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Toxic Analysis of Leaf Protein Concentrate Regarding Common Agricultural Residues

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HIGHLIGHTS

- An open-source toolchain for non-targeted screening of toxins from high-resolution mass spectrometry data was used.
- Average yield ranged from about 7 to 14.5% for the nine Leaf Protein Concentrates investigated.
- Leaf Protein Concentrate from agricultural residue could be a valuable food source.

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Acronyms and abbreviations
ASRS=Abrupt Sunlight Reduction Scenario
ESI=Electrospray Ionization
LC/MS=Liquid Chromatography/ Mass Spectrometry
LPC=Leaf Protein Concentrate

ABSTRACT

Background: Potential resilient foods which help reduce hunger are converting the ~998 million tons of agricultural residue generated each year into human edible food. Although it is possible to extract Leaf Protein Concentrate (LPC) from agricultural residues, it is not widely practiced because both toxicity and yields of the protein concentrates have not been widely investigated in the most common agricultural residues.

Methods: To fill this knowledge gap, this study uses high-resolution mass spectrometry and an open-source toolchain for non-targeted screening of toxins of nine agricultural plant residues in October 2021; it included seven agricultural residues: corn/maize, wheat, barley, alfalfa, yellow pea, sunflower, canola/rapeseed, and two weeds/agricultural residues of kochia, and round leaf mallow.

Results: The average yield ranged from about 7 to 14.5% for the nine LPCs investigated. According to the results, yellow pea, round leaf mallow, and canola are recommended for further investigation and scaling as they appear to be fit for human consumption based on the lack of dangerous toxins found in the analysis performed in this study.

Conclusion: All the compounds identified in these samples have either been approved by international regulatory boards for safe consumption or are known to be present in common beverages. The other agricultural residues require additional quantification of the toxins identified as it will determine the actual risk for human consumption. Overall, the potential for LPC to provide more needed calories from existing agricultural practices is extremely promising, but substantial amount of future work is needed to screen LPCs in all the agricultural residues depending on harvesting, handling, and storage conditions.

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Introduction

World hunger is still an important issue and will become more challenging in many areas due to conflicts, civil unrest and economic shocks, crop failures, weather extremes, and climate change (FAO et al., 2017; FSIN and Global Network Against Food Crises, 2020). Resilient or alternative foods may lessen the severity of hunger/malnutrition worldwide (McClements et al., 2021; Pham et al., 2022; Tzachor et al., 2021). According to several studies, it is possible to support global population with no conventional agriculture if resilient foods are ramped up quickly (Denkenberger and Pearce, 2014, 2015). This can be done, in part, by extracting additional calories from agricultural residue. Agricultural byproducts are generally used as food in animal agriculture (Reddy and Yang, 2005); however, it is well established that widespread animal agriculture is unsustainable (Eshel et al., 2016; Foley et al., 2011; Gerber et al., 2013; Scarborough et al., 2014). Animal agriculture alone produces 18% of all anthropogenic greenhouse gas emissions – more than all transportation related emissions (Gerber et al., 2013; Koneswaran and Nierenberg, 2008). Eating plant products instead of feeding them to livestock also requires much less water and land than traditional animal agriculture (Eshel et al., 2016; Mekonnen and Hoekstra, 2012).

Not all plant material is edible directly, and this generates an estimated 998 million tons of agricultural residue per year globally (Cherubin et al., 2018; Obi et al., 2016); such amount may be a potential source of resilient food. For example, the leaves of plants which are not normally eaten can be ground, pressed, and then coagulate the resultant liquid as leaf concentrate, which contains ~8% of the dry matter of the original leaves mostly in the form of edible protein (Kennedy, 1993). Although it is possible to extract this Leaf Protein Concentrate (LPC) from agricultural residue, it has not been studied in detail, and the toxicity of the protein concentrate for humans from the most common agricultural residues has not been investigated. High-resolution mass spectrometry is used for non-targeted screenings when no previous information is available for identification of unknown chemicals (Schymanski et al., 2014). The quantity and quality of evidence available lead to a range of confidence levels for compound identification (Sumner et al., 2007; Schymanski et al., 2015). This approach has been used successfully for rapid toxic screening process of the concentrates of the most common leaf in North America, red maple (acer rubrum) (FIA, 2022), concentrates to be used for resilient foods (Pearce et al., 2019). A completely new open-source toolchain has been developed to automate non-targeted screening of toxins (Breuer et al., 2021), which is an effective method to identify potentially harmful compounds in either alternative or resilient foods. This method is used here to do initial screening on nine agricultural plant residues including seven agricultural residues: corn/maize, wheat, barley, alfalfa, yellow pea, sunflower, canola/rapeseed, and two weeds/agricultural residues: kochia and round leaf mallow.

