of cereal, its global production reached 770 million tonnes in 2017, of which 150 million tonnes were harvested in Europe, thus representing 33% of the total production. (FAO, 2018). During the milling process, the wheat undergoes a series of treatments aimed at separating the outer fractions of the seed from the endosperm, i.e., the heart of the caryopsis intended for processing and transformation into cereal based-products. Wheat husks are generated as waste material and represent in terms of weight (%) about 20% of the processing output (Đorđević et al., 2016). We are therefore able to estimate that approximately 30 million tonnes of wheat husks are produced in the European Union each year. Wheat husks are often incinerated to generate electricity or heat, generating severe air pollution, or they are left to natural organic decomposition, thus contributing to greenhouse gas emissions (Sánchez et al., 2015). The valorisation of agricultural by-products through the extraction and recovery of molecules with high nutritional value (e.g., polyphenols, antioxidants, carotenoids, etc.), as a new resource to be reused in other production processes, could represent an alternative to incineration or composting (FAO, 2017). Bioactive compounds are studied for their biological properties, which can provide multiple health benefits (antihypertensive, anti-cancer, anti-inflammatory, hypoglycaemic, antimicrobial, antiviral, anti-tumour, antithrombotic, cholesterol-lowering, etc.). One problem related to the extraction of these bioactive compounds is the organic solvents generally used for their recovery (methanol, acetone, chloroform and hexane). These solvents are often discarded because they are flammable, explosive and poorly biodegradable. One solution to this problem is the use of alternative solvents: deep eutectic solvents (DES) and natural eutectic solvents (NADES), generally referred to as green solvents. DES are homogeneous liquids formed from at least one hydrogen bond acceptor (HBA) and one hydrogen bond donor (HBD), which when combined in a certain molar ratio their melting points are lower than the melting points of the individual components in the mixture (Rebocho et al., 2022). When DES are formed from natural eutectic compounds from plant metabolites or their derivatives (amino acids, organic acids, sugars, or choline derivatives), they are called NADES. NADES are easily biodegradable and due to their natural composition, the extract obtained from NADES can be used directly in food, pharmaceuticals, and cosmetics without the need to remove the solvent, thus avoiding costly downstream processing and purification. In this study, the extraction of polyphenols and antioxidant from wheat husk was investigated by using different NADES. The extraction efficiency of BCs was compared with conventional solvent (Ethanol in aqueous solution) and water, and was achieved by means of UV-Vis spectrophotometric analysis. Furthermore, the application of the Life Cycle Assessment (LCA) methodology [9-10] would allow a sustainability assessment for the recovery of bioactive components in comparison with wheat by-products.

# Material and methods

* + 1. ***Chemicals***

Folin-Ciocâlteu reagent, gallic acid (analytical standard), rutin (analytical standard), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), choline chloride, betaine, fructose, glucose, sodium carbonate (Na2CO3), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), Ethanol (HPLC-grade), sodium nitrite (NaNO2), aluminum chloride (AlCl3), sodium hydroxide (NaOH), phosphate-buffered saline (PBS), methanol (HPLC-grade), and ultrapure water were purchased from Sigma Aldrich Chemical Co, Saint Louis, MO, USA.

* + 1. ***Instruments***

The following instruments were used: Bandelin Sonorex RK100H water and ultrasonic thermostatic bath, Tehtnica Rotamix 500 MMH magnetic stirrer, NEYA 10R refrigerate centrifuge (Exacta Optech, Modena, Italy), and UV-Vis spectrophotometer (Jenway, Stone, UK).

* + 1. ***Natural Deep Eutectic Solvents (NADES) synthesis***

NADES synthesis was performed according to the Heating and Stirring method (Florindo, C. et al., 2014). According to this method, the NADES components are placed in a closed bottle and heated at T= 60 °C under magnetic agitation, and stirred for around 30 min to 1 hour, until a clear liquid solvent is formed. Specific molar ratios between the hydrogen bond acceptor (HBA) [Betaine (Bet), and Choline Chloride (ChCl)] and hydrogen bond donor (HBD) [Fructose (Fru) and Glucose (Glu)] were synthesized as indicated in Table 1. All of the solvents were diluted with 30% of water.

**Table 1.** NADES Components, Molar Ratios, and Water Content

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name | Component 1 (HBA) | Component 2 (HBD) | Molar ratio | Water Content (%) |
| NADES1 | ChCl | Glu | 1:1 | 30 |
| NADES2 | ChCl | Fru | 1:1 | 30 |
| NADES3 | Bet | Glu | 1:1 | 30 |
| NADES4 | Bet | Fru | 1:1 | 30 |

* + 1. ***Extraction of polyphenols from the wheat husk*** 
       1. *Preparation of NADES and hydroalcoholic-based extracts*

NADES and hydroalcoholic-based extraction in wheat husk was performed according to Manuela et al. 2020, with some modifications for wheat husk. Solid-liquid ratios of 0.5 g (±0.01g) of homogenized wheat husk per 10 mL of NADES, Ethanol in aqueous solution (80:20), and aqueous solution were used for the extraction of polyphenols. Extraction was carried out in an ultrasonic and thermostatic bath (power 160 W) at constant temperature (T= 70 °C), for 40 min. The samples were then centrifuged at 2,900x g for 10 min at T= 25 °C. The supernatant was collected in a 10-mL amber flask and stored at T= + 4 °C until the day of analysis. All analyses were conducted in triplicate.

* + 1. ***Spectrophotometric analysis for Total Phenolic Content (TPC), Total Flavonoids Content (TFC), and ABTS radical scavenging assay***

*1.2.5.1 Total Phenolic Content by Folin-Ciocâlteu Assay*

Total phenolic content (TPC) was performed by spectrophotometric analysis using the Folin-Ciocâlteu method (Preti et al., 2017). The absorbance was measured at λ=750 nm in 1-cm path length cuvettes against the blank solution. The total content of phenols was expressed as milligrams of Gallic Acid Equivalent (GAE) per g of wheat husk. The results were obtained through a calibration curve ranging from 10 to 100 mg/L (R2 = 0.9997).

*1.2.5.2 Total Flavonoids Content by Aluminum Chloride Assay*

The total flavonoids content was determined with the aluminum chloride method, as reported by Zargar et al., 201. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The results were expressed as milligrams of Rutin Equivalents (RE) per g of wheat husk samples (R2= 0.9959).

*1.2.5.3* *Antioxidant Activity by ABTS radical scavenging Assay*

The Trolox equivalent antioxidant capacity (TEAC) of wheat husk extracts was estimated by the ABTS radical scavenging assay, according to Omono et al., 2015 with some modifications. The decolorization of ABTS●\* was expressed as µM Trolox Equivalent (TE) per g of wheat husk, obtained by a calibration curve ranging from 0.5 µM TE to 200 µM TE (R² = 0.9963).

## Life cycle Assessment

The study evaluates the sustainability assessment associated with wheat by-products, which account for 17-20% of the total production, through the application of the Life Cycle Assessment methodology (ISO 14040, 2006; ISO 14044, 2006).

### Goal and scope definition

This study was conducted to compare the environmental impacts of wheat production with wheat by-products for the recovery of bioactive compounds. The system considered the phases from land preparation to harvesting the raw cereal (from cradle to farmgate). The functional unit considered was the cultivation of one hectare (ha) of wheat.

### Life cycle inventory

In this study, wheat cultivation involves the present inputs shown in Table 2 (i.e. seeds, fertilizers, pesticides, diesel consumption for agricultural operations) and the grain yield obtained.

Table 2. LCI data

|  |  |  |
| --- | --- | --- |
| Inputs/outputs | Unit | Value |
| Input |  |  |
| Diesel | Kg | 161,8 |
| Lubricating oil | Kg | 0,8 |
| Water | Kg | 900 |
| Mineral superphosphate (19% P2O5) | Kg | 57 |
| Ammonium nitrate (26% N) | Kg | 78 |
| Urea(46% N) | Kg | 92 |
| PP(sacks) | Kg | 7,1 |
| Paper(container) | Kg | 3,1 |
| Seed | Kg | 200 |
| Herbicide | Kg | 0,044 |
| Insecticide | Kg | 0,378 |
| Output |  |  |
| Wheat | t | 154,3 |
| Wheat by-products | t | 34,8 |

### Life Cycle Impact Assessment (LCIA)

This phase was aimed at evaluating the contribution of the wheat by-products in terms of individual impact categories, by using SimaPro 9.2.2. software. The ReCiPe 2016 Midpoint (H) V1.05 method was used for the impact calculations.

