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Yield and Toxin Analysis of Leaf Protein Concentrate from Common North American Coniferous Trees

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Abstract: In the event of an abrupt sunlight reduction scenario, there is a time window that occurs between when food stores would likely run out for many countries (~6 months or less) and ~1 year when resilient foods are scaled up. A promising temporary resilient food is leaf protein concentrate (LPC). Although it is possible to extract LPC from tree biomass (e.g., leaves and needles), neither the yields nor the toxicity of the protein concentrates for humans from the most common tree species has been widely investigated. To help fill this knowledge gap, this study uses high-resolution mass spectrometry and an 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. The yields for LPC extraction from the conifers ranged from 1% to 7.5%. The toxicity screenings confirm that these trees may contain toxins that can be consumed in small amounts, and additional studies including measuring the quantity of each toxin are needed. The results indicate that LPC is a promising candidate to be used as resilient food, but future work is needed before LPCs from conifers can be used as a wide-scale human food.

Keywords: alternative food; resilient food; distributed production; edible plants; existential risk; food security; global catastrophic risk; leaf concentrate; leaf protein; non-target screening; resilience; sustainable food systems; toxins

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1. Introduction

In the event of a naturally occurring abrupt sunlight reduction scenario (ASRS), such as super volcanic eruption and asteroid or comet impact [1], or an anthropogenic cause, such as large-scale nuclear winter [2], numerical simulations indicate an anticipated reduction in crop-produced food calories of 90% [3]. If this occurs, there is likely to be widespread human starvation unless appropriate actions are taken [4]. This could negatively affect the long-run trajectory of humanity [5]. Planned responses from The Alliance to Feed the Earth in Disasters (ALLFED) and other groups include a rapid deployment of resilient or alternative foods, [6] such as natural gas consuming single celled protein (SCP) [7], conversion of fiber (plant material that humans cannot digest) via industrial methods such as cellulosic sugar [8], macroalgae production, a two-step process involving partial decomposition from bacteria or fungi fed to animals and greenhouses to expand the potentially acceptable growing conditions [9] could feasibly address a large portion of this food deficit [10], providing a cost-effective means to save lives [4]. Additional resilient foods include wild edible plants [11], hydrogen single-cell protein [12], electroactive

bacteria-produced vinegar [13], chemically synthesized glycerine [14], rabbits [15], and petroleum wax transformed into edible fat [16].

Unfortunately, there is a difficult-to-overcome time window that occurs between when food stores would be likely to run out in many countries (~6 months or less depending on when the ASRS occurred) and ~1 year when the resilient foods are scaled up. In this gap of time, leaf protein concentrate (LPC) has been proposed as a solution [6]. The low-tech nature of LPC production and the potential for large amounts of tree-based biomass to be immediately available depending on the time of year at which the ASRS occurred indicates LPC could provide a source of calories to meet short-term deficits until resilient food systems are further developed. The vitamins of LPC would be a valuable addition to a resilient food diet [17]. In summation, LPC from trees 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 start of ASRS. Furthermore, since low-tech methods can suffice, LPC could be valuable in scenarios where there is loss of electricity/industry [18]. Although there are several species, the predominant type of tree that would have non-wood biomass during all of the year outside the tropics is conifers. They are the dominant plants over large areas of land in the taiga of the Northern Hemisphere, but also in similar cool climates in mountains further south. Boreal conifers have adapted to survive in winter by shedding snow due to their shape and limb hanging angles. They also alter their biochemistry to make them more resistant to freezing. The immense conifer forests of the world represent the largest terrestrial carbon sink and thus the largest potential source of LPC.

Although it is possible to extract leaf protein concentrate (LPC) from tree biomass (e.g., leaves and needles), neither the yields nor the toxicity of the protein concentrates for humans from the most common tree species has been widely investigated. To help fill this knowledge gap, the aim of this study is to use high-resolution mass spectrometry and an open-source toolchain for non-targeted screening of toxins of five common North American coniferous, including: (1) Western Cedar (WC), (2) Douglas Fir (DF), (3) Ponderosa Pine (PP), (4) Western Hemlock (WHL), and (5) Lodgepole Pine (LPP). The yields for LPC extraction from the conifers are quantified, and the LPCs are screened for toxins. This analysis is novel and is the first original comprehensive investigation of conifer LPC toxicity. The objective of this work is to lay the technical foundation for treating conifer LPCs as resilient foods. The results are presented and discussed in terms of the potential for coniferous trees to provide resilient food in a disaster and during normal times to reduce hunger and starvation. Future work is outlined for what needs to occur before LPCs from conifers can be used as a human food.

2. Materials and Methods

2.1. Materials

Raw materials were acquired from the five common North American conifer trees [19]:

(1) Western Cedar (WC) is a native tree in North America [10]. This tree is widespread from northern California to southeastern Alaska, and from McGregor River to western Montana and northern Idaho [20]. Western cedar represented about 750 million cubic meters stock in British Columbia, 5 million seedlings were planted, and 1 percent of the stock was harvested annually in 2003 [21].
(2) Douglas Fir (DF) is a native tree in temperate regions of western North America and is non-native but widely planted in Europe [22,23]. This tree covered 2–3% of forests in several European countries in 2008 [24] and is found in British Columbia [25].
(3) Ponderosa Pine (PP) trees grow in the driest and warmest zones and are food for a wide range of animals that consume the tree seeds [26]. They are one of the native widely distributed pine trees in North America [27].

(4) Western Hemlock (WH) is a large evergreen native tree in North America and distributed in temperate rainforests [28]. This tree is commercially famous because of its timber quality. It is used for construction and pulp, and represented 34% of the log harvest volume in 2015 [20].
(5) Lodgepole Pine (LPP) is one of the native trees in North America [29]. About 75 million lodgepole trees were planted in British Columbia in 2002 [30]. Native people used to consume the sweet succulent bark freshly or stored it. Also, the pitch was used in many medicines in the past [31].

2.2. Material Processing

The following procedure was followed for all of the five conifer species. Needles were cut into shorter lengths and either blended in a food processor with room-temperature water for 3 min (Lodgepole pine, Ponderosa pine, Western Red Cedar run 1, Western Hemlock) or passed through a meat grinder(Douglas Fir, Western Red Cedar run 2). Processed residue, and liquid and flushing water were retained . Liquid from processed samples was separated from the mixture by passing through a woven polyester bag to yield a green liquid. The liquid was boiled for 3 min until a curd formed on the surface. The majority of coagulated leaf protein was collected with a spoon. Smaller amounts of LPC remained in suspension and were left to sediment for 30–60 min before liquid was decanted. Sedimented LPC was combined with skimmed LPC and dried using a Crawford Kitchen-Professional Dehydrator (Alternative methods for drying could be used, including a conventional oven, food dehydrator or open source vacuum drier [32]). The curd was skimmed off and put into a dehydrator set at 66 °C (152 °F) for 16 h to yield a green solid. The following equation was used to calculate the yield (Y):

$$Y = M_c/M_l \quad (1)$$

In this equation M_c is the mass of dried concentrate (g), and M_l is the mass of dried leaves (g).

2.3. LC/MS Instrumentation

An ultra-high-resolution hybrid ion trap orbitrap mass spectrometer (MS) instrument (Thermo Scientific Orbitrap Elite equipped with electrospray ionization (ESI)) coupled with an ultra-high-pressure two-dimensional liquid chromatograph (HPLC) system (Thermo Scientific Dionex Ultimate 3000 standard system) was used for the non-targeted toxic screening approach.

The HPLC was calibrated with Thermo Pierce calibration solution followed by diluting the LPC in water acetonitrile 80:20 (*v*:*v*) and filtering with a 0.2 μm quartz filter for LC/MS analysis. The analytical column was Phenomenex reversed-phase Kinetex XB-C18, "150 × 2.1" mm, 100 Å , with 1.7 μm particle size [33].

Mobile phase A and mobile phase B were 0.1% formic acid in 100% LC/MS grade water, and 0.1% formic acid in LC/MS grade acetonitrile–water 95:5 (*v*:*v*) solution respectively. The flow rate was constant, equal to 0.2 mL/min and the mobile phase gradient was 0 min; 5%B, 5 min; 5%B, 65 min; 90%B, 70 min; 90%B. Mobile phase A was used to calibrate the column for 15 min. The column oven temperature was 35 °C and the full loop injection volume was 5 μL. The resolving power was set at 120 K at mass to charge ratio (m/z) 400. Positive and negative electrospray ionization modes (ESI+ and ESI−) were used for two separate LC/MS runs for samples. Full screen mode was used for recording the masses in 100–600 m/z ranges. Also, to identify co-eluting compounds, data-dependent MS/MS fragmentation was also recorded for the 5 tallest peaks on each spectral scan with a collision energy of 25 (arbitrary unit).

2.4. Data Analysis

An open-source software toolchain was used to analyze the samples. The software included mass spectrometry analysis with MZmine 2, formula assignment with MFAssignR, and data filtering with ToxAssign, which has been previously described in detail in [34]. ToxAssign worked by comparing the existing database of the OpenFoodTox Chemical Hazards achieved by the European Food Safety Authority [35].

Based on the toxicity of toxic components, four classes are defined by the U.S. EPA. These classes include oral and dermal LD50 (lethal dose in 50% of test animals), inhalation LC50 (lethal air concentration in 50% of test animals), and the level of eye or skin irritation after contact [36]. In Appendix B, the class of characteristics for pesticides and other agents is shown. Agents in Category I are highly toxic and harmful by exposure [36]. Those in category II are moderately toxic and harmful in the case of consuming or absorbing by the body [36]. Those in category III are slightly toxic and could be harmful if exposed to a large quantity. Also, substrates in category IV have very low toxicity and are not harmful when not consumed in large quantities [36]. Since Category I and II are more dangerous and toxic, the focus in this study is on these categories.

The toxicity tests were run for all five tree samples twice. In addition, for Western Cedar both the LPC and the viscous liquid were also tested. Finally, to start to gauge the repeatability of LPC extraction, it was repeated for the Ponderosa Pine samples. Differing mass spectra for the sample (shown in Appendix A) generally resulted from small changes in sensitivity parameters. Assuming the distinct peaks (those with listed values) are the same across samples, high confidence can be had in the initial mass spectrometry analysis.

3. Results

The results for each of five trees for both yield and toxic analysis are detailed below. The total ion chromatograms are available in Appendix A for all LPC analyzed in Figures A1–A22 for both the positive and negative ionization. There are also two runs of tests for each tree. Analysis of each tree and differences for each run are explained in separated sections in the following.

3.1. Western Cedar

The parameters considered for cedar are shown in Table 1. The dry yield is 2.07% of the original dry mass.

The first run was performed using a food processor type blender. When separating the liquid from the fiber mass, the liquid obtained appeared more viscous, having a slightly gelatinous/syrupy consistency. When boiled, the ability for the protein to coagulate into a layer was significantly reduced, yielding little to no LPC. Attempts to isolate protein through cooling and resting to induce sedimentation were also ineffective, resulting in minimal isolated protein. A meat grinder type processor was used in the second run. The second run also failed to show coagulation of leaf protein. A combination of partially sedimented large portion of protein and liquid was dried to yield a crystalline solid. This crystalline solid likely contained protein and water-soluble solids and rheology-altering compounds.

Table 1. Yield and sample preparation data for WC #1.

Sample	WC #1
Wet weight of leaf (g wet biomass)	50.01
LPC drying paper weight (g)	2.33
Fiber Mass drying paper weight (g)	1.79
Drying time (hours)	16
Heating time (minutes)	3.0
Blending time (minutes)	3.0

Paper + Fiber Mass (g dry)	23.59
Fiber Mass (g dry)	21.8
Fiber Mass yield (% dry fiber mass to dry leaf weight)	81.89%
Paper + LPC (g dry)	2.88
LPC (g dry)	0.55
LPC yield % (dry LPC to dry leaf weight)	2.07%

Cedar returned multiple toxins in class 1 and 2 seen in Table 2 for the first run and the second run.

Table 2. Toxic compound screening results for WC #1 samples.

Material	Class	Toxin (in ESI+ or ESI–)	Run 1	Run 2
WC #1	Toxic Class 1	Alternariol monomethyl ether (+,–)	✓	✓
		4-Vinylphenol (–)	✓	✓
		HT-2 toxin (–)	✓	✓
		T-2 Toxin tetraol (–)	✓	-
	Toxic Class 2	Deoxynivalenol 3-glucoside (–)	✓	✓

Alternariol monomethyl ether found in the results is a mycotoxin which is known to be mutagenic and genotoxic, but it is not very acutely toxic [37]. Mycotoxins can cause a range of symptoms based on the dosage, exposure, and other conditions [38]. 4-Vinylphenol is a natural product driven from hydride of a styrene [39] and has a potent cytotoxicity [40]. This chemical is found in wine and because of that, it is assumed that it is not harmful for humans when consumed in small amounts [41], but needs to be quantified. Moreover, T-2 Toxin tetraol and HT-2 are mycotoxins and are byproducts of naturally occurring fungi in soil and on plants. The concentration of HT-2 toxin is two-thirds the sum of T-2 toxin tetraol and HT-2 toxin concentration [42]. These two chemicals are potentially harmful for humans if they are orally consumed, inhaled, and dermally exposed [42]. They, however, unavoidably exist in human foods, including wheat, rye, and cereals, and the permitted content of these toxins in human food are determined in some countries [43]. In this regard, the daily exposure, E, level to T-2 and HT-2 toxins are calculated as shown in equation 2 [43]:

$$E\ (kg/bw/day) = \frac{\mathrm{c} \times \mathrm{d}}{\mathrm{w}} \quad (2)$$

where c is the toxin concentration in samples (μg/kg), d is the daily consumption of the sample (g/day/person), and w is the mean body weight of the person (kg/person).

Deoxynivalenol 3-glucoside, a toxin from class 2, is a masked mycotoxin that has a drastically decreased toxicity in the intestines [44,45].

Western Cedar and Viscose Liquid (WC #2 LPC (+))

The parameters used for Western Cedar and viscose liquid are shown in Table 3.

Table 3. Toxic compound screening results for WC #2 samples.

Material	Class	Toxin (in ESI+ or ESI–)	Run 1	Run 2
WC #2 LPC (+)	Toxic Class 1	4-Vinylphenol (+,–)	✓	✓
		Alternariol monomethyl ether (+,–)	✓	✓
		Aflatoxin M1 (–)	✓	✓
		HT-2 toxin (+)	✓	✓
		T-2 Toxin tetraol (–)	✓	-
		Altenuene (+)	✓	-
		Neosolaniol (+)	-	✓
	Toxic Class 2	Deoxynivalenol 3-glucoside (+,–)	✓	✓
		Aflatoxin G1 (–)	✓	✓
		15-Acetyldeoxynivalenol (–)	✓	✓
		Glutaraldehyde (+)	✓	✓
		Carbofuran (+)	✓	✓
		2-Acetylfuran (+)	✓	-
		Anguidine (–)	-	✓
		Nivalenol (+)	-	✓

As can be seen by comparing Tables 3 and 4, there are far more toxic compounds found in the viscose liquid than in the LPC.