Materials and methods

Materials

Seven of the nine agricultural residues (e.g., normally non-harvested parts of edible plants) were obtained in southern Alberta, Canada, in early October 2021. The biomass utilized was at various stages of maturity. Wheat, barley, canola, and yellow pea were second growth; alfalfa and round leaf mallow were cover crops, and kochia was an untended field. Samples were harvested and stored in zip lock bags for between 3-7 days under refrigeration while being processed. Sunflower and corn were obtained from Nelson, British, Columbia, in mid-September and stored in refrigerator for less than 48 h before being processed. For barley, an additional experiment was run where fresh LPC was compared against barley residue; it was dried and partially exposed to sunlight for two months before being processed to simulate how protein yield might degrade for crop residue left to dry in the field.

Material processing

To determine the yield, a known mass of agricultural residue product (20-100 g) was dried using a Crawford Kitchen – Professional Dehydrator (which could also be processed with a conventional oven, food dehydrator, or open-source vacuum drier (Hubbard et al., 2021)). The dried material was massed and set aside. A known mass of fresh leaves (20-100 g) were then blended with ample water to create a thin paste, which varied by the sample. When fully blended, the mixture was extracted from the blender with a spoon or spatula.

Liquid was separated from pulp and captured by passing through a finely woven polyester bag under compression. Juice was heated to the boiling point while being stirred gently. Any curd formed on the surface of the liquid was skimmed off and collected in a separate vessel and transferred to the dehydrator set at 66 °C (152 °F) for 16 h, yielding a dry green solid.

To calculate the yield, the following equation was used:

$$Y = M_c / M_l$$

Where Y represents the yield of leaf concentrate, M_c, the mass of dried (dehydrated) concentrate (g), and M_l, the mass of dried leaves (g). For all samples, the blending and

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boiling times used were 3 min each, and the drying time was 16 h at 66 °C.

Liquid Chromatograph / Mass Spectrometer (LC/MS) instrumentation

The non-targeted approach used an ultra-high-resolution hybrid ion trap orbitrap MS instrument (Thermo Scientific Orbitrap Elite equipped with Electrospray Ionization (ESI)) coupled to an Ultra-High-Pressure two-dimensional Liquid Chromatograph (HPLC) system (Thermo Scientific Dionex Ultimate 3,000 standard system).

LC/MS grade acetonitrile and water were purchased from Fisher Scientific (Waltham, MA, USA) and HoneyWell (Morris Plains, NJ, USA), respectively. LC/MS grade formic acid was purchased from Sigma Aldrich (St. Louis, MO, USA).

For LC/MS analysis, samples underwent the following procedure similar to Breuer et al. (2021); The agricultural residue concentrate was diluted 12 times in water–acetonitrile 80:20 (v:v). Then, it was filtered with a 0.2 µm quartz filter. The HPLC was calibrated externally with Thermo Pierce calibration solution before LC/MS runs. Following Pearce et al. (2019), the analytical column was Phenomenex reversed-phase Kinetex XB-C18, 150×2.1 mm, 100 A, with 1.7 µm particle size. Mobile phase A was 0.1% formic acid in 100% LC/MS grade water, and mobile phase B was 0.1% formic acid in LC/MS grade acetonitrile–water 95:5 (v:v) solution. A constant flow rate (0.2 ml/min) was used due to the small particle size of the column (1.7 um), and the mobile phase gradient included: 0 min: 5%B, 5 min: 5%B, 65 min: 90%B, and 70 min: 90%B. The column was equilibrated with mobile A for 15 min before the next injection. The column oven was set at 35 °C, and the full loop injection volume was set at 5 µl.

The resolving power for accurate mass measurement during the LC/MS run was 120 K defined at mass to charge ratio (m/z) 400. The sample was run with both positive and negative electrospray ionization modes under two separate LC/MS runs and was abbreviated to ESI+ and ESI-. All the masses in the range of 100-600 m/z were recorded with full scan mode. Samples underwent a full scan. In addition, data-dependent MS/MS fragmentation was recorded for the 5 tallest peaks on each spectral scan with collision energy of 25 (arbitrary unit). This was done to identify co-eluting compounds (Breuer et al., 2021).

Analysis

The samples were then analyzed using an open-source software tool chain consisting of mass spectrometry analysis with MZmine 2, formula assignment with MFAssignR, and data filtering with ToxAssign (Breuer et al., 2021). ToxAssign compares the compounds to those listed in the OpenFoodTox Chemical Hazards Database maintained by the European Food Safety Authority (Dorne et al., 2021; EFSA, 2022).