**2. Results and Discussion**

**2.1 Bioactive compounds determination in wheat husk**

Table 3. showed quantitative results for colorimetric assays for the determination of bioactive compounds in wheat husk.

**Table 3. Bioactive compounds result in different wheat husk extracts.** *Data are expressed as mg/g of sample ± standard deviation (SD).*

|  |  |  |  |
| --- | --- | --- | --- |
| Wheat husk extracts | TPC (mg GAE/g) | TFC  (mg RE/g) | ABTS (µmol TE/g) |
| EtOH:H2O (80:20, v/v) | 1.60±0.06 | 4.71±0.05 | 4.92±0.04 |
| H2O | 2.44±0.26 | 2.86±0.03 | 5.32±0.12 |
| Bet/Glu (1:1) | 2.68±0.05 | 4.86±0.19 | 4.75±0.09 |
| Bet/Fru (1:1) | 3.42±0.28 | 6.59±0.23 | 4.76±0.07 |
| ChCl:Glu (1:1) | 1.54±0.13 | 3.38±0.02 | 5.17±0.11 |
| ChCl:Fru (1:1) | 1.46±0.14 | 6.15±0.05 | 4.51±0.08 |
| *EtOH: Ethanol; H2O: Water; Bet: Betaine; Glu: Glucose; Fru: Fructose; ChCl: Choline Chloride. GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; TE: Trolox Equivalent.* | | | |

The results showed a variability among spectrophotometric assays depending on the composition of the extracting solvent, thus indicating the importance in the selection of suitable NADES. For TPC and TFC respectively, NADES 4 resulted in the best solvent (3.42±0.28 mg GAE/g; 6.59±0.23 mg RE/g), thus showing an extraction efficiency about 50% higher than conventional extraction (1.60±0.06 mg GAE/g; 4.71±0.05 mg RE/g). This could probably be related to the physico-chemical characteristics of NADES indicating that intermolecular interactions between NADES constituents and phenolic compounds and flavonoids play an important role in solubility (Dai et al., 2010). Our results are in agreement with the study by Cherif et al. (2020), the only study in the literature that evaluated the potential of polyphenol extraction by DES from wheat production waste. Specifically, a NADES composed of glycerol/citric acid/glycine (molar proportion of 4:1:1) was used in the study for the extraction of total polyphenols expressed as mg ferulic acid equivalent per g dry mass of wheat bran. The results show that extraction by NADES resulted in a total polyphenol yield of 6.31 ± 0.06 mg ferulic acid equivalent g-1 dry weight. This value was found to be higher than the use of conventional solvents such as 60% ethanol and water, with more efficient extraction of about 40% and 50% respectively. Antioxidant activity was tested by ABTS, which is an *in vitro* anti-radical assay, soluble in both aqueous and organic solvents, so can be used to determine both hydrophilic and lipophilic antioxidant capacity of extracts. The results of this assay are in the range of 4.51±0.08 µmol TE/g to 5.32±0.12 µmol TE/g. In the work of Fogarsi et al. 2015, the ABTS scavenging capacity of eight wheat varieties was evaluated and the content ranged from 0.72 and 2.83 μmol TE/g.

## LCIA results

The application of the LCA methodology allowed the study of wheat production and its waste, the wheat husk, highlighting the stages with the greatest impact. The results of the assessment are shown in Table 4.

**Table 4**. LCIA of Wheat Production and Wheat by-products

|  |  |  |  |
| --- | --- | --- | --- |
| **Impact categories** | **Unit** | **Wheat Production** | **Wheat by-products** |
| **Global warming** | **kg CO2 eq** | 3,29x10-1 | 1,05x10-1 |
| **Stratospheric ozone depletion** | **kg CFC11 eq** | 6,10x10-6 | 9,91x10-7 |
| **Ionizing radiation** | **kBq Co-60 eq** | 5,29x10-3 | 6,98x10-3 |
| **Ozone formation, Human health** | **kg NOx eq** | 2,10x10-3 | 4,34x10-4 |
| **Fine particulate matter formation** | **kg PM2.5 eq** | 8,82x10-4 | 2,54x10-4 |
| **Ozone formation, Terrestrial ecosystems** | **kg NOx eq** | 2,11x10-3 | 4,37x10-4 |
| **Terrestrial acidification** | **kg SO2 eq** | 4,64x10-3 | 8,83x10-4 |
| **Freshwater eutrophication** | **kg P eq** | 1,63x10-4 | 5,05x10-5 |
| **Marine eutrophication** | **kg N eq** | 1,97x10-3 | 3,27x10-4 |
| **Terrestrial ecotoxicity** | **kg 1,4-DCB** | 2,19x10-1 | 1,14x10-1 |
| **Freshwater ecotoxicity** | **kg 1,4-DCB** | 1,15x10-2 | 3,88x10-3 |
| **Marine ecotoxicity** | **kg 1,4-DCB** | 4,64x10-3 | 3,80x10-3 |
| **Human carcinogenic toxicity** | **kg 1,4-DCB** | 2,02x10-3 | 2,56x10-3 |
| **Human non-carcinogenic toxicity** | **kg 1,4-DCB** | 5,26x10-1 | 1,39x10-1 |
| **Land use** | **m2a crop eq** | 1,80 | 3,11x10-1 |
| **Mineral resource scarcity** | **kg Cu eq** | 2,75x10-4 | 4,17x10-4 |
| **Fossil resource scarcity** | **kg oil eq** | 5,94x10-2 | 2,25x10-2 |
| **Water consumption** | **m3** | 1,02x10-2 | 2,04x10-3 |

On a life cycle analysis basis, the production of wheat by-products generates lower negative environmental impacts than the production of wheat, the production of which, on the other hand, impacts 15 out of 18 categories. From the results obtained, the macro area in which wheat production has the greatest impact is that of the environment specifically we find high values in the categories: Global warming (3,29x10-1 kg CO2 eq), Ozone formation Terrestrial ecosystems (2,11x10-3kg NOx eq**)**, Terrestrial acidification (4,64x10-3kg SO2 eq), Freshwater eutrophication (1,15x10-2 kg P eq), Marine eutrophication (1,97x10-3 kg N eq). All of these values of grain production turned out to be 70% higher than the results of grain by-products as can be seen in Figure 1 where the results were characterized and expressed as a relative impact, where the scenario with the highest value in the impact category is set as the reference value (100) and the other is calculated accordingly.

Figure 1. Characterized results of Wheat production in relation to Wheat by-products

Furthermore, after demonstrating how wheat by-products have less impact than wheat itself by extracting polyphenols using NADES, these wastes could be valorized by incorporating these theoretically edible extracts into foods.

This possibility would also generate a gain from an economic point of view considering a yield of 2.2% in polyphenols from the wheat husk matrix, and that phenolic acid is the polyphenol most present in wheat (Shahidi, F. and Ambigaipalan, P., 2015) and that its cost in pure form is 169€ per 0.1 g (Sigma-Aldrich).

**3. Conclusion**

This study showed that new natural deep eutectic solvents (NADES) can be an exceptionally effective means of recovering polyphenolic antioxidants from the by-products of wheat processing, particularly husks. Furthermore, through the application of LCA methodology, it was possible to assess the sustainability of the recovery of bioactive components from wheat by-products. Our study demonstrates that the use of husks can be useful in mitigating a number of environmental impacts including the potential for global warming. Moreover, thanks to its polyphenol content and its extraction by means of green and naturally based solvents such as NADES, it is possible to use them for the formulation of functional foods, also generating a profit in a circular economic optics.

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