3.2. Douglas Fir (DF)

Douglas Fir LPC parameters can be seen in Table 4. The LPC yield is 1.06%.

Table 4. Yield and sample preparation data for DF.

Sample	DF
Wet weight of leaf (g wet biomass)	50.2
LPC drying paper weight (g)	1.27
Fiber Mass drying paper weight (g)	1.55
Drying time (hours)	16
Heating time (minutes)	3.0
Blending time (minutes)	3.0
Paper + Fiber Mass (g dry)	19.6
Fiber Mass (g dry)	18.05
Fiber Mass yield (% dry fiber mass to dry leaf weight)	61.65%
Paper + LPC (g dry)	1.58
LPC (g dry)	0.31
LPC yield % (dry LPC to dry leaf weight)	1.06%

The toxic compounds are shown in Table 5.

Table 5. Toxic compound screening results for DF samples.

Material	Class	Toxin (in ESI+ or ESI–)	Run 1	Run 2
DF	Toxic Class 1	Alternariol monomethyl ether (+,–)	✓	✓
		4-Vinylphenol (+,–)	✓	✓
	Toxic Class 2	T-2 Toxin tetraol (–)	-	✓
		15-Acetyldeoxynivalenol (–)	✓	✓
		Dimethyl dicarbonate (–)	✓	-

Except HT-2 toxin and Deoxynivalenol 3-glucoside, the Douglas Fir has the same toxins as Western Cedar along with 15-Acetyldeoxynivalenol and Dimethyl dicarbonate. 15-Acetyldeoxynivalenol is a mycotoxin related to a deoxynivalenol with acute toxicity [46] Dimethyl dicarbonate is an acute toxin chemical which is a organooxygen compound. This compound is used for sterilizing soft drinks and wines [47].

3.3. Ponderosa Pine (PP)

The Ponderosa Pine LPC parameters are shown in Table 6 and the average of LPC yield is calculated as 3.05%. The LPC yield is shown to have a large variation in values indicating that future work should consider a high number of experiments under various conditions and tool sets to get a reliable estimate for the average LPC yield that could be expected globally during a disaster.

Table 6. Yield and sample preparation data for PP.

Sample	#1	#2	Average
Wet weight of leaf (g wet biomass)	50	50	50
LPC drying paper weight (g)	2.1	1.9	2.0
Fiber Mass drying paper weight (g)	2.12	2.60	2.36
Drying time (hours)	14	14	14
Heating time (minutes)	3.0	3.0	3.0
Blending time (minutes)	3.0	3.0	3.0
Fiber Mass (g dry)	20.58	20.58	20.58
Fiber Mass yield (% dry fiber mass to dry leaf weight)	78.12%	78.12%	78.12%
Paper + LPC (g dry)	3.01	2.59	2.85
LPC (g dry)	0.91	0.7	0.80
LPC yield % (dry LPC to dry leaf weight)	3.45%	2.66%	3.05%

Ponderosa Pine returned multiple toxic compounds in class 1 and class 2 which are shown in Table 7.

Table 7. Toxic compound screening results for PP samples.

Material	Class	Toxin (in ESI+ or ESI−)	Run 1	Run 2
PP	Toxic Class 1	4-Vinylphenol (+,−)	✓	-
		Alternariol monomethyl ether (−)	✓	✓
		Altenuene (−)	-	✓
	Toxic Class 2	T-2 Toxin tetraol (+)	-	✓
		Deoxynivalenol 3-glucoside (−)	✓	✓
		Dimethyl dicarbonate (−)	-	✓

Except H-2 Toxin tetraol, the same toxins found in Western Cedar were found in Ponderosa Pine along with Altenuene and Dimethyl dicarbonate. Altenuene, a metabolite of alternariol, is a mycotoxin and is not very acutely toxic [37].

3.4. Western Hemlock (WHL)

The LPC parameters for Western Hemlock samples are shown in Table 8. The LPC yield was 7.29%.

Table 8. Yield and sample preparation data for WHL.

Sample	WHL
Wet weight of leaf (g wet biomass)	42.7
LPC drying paper weight (g)	0.97
Fiber Mass drying paper weight (g)	2.52
Drying time (hours)	16
Heating time (minutes)	3.0
Blending time (minutes)	3.0
Paper + Fiber Mass (g dry)	18.4
Fiber Mass (g dry)	15.88
Fiber Mass yield (% dry fiber mass to dry leaf weight)	74.71%
Paper + LPC (g dry)	2.52
LPC (g dry)	1.55
LPC yield % (dry LPC to dry leaf weight)	7.29%

Table 9 represents the toxic compounds in Western Hemlock.

Table 9. Toxic compound screening results for PP samples.

Material	Class	Toxin (in ESI+ or ESI−)	Run 1	Run 2
Western Hemlock	Toxic Class 1	Alternariol monomethyl ether (+,−)	✓	✓
		4-Vinylphenol (+,−)	✓	✓
	Toxic Class 2	Carbofuran (+)	✓	
		Aflatoxin B2 (−)	-	✓
		Deoxynivalenol 3-glucoside (−)	-	✓
		Dimethyl dicarbonate (−)	-	✓

Except T-2 Toxin and Altenuene, Western Hemlock has the same toxin as Ponderosa Pine along with Carbofuran and Aflatoxin B2. All of aflatoxins are mycotoxins. One of the most common aflatoxins, Aflatoxin B2, is considered to be much less toxic than Aflatoxin B1 [48]. Generally, aflatoxins are unsafe only for people who are exposed to this chemical continuously, including agricultural workers [49]. Carbofuran is a common pesticide and one of the most toxic pesticides of its class. It is "fairly persistent in soil and water ecosystems" [50].

3.5. LodgePole Pine (LPP)

The parameters used for Lodgepole Pine are shown in Table 10 with an LPC yield of 2.55%.

Table 10. Yield and sample preparation data for LPP.

Sample	LPP
Wet weight of leaf (g wet biomass)	50.27
LPC drying paper weight (g)	1.26
Fiber Mass drying paper weight (g)	2.07
Drying time (hours)	16
Heating time (minutes)	3.0
Blending time (minutes)	3.0
Paper + Fiber Mass (g dry)	22.1
Fiber Mass (g dry)	20.03
Fiber Mass yield (% dry fiber mass to dry leaf weight)	23%
Paper + LPC (g dry)	2

LPC (g dry)	0.74
LPC yield % (dry LPC to dry leaf weight)	2.55%

The toxic compound results for LPP are shown in Table 11.

Table 11. Toxic compound screening results for LPP samples.

Material	Class	Toxin (in ESI+ or ESI–)	Run 1	Run 2
LPP	Toxic Class 1	Alternariol monomethyl ether (+,–)	✓	✓
		4-Vinylphenol (–)	✓	✓
		Aflatoxin M1 (–)	-	✓
	Toxic Class 2	Dimethyl dicarbonate (–)	✓	✓
		Deoxynivalenol 3-glucoside (+)	✓	✓
		Aflatoxin G1 (–)	-	✓
		2-Acetylfuran (+)	✓	-

Except Altenuene and T-2 Toxin found in Ponderosa Pine, the same toxins are found in LodgePole Pine along with Aflatoxin M1, Aflatoxin G1, and 2-Acetylfuran. Aflatoxin G1, which is one of the most common aflatoxins is considered to be much less toxic than Aflatoxin B1. Also, Aflatoxin M1, which is a common metabolite, is even less toxic than Aflatoxin G1 [48]. 2-Acetylfuran, which is returned in the second run and is a toxic class 2, is toxic if consumed [51].

4. Discussion

All of the yields for LPCs are summarized in Table 12, where values ranged from 1% to 7%. These are somewhat low as compared to agriculture residue LPC and other LPCs. In one study of agricultural residue LPC, yield values ranged from 7 to 14.5% [52]. Additional studies of various agricultural products and other common tropical, subtropical, and temperate vegetation showed yield values ranging from 2% to 9% [53]. Overall, needles (fir, ponderosa, lodgepole) were extremely difficult to blend using a food processor, because of the length and cylindrical nature of the needles as well as the extreme toughness and fibrousness of the needle fibers. This often resulted in needles bouncing off the yield blades, rendering them non-processible with the meat grinder. The cedar was comparatively easy to blend, but has some compounds with rheological modifying properties because they formed a soapy, gelatinous type texture that was difficult to separate. The consistency of the liquid ended up making it difficult to isolate the protein, which appeared to be suspended as very small coagulate chunks in the liquid. The hemlock's small leaves were much more like 'crop' leaves in terms of toughness and texture, though they had to be stripped from small branches, which was time intensive.

Table 12. Summary of residues yields and average yields.

Tree	Yield % (Dry LPC/Dry Leaf Weight)
Western Cedar	2.07
Douglas Fir	1.06
Ponderosa Pine	3.05 (2.66–3.45)
Western Hemlock	7.29
Lodgepole Pine	2.55

Western Cedar contains several toxins. Many of these chemicals are not considered as acutely toxic if consumed in low concentrations. HT-2 toxin along with T-2 toxin tetraol, however, are of greater concern and they could be harmful for human consumption. It should be noted that they are found in many foods which are already used. Thus, the dosage of these chemicals should be considered and the LPC could be consumed only if

the dosage does not exceed that which can be measured from Equation 1 [43]. Similarly, toxins in Douglas Fir and Ponderosa Pine are mostly mycotoxins, which can already be found in many foods, and they can be consumed if their dosage is less than specific levels. Conifer pine needles are already eaten regularly [53] in a number of recipes, in pine needle tea, which is high in vitamin C, and they are available commercially [54]. The majority of the substances found in Western Hemlock and Lodgepole Pine do not preclude the use of these trees as a candidate for food. However, they do contain Aflatoxins. The amount of this toxin should be measured since large amounts can be dangerous, but again it should be noted small dosages of them are not harmful and they already exist in many cereals and grains. These trees may, however, require significant decontamination before human consumption if they contain high concentrations of aflatoxins [35]. As the results show, potential toxins were found in all leaves. These are only potential toxins and future work is necessary to quantify them to determine if consuming large quantities of conifer LPC represents a danger. This can be done by running the identified potential toxins through the same system with calibration standards and repeating the measurements. In addition, animal studies can be run on the LPCs eaten in high volumes to help ensure no toxicity to obtain FDA approval. As some of these species are already commercially available for food (e.g., pine needle tea) [55], work can already be done to determine the potential cost effectiveness of using conifer LPCs as a resilient food and compare it to other approaches [56–58].

5. Conclusions

The average yield ranging from 1% to 7% is investigated for five coniferous LPCs. While this range is lower than that found in other LPC analyses (e.g., agricultural waste), coniferous LPC provides a viable option for protein in forested areas in the event of extreme food shortage. Moreover, this study provided the first original high-resolution mass spectrometry of coniferous LPC samples and ran them through an open-source toolchain for non-targeted screening of five tree residues. The results confirm that these trees may contain toxins that can be consumed in small amounts, and additional studies including measuring the quantity of each toxin and determining the conditions that enable these toxins to grow are required (e.g., were some of them caused by the environment in which they were found?) Generally, the objectives of this study was met, and LPC was shown to be a promising candidate to be used as a resilient food; however, this field requires a wider range of studies to screen the LPC based on different conditions in which they are harvested, handled and stored.

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Appendix A

Total ion chromatograms are shown for all needles analyzed in Figures A1–A24 for both the positive and negative ionization.

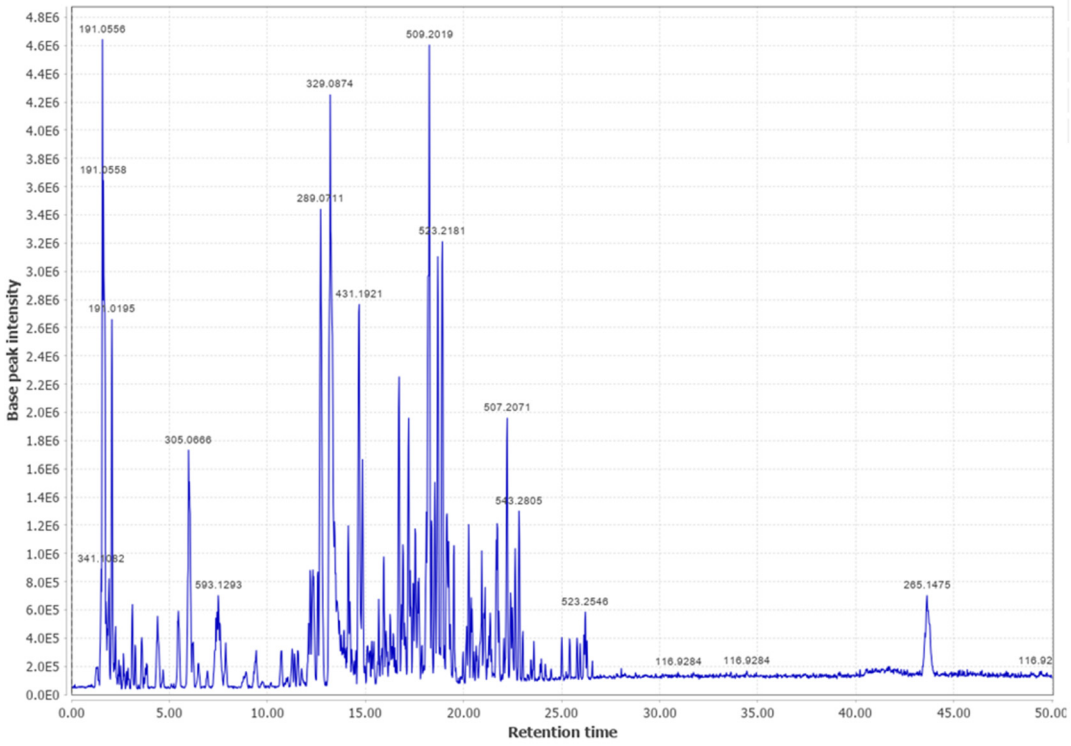

Figure A1. First run WC #1 negative.

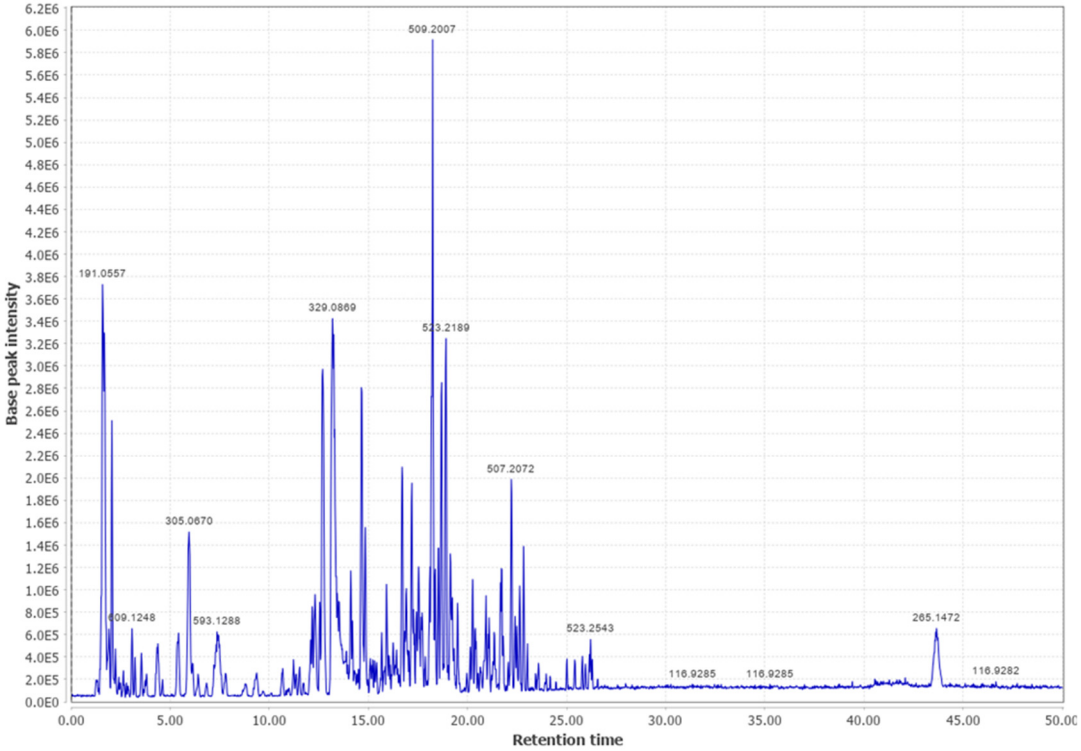

Figure A2. Second run WC #1 negative.

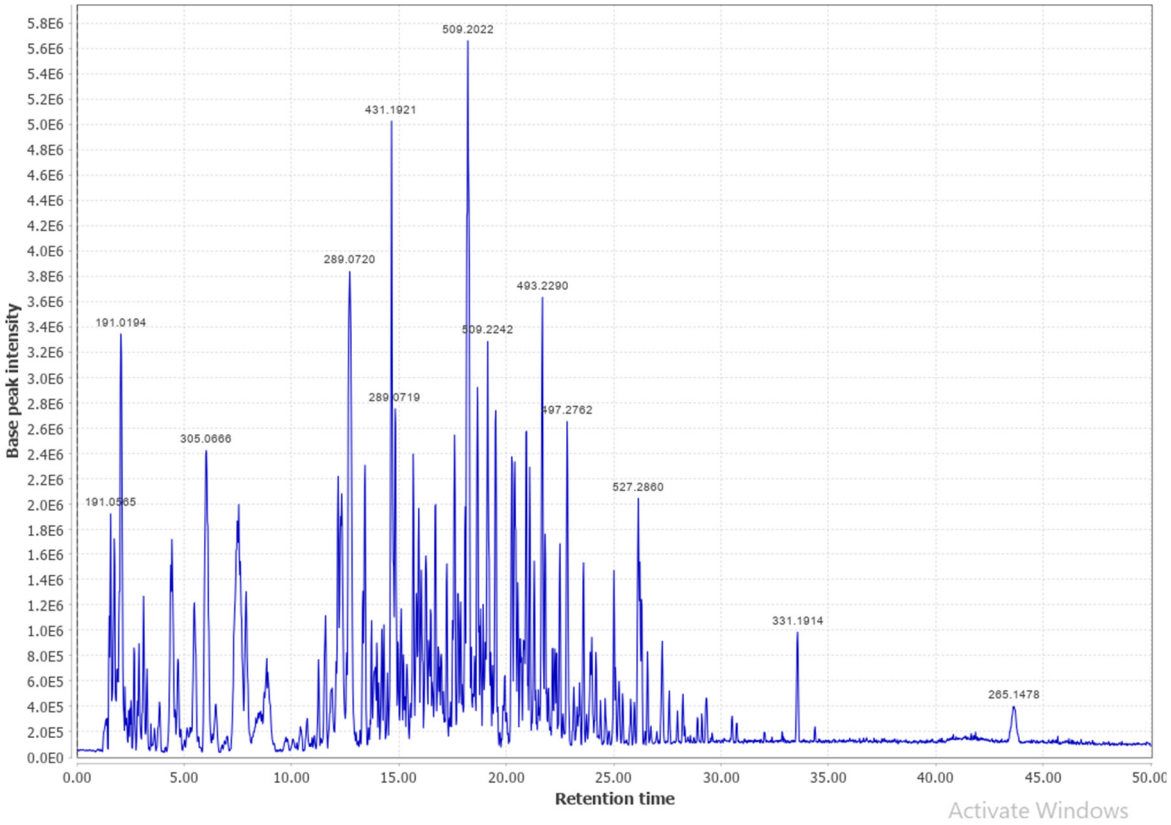

Figure A3. First run WC #2 LPC(+) negative.

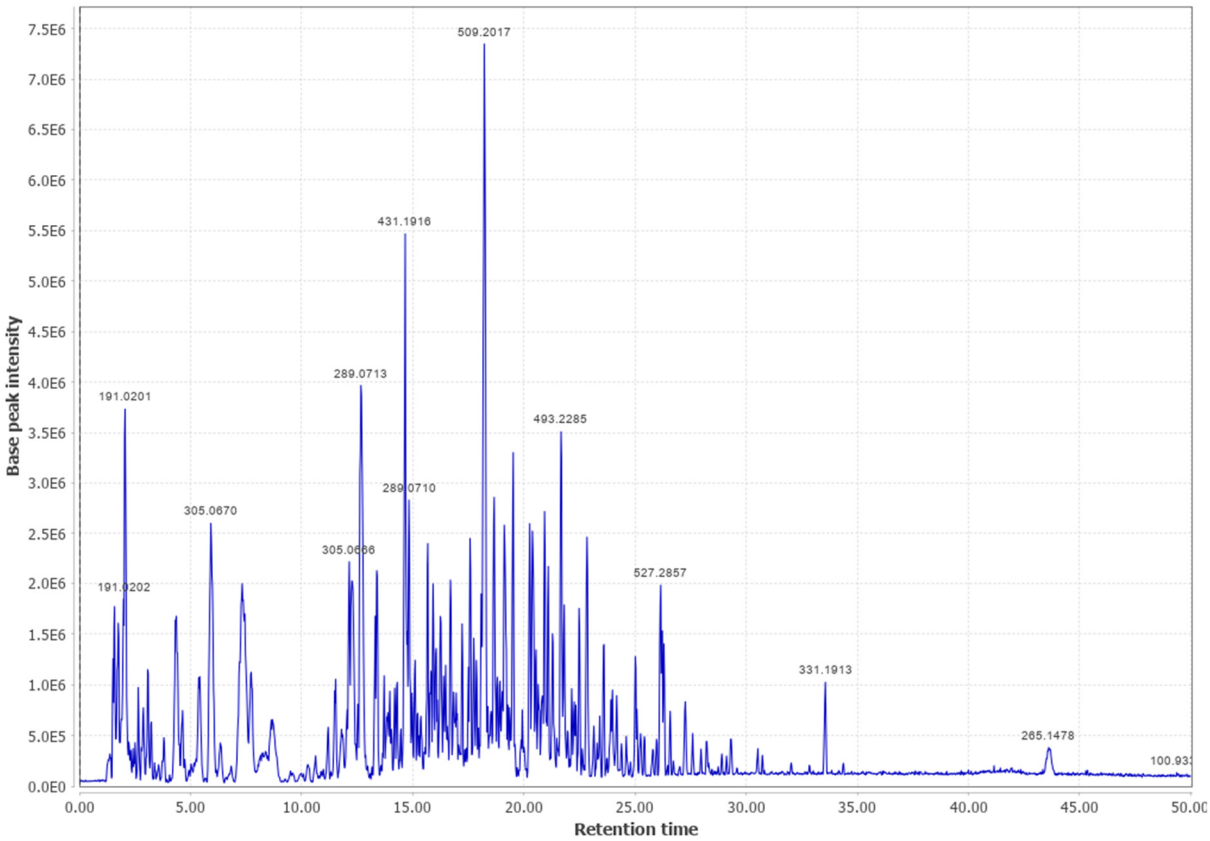

Figure A4. Second run WC #2 LPC (+) negative.

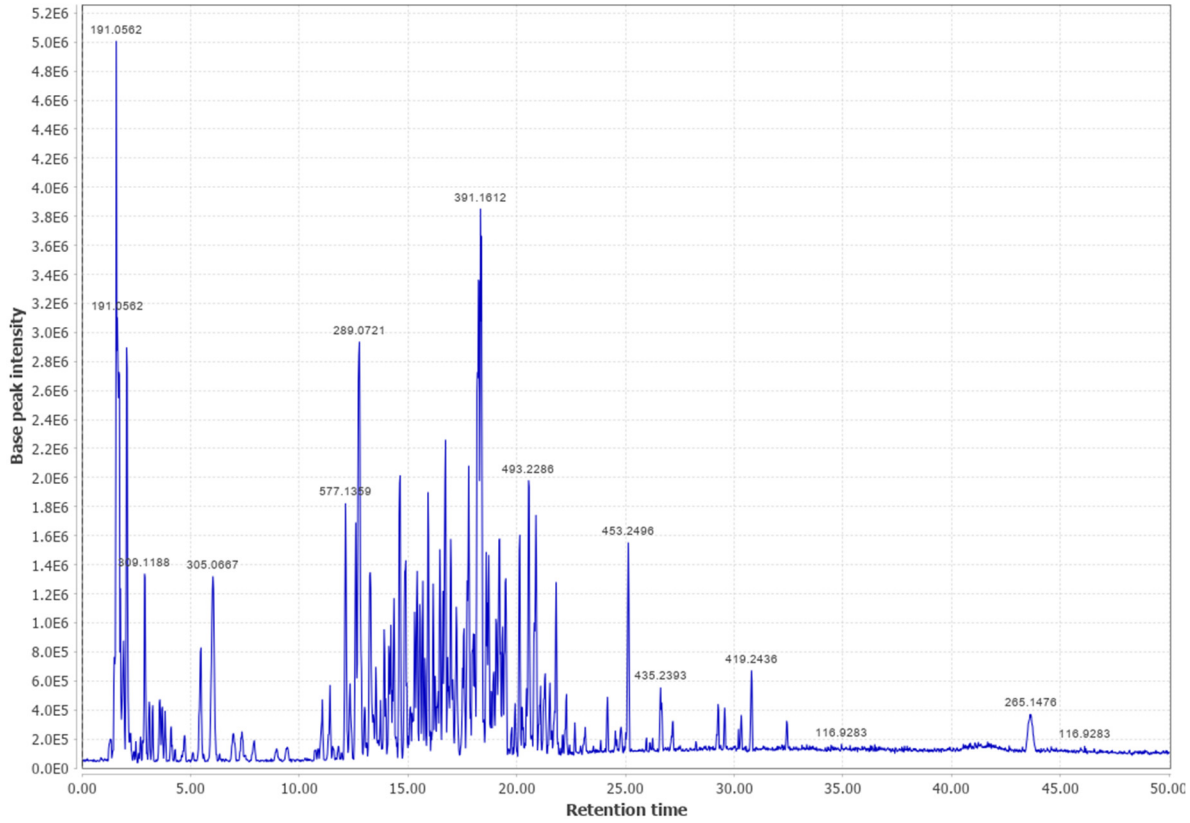

Figure A5. First run DF negative.

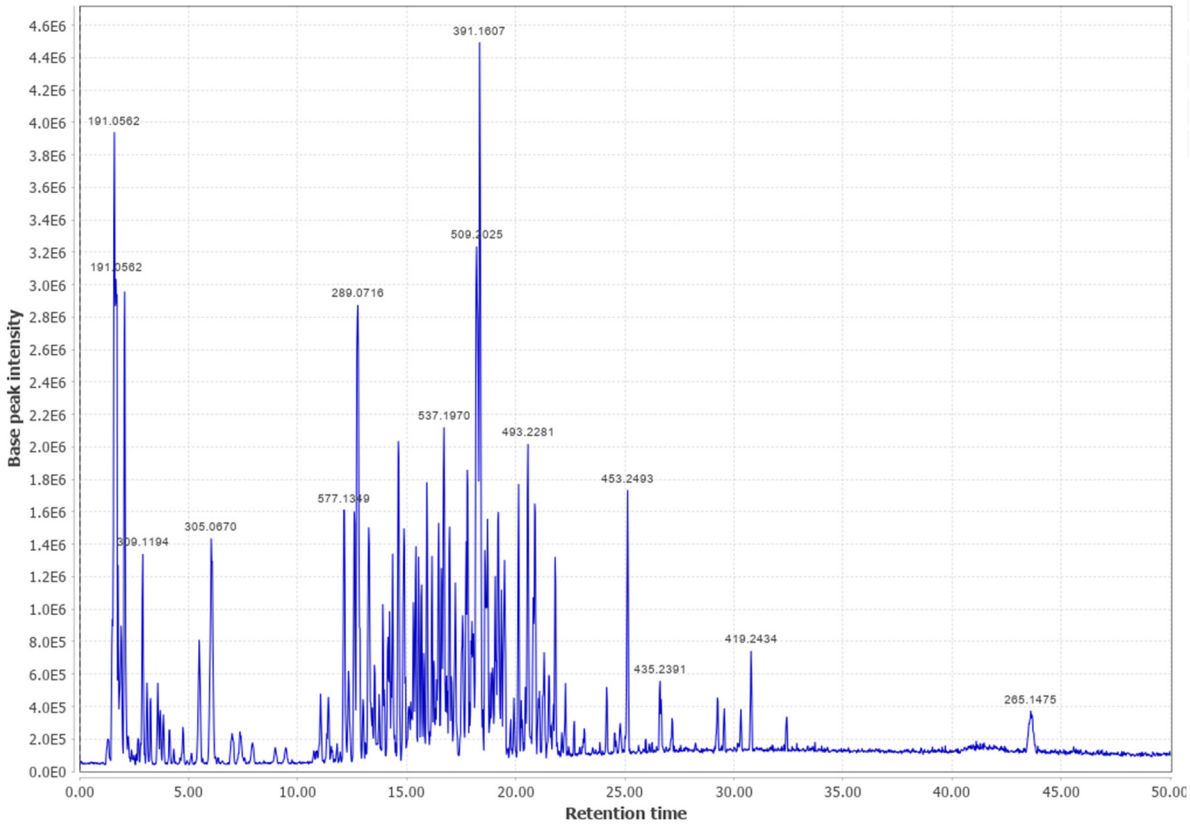

Figure A6. Second run DF negative.

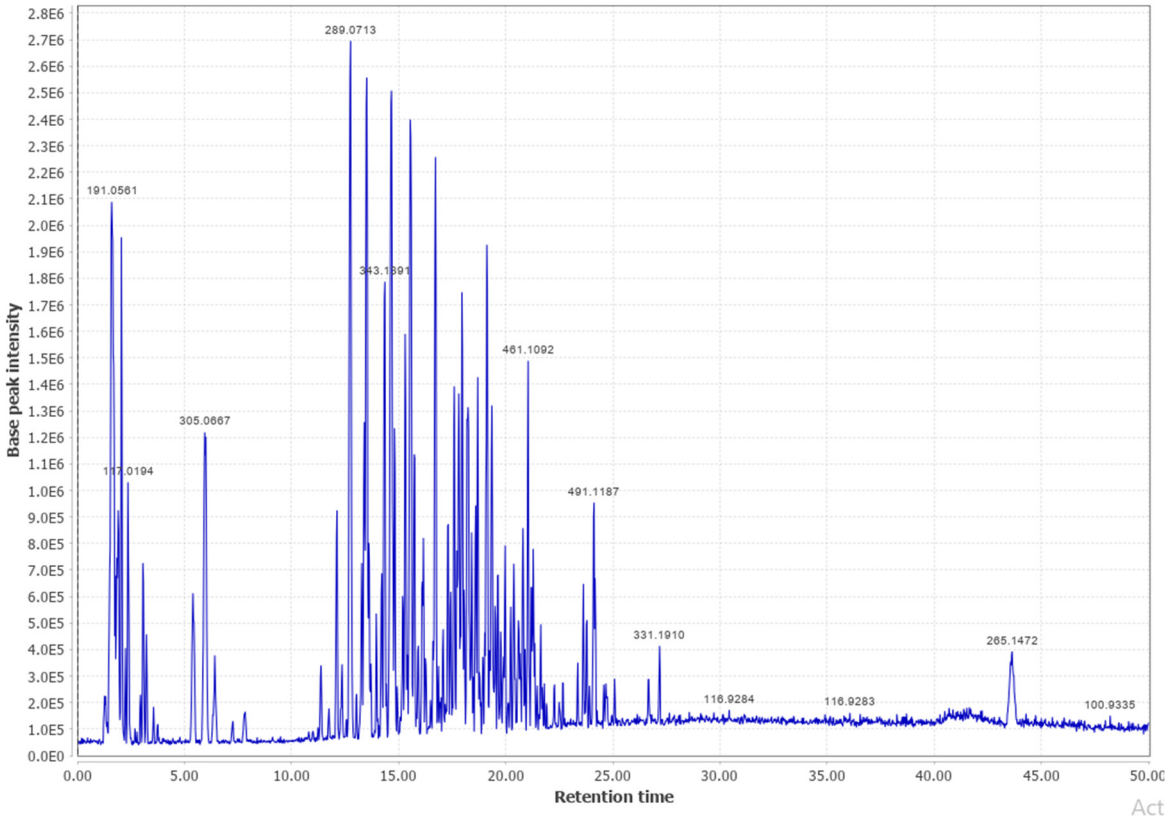

Figure A7. First run PP negative.

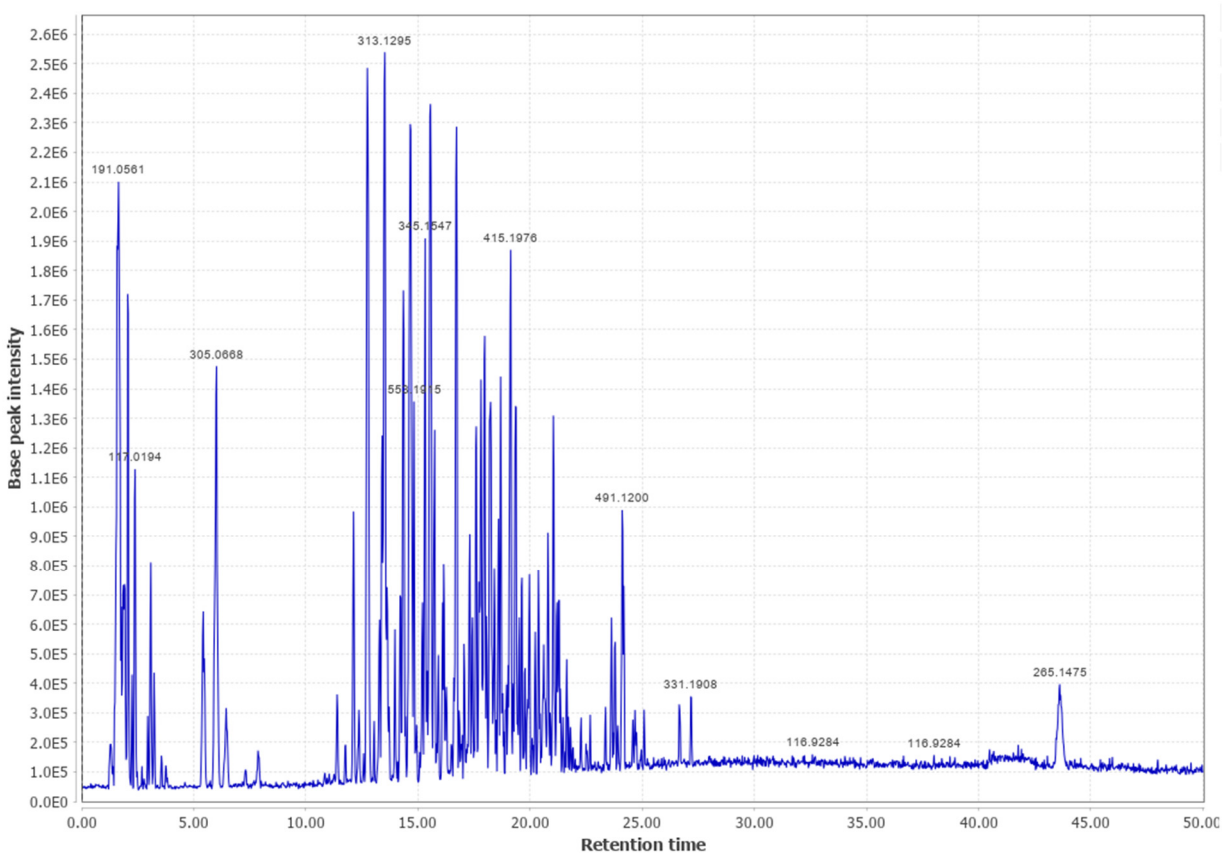

Figure A8. Second run DP negative.

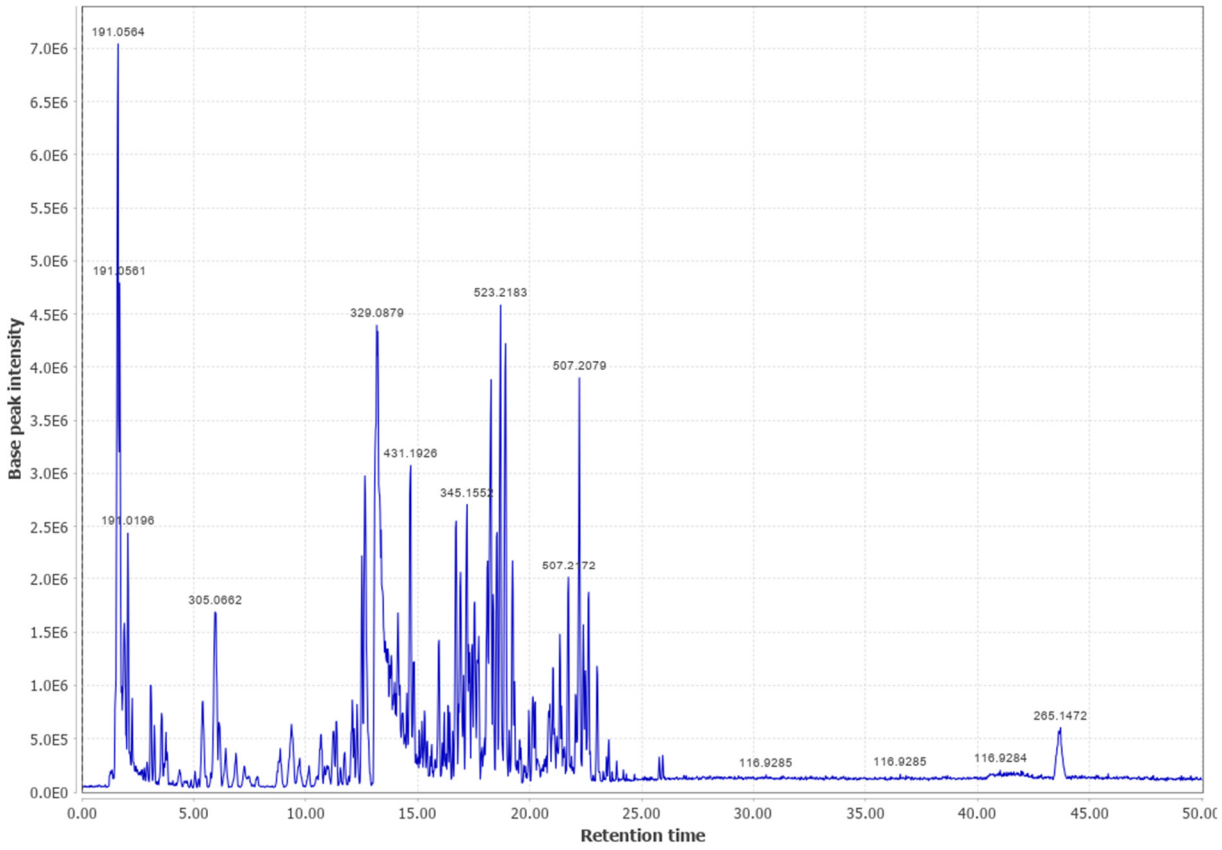

Figure A9. First run WHL negative.

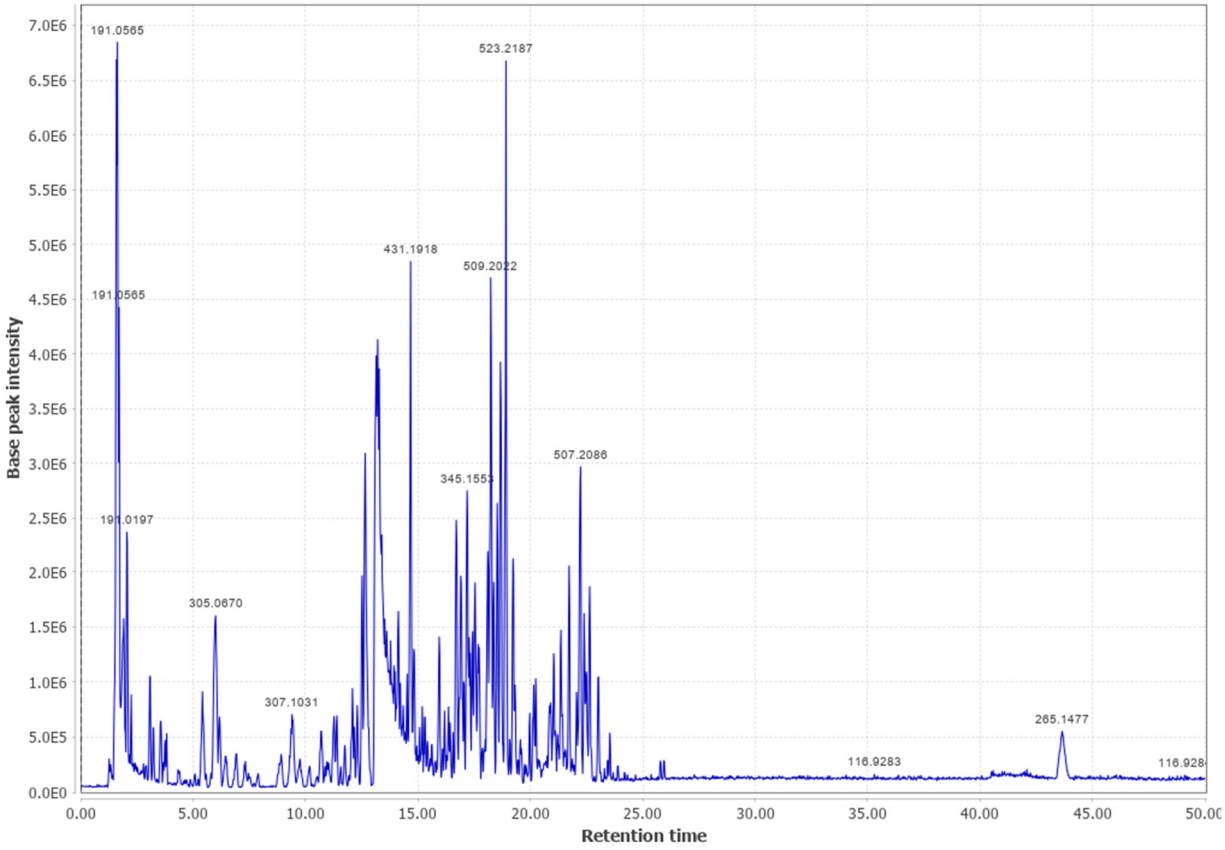

Figure A10. Second run WHL negative.

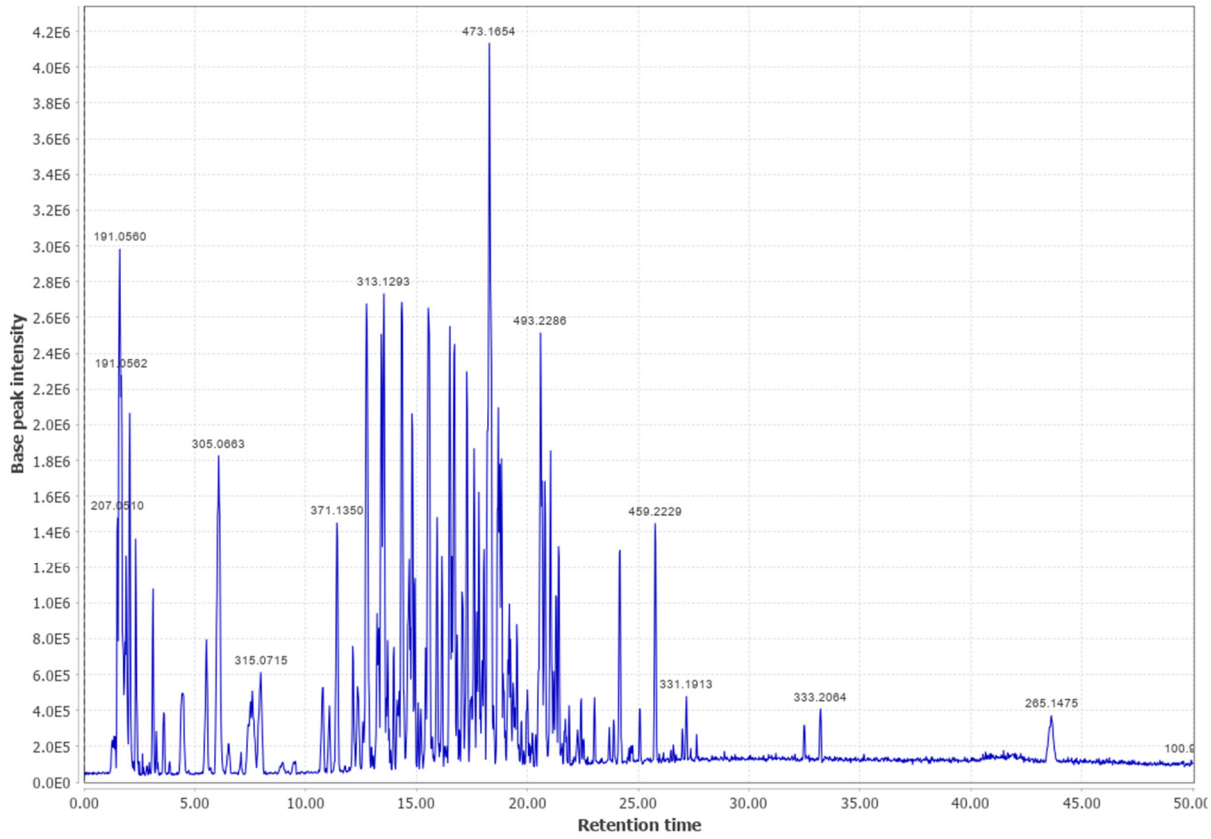

Figure A11. First run LPP negative.

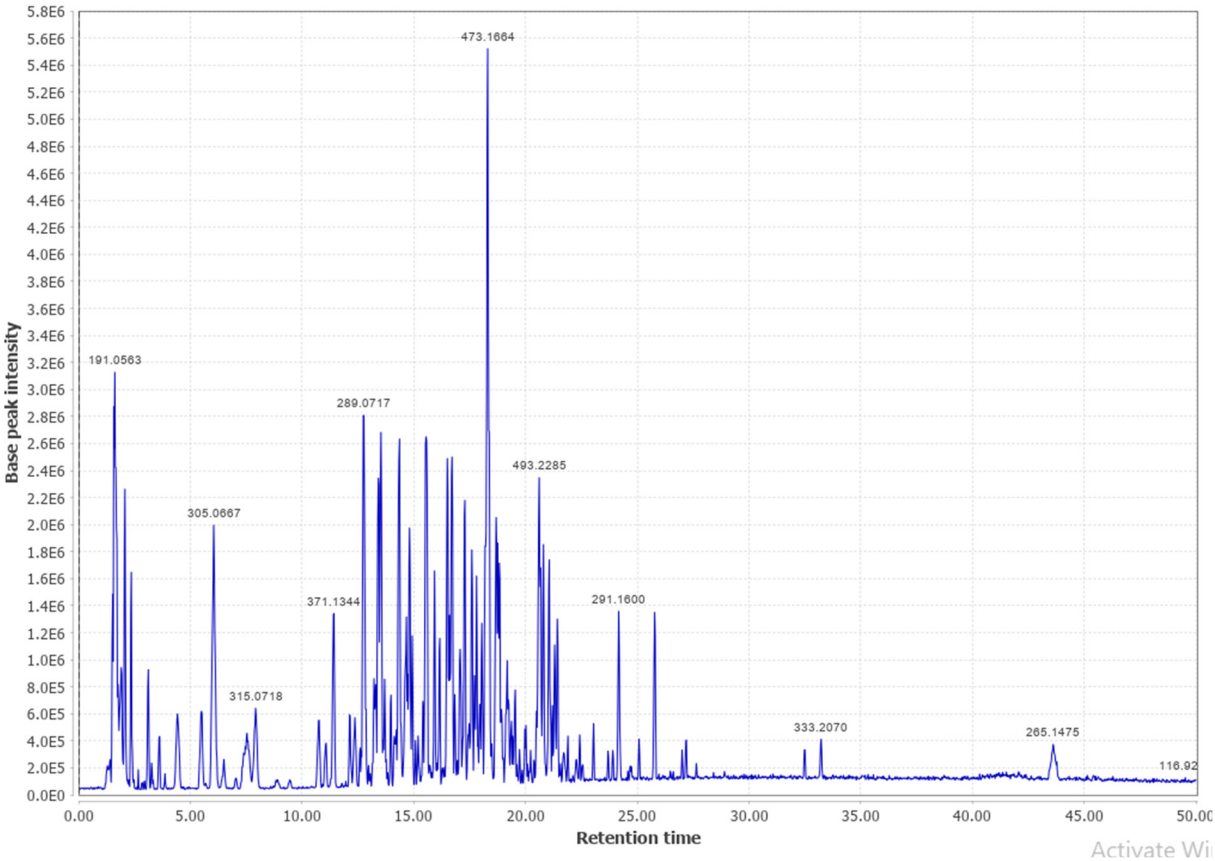

Figure A12. Second run LPP negative.

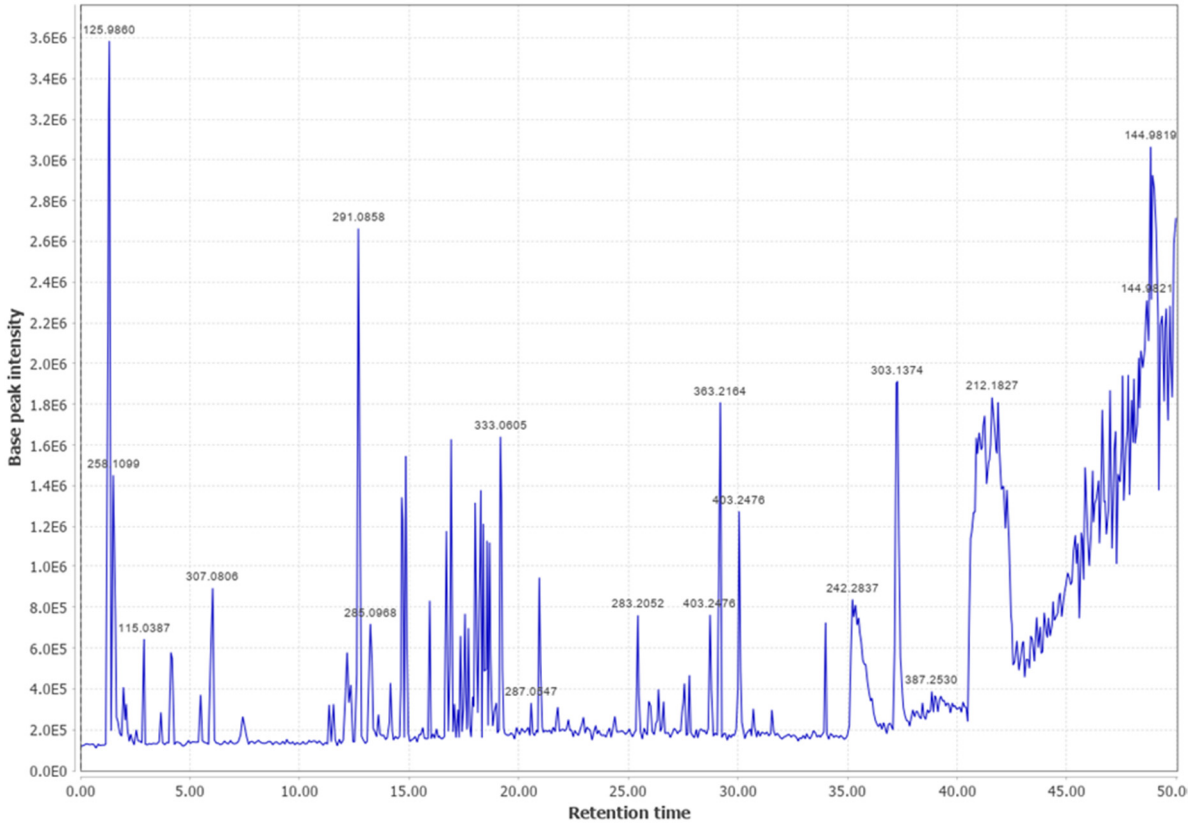

Figure A13. First run WC #1 positive.

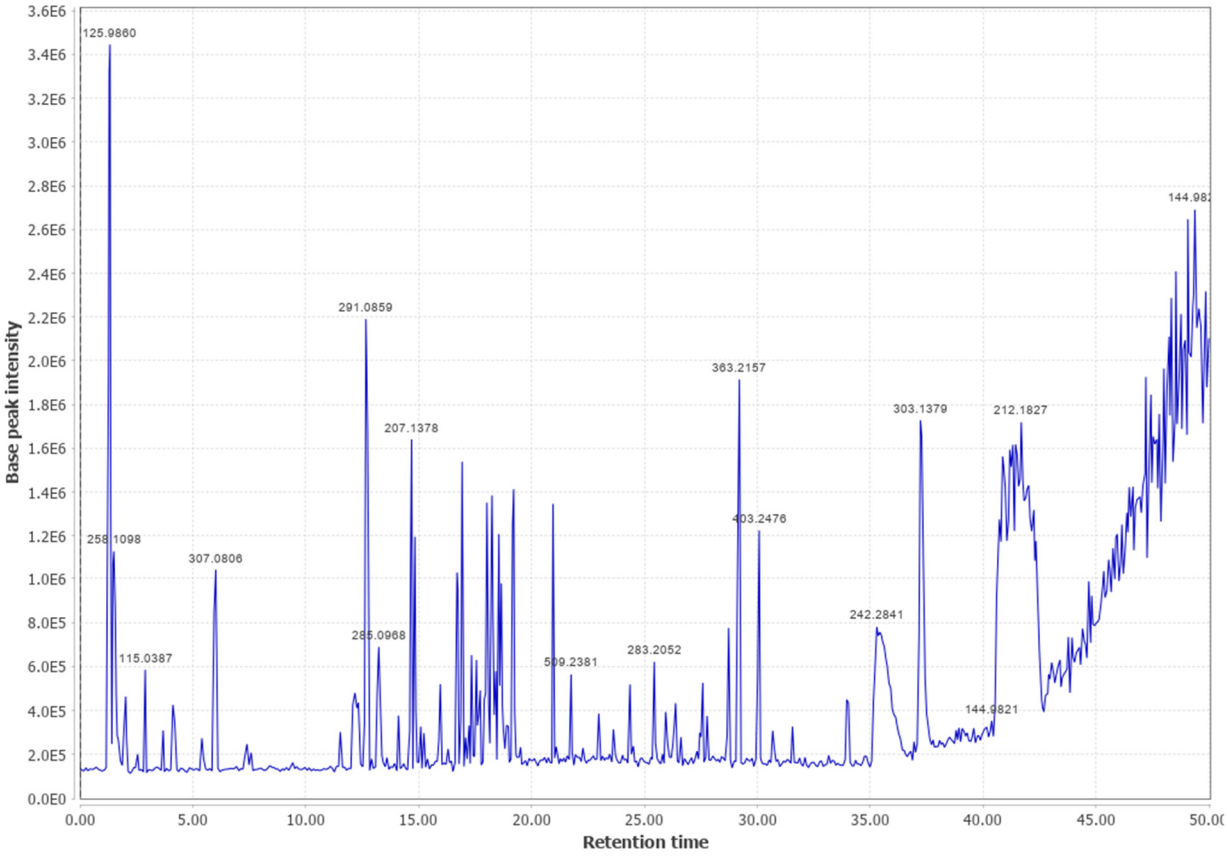

Figure A14. Second run WC #1 positive.

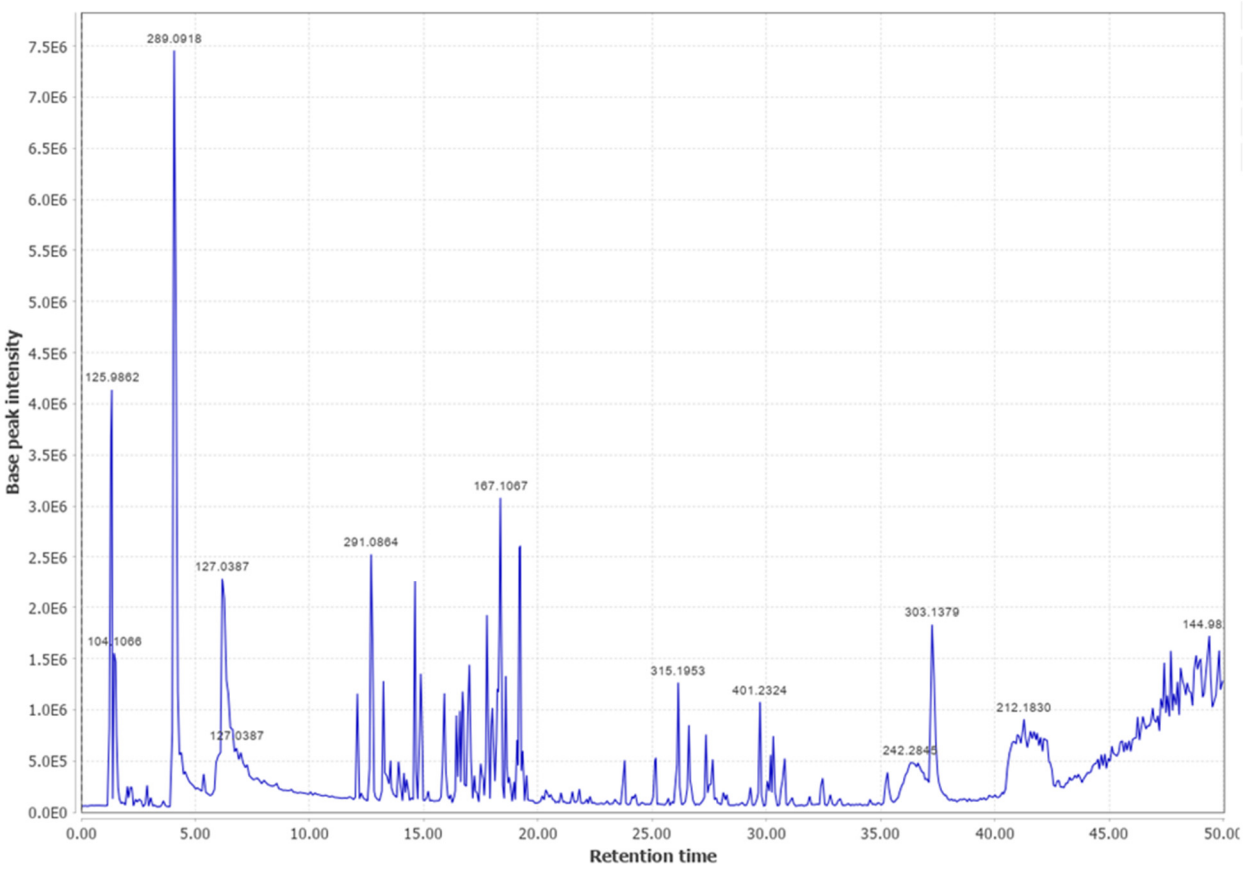

Figure A15. First run DF positive.

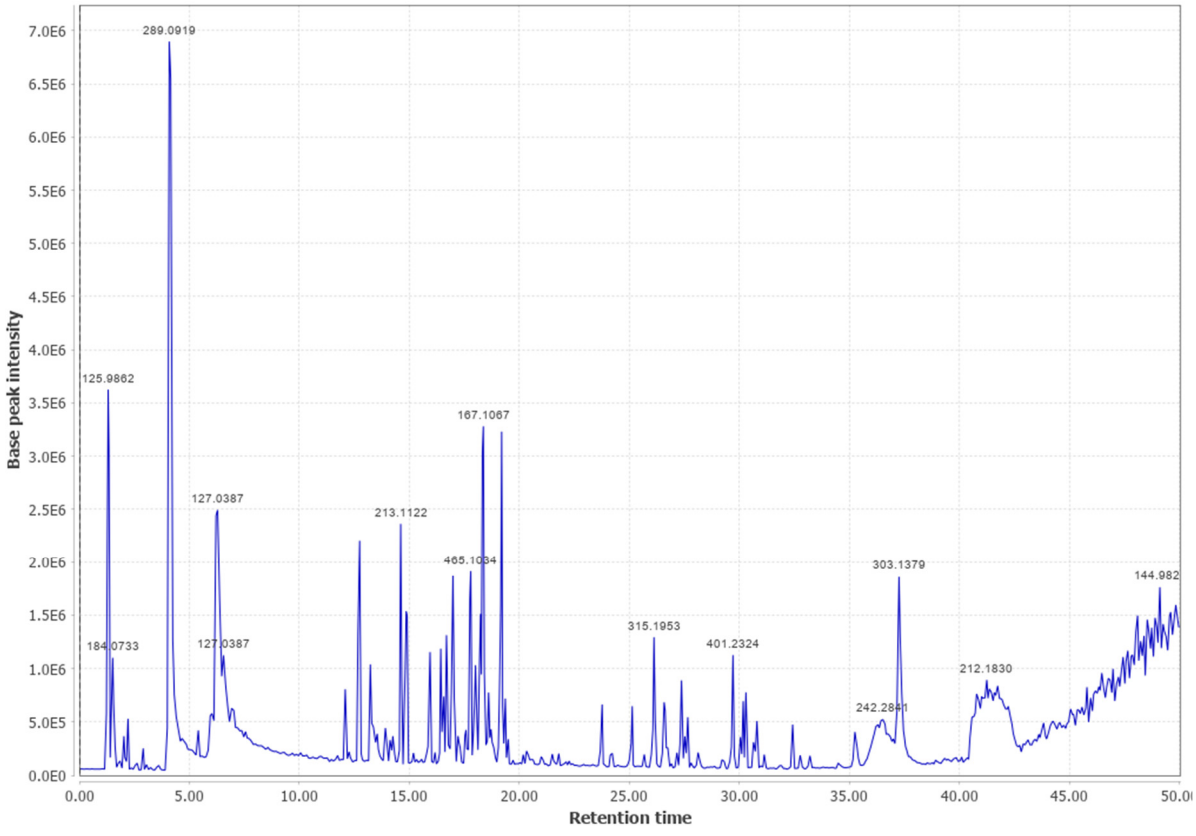

Figure A16. Second run DF positive.

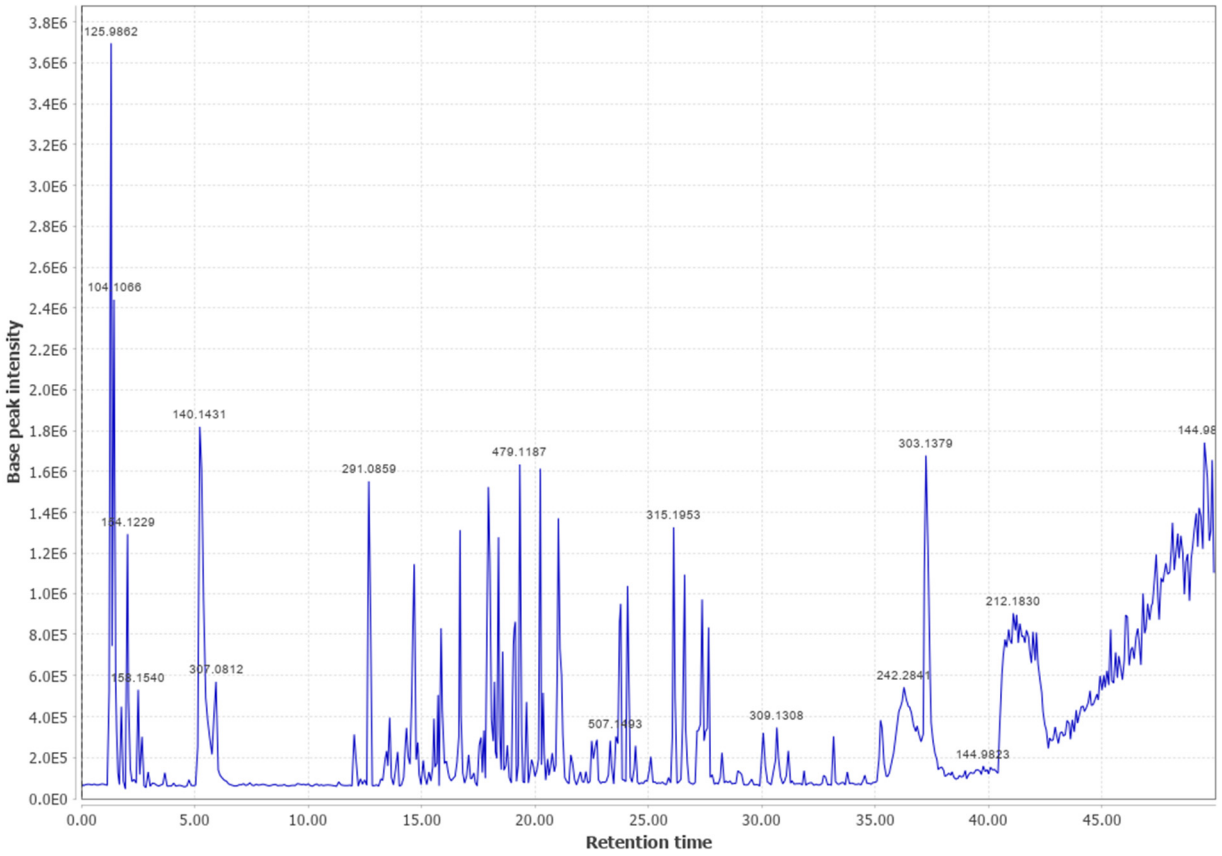

Figure A17. First run PP positive.

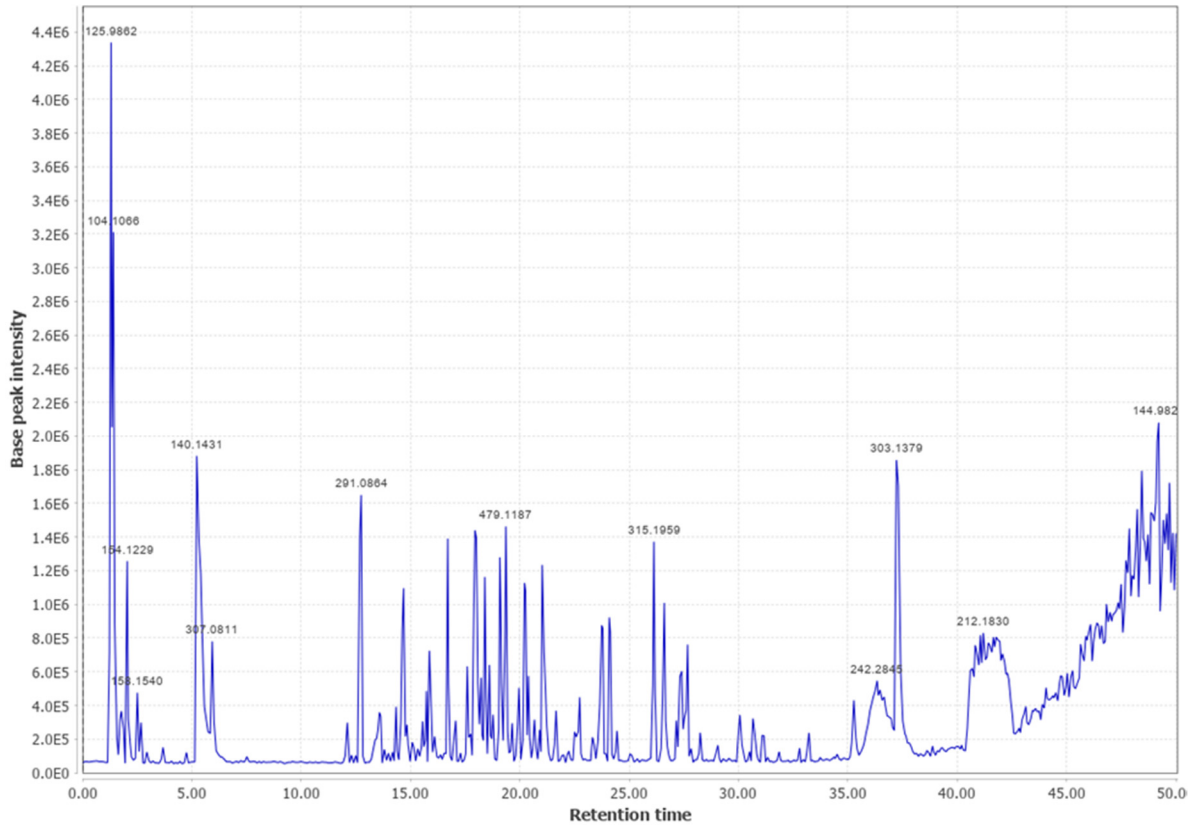

Figure A18. Second run PP positive.

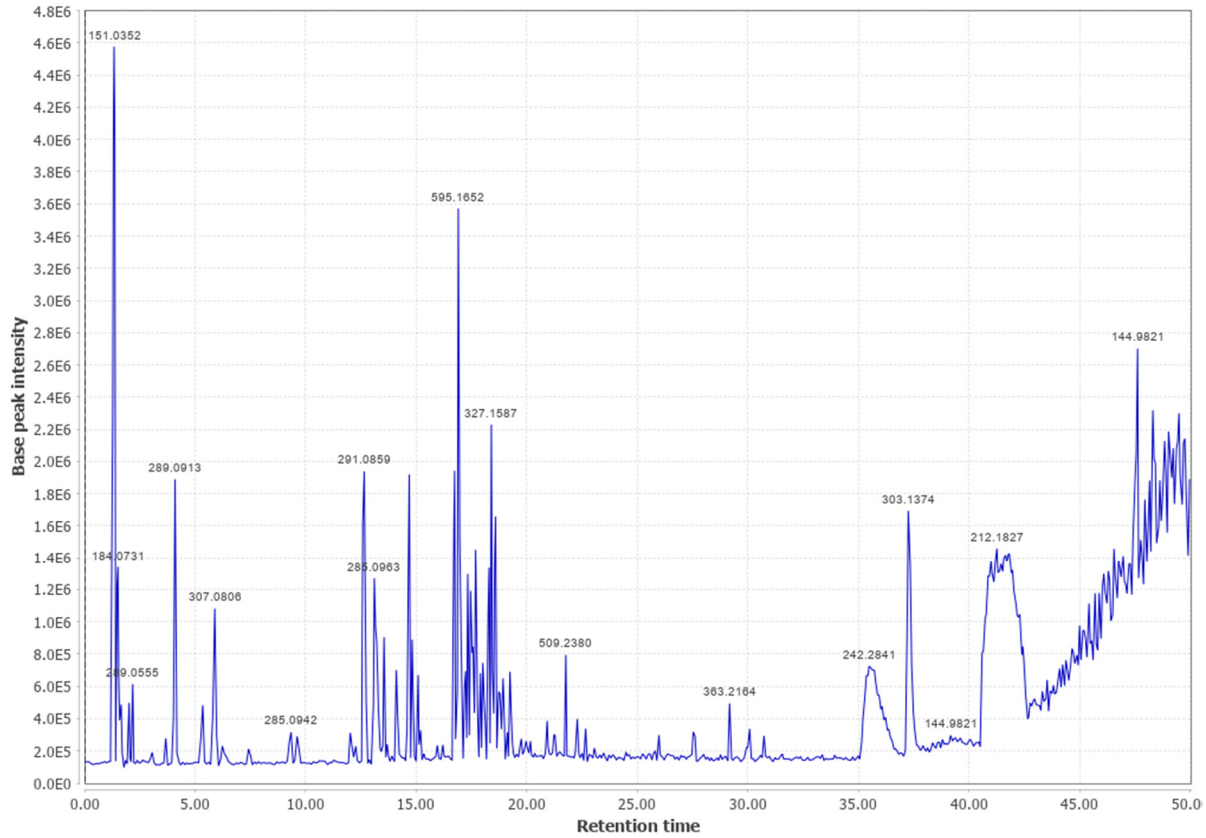

Figure A19. First run WHL positive.

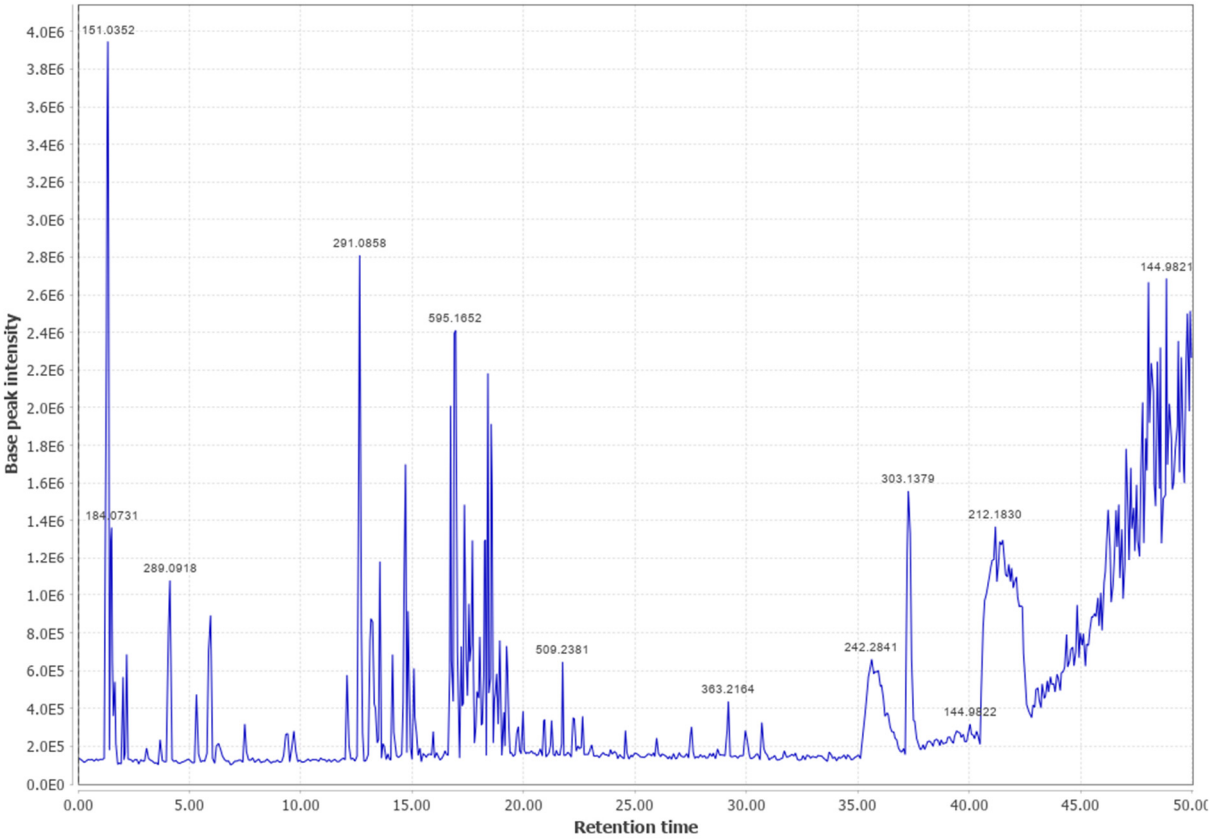

Figure A20. Second run WHL positive.

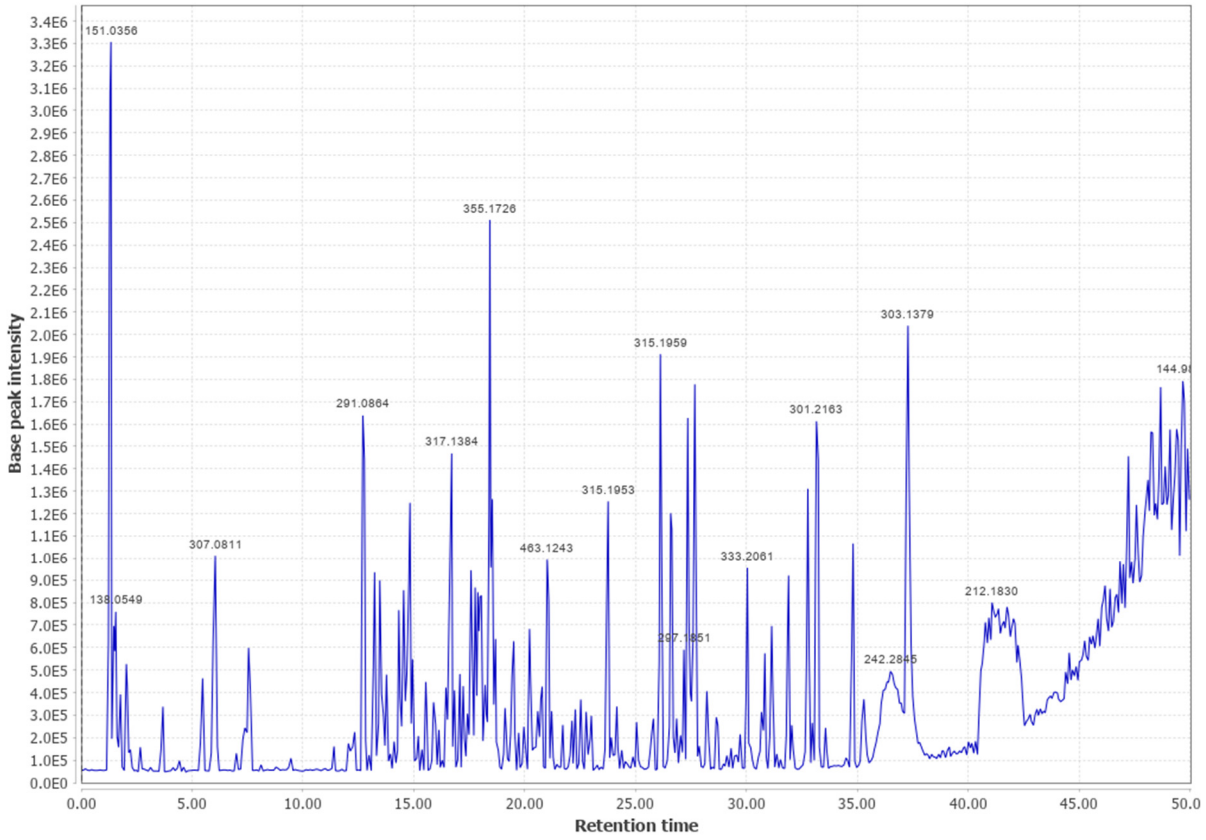

Figure A21. First run LPP positive.

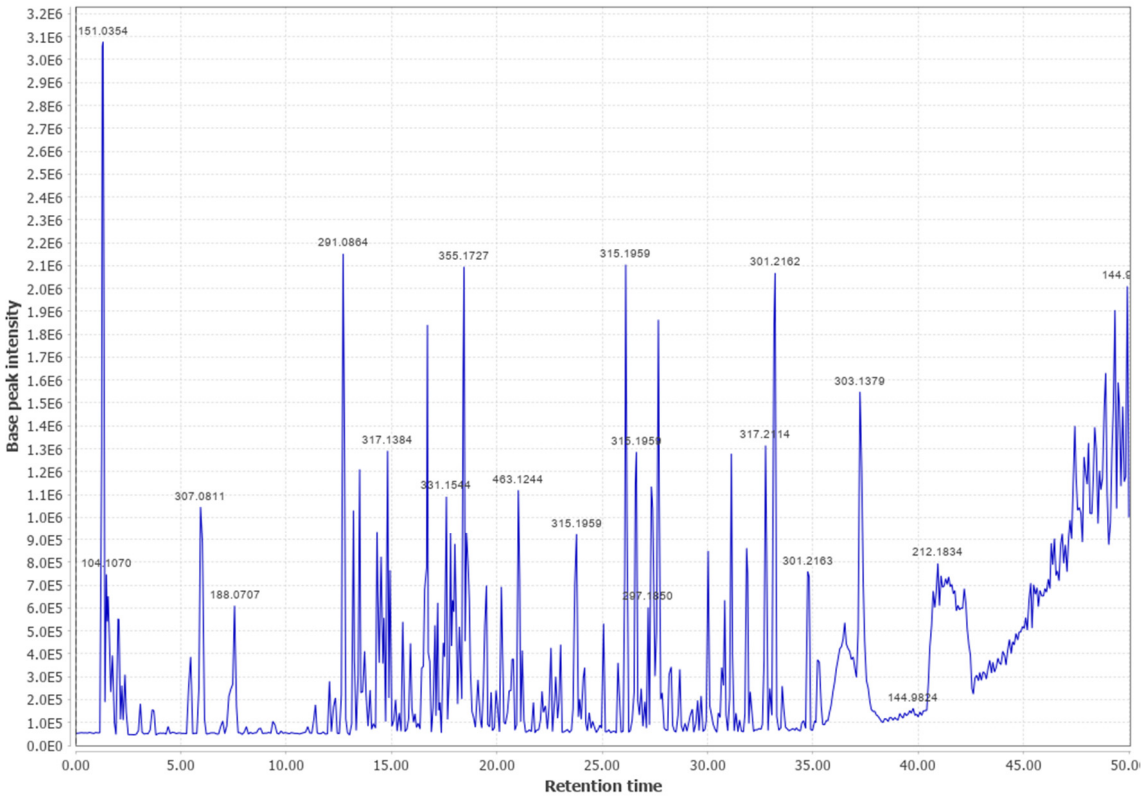

Figure A22. Second run LPP positive.

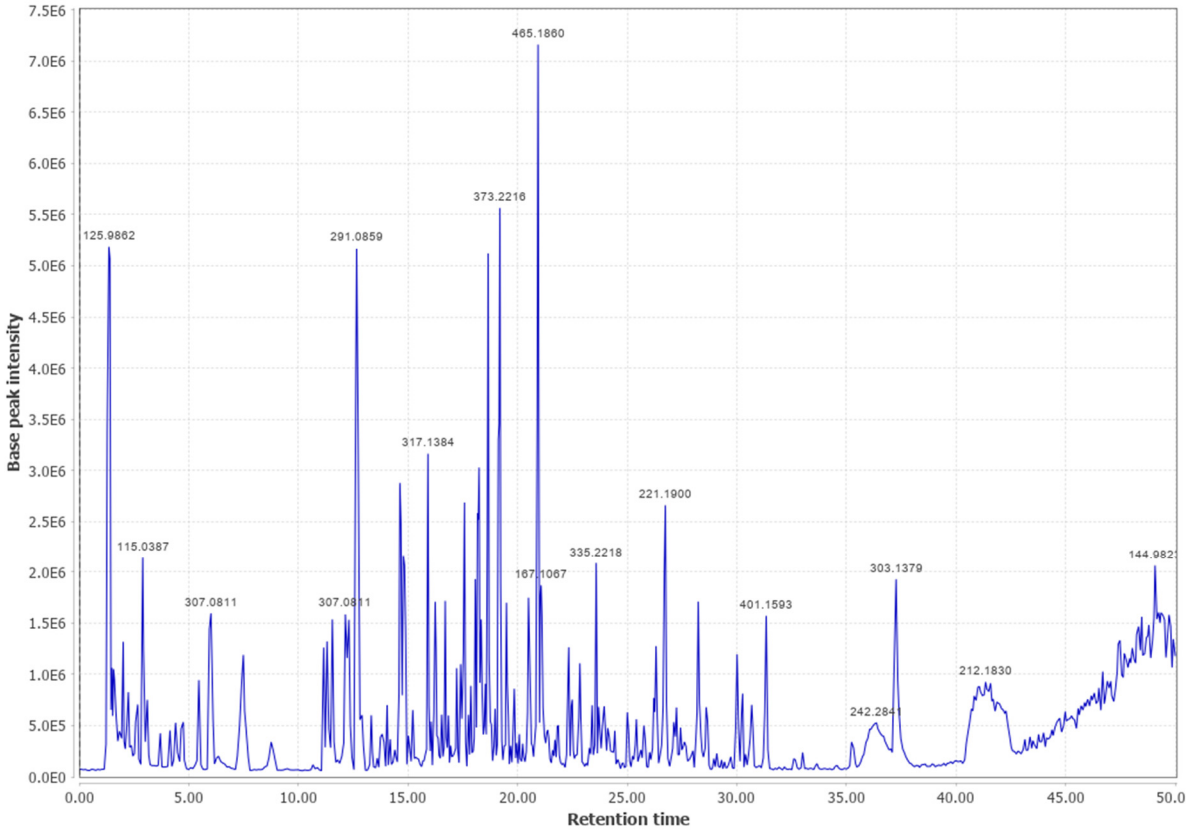

Figure A23. First run WC #2 LPC (+) positive.

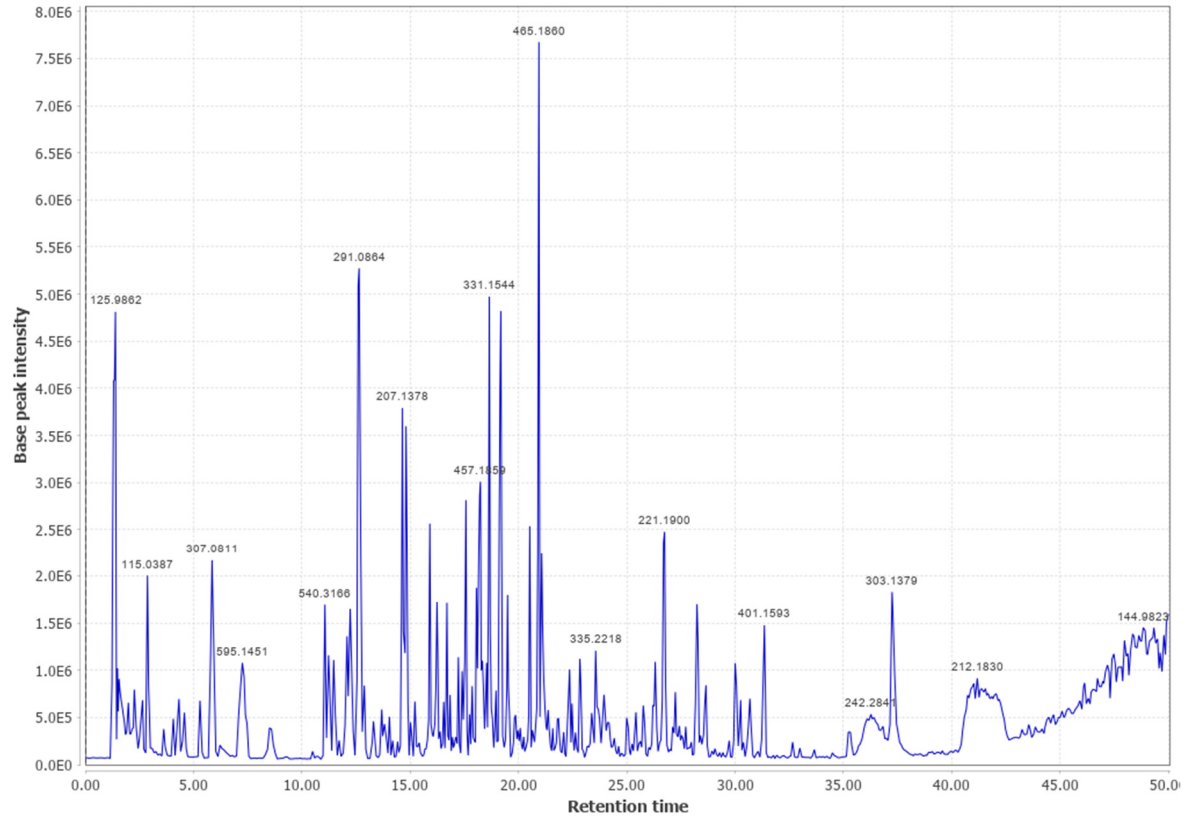

Figure A24. Second run WC #2 LPC (+) positive.

Appendix B

Table A1. Acute Toxicity Categories for Pesticide Products [36].

Hazard Indicators	I	II	III	IV
Oral LD50	Up to and including 50 mg/kg	>50 thru 500 mg/kg	>500 thru 5000 mg/kg	>5000 mg/kg
Dermal LD50	Up to and including 200 mg/kg	>200 thru 2000 mg/kg	>2000 thru 20,000 mg/kg	>20,000 mg/kg
Inhalation LC50	Up to and including 0.2 mg/liter	>0.2 thru 2 mg/liter	>2 thru 20 mg/liter	>20 mg/liter
Eye irritation	Corrosive; corneal opacity not reversible within 7 days	Corneal opacity reversible within 7 days; irritation persisting for 7 days	No corneal opacity; irritation reversible within 7 days	No irritation
Skin irritation	Corrosive	Severe irritation at 72 h	Moderate irritation at 72 h	Mild or slight irritation at 72 h

References

1. Bostrom, N.; Cirkovic, M.M. (Eds.). *Global Catastrophic Risks*; Oxford University Press: Oxford, UK; New York, NY, USA, 2011.
2. Robock, A.; Oman, L.; Stenchikov, G.L. Nuclear winter revisited with a modern climate model and current nuclear arsenals: Still catastrophic consequences. *J. Geophys. Res.* **2007**, *112*, D13. https://doi.org/10.1029/2006JD008235.
3. Xia, L.; Robock, A.; Scherrer, K.; Harrison, C.S.; Bodirsky, B.L.; Weindl, I.; Jägermeyr, J.; Bardeen, C.G.; Toon, O.B.; Heneghan, R. Global food insecurity and famine from reduced crop, marine fishery and livestock production due to climate disruption from nuclear war soot injection. *Nat. Food* **2022**, *3*, 586–596. https://doi.org/10.1038/s43016-022-00573-0.
4. Denkenberger, D.C.; Pearce, J.M. Feeding everyone: Solving the food crisis in event of global catastrophes that kill crops or obscure the sun. *Futures* **2015**, *72*, 57–68. https://doi.org/10.1016/j.futures.2014.11.008.
5. Denkenberger, D.; Sandberg, A.; Tieman, R.J.; Pearce, J.M. Long term cost-effectiveness of resilient foods for global catastrophes compared to artificial general intelligence safety. *Int. J. Disaster Risk Reduct.* **2022**, *73*, 102798. https://doi.org/10.1016/j.ijdrr.2022.102798.
6. Denkenberger, D.; Pearce, J. *Feeding Everyone No Matter What: Managing Food Security After Global Catastrophe*; Academic Press: Cambridge, MA, USA, 2014.
7. García Martínez, J.B.; Pearce, J.M.; Throup, J.; Cates, J.; Lackner, M.; Denkenberger, D.C. Methane Single Cell Protein: Potential to Secure a Global Protein Supply Against Catastrophic Food Shocks. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1125.
8. Throup, J.; García Martínez, J.B.; Bals, B.; Cates, J.; Pearce, J.M.; Denkenberger, D.C. Rapid repurposing of pulp and paper mills, biorefineries, and breweries for lignocellulosic sugar production in global food catastrophes. *Food Bioprod. Process.* **2022**, *131*, 22–39. https://doi.org/10.1016/j.fbp.2021.10.012.
9. Alvarado, K.A.; Mill, A.; Pearce, J.M.; Vocaet, A.; Denkenberger, D. Scaling of greenhouse crop production in low sunlight scenarios. *Sci. Total Environ.* **2020**, *707*, 136012. https://doi.org/10.1016/j.scitotenv.2019.136012.
10. Stewart, H. *Cedar: Tree of Life to the Northwest Coast Indians*; Douglas & McIntyre: Vancouver, BC, Canada, 1984.
11. Winstead, D.J.; Jacobson, M.G. Food resilience in a dark catastrophe: A new way of looking at tropical wild edible plants. *Ambio* **2022**, *51*, 1949–1962. https://doi.org/10.1007/s13280-022-01715-1.
12. García Martínez, J.B.; Egbejimba, J.; Throup, J.; Matassa, S.; Pearce, J.M.; Denkenberger, D.C. Potential of microbial protein from hydrogen for preventing mass starvation in catastrophic scenarios. *Sustain. Prod. Consum.* **2021**, *25*, 234–247. https://doi.org/10.1016/j.spc.2020.08.011.
13. García Martínez, J.B.; Brown, M.M.; Christodoulou, X.; Alvarado, K.A.; Denkenberger, D.C. Potential of microbial electrosynthesis for contributing to food production using CO_2 during global agriculture-inhibiting disasters. *Clean. Eng. Technol.* **2021**, *4*, 100139. https://doi.org/10.1016/j.clet.2021.100139.
14. García Martínez, J.B.; Alvarado, K.A.; Christodoulou, X.; Denkenberger, D.C. Chemical synthesis of food from CO2 for space missions and food resilience. *J. CO_2 Util.* **2021**, *53*, 101726. https://doi.org/10.1016/j.jcou.2021.101726.
15. Meyer, T.K.; Pascaris, A.; Denkenberger, D.; Pearce, J.M.U.S. Potential of Sustainable Backyard Distributed Animal and Plant Protein Production during and after Pandemics. *Sustainability* **2021**, *13*, 5067. https://doi.org/10.3390/su13095067.
16. García Martínez, J.B.; Alvarado, K.A.; Denkenberger, D.C. Synthetic fat from petroleum as a resilient food for global catastrophes: Preliminary techno-economic assessment and technology roadmap. *Chem. Eng. Res. Des.* **2022**, *177*, 255–272. https://doi.org/10.1016/j.cherd.2021.10.017.

17. Denkenberger, D.; Pearce, J. Micronutrient Availability in Alternative Foods During Agricultural Catastrophes. *Agriculture* **2018**, *8*, 169. https://doi.org/10.3390/agriculture8110169.
18. Denkenberger, D.C.; Cole, D.D.; Abdelkhaliq, M.; Griswold, M.; Hundley, A.B.; Pearce, J.M. Feeding everyone if the sun is obscured and industry is disabled. *Int. J. Disaster Risk Reduct.* **2017**, *21*, 284–290. https://doi.org/10.1016/j.ijdrr.2016.12.018.
19. Fist, T.; Adesanya, A.A.; Denkenberger, D.; Pearce, J.M. Global distribution of forest classes and leaf biomass for use as alternative foods to minimize malnutrition. *World Food Policy* **2021**, *7*, 128–146. https://doi.org/10.1002/wfp2.12030.
20. Addo-Danso, S.D. *Responses of Western Hemlock, Western Redcedar, and Amabilis Fir to Fertilization: A Synthesis*; Province of British Columbia: Victoria, BC, Canada, 2019.
21. BC Market Outreach Network. *BC Forest Information. Managing B.C. Cedar for the Future*; BC Market Outreach Network: Vancouver, BC, Canada, 2003.
22. McArdle, R.E.; Meyer, W.H.; Bruce, D. *The Yield of Douglas Fir in the Pacific Northwest*; IDEAS Working Paper Series from RePEc; *US Department of Agriculture*1930.
23. Gazol, A.; Valeriano, C.; Cantero, A.; Vergarechea, M.; Camarero, J.J. Douglas Fir Growth Is Constrained by Drought: Delineating the Climatic Limits of Timber Species under Seasonally Dry Conditions. *Forests* **2022**, *13*, 1796. https://doi.org/10.3390/f13111796.
24. Schmid, M.; Pautasso, M.; Holdenrieder, O. Ecological consequences of Douglas fir (*Pseudotsuga menziesii*) cultivation in Europe. *Eur. J. For. Res.* **2014**, *133*, 13–29. https://doi.org/10.1007/s10342-013-0745-7.
25. Nuszdorfer, F.C. *Old and Large Douglas-Fir and Western Redcedar in the Squamish Forest District, British Columbia, Canada [microform]*; Vancouver Forest Region: Nanaimo, BC, Canada, 2000.
26. Alldritt, M.J. *The Ecology of the Ponderosa Pine Zone*; British Columbia Ministry of Forests: BC, Canada, 1998.
27. Safford, H.D.; Stevens, J.T. *Natural Range of Variation for Yellow Pine and Mixed-Conifer Forests in the Sierra Nevada, Southern Cascades, and Modoc and Inyo National Forests, California, USA*; General Technical Report; Pacific Southwest Research Station, USDA Forest Service: 2017.
28. Arno, S. *Northwest Trees: Identifying and Understanding the Region's Native Trees*; Mountaineers Books: 2020.
29. Schuler, J.L. National Wildlife Federation Field Guide to Trees of North America. *J. Environ. Qual.* **2009**, *38*, 1330–1330. https://doi.org/10.2134/jeq2008.0025br.
30. *Final Report of the Lodgepole Pine Seed Set Task Group, Interior Technical Advisory Committee, Forest Genetics Council of British Columbia*; Forest Genetics Council of British Columbia: Victoria, BC, Canada, 2002.
31. Lodgepole Pine 2022. Available online: https://www.for.gov.bc.ca/hfd/library/documents/treebook/lodgepolepine.htm (accessed on 18 November 2022).
32. Hubbard, B.R.; Putman, L.I.; Techtmann, S.; Pearce, J.M. Open Source Vacuum Oven Design for Low-Temperature Drying: Performance Evaluation for Recycled PET and Biomass. *J. Manuf. Mater. Process.* **2021**, *5*, 52. https://doi.org/10.3390/jmmp5020052.
33. Pearce, J.M.; Khaksari, M.; Denkenberger, D. Preliminary Automated Determination of Edibility of Alternative Foods: Non-Targeted Screening for Toxins in Red Maple Leaf Concentrate. *Plants* **2019**, *8*, 110. https://doi.org/10.3390/plants8050110.
34. Breuer, S.W.; Toppen, L.; Schum, S.K.; Pearce, J.M. Open source software toolchain for automated non-targeted screening for toxins in alternative foods. *MethodsX* **2021**, *8*, 101551. https://doi.org/10.1016/j.mex.2021.101551.
35. Dorne, J.L.C.M.; Richardson, J.; Livaniou, A.; Carnesecchi, E.; Ceriani, L.; Baldin, R.; Kovarich, S.; Pavan, M.; Saouter, E.; Biganzoli, F.; et al. EFSA's OpenFoodTox: An open source toxicological database on chemicals in food and feed and its future developments. *Environ. Int.* **2021**, *146*, 106293. https://doi.org/10.1016/j.envint.2020.106293.
36. Code of Federal Regulation 2022. Available online: https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-156/subpart-D/section-156.62 (accessed on 18 November 2022).
37. Ostry, V. *Alternaria* mycotoxins: An overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin J.* **2008**, *1*, 175–188. https://doi.org/10.3920/WMJ2008.x013.
38. Case Definition: Trichothecene Mycotoxin 2018. Available online: https://emergency.cdc.gov/agent/trichothecene/casedef.asp (accessed on 25 November 2022).
39. Dionisio, K.L.; Phillips, K.; Price, P.S.; Grulke, C.M.; Williams, A.; Biryol, D.; Hong, T.; Isaacs, K.K. The Chemical and Products Database, a resource for exposure-relevant data on chemicals in consumer products. *Sci. Data* **2018**, *5*, 180125. https://doi.org/10.1038/sdata.2018.125.
40. Watabe, T.; Hiratsuka, A.; Aizawa, T.; Sawahata, T.; Ozawa, N.; Isobe, M.; Takabatake, E. Studies on metabolism and toxicity of styrene IV. 1-Vinylbenzene 3,4-oxide, a potent mutagen formed as a possible intermediate in the metabolism in vivo of styrene to 4-vinylphenol. *Mutat. Res./Fundam. Mol. Mech. Mutagen.* **1982**, *93*, 45–55. https://doi.org/10.1016/0027-5107(82)90124-5.
41. Chatonnet, P.; Dubourdie, D.; Boidron, J.; Pons, M. The origin of ethylphenols in wines. *J. Sci. Food Agric.* **1992**, *60*, 165–178. https://doi.org/10.1002/jsfa.2740600205.
42. Haschek, W.M.; Voss, K.A. *Mycotoxins. Haschek and Rousseaux's Handbook of Toxicologic Pathology*; Elsevier: Amsterdam, The Netherlands, 2013, pp. 1187–1258. https://doi.org/10.1016/B978-0-12-415759-0.00039-X.
43. Adhikari, M.; Negi, B.; Kaushik, N.; Adhikari, A.; Al-Khedhairy, A.A.; Kaushik, N.K.; Choi, E.H. T-2 mycotoxin: Toxicological effects and decontamination strategies. *Oncotarget* **2017**, *8*, 33933–33952. https://doi.org/10.18632/oncotarget.15422.

44. Berthiller, F.; Crews, C.; Dall'Asta, C.; Saeger, S.D.; Haesaert, G.; Karlovsky, P.; Oswald, I.P.; Seefelder, W.; Speijers, G.; Stroka, J. Masked mycotoxins: A review. *Mol. Nutr. Food Res.* **2013**, *57*, 165–186. https://doi.org/10.1002/mnfr.201100764.
45. Pierron, A.; Mimoun, S.; Murate, L.S.; Loiseau, N.; Lippi, Y.; Bracarense, A.-P.F.L.; Liaubet, L.; Schatzmayr, G.; Berthiller, F.; Moll, W.D.; et al. Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-d-glucoside. *Arch. Toxicol.* **2016**, *90*, 2037–2046. https://doi.org/10.1007/s00204-015-1592-8.
46. Rutz, A.; Sorokina, M.; Galgonek, J.; Mietchen, D.; Willighagen, E.; Gaudry, A.; Graham, J. G.; Stephan, R.; Page, R.; Vondrášek, J.; Steinbeck, C.; Pauli, G. F.; Wolfender, J.-L.; Bisson, J.; Allard, P.-M. The LOTUS Initiative for Open Knowledge Management in Natural Products Research. eLife 2022, 11, e70780. https://doi.org/10.7554/eLife.70780.
47. PubChem. Dimethyl Dicarbonate 2022. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/3086 (accessed on 25 November 2022).
48. Yu, J.; Proctor, R.H.; Brown, D.W.; Abe, K.; Gomi, K.; Machida, M.; Hasegawa, F.; Nierman, W.C.; Bhatnagar, D.; Cleveland, T.E. Genomics of Economically Significant Aspergillus and Fusarium Species. *Appl. Mycol. Biotechnol.* **2004**, *4*, 249–283. https://doi.org/10.1016/S1874-5334(04)80013-3.
49. Boonen, J.; Malysheva, S.V.; Taevernier, L.; Diana Di Mavungu, J.; De Saeger, S.; De Spiegeleer, B. Human skin penetration of selected model mycotoxins. *Toxicology* **2012**, *301*, 21–32. https://doi.org/10.1016/j.tox.2012.06.012.
50. Mishra, S.; Zhang, W.; Lin, Z.; Pang, S.; Huang, Y.; Bhatt, P.; Chen, S. Carbofuran toxicity and its microbial degradation in contaminated environments. *Chemosphere* **2020**, *259*, 127419. https://doi.org/10.1016/j.chemosphere.2020.127419.
51. Substance Information—ECHA 2022. Available online: https://echa.europa.eu/substance-information/-/substanceinfo/100.013.416 (accessed on 18 November 2022).
52. Meyar, T.K.; Tieman, R.J.; Breuer, S.W.; Denkenberger, D.; Joshua, M.P. Yield and Toxic Analysis of Leaf Protein Concentrate of Common Agricultural Residues. *to be published*.
53. Kennedy, D. Leaf for Life. Leaf Concentrate: A Field Guide for Small Scale Programs. Available online: https://www.leafforlife.org/gen/leaf_concentrate.html (accessed on 9 January 2023).
54. Can You Eat Pine Needles? 2022. Available online: https://www.primalsurvivor.net/can-you-eat-pine-needles/ (accessed on 25 November 2022).
55. Amazon.ca : Pine Needle Tea 2022. Available online: https://www.amazon.ca/s (accessed on 25 November 2022).
56. Denkenberger, D.; Pearce, J.; Taylor, A.R.; Black, R. Food without sun: Price and life-saving potential. *Foresight* **2019**, *21*, 118–129. https://doi.org/10.1108/FS-04-2018-0041.
57. Denkenberger, D.C.; Pearce, J.M. Cost-effectiveness of interventions for alternate food in the United States to address agricultural catastrophes. *Int. J. Disaster Risk Reduct.* **2018**, *27*, 278–289. https://doi.org/10.1016/j.ijdrr.2017.10.014.
58. Denkenberger, D.C.; Pearce, J.M. Cost-Effectiveness of Interventions for Alternate Food to Address Agricultural Catastrophes Globally. *Int. J. Disaster Risk Sci.* **2016**, *7*, 205–215. https://doi.org/10.1007/s13753-016-0097-2.