The United States Environmental Protection Agency sets four classes for toxic substances based on their acute toxicity. Class-determining characteristics include oral and dermal LD_{50} (lethal dose in 50% of test animals), inhalation LC_{50} (lethal air concentration in 50% of test animals), and the level of eye or skin irritation that results with contact (Code of Federal Regulations, 2022). The class determining characteristics for pesticides and other agents are shown in supporting material in the Open Science Framework in Table A1. Category I (class 1) agents are highly toxic by at least one route of exposure (NPIC, 2022). Category II (class 2) agents are considered moderately toxic if consumed or otherwise absorbed into the body (NPIC, 2022). Category III (class 3) substances are classified as “slightly toxic” and generally need to be consumed in large doses to produce harmful effects, and Category IV (class 4) agents have very low toxicity and must be consumed in very large quantities to produce harmful effects (NPIC, 2022). Thus, in this preliminary analysis, the results for only toxins in Category I and II are reported.

Results

The results for each of the nine agricultural residues for both yield and toxic analysis are detailed below.

Corn

The parameters for LPC of corn are shown in Table 1 with an average yield of 8.16%. Due to highly fibrous nature, leaves were comparatively tough compared to wheat, rye, etc. Corn returned multiple toxins in each class, but results were dominated by aflatoxins. Aflatoxin G_2, a class 1 toxin, was present in both ESI+ and ESI- modes. Class 1 toxins, aflatoxin B_1 were also present in ESI+ modes. Two class 2 toxins were also identified: aflatoxin G_1 in ESI+ and dimethyl dicarbonate in ESI-.

Wheat

Wheat LPC parameters are shown in Table 2 with an average LPC yield of 15.16%. Wheat samples also returned multiple compounds in class 1 and 2. Class 1 toxins included 4-vinylphenol (ESI+), T-2 Toxin (ESI+), aflatoxin G_2 (ESI+ and ESI-), aflatoxin B_1 (ESI+). Class 2 toxins included aflatoxin G_1 (ESI+) and dimethyl dicarbonate (ESI-).

Barley

Barley LPC parameters are shown in Table 3 with an average LPC yield of 7.46%. Two different samples containing barley at the same location were analyzed. One sample was the processed fresh barley. The second one was

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artificially dried, and was left partially exposed to sunlight for two months before being processed to simulate how protein yield might degrade for samples left to dry in the field. In both samples, barley returned multiple class 1 toxins and one class 2 toxin. It should be noted that the toxic profile also changed between these samples, indicating that substantial future work may be needed to evaluate toxicity based on handling conditions including weather, humidity, and photodegradation.

In the fresh barley samples, class 1 toxins Aflatoxin G_2, Aflatoxin B_1, and HT-2 Toxin were all identified in ESI+ mode. Class 2 toxin dimethyl decarbonate was also found in fresh barley samples using ESI-. In dried barley samples, class 1 toxins 4-vinylphenol (ESI+ mode), T-2 toxin tetraol (ESI+ and ESI- modes), and deoxynivalenol (ESI- mode) were identified. Class 2 toxin dimethyl dicarbonate was also identified in ESI- mode.

Alfalfa

Alfalfa LPC parameters are shown in Table 4 with an average LPC yield of 11.41%. Alfalfa samples returned multiple toxins in each class. Class 1 toxins aflatoxin G_2 and 4-vinylphenol were both identified using ESI+ mode. Class 2 toxins included aflatoxin B_2 (ESI+ and ESI- modes), nivalenol (ESI- mode), T-2 toxin tetraol (ESI- mode), and dimethyl dicarbonate (ESI- mode).

Yellow pea

Yellow pea LPC parameters are shown in Table 5 with an average LPC yield of 10.35%. Yellow pea only returned one class 2 toxin, dimethyl dicarbonate, which was identified in ESI- mode.

Sunflower

Sunflower LPC parameters are shown in Table 6 with an average LPC yield of 9.84%. Fiber mass portion was not retained. Testing on sunflower samples presented multiple class 1 and class 2 toxins. Class 1 toxins included altenuene (ESI+ mode), aflatoxin G_2 (ESI+ and ESI- modes), and 4-vinylphenol (ESI+ mode). Class 2 toxins included dimethyl dicarbonate (ESI- mode), deoxynivalenol 3-glucoside (ESI+ mode), aflatoxin B_2 (ESI+ and ESI- modes), altenuene (ESI+ mode), anguidine (ESI+ mode), and nivalenol (ESI- mode).

Canola

Canola LPC parameters are shown in Table 7 with a LPC yield of 9.84%. Only a single sample of canola was processed.

Canola returned one class 2 toxin, dimethyl dicarbonate, which was identified in ESI- mode.

Kochia

Kochia LPC parameters are shown in Table 8 with an average LPC yield of 4.21%. Stem and leaves of kochia were blended. The stems, although green, were tough, making them difficult to blend. Separation of leaves from stems was not feasible due to small size. This made processing comparatively labor intensive in the context of other samples.

Kochia returned two class 1 toxins and one class 2 toxin. Class 1 toxins included 4-vinylphenol (ESI+ mode) and alternariol monomethyl ether (ESI+ and ESI- modes). The identified class 2 toxin was dimethyl dicarbonate (ESI- mode).

Round leaf mallow

Round leaf mallow LPC parameters are shown in Table 9 with an average LPC yield of 7.39%. Fiber mass was not retained, and round leaf mallow returned one toxin in each class. Toxic class 1 substance, 4-vinylphenol, and toxic class 2 substance, dimethyl dicarbonate, were both identified in ESI- mode.

The plant component used maturity and average yield which ranged from about 7 to 14.5% for the nine agricultural residues investigated; this is summarized in Table 10.

All data for this study including the total ion chromatograms for both the positive and negative ionization and digital photographs of selected agricultural residues are available at https://osf.io/h5vse/ (Pearce, 2023).

Table 1: Yield and sample preparation data for corn

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	99.98	100.15	100.12	100.08
LPC drying paper weight (g)	1.12	1.10	0.90	1.04
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	18.06	19.10	18.47	18.54
Paper+LPC (g dry)	2.43	2.71	2.14	2.43
LPC (g dry)	1.31	1.61	1.24	1.39
LPC yield % (dry LPC to dry leaf weight)	7.66%	9.39%	7.24%	8.10%

LPC=Leaf Protein Concentrate

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Table 2: Yield and sample preparation data for wheat

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	50.05	50.01	50.20	50.09
LPC drying paper weight (g)	0.74	0.63	0.63	0.67
Fiber Mass drying paper weight (g)	0.69	0.63	0.55	0.62
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	5.68	5.57	5.48	5.58
Fiber mass (g dry)	4.99	4.94	4.93	4.95
Fiber mass yield (% dry fiber mass to dry leaf weight)	0.55	0.54	0.54	0.54
Water soluble solids	2.83	2.78	2.88	2.83
Water soluble solids yield % (g dry solids from water to dry leaf weight)	0.31	0.31	0.32	0.31
Paper+LPC (g dry)	2.02	2.00	1.95	1.99
LPC (g dry)	1.28	1.37	1.32	1.32
LPC yield % (dry LPC to dry leaf weight)	14.07%	15.07%	14.46%	14.53%

LPC=Leaf Protein Concentrate

Table 3: Yield and sample preparation data for barley

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	50.11	50.13	50.18	50.14
LPC drying paper weight (g)	0.71	0.72	0.80	0.74
Fiber mass drying paper weight (g)	0.68	0.59	0.66	0.64
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	5.09	4.75	4.49	4.78
Fiber mass (g dry)	4.41	4.16	3.83	4.13
Fiber mass yield (% dry fiber mass to dry leaf weight)	43.18%	40.72%	37.45%	40.45%
Water soluble solids	4.90	5.33	5.75	5.33
Water soluble solids yield % (g dry solids from water to dry leaf weight)	48.00%	52.14%	56.19%	52.11%
Paper+LPC (g dry)	1.61	1.45	1.45	1.50
LPC (g dry)	0.90	0.73	0.65	0.76
LPC yield % (dry LPC to dry leaf weight)	8.81%	7.15%	6.36%	7.44%

LPC=Leaf Protein Concentrate

Table 4: Yield and sample preparation data for alfalfa

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	50.15	50.43	50.48	50.35
LPC drying paper weight (g)	0.76	0.83	0.76	0.78
Fiber mass drying paper weight (g)	0.76	0.66	0.81	0.74
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	8.65	8.82	8.68	8.72
Fiber mass (g dry)	7.89	8.16	7.87	7.97
Fiber mass yield (% dry fiber mass to dry leaf weight)	65.64%	67.51%	65.05%	66.07%
Water soluble solids	2.77	2.74	2.67	2.72
Water soluble solids yield % (g dry solids from water to dry leaf weight)	23.04%	22.64%	22.06%	22.58%
Paper+LPC (g dry)	2.12	2.02	2.32	2.15
LPC (g dry)	1.36	1.19	1.56	1.37
LPC yield % (dry LPC to dry leaf weight)	11.31%	9.85%	12.89%	11.35%

LPC=Leaf Protein Concentrate

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Table 5: Yield and sample preparation data for yellow pea

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	25.01	25.10	25.19	25.10
LPC drying paper weight (g)	0.74	0.70	0.62	0.69
Fiber mass drying paper weight (g)	0.79	0.62	0.86	0.76
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	3.97	3.43	4.00	3.80
Fiber mass (g dry)	3.18	2.81	3.14	3.04
Fiber mass yield (% dry fiber mass to dry leaf weight)	55.00%	48.43%	53.92%	52.45%
Water soluble solids	2.04	2.37	2.04	2.15
Water soluble solids yield % (g dry solids from water to dry leaf weight)	35.31%	40.88%	35.09%	37.09%
Paper+LPC (g dry)	1.30	1.32	1.26	1.29
LPC (g dry)	0.56	0.62	0.64	0.61
LPC yield % (dry LPC to dry leaf weight)	9.69%	10.69%	10.99%	10.45%

LPC=Leaf Protein Concentrate

Table 6: Yield and sample preparation data for sunflower

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	100.10	99.99	100.00	100.03
LPC drying paper weight (g)	1.22	1.20	1.24	1.22
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min.)	3.00	3.00	3.00	3.00
Paper+LPC (g dry)	2.75	3.17	3.38	3.10
LPC (g dry)	1.53	1.97	2.14	1.88
LPC yield % (dry LPC to dry leaf weight)	8.01%	10.32%	11.21%	9.84%

LPC=Leaf Protein Concentrate

Table 7: Yield and sample preparation data for canola

Sample	1
Wet weight of leaf (g wet biomass)	20.25
LPC drying paper weight (g)	0.38
Fiber mass drying paper weight (g)	0.78
Drying time (h)	16.00
Heating time (min)	3.00
Blending time (min)	3.00
Paper+fiber mass (g dry)	1.94
Fiber mass (g dry)	1.16
Fiber Mass yield (% dry fiber mass to dry leaf weight)	46.41%
Water soluble solids	1.07
Water soluble solids yield % (g dry solids from water to dry leaf weight)	42.79%
Paper+LPC (g dry)	0.65
LPC (g dry)	0.27
LPC yield % (dry LPC to dry leaf weight)	10.80%

LPC=Leaf Protein Concentrate

Table 8: Yield and sample preparation data for kochia

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	100.25	100.15	100.12	100.17
LPC drying paper weight (g)	0.75	0.80	0.76	0.77
Fiber mass drying paper weight (g)	0.75	0.80	0.76	0.77
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+fiber mass (g dry)	12.49	13.70	13.50	13.23
Fiber mass (g dry)	11.74	12.90	12.74	12.46
Fiber Mass yield (% dry fiber mass to dry leaf weight)	56.06%	61.66%	60.92%	59.55%
Water soluble solids	8.55	7.03	7.17	7.58
Water soluble solids yield % (g dry solids from water to dry leaf weight)	40.83%	33.60%	34.30%	36.25%
Paper+LPC (g dry)	1.40	1.79	1.76	1.65
LPC (g dry)	0.65	0.99	1.00	0.88
LPC yield % (dry LPC to dry leaf weight)	3.10%	4.73%	4.78%	4.21%

LPC=Leaf Protein Concentrate

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Table 9: Yield and sample preparation data for round leaf mallow

Sample	1	2	3	Average
Wet weight of leaf (g wet biomass)	50.03	50.01	50.32	50.12
LPC drying paper weight (g)	0.82	0.80	0.64	0.75
Fiber mass drying paper weight (g)	0.78	0.80	0.67	0.75
Drying time (h)	16.00	16.00	16.00	16.00
Heating time (min)	3.00	3.00	3.00	3.00
Blending time (min)	3.00	3.00	3.00	3.00
Paper+LPC (g dry)	1.55	1.60	1.37	1.51
LPC (g dry)	0.73	0.80	0.73	0.75
LPC yield % (dry LPC to dry leaf weight)	7.17%	7.86%	7.12%	7.38%

LPC=Leaf Protein Concentrate

Table 10: Summary of residues average yields

Residue	Maturity	Plant component used	Average Yield % (Dry LPC/Dry Leaf weight)
Sunflower	Seed development 90-100 days	Leaves	9.84%
Wheat	Tillering 28-35 days	Entire above ground portion	14.53%
Barley	Tillering 28-35 days	Entire above ground portion	7.44%
Alfalfa	35-42 days	Entire above ground portion	11.35%
Yellow pea	21-28 days	Entire above ground portion	10.45%
Round leaf mallow	21-28 days	Entire above ground portion	7.38%
Canola	14-21 days	Entire above ground portion	10.80%
Corn	110-130 days	Leaves	8.10%

LPC=Leaf Protein Concentrate

Discussion

First, the identified toxins will be discussed individually, and then, the results will be compared to identify the most promising resilient food sources from the agricultural residues targeted.

Trichothecene mycotoxins represent a wide category of fungal toxins which can cause a range of symptoms depending on dose, exposure type, and other conditions (CDC, 2018). Many of the toxins found in these agricultural residue products are a part of this group. Contamination with these types of toxins is virtually unavoidable and is present in low doses in many food products (e.g., grains, cereals). Mycotoxins include altenuene, all aflatoxin compounds, HT-2 and T-2 toxins, nivalenol, and alternariol monomethyl ether.

Altenuene is a mycotoxin with antioxidant properties (Bhagat et al., 2016). It has not shown toxicity in preliminary testing (Cayman Chemical, 2022a), but there is limited literature exploring its toxicity or presence in food products.

Aflatoxins are among the major groupings of mycotoxins (Varga et al., 2011) and are produced by common molds such as *Aspergillus flavus* and *A. parasiticus* (Fratamico et al, 2006). They are often found in wheat, corn, various other grains, and soil where decay is present (Dhakal et al., 2022; Health Matters, 2022). The United States Food and Drug Administration (FDA) considers aflatoxins "an unavoidable contaminant of foods" (Dhakal et al., 2022). While aflatoxins are considered toxigenic and are known to have carcinogenic effects, the dose and duration of exposure are also considered to be major determinants of the toxicology (Dhakal et al., 2022). Aflatoxins may also have genotoxic effects through dermal exposure, but only in levels that would be unsafe for those in constant contact such as agricultural workers (Boonen et al., 2012).

In this study, aflatoxins B_1, B_2, G_1, and G_2 were found, as were two metabolites of aflatoxins – M_1 and M_2. Aflatoxins G_2 and B_2 were present in both positive and negative samples. Aflatoxins B_1, M_1, and G_1 were present only in positive samples. While aflatoxin B_1 is considered the most toxic compound in the aflatoxin category (Health Matters, 2022), there is significant evidence that mixes of aflatoxins are carcinogenic in humans (National Center for Biotechnology Information, 2022a). Aflatoxin B_1 and G_1 have also been shown to have fetotoxic effects in animal studies, and there is evidence that aflatoxins are able to cross placental barrier in humans (Gupta, 2012). High levels of oral exposure can also lead to liver failure and various types of cancer (Health Matters, 2022).

HT-2 toxin is a fungal compound found on corn, wheat, and other grains and its oral consumption, inhalation, and dermal exposure have potentially toxic effects for humans (Haschek et al., 2013).

T-2 toxin tetraol, generally present alongside HT-2 toxin, is a trichothecene mycotoxin which occurs as a

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byproduct of naturally occurring fungi in soil and on plants (Haschek et al., 2013). It is considered toxic to humans and can have harmful dermal (Boonen et al., 2012) and oral effects (Haschek et al., 2013).

Nivalenol is a trichothecene mycotoxin commonly found in cereals (i.e., wheat, corn, and other grains) and nuts (Coker, 2000). Deoxynivalenol is a broad category of mold toxins including nivalenol and represents the largest category of trichothecene in the world (Haschek et al., 2013). Toxins in this category are known to cause a variety of acute health issues in humans and can be lethal for mice (Cayman Chemical, 2022b). Nivalenol specifically has been known to cause vomiting in animals (Coker, 2000). In humans, scientific literature suggests that nivalenol is unlikely to be genotoxic, but immunotoxicity and haematotoxicity are significant concerns in high doses (EFSA, 2022). However, available data sets the tolerable daily intake of nivalenol at 1.2 µg/kg body weight per day which allows for average consumption of cereals and other grain products (EFSA, 2022). Alternariol monomethyl ether was found in both positive and negative samples. It is a common fungus on grain products in low concentrations (up to 12 ng/g) (Scott et al., 2012). It is considered mutagenic in vitro, but there is limited evidence supporting a carcinogenic classification (Scott et al., 2012).

4-Vinylphenol is a minor metabolite of styrene (used in latex and various plastic packaging products) (CDC, 2017). Naturally, 4-vinylphenol is most commonly found in wine (Chatonnet et al., 1992). Metabolites of 4-vinylphenol have been shown to cause pneumotoxicity and hepatotoxicity in mice (Carlson et al., 2002). However, because of its presence in alcoholic beverages, small amounts in dilute solutions are theoretically safe for human consumption.

Dimethyl dicarbonate is a compound used in food preservation, specifically in the stabilization of beverages (FDA, 2022). The EU Scientific Committee on Food, United States FDA, and Joint FAO/WHO Expert Committee on Food Additives (JECFA) have approved its safety for use in non-alcoholic beverages up to a concentration of 250 mg/L and in wine, up to a concentration of 200 mg/L (FDA, 2022; Government of Canada, 2012; National Center for Biotechnology Information, 2022b).

Based on these results, yellow pea, round leaf mallow, and canola are recommended for further investigation as they appear to be fit for human consumption based on the lack of dangerous toxins found in the analysis performed in this study. All compounds identified in these samples have either been approved by international regulatory boards for safe consumption or are known to be present in common beverages.

The other agricultural residues require additional study including quantification of the toxins identified as the quantity of the toxin will determine the actual risk for human consumption. The preliminary study here identified the toxic compounds that would need testing against specific standards, and then, run through the same chemical analysis protocol with each agricultural residue sample. In addition, as many of the toxins were found to be fungal in origin, the same agricultural residues should also be tested as a function of decay time on the fields (e.g., from fresh to just before the spring planting). The time taken to process several samples, wheat, barley, alfalfa, and kochia could have contributed to the time that fungi had to grow. Decreasing the time from harvesting to processing may reduce toxicity and warrants further investigation. This study is most similar to a recent study which used high-resolution mass spectrometry and the same open-source toolchain for non-targeted screening of toxins on five common North American coniferous species: Western Cedar, Douglas Fir, Ponderosa Pine, Western Hemlock, and Lodgepole Pine (Mottaghi et al., 2023). The yields for LPC extraction from the conifers ranged from 1 to 7.5%, where, as in this study, the yields from the agricultural residues ranged from 7 to 14.5% for the nine LPCs investigated. The results in this study are much better due to the relatively easy processing of the agricultural residues as compared to the conifers. It should be pointed out, however, that the agricultural residues from this study are only available during restricted times of the year, while the conifer biomass would be available year round in an emergency. This would make the conifer LPC possibly the only option in certain parts of the world at certain times (e.g. northern areas in the winter). This was while the LPC from agricultural waste studied in this paper could be used to supplement calories on an annual basis in areas that need them. For example, African countries have been severely affected by food insecurity; 54% of the population (73 million people) are acutely food insecure or in crisis (FSIN and Global Network Against Food Crises, 2020). A recent study, determined the potential for adopting agricultural residue (especially crop leaves) as food in food-insecure areas at the community scale (Ugwoke et al., 2023). Ugwoke et al. (2023) performed two residue utilization cases including a pessimistic and an optimistic case for human-edible calories in 13 communities in Nigeria to compare national level values. Overall, the study found that between 3.0 and 13.8 million Gcal are available in Nigeria per year from harvesting agricultural residue as alternative food; this could feed between 3.9 and 18.1 million people per year, covering from 10 to 48% of Nigeria's current estimated total food deficit. For this purpose, all the food residues need to be free of toxins and will need to be tested following the procedure detailed in this study.

All agricultural residue products containing aflatoxins

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(corn, wheat, barley, alfalfa, and sunflower) could require significant decontamination or processing before human consumption due to highly carcinogenic and otherwise toxic properties. Many of these toxins are caused by fungus, which could be prevented by processing the agricultural residue immediately before fungus is able to grow (Gibson and Hocking, 1997). Further decontamination may be required for the remaining agricultural residue products as many of the contaminants can have mutagenic or carcinogenic properties. More information is also required regarding the level of contamination of these compounds, as small amounts of the compounds detected are suitable for consumption and are common in many cereal and grain products (Chatonnet et al., 1992; EFSA, 2022). The toxicity screenings from (Mottaghi et al., 2023) confirm that the trees they investigated may contain toxins that can be consumed in small amounts; thus, additional studies including measuring the quantity of each toxin are needed. These results are similar to those found in the present study. Overall, the results indicated that LPC is a promising candidate for resilient food, but future work is needed before LPCs from either sources (conifers or agricultural waste). This can be used as a wide-scale human food for some of the LPCs which are not already derived from known-human edible foods.

Wheat, barley, yellow pea, canola, round leaf mallow, and alfalfa were all juvenile plants ranging from 14 to 42 days of age. The most readily available residue by mass from such crops will be post- combine (after grain heads have been removed); such biomass will likely be drier and less green, but it is highly dependent on the situation when the food is needed. Future research should rerun such samples at this later state of maturity as it may be a viable form of food during non-disasters. Additionally, it should be pointed out that such crops will have stayed longer on the field which may decrease protein content and increase the potential for microbial contamination. Next, it should be determined if protein can be obtained from post-combine chaf, leaves, and stalks, as this is where the bulk of the waste will be. Moreover, this might reduce processing costs if it goes straight from the combine into a truck to be harvested, or if there can be a portable extruder for the open source design (Oberloier and Pearce, 2018; Pearce, 2022) made from low-cost, easily obtained or digitally manufactured components (Woern et al., 2018); this is a rich area of further research.

Future work in this field also includes experiments to quantify toxins found in each sample as noted above to ensure that the agricultural residues have the same toxic profile regardless of geographic location. For the agricultural residues found in the future to be safe, further information on the ratio of conventional crop yield and residue produced, combined with the yield analysis in this study, will provide information to quantify the total potential of this approach. Finally, any negative impacts of harvesting residues for LPCs should be explored (e.g., the potential to deplete soils depending on the agricultural practice) (Lal, 2009). This study showed promise not only for providing additional sources of human calories from existing crops; the potential alternative or resilient foods for global catastrophic risks may make conventional agricultural practices unusable similar to that of tree residue (Mottaghi et al., 2023).

In the event of an Abrupt Sunlight Reduction Scenario (ASRS) such as a supervolano eruption, modeling indicates an anticipated 90% reduction in crop produced food calories (Xia et al., 2022). A planned response including a rapid deployment of resilient foods, such as single celled protein (García Martínez et al., 2022), cellulosic sugar (Throup et al., 2022), macroalgae production and greenhouses (Alvarado et al., 2020) could feasibly address a large portion of this food deficit (Rivers et al., 2022) providing a cost-effective means to save lives (Denkenberger et al., 2022). Although a diet consisting of resilient foods would meet a significant portion of nutritional requirements, several key nutrients, vitamin D, E, and K were critically low (Pham et al., 2022). LPC from lucerne contains a variety of vitamins principally b-carotene, vitamins B6, B9, E, and K, plus iron, calcium, and magnesium (Davys et al., 2011), indicating LPC can potentially address vitamin deficits in resilient food diets if available, though future research determining micronutrient content of key residues would be required to confirm this. The low-tech nature of LPC production and the potential for large amounts of unused residues to be immediately available depends on the time of year at which the ASRS occurred. This indicates LPC could provide a source of calories to meet short term deficits while resilient foods scale up to meet all human needs. In terms of potential scale of LPC, ~11.6 Gt/year, dry matter of unused residues are produced globally per year (see Table 1 in Alexander et al. (2017)) which produce ~800 Mt/year dry matter of LPC, assuming LPC yields reported in this paper. This would provide approximately 3.2×10^9 petacalories of energy, assuming 4 kcal/g dry matter of LPC as per general value for protein (FAO Food and Nutrition Paper, 2003). In summary, LPC from agricultural residues would complement existing resilient foods for an ASRS response by providing critical nutrients and access to a short-term food source during a high vulnerability period after the onset of ASRS (García Martínez et al., 2022).

Conclusion

The average yield ranged from about 7 to 14.5% for the nine LPCs investigated, which is in the range of LPCs

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found in the literature for a wider array of leaves. In addition, this study successfully used an open-source toolchain for non-targeted screening of toxins from high-resolution mass spectrometry of nine agricultural plant residues. The results showed that yellow pea, round leaf mallow, and canola are extremely promising for scaling because they appear to be fit for human consumption as safe foods based on the lack of dangerous toxins found. The other agricultural residues including corn/maize, wheat, barley, alfalfa, sunflower, and kochia require additional quantification of the toxins identified under various handling and maturation conditions. Overall, the potential for LPC to provide more needed calories from existing agricultural practices is extremely promising as a means of providing resilient safe food, but substantial amount of future work is needed to screen LPCs from all the agricultural residues depending on harvesting, handling, and storing conditions.

Authors' contributions

D.D. and J.M.P. designed the study; T.K.M., R.J.T., and S.W.B. conducted the experimental work; T.K.M., R.J.T., S.W.B., D.D., and J.M.P. analyzed the data; T.K.M., R.J.T., S.W.B., D.D., and J.M.P. wrote and edited the manuscript. All authors read and approved the final manuscript.

Conflict of interests:

All the authors declared no conflict of interes.

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