Sustainable Agriculture Research; Vol. 12, No. 2; 2023
ISSN 1927-050X E-ISSN 1927-0518
Published by Canadian Center of Science and Education

High-intensity, Low-frequency Ultrasound Treatment as Sustainable Strategy to Develop Innovative Biomaterials from Agri-food Byproducts and Wastes

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Received: July 2, 2023 Accepted: August 5, 2023 Online Published: August 12, 2023

doi:10.5539/sar.v12n2p45 URL: https://doi.org/10.5539/sar.v12n2p45

Abstract

Bioconversion is an important avenue for finding value from biomass waste produced by the agricultural industry. One avenue of conversion is the development of upcycling byproducts and waste from food processing to value-added products. This includes degradable biomaterials which have real potential to reduce waste, improving economic, social and environmental impacts. As such, this research paper was focused on exploring two avenues of bioconversion from waste products of tomato skin, hemp meal and hops vines: identification of phytochemicals and the development of bioplastic. Combined to these researches, the effect of Ultrasound as a green technology was studied in both contexts. It was found that Ultrasound treatment reduced extraction time for saponin and phenolic acid from tomato skin, hemp meal and/or hops vines from 24h to 30 min. However, Ultrasound Assisted Extraction (UAE) was shown to affect the phenolic acid and saponin profiles of certain extracts.

Ultrasound treatment was shown to positively impact the overall microscopic structure and qualities of bioplastic such as water activity, percentage moisture, hardness, cohesiveness, resilience, and springiness index. This study suggests that Ultrasound can be used as sustainable non-thermal method for extraction of active saponins and phenolics but also in bioplastic formulation to enhance physico-chemical characteristic.

Keywords: agricultural waste, biomaterial, bioplastic, HPLF-US, texture analysis, UAE

Abbreviations

AUC - Area Under the Curve
HPLF- High Power Low Frequency
HPLF-US - High Power Low Frequency Ultrasound
PLM - Polarized-light microscopy
RPM - Rotation Per Minute
SEM - Scanning Electron Microscopy
TFC-Total flavonoid Content
TPC- Total Phenolic Content
TSC-Total Saponin Content
US - Ultrasound
24h - Samples extracted mechanically for 24h using acidified ethanol as solvent
US 30 min - Samples extracted using HPLF US for 30 minutes and acidified ethanol as a solvent

1. Introduction

Agriculture is a pillar of human activities which allows for production of most if not all the food consumed. However, the agricultural sector is also responsible for the production of different wastes(Obi et al., 2016). The food/agricultural industry produces large amounts of plant materials a portion of which through improper

management, overproduction or simply necessity ends up as waste. In Canada, it is estimated that about 40% of the food produced in farms is not consumed every year (Ishangulyyev et al., 2019). Furthermore, the number doesn't account for the by-products linked to the processing of the other 60 percent.

Finding value in waste is a sound economical proposition. Creating and implementing systems to process waste could lead to the development of new economic sectors (Romero-Hernández & Romero, 2018). By utilizing waste, the mass of waste produce diminished which is an important aspect of developing more sustainable practices. Biorefineries offer a solution by producing cellulose, lignin, hemicellulose, and other bio-product from the biomass. However, they can be complex processes with limitation in their outputs and further transformation of certain of these products when other than biofuels would need to happen (Clark & Deswarte, 2015). As such, the important of exploring other methods of bioconversion should not be dismissed. A perfect example of this mindset can be seen in the various researches dedicated to utilizing fruit waste for the recovery of pectin (Kluczkovski et al., 2020; Sarangi et al., 2023).

Creating value-added products like packaging products made directly from grinded left-over biomass could offer a simple but effective method (Menon & Rao, 2012). Similarly, Heinz recently started to find innovative ways to upcycle peels, stems and seeds from more than two million tons of tomatoes to develop sustainable composite materials for diverse utilizations (Ontario Processing Vegetable Growers, 2023). Other companies such as Ford joined up with Heinz and other companies such as Coca-Cola Company, NIKE Inc., to use the above composite materials to be used in vehicle manufacturing and alternative to traditional polyethylene terephthalate (PET) plastic bottles. To fill these huge gaps, developing degradable biomaterials containing phenolic antioxidants, in addition to finding new use for Ultrasound application as a green technique were the major aim of this study.

As this need to find value from waste is clearly identified, it is also important to note the importance of finding alternative sustainable converting processes (Vilkhu et al., 2008).

Ultrasound assisted extraction is a sustainable technique that has become of interest for its capacity to increase extraction efficiency while having a relatively low energy use (Herrera & Luque De Castro, 2004; Mazza et al., 2019). High-Power Low-Frequency Ultrasound application has been shown to decrease the time needed for extraction of a variety of samples through use of cavitation and detexturation of samples (Chemat et al., 2017). Through application of micro jet forces, Ultrasound breaks down the surface of plant matrix allowing for the different compounds to dissolve in the solvent in a more efficient matter than with regular stirring. Furthermore, Ultrasound treatment has been shown to allow the development of stronger double emulsion (Dornan, 2021).

On the other hand, plastic production and packaging are intrinsically linked as plastic is one of its mainstay components. In 2015, the global production of plastic was estimated at 350 million tons (Geyer et al., 2017). Although plastics are so commonly used and produced in enormous quantities, proper disposal systems are rarely put in place. Up to 2015, up to 55% of plastics were still disposed of mainly through landfills and although trends show a slow increase in the proportion of plastics properly disposed of through incineration and recycling, a lot of environmental damage has/is still taking place (Eriksen et al., 2014; Okunola et al., 2019) Plastics have slow degradation processes and the by-product of these breakdowns are often toxic for the environment (Arvanitoyannis, 2013; Gewert et al., 2015; Lebreton et al., 2017; Webb et al., 2013) Plastic are almost always made of non-biodegradable polymers making them non-biodegradable materials. Biopolymers and biomaterials, which are both by definition biodegradable present themselves as a powerful area of scientific exploration as they could potentially replace all together traditional plastic (Kanmani & Rhim, 2014).

It is in this optic that the focus of this study was to work on finding different avenues of utilization of cellulosic waste material. With this goal in mind, this study had a two pronged approached to further extract value from waste. The first aspect of the research focused on extracting valuable plant component such as saponins and phenolic acids which find use in the cosmetic industry or in the pharmaceutical industry. This was performed utilizing the previously discussed green technology of HPLF-US to increase the efficiency of these extractions.

The second aspect of our research focus on using the left over cellulosic material resulting from the UAE, as bulking component to develop bioplastics made entirely of biodegradable component. as to find alternative solution to improving sustainable production and future commercialization. To develop analytical protocols that can be used on different plant-based materials, it was important to assess the experimental methods on different plant sources. Therefore, working on diverse plant materials permitted to develop a suitable methodology and analytical protocols. Raw materials were not purchased from commercial sources but were obtained through collaboration with Agriculture and Agri-Food Canada (AAFC) from whom three available plants waste materials including tomato skin, hemp meal, and hops vines were provided.

2. Material and Methods

2.1 Materials

Tomato skin, Hemp meal and hops vines were collected from a tomato canner, a hemp farmer and a hop farmer, respectively that collaborated with AAFC. Acetonitrile, HPLC grade methanol and acetic acid were obtained from Fisher Chemical (Ontario, Canada). Ethanol (100%) was from Commercial Alcohols (Ontario, Canada). Acetic acid was from Aldrich Chemical Company INC. Flavonoid, Phenolic compound standard, Aluminum Chloride powder and Folin-Ciocalteu's reagent were ordered from Sigma-Aldrich (Ontario, Canada) as was Sodium Acetate and Sodium Carbonate Anhydrous were from Bioshop (Ontario, Canada). Saponin standard of Tomatine was purchased from Biopurify (Sichuan, China). Glycerol, Phosphoric acid, and Calcium hydroxide were obtained from Fisher Chemical (Ontario, Canada). Gelatin was received from Sigma-Aldrich (Ontario, Canada). Sodium Alginate was sourced from Landor Trading Co. (Quebec, Canada). Casein Sodium was ordered from MP biomedicals (CA, USA).

2.2 Traditional Extraction of Tomato Skin, Hemp Meal and Hops Vines

A modified version of Dornan et al., 2020 was used to perform extractions. Grinded tomato skin, hemp meal or hops vines were extracted (1/20 w/w) using mechanical stirring or for 30 minute using UAE, set with a power of 90W and a frequency of 20 kHz in 5% acetic acid in ethanol.

2.3 Phenolic Analysis using HPLC-PDAD

Methanolic extracts were analyzed using a Waters e2695 HPLC system equipped with aa 2998 Photodiode array detector (PDA) (Waters, Milford, MA, USA) and Synergi-Max-RP column (250X4.6mm, 5 mm). 10µL was injected at a temperature of 23℃. The column temperature was 35 ℃ and flow rate 1mL/min. The solvents used were 0.05% formic acid/water (v/v) and solvent B: 100% acetonitrile. Gradient was set as such: from 0-35 minutes: 90% 0.05% formic acid/water, 10% 100% acetonitrile, 35-40minutes: 50% 0.05% formic acid/water, 50% 100% acetonitrile, 40-50minutes: 90% 0.05% formic acid/water, 10% 100% acetonitrile. Detection of phenolic and flavonoids was done at 280nm and 320nm.

2.4 Total Phenolic Content

The total phenolic content was measured using Folin-Ciocalteu assay (Gunenc et al., 2015). Absorbance was read at 725nm using Biotek Cytation Hybrid Multi-Mode Reader (Biotek, Winooski, VT, USA). The TPC values were expressed as mg of gallic acid equivalent per 100g of samples.

2.5 Total Flavonoid Content

The Total flavonoid content was measured through aluminium chloride colorimetric assay (Yang et al., 2012). Standard concentration of quercetin was prepared from a stock solution of 1mg/mL (0.01mg/mL, 0.02mg/mL, 0.04mg/mL, 0.05mg/mL, 0.06mg/mL, 0.08mg/mL and 0.1mg/mL). A volume of 1 mL of standard or sample were mixed with 50 mL of aluminum chloride solution (0.1mM) and 50 mL sodium acetate solution (0.1nM).

2.6 Saponin analysis using HPLC-ELSD

A methodology for the analysis of saponin was developed from Tenon et al., 2017. Methanolic extracts were analyzed by a Waters e2695 HPLC system equipped with a w2424 Evaporating Light Scattering Detector (ELSD) (Waters, Milford, MA, USA). The column temperature was 40 ℃. Solvent used were 0.1% formic acid/water (v/v) and 0.1%formic acid/acetonitrile (v/v). ELSD was set as follow nebulizer :67%, drift tube: 55 °, gain: 500, pressure:51.0 psi. The solvent gradient was set as followed:

0-40 minutes: 2% 0.1% formic acid/water, 98% 0.1%formic acid/acetonitrile 40-45minutes: 98%0.1% formic acid/water, 2% 0.1%formic acid/acetonitrile

2.7 Total Saponin Content

Total saponin content was measured using a modified version of the method (Makkar et al., 2007; Navarro del Hierro et al., 2018) A solution of vanillin (80mg/mL) and standard solution of diosgenin of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL and 0.5mg/mL were prepared. 100mL of standard or samples were mixed with 100mL of vanillin solution and 1000mL of a 70% sulfuric acid solution. The solution was vortexed and incubated at 60℃ for 10 minutes. Absorbance was read at 544 nm using Biotek Cytation Hybrid Multi-Mode Reader (Biotek, Winooski, VT, USA).

2.8 Oxygen Radical Absorbance Capacity

Oxygen Radical Absorbance Capacity assay were performed as outlined in Huang et al. (2002). Standard Trolox solutions (50 µM, 25 µM, 12.5µM and 6.25µM) in a K2HPO4 buffer (0.75M) were prepared. On a microplate

20 µLof Trolox standard or diluted sample solutions were platted with 120 µLof fluorescein solution. After incubating at 37 ℃ for 30 minutes, 60 µLof AAPH solution (153 mM). Absorbance was recorded each second for 60mins to create a curve of the absorbance in relation to time using a Biotek Cytation Hybrid Multi-Mode Reader (Biotek, Winooski, VT, USA). This functionality is partially resulting from the presence of phenolic compounds which were measured as described in section 2.4, by the total phenolic content/TPC assay.

2.9 Developing Bioplastic Formulation

Bioplastic were formulated based on previous research (Freire, 2019). Mass and role of each ingredient are outlined in Table 1. Biowaste materials were mixed casein, calcium hydroxide, canola oil, glycerol phosphoric acid. A previously-prepared mixture of boiling water, sodium alginate and gelatin were added to the biowaste material preparation. The bio-material paste was treated with US before being extruded or extruded directly. All resulting material were left to dry for 24h.

Table 1. Overall formulation of bioplastic per 100g

	Mass (g)	Role
Biowaste Material	15.0	Bulking Agent/ Additive
Casein	9.0	Biopolymer
Calcium Hydroxide	0.1	Plasticizer
Canola Oil	8.0	Plasticizer
Glycerol	8.0	Plasticizer
Phosphoric Acid	2.0	Plasticizer
Gelatin	3.0	Biopolymer
Sodium Alginate	0.5	Biopolymer
Water	54.4	Solvent

2.10 Texture Profile Analysis

Texture Profile Analysis was performed on the biomaterials using a CT3 Brooksfield Texture Analyzer set with a TA3/100 probe. Targer deformation was set at 0.15cm, trigger load at 6.8g, test speed and return speed at 0.05cm/s and recovery time was set a 60s. Hardness, Springness, Cohessiness, Resilience and Adhessiveness were measured as described by (Breene, 1975; Roopa & Bhattacharya, 2008). Each tests were performed in triplicates.

2.11 Water Activity and % Moisture

Biomaterial samples were analyzed using an Aqualab Model Series 3 water activity meter (Decagon Devide Inc, WA, US) and % moisture was also obtained after proper drying time. Each sample was measured as to obtain triplicates values.

2.12 Scanning Electron Microscopy

SEM images Biomaterial samples were obtained using a Tescan Scanning Electron Microscope at the Carleton Nano Imaging Facility. The most representative picture was selected from each triplicate.

2.13 Polarized Light Microscopy

PLM images of Biomaterial samples were determined by polarized light microscope (Axioplan 2 imaging and Zeiss Axiophot 2 universal microscope, Carl Zeiss Inc., Jena, Germany). The images were taken with a Retiga 1300 camera linked to Northern eclipse software. The distributed size from the images was analyzed via Image J software.

2.14 Statistical Analysis

Results were analyzed using IBM SPSS Statistics software (IBM corp, Armonk, New York, USA). Significant difference between triplicate values was determined using a statistical analysis of variance ANOVA. A P-value inferior to 0.05 indicated a significant difference between the triplicate values. The mean values were compared using Duncan's Multiple Range

3. Results and Discussion

3.1 Effect of UAE on yield of extract

The percentage yield of final extracts using mechanical stirring for 24h and 30 minutes HPLF-US treatment as extraction methods were compared for each of the samples obtained as seen in Table 2. When looking at the

difference between the sample types, there was no significant difference in final yield between tomato skin, hemp meal and hops vines indicating that for this extraction methodology, yield was independent of sample type. Despite slightly lower yield of samples for both tomato skin and hops vines, extraction methods did not affect the TPC, TSC and the TFC as will be discussed in the next sections.

3.2 Effect of UAE on TPC

The Total phenolic content (TPC) of Ultrasound extracted (US 30 min) and traditional mechanically extracted (24h), tomato skin, hemp meal and hops vines are shown in Table 2. There was no significant difference ($p>0.05$) between the US treatment (US 30 min) and 24h extraction (24h) for any of the three samples (tomato, hemp, and hops). However, there was a significant difference in average TPC between the hop vines extraction and the tomato and hemp extractions. No significant difference was found between tomato skin TPC and hemp meal TPC. As such, hops vines resulted in higher TPC than both hemp meal and tomato skin. Furthermore, from the lack of significant difference mentioned between the TPC associated to US and traditional extraction of any of the sample, it can be mentioned that UAE doesn't influence the TPC when compared to a 24h traditional extraction.

3.3 Effect of UAE on TFC

The total flavonoid content in tomato skin, hemp meal and hops vines are shown in Table 2. Values for the TFC of US Samples, 24h samples for both hemp meal and tomato skin were observed to be respectively 19.92 ±2.24 mg of QE/g of sample, 15.08 ±1.37 mg of QE/g of sample, 31.75 ±6.30 mg of QE/g of sample and 51.31 ±3.52 mg of QE/g of sample. Tomato skin and hemp meal extractions showed no statistical difference ($p> 0.5$). However, both hop sample extraction had significantly higher TFC, 171.10 ±2.66 mg of QE/g of sample for the US extraction and 131.80 ±13.79 mg of QE/g of sample for the 24h extraction, when compared to all other extractions. This is in accordance with the results in Table 2, which showed significantly higher results for the hops extraction. Samples with significantly higher phenolic concentration also showed a significantly higher concentration of flavonoids. There was no significant difference in TFC between 24h and US extracts of any of the sample tomato, hemp or hops samples indicating that TFC was not influenced by the difference in extraction techniques.

Table 2. Phenolic Profile and Antioxidant activity of plant extracts

Sample	Amount	Tomato US 30min	Tomato 24h	Hemp US 30min	Hemp 24h	Hops US 30min	Hops 24h
Yield	*mg/100g	12.51 ±0.51^{a}	18.65 ±3.0b	8.86 ±0.74^{a}	8.49- ±0.03^{a}	13.08 ±2.63^{a}	16.75 ±5.56^{b}
TPC	mg of **GAE/100g	87.22 ±21.12^{a}	89.14 ±11.61^{a}	147.39 ±16.92^{a}	159.42 ±28.20^{a}	450.32 ±26.47^{b}	460.95 ±48.57^{b}
TFC	mg of ***QE/100g	19.92 ±2.24^{a}	15.08 ±1.37^{a}	31.75 ±6.30^{a}	51.31 ±3.52^{a}	171.10 ±2.66^{b}	131.80 ±13.79^{b}
ORAC	μg of ****TE/g	733 ±161^{a}	1070 ±167^{ab}	1273 ±240^{ab}	1143 ±240^{ab}	1625 ±433^{b}	1650 ±307^{b}
Gallic	mg/g	5.02 ±0.09^{a}	4.89 ±0.03^{a}	-	-	3.14 ±0.09^{b}	3.20 ±0.20^{b}
Protocatechuic	mg/g	4.83 ±0.09^{a}	4.86 ±0.01^{a}	28.12 ±0.60^{b}	29.40 ±0.31^{c}	12.79 ±0.24^{d}	7.92 ±0.34^{e}
P-OH Benzoic	mg/g	6.17 ±0.36^{a}	6.24 ±0.47^{a}	2.44 ±0.04^{b}	8.78 ±0.08^{c}	19.04 ±1.32^{c}	10.60 ±0.96^{d}
Chlorogenic	mg/g	5.38 ±0.05^{a}	5.50 ±0.06^{a}	3.00 ±0.07^{b}	3.49 ±0.01^{b}	17.90 ±0.44^{d}	12.50 ±0.96^{e}
Vanillic	mg/g	-	-	-	1.35 ±0.04^{a}	11.82 ±0.09^{b}	5.67 ±0.31^{c}
Syringic	mg/g	10.95 ±0.2^{a}	10.90 ±0.02^{a}	-	4.61 ±0.03^{b}	13.43 ±0.35^{a}	10.60 ±2.32^{a}
P-Coumaric	mg/g	19.68 ±0.01^{a}	19.67 ±0.01^{a}	9.99 ±0.01^{b}	10.03 ±0.01^{b}	13.70 ±0.04^{c}	13.78 ±0.28^{c}
Ferulic	mg/g	4.59 ±0.07^{a}	4.29 ±0.02^{a}	2.15 ±0.003^{b}	2.45 ±0.02^{b}	8.00 ±0.16^{c}	6.90 ±0.59^{c}
O-Coumaric	mg/g	-	-	-	-	5.68 ±0.21	4.48 ±0.19
Pyrogallol	mg/g	-	-	-	-	23.01 ±0.67	-
Catechin	mg/g	6.27 ±0.04^{a}	6.25 ±0.23^{a}	2.03 ±0.28^{b}	3.22 ±0.12^{c}	23.19 ±0.56^{d}	21.66 ±0.54^{e}
Rutin	mg/g	4.07 ±0.21^{a}	3.56 ±0.25^{a}	1.85 ±0.15^{b}	1.80 ±0.01^{b}	10.18 ±0.84^{c}	14.11 ±0.67^{d}
Myricetin	mg/g	-	-	-	-	10.67 ±0.29^{a}	11.28 ±0.30^{a}
Quercetin	mg/g	2.96 ±0.05^{a}	2.93 ±0.02^{a}	3.95 ±0.6^{b}	6.00 ±0.2^{c}	15.12 ±0.05^{d}	13.46 ±0.4^{e}
Kaempherol	mg/g	-	-	6.87 ±0.86^{a}	5.68 ±.19^{a}	15.03 ±0.87^{b}	13.11 ±2.16^{b}

Results are represented as mean values ± SEM with differing subscript letter representing statistical difference. *Calculated per 100g of base materials. ** Gallic Acid Equivalent *** Quercetin Equivalent ****Trolox equivalent

3.4 Influence of UAE on Phenolic Acid Profile

Protocatechuic acid, hydroxybenzoic acid, chlorogenic acid, syringic acid, p-coumaric and ferulic acid were all present in both extracts. Furthermore, no statistical difference ($p>0.05$) can be noted for the concentration of any of the phenolic present in both samples. Hemp extract on the other hand show significant difference in both

composition and concentration of phenolic acids. 24 hours extracted hemp sample (Hemp 24h) shows a higher diversity of phenolic acid when compared to the 30 minutes US extracted samples. Both hemp seed TPC and phenolic profile is also in accordance to previously obtained results such as the one presented in (Teh et al., 2014). As such, two more phenolic acid are present in the 24h extract vanillic acid and syringic acid. In both samples, chlorogenic acid, p-coumaric, and ferulic acid were present in concentration that were not shown to be statistically different. On the other hand, the concentration of protocatechuic acid and hydroxybenzoic acid was shown to be statistically different ($p<0.05$) in both hemp extract. The 24 hours extraction showing higher average concentration of both these compounds. Hops vines extract had similar phenolic acid profile. In both extracts the following phenolic acid were detected: gallic acid, protocatechuic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid and o-coumaric acid. Gallic acid, p-coumaric acid, syringic acid and ferulic acid were all found to be similar concentration in Hops 24h and Hops US 30 min, meaning there was no statistical difference between the concentration resulting from the analysis. Alternatively, protocatechuic acid, hydroxybenzoic acid, chlorogenic acid and o-Coumaric acid were present in statistically higher concentration in both the 24h hop extract and the US extract. As such hops showed the most diversity in phenolic acid composition between all 3 plant samples. UAE extraction for both tomato skin and hops vines increases the concentration of phenolic compound extracted. However, the same cannot be said for the extraction of hemp where traditional method showed overall higher diversity and concentration of phenolic. This could indicate a need to increase time of extraction for hemp sample as to increase the efficiency of US as has been previously reported in (Medina-Torres et al., 2017).

3.5 Influence of UAE on Flavonoid Profile

The flavonoid profile of the various tomato skin, hemp meal and hop vines extracts are summarized in Table 2. Tomato skin extracts showed the same flavonoid profile on both Tomato US 30 min or Tomato 24h. Catechin, Rutin and Quercetin were all found in both tomato extracts. These results are in accordance to Dumitrash et al., 2016. Where UAE extraction was shown to decrease the time of extraction in tomato seeds. The concentrations of each of those phenolic compounds were not statistically different. Hemp US 30 min and Hemp 24h showed a significant difference when it came to the concentration of the various flavonoid identified. Both extracts showed the presence of the same 4 flavonoids: catechin, rutin, quercetin and kaempherol. All these compounds were found in higher concentration in the 24h extract aside from kaempherol. Kaempherol concentration was not found to be statistically different between US extracts and the 24h extracts. Hops vines extracts showed difference in the flavonoid profile. As such, Hops US 30 min had a broader diversity of flavonoid showing the presence of pyrogallol, catechin, rutin, myricetin, quercetin and kaempherol. Comparatively, pyrogallol was not found to be present in the 24h extracts. The concentration of catechin and quercetin was found to statistically higher in the US extract. Rutin concentration appeared to be significantly lower in the UEA extracted sample than the 24h extracts. The concentrations of myricetin and kaempherol of both hop extracts were not found to be statistically different. Additionally, Hops was found to have the highest diversity of flavonoids of the three plant samples. As such, the total flavonoid content were not affected by UAE for both tomato and hops extract slight difference could be noticed in hemp extracts. This could be remedied by performing longer US extraction as to ensure further cavitation and sonoporation of the sample which would logically lead to further leaching of bioactive compounds still present in the plant matrix.

3.6 Effect of UAE on TSC

The total saponin content was measured for each of the samples (tomato skin, hemp meal and hops vines) extracts.). There was no significant difference found between the TSC of US extracts and the 24h extracts ($p >0.05$) in tomato extracts. Hemp extracts also did not show any significant difference in TSC when extracted with US vs the traditional method, the measured TSC was respectively found to be 1511.25 ± 136.98 mg of DE/ 100g of sample and 1618.93 ± 58.90 mg of DE/ 100g of sample. Hops extracts similarly showed no significant difference between TSC of the US extraction and the traditional extraction where values were respectively 8037.83 ± 885.45 mg of DE/ 100g of sample and 9847.34 ± 2063.63 mg of DE/ 100g of sample. Both Hops extraction had total saponin concentration significantly higher than hemp and tomato extracts ($p<0.05$). A such, UAE did not influence the final concentration of saponin when compared to traditional extraction.

Table 3. TSC and tomatine content in mg/g of dried grinded tomato skin, hemp meal and hops vines after extraction

Sample		Tomato US 30min	Tomato 24h	Hemp US 30min	Hemp 24h	Hops US 30min	Hops 24h
TSC	mg of DE*/100g	1444 ± 126^{a}	1338 ± 240^{a}	1511 ± 137^{a}	1619 ± 59^{a}	8038 ± 886^{b}	9847 ± 2064^{b}
Tomatine	mg/g	1476 ± 243^{a}	1416 ± 68^{a}	-	-	-	-

Results are represented as mean values ± SEM with differing subscript letter representing statistical difference. * DE: Diosgenine equivalent

3.7 Effect of UAE on Saponin Profile

Table 3 shows the concentration of Tomatine present in the different extracts. Tomatine was found only in tomato extracts. Tomato extracts show the presence of tomatine which is in accordance with previous literature (Friedman, 2002). Furthermore, US treatment leads to a decrease in the time necessary for extraction from 24h to 30 minutes to extract saponins.

3.8 Influence of UAE on ORAC

The Oxygen radical absorbance capacity (ORAC) was measured for each extraction method and each sample. Tomato extracts showed values of 733.44 ±161.73 mg of Trolox/g of sample for UAE and 1070.28 ±166.83mg of Trolox/g of sample and the traditional extraction. Hemp US and Hemp, No US had values of 1273.16 ±240.06 and 1142.86 ±240.06 mg of Trolox/g of sample. Hops on the other hand showed values of 1624.59 ±432.66 and 1649.57 ±306.86 mg of Trolox/g of sample for the US extracts and 24h extracts. The method of extraction did not influence ORAC as there was no significant difference between US extracted sample results and 24h extracted sample results. As ORAC values are related to the antioxidative activity of phenolic and flavonoid compounds, these results correspond to the data measured in section 3.3 to 3.5. Other studies come to support that UAE does not lead to lower antioxidant activity of extracts as long as these values correlate with the phenolic content of a sample extracted in (Khan et al., 2010) where similar results were observed when comparing the standard and Ultrasound assisted extraction of orange peels.

3.8 Overall Appearance of Bioplastics

In Figure 1, little difference between No US samples and US samples. However, US treated samples of both Hemp and tomato were slightly oily toward the center of the sample surface when compared to the No US samples. This oily sheen could potentially be canola oil separating from the bioplastic structure. As canola oil was used as an external plasticizer it is possible that US treatment increased the rate of separation of canola oil from the biopolymer structure (Tyagi & Bhattacharya, 2019). Hops-based bioplastics were much drier and rougher in appearance

Overall, US treatment appeared to influence the overall appearance of both hemp and tomato sample only slightly.

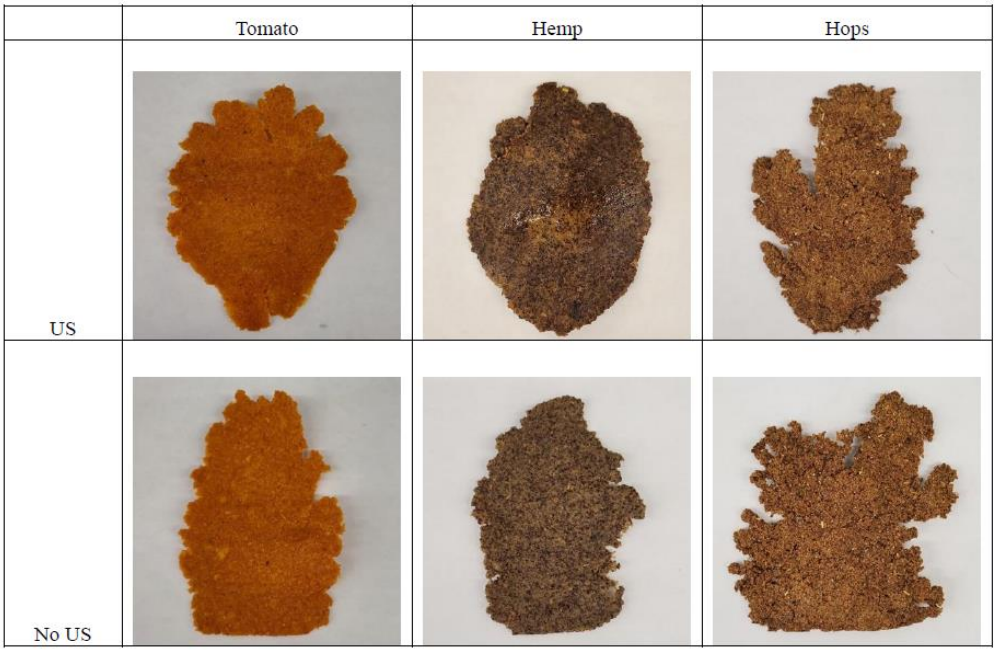

Figure 1. Overall appearance of bioplastic made from tomato, hemp and hops with Ultrasound treatment and no Ultrasound treatment

3.9 Microscopic Imaging of Biomaterials

Looking at the tomato-based and tomato-based bioplastic in Figure 2, the inclusion of tomato/hemp particles can clearly be seen in the US treated samples. No US samples show clusters of bubble trapped in the matrix of the biopolymer. In US samples, these clusters are replaced by a few single bubbles distributed unevenly. From the

60x pictures in Figure 3, the surface of the bioplastic treated with US present a rougher appearance than the No US samples. In the 400X magnification pictures US-treated samples showed clear micro crater indicating potential proof of microcavitation. Comparatively, the No US samples had a more uniform surface.

Hops-based bioplastic had a different appearance from the other samples. No bubbles were caught in the matrix of the biopolymer and the overall appearance looks jagged and rougher. These finding can also be noted in the SEM pictures of hops-based samples. US treatment increases the roughness of the biopolymer surface. Small crater like structures are present on surface of the biopolymer gel hinting at US induced cavitation. This indicate the lack of biopolymer to coat completely the hops vines particles as uncoated fiber of cellulosic material as can be seen in the 40X pictures of most samples. In US samples, small tunnel like structure are present. These results support the hypothesis that sonoporation/cavitation influences the surface structure of the developed bioplastics.

These results indicate that US treatment influence both the internal structure of the gel emulsion and the surface texture of our samples.

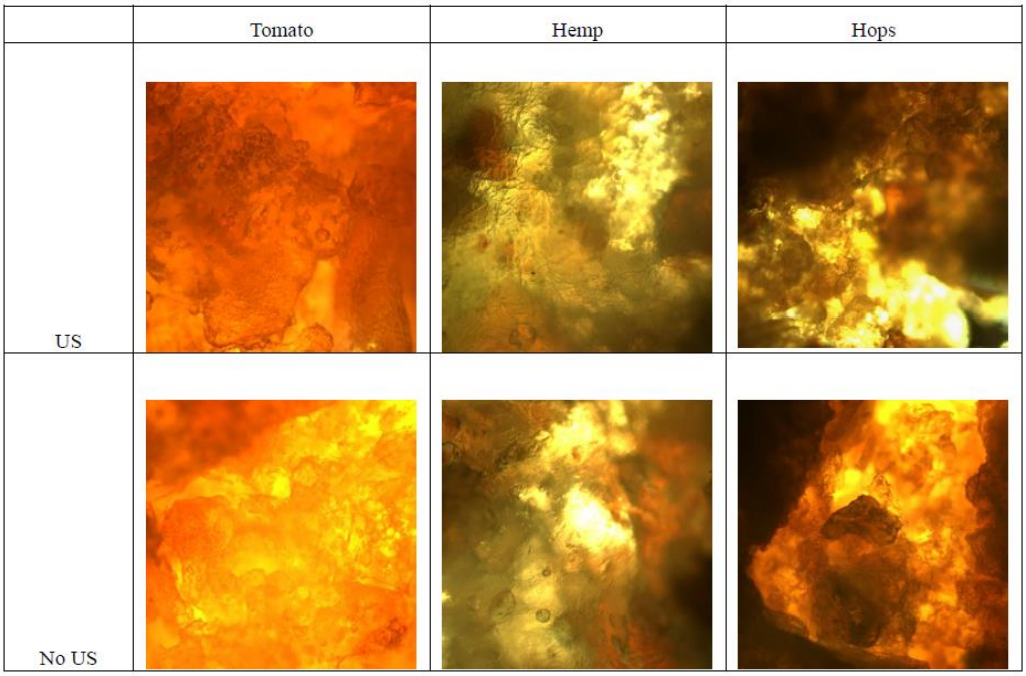

Figure 2. Polarized Light Microscopy images of biomaterial with Ultrasound treatment and no Ultrasound treatment under a magnification of 10x

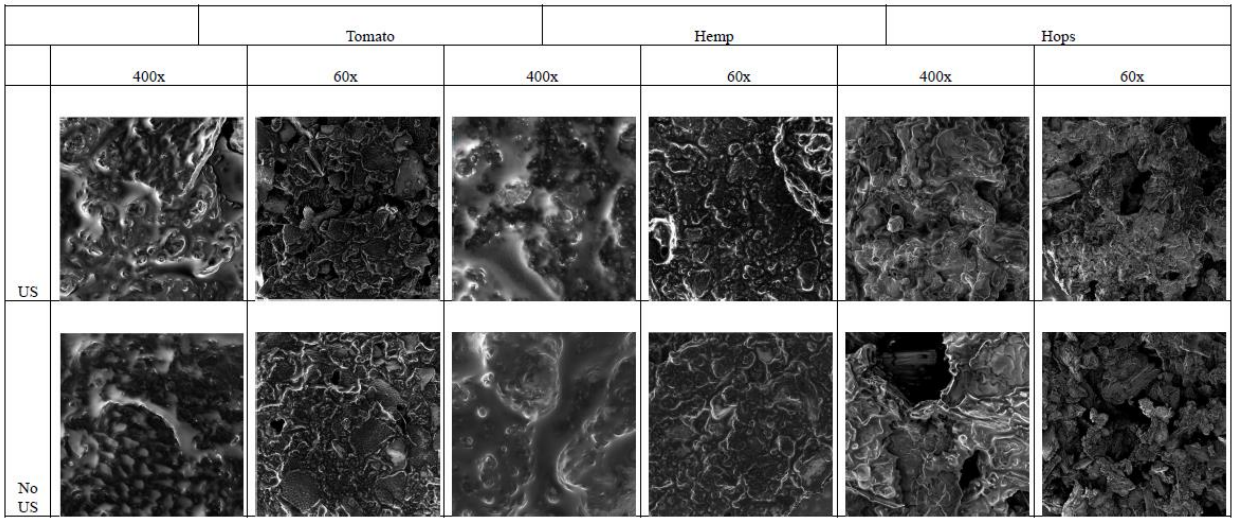

Figure 3. Scanning Electron Microscopy images of biomaterial with Ultrasound treatment and no Ultrasound treatment under magnification of 400 and 60

3.10 Rheological Properties of Biomaterials

Table 4. Texture profile analysis, water activity and moisture content of biomaterial made from tomato skin, hemp meal and hops vines with and without Ultrasound (US, NO US) treatment. Results are represented as mean values ± SEM of triplicates with differing subscript letter representing statistical difference

	Tomato		Hemp		Hops	
	US	No US	US	No US	US	No US
Hardness	2498±450a	4529±447b	1509±782a	2347±1711a	377±52a	4728±138b
Cohesiveness	0.91±0.01a	0.71±0.02b	0.68±0.01c	0.69±0.09bc	0.91±0.04c	0.66±0.05b
Resilience	0.45±0.04a	0.25±0.03b	0.23±0.04b	0.14±0.03c	0.45±0.02a	0.25±0.03b
Springiness	0.94±0.01b	0.89±0.03a	0.93±0.01b	0.82±0.03a	0.95±0.03b	0.86±0.04a
Moisture content (%)	5.20±0.72a	2.18±0.38b	12.10±1.71c	5.29±1.02a	0.69±0.19b	1.03±0.89b
Water activity	0.518±0.008a	0.549±0.018b	0.679±0.022c	0.513±0.009b	0.347±0.058d	0.532±0.102bc

US treatment statistically reduces the hardness of tomato-based and hop-based samples. Hardness of tomato, hemp and hop based biomaterial treated with US and non-treated is shown in Table 4. Tomato US and Tomato No US had hardness of 2498 ±450g and 4529 ±447g.These results hint at the fact that US treatment leads to an overall reducing of hardness of samples of bioplastic.

A clear trend can be noticed where US treated samples had higher resilience than their non-US treated. Resilience values for Tomato US and Tomato No US were found to have value of 0.45 ±0.04 and 0.25 ±0.03. Hemp based samples showed values of 0.23 ±0.04 and 0.14 ±0.03. Hops US and Hops No US had values of 0.45 ±0.02 and 0.25 ±0.02. As such, all samples were statistically different when comparing the US treated sample to the non-US treated samples. This indicates that US treatment increased the resilience of the formulated biomaterials.

Similarly, to the tomato-based samples, hops-based samples showed an increase in cohesiveness when US was applied. The values measured were 0.91 ±0.04 for Hops US and 0.66 ±0.05 for Hops No US.

Tomato US and Tomato No US showed a springiness index of 0.94 ±0.01 and 0.89 ±0.03. US treatment did not influence the springiness index of the biomaterial. Hemp No US and Hemp No US showed a springiness index of 0.93 ±0.01 and 0.82 ±0.03. For all samples, results show that US treatment increases the springiness index of our bioplastic. Springiness is a measurement of a material to regain its form. As such, it can be said that US treatment increases the capacity of our bioplastic to regain form after deformation. Overall, these results show that US treatment does impact biomaterials rheological properties.

3.11 Moisture Content and Water activity

Percentage (%) Moisture was measured for samples of biomaterial and results were in Table 4. Tomato-based based sample treated with US had a 5.20 ±0.72% moisture. On the other hand, non-treated Tomato-base sample (Tomato No US) showed a percentage moisture of 2.18 ±0.38. These values were found to be significantly different ($p<0.05$) when compared to each other. Similarly, a statistical difference was found between both Hemp-based bioplastic sample treated with US (Hemp US) and non-treated (Hemp No US). Hops is the only sample where US treatment did not influence the percent moisture as there was no statistical difference between US treated samples (Hops US) which had a value of 0.69% ±0.19 and the non-treated samples (Hops No US) with a value 1.03 ±0.89%.

Water activity was measured at 0.518 ±0.008 for US treated tomato-based bioplastic samples (Tomato US) and 0.549 ±0.018 for non-treated tomato-based bioplastic samples (Tomato No US). These values were statistically different indicating that US treatment decreased the water activity of our tomato-based bioplastic. These values were also found to be statistically different ($p<0.05$). Hemp-based biomaterial did not show any significant difference ($p>0.05$) in water activity values as values where respectively 0.679 ±0.022 and 0.513 ±0.009 for US (Hemp US) treated samples and non-treated hemp samples (Hemp No US).

4. Conclusion

Overall, these results prove that UAE is a more efficient alternative to traditional extraction. Hemp, hops and tomato extract all showed statistically similar, TPC, TFC, TSC, phenolic and saponin profiles as well as ORAC results. Furthermore, Bioplastic formulated in this study showed a textural difference based on the bulking agent used (tomato skin, hemp meal or hops vines). Ultrasound treatment was proven to have an impact on the microstructure of these bioplastic. This impact can be perceived as positive as it improves most rheological properties of these bioplastic samples. This could be due to an improved capacity at keeping moisture trapped in the gel matrix of the biomaterial. Overall US treatment has shown to have positive effects on the development of

bioplastics made from agricultural waste materials.

Funding

This work was funded by Natural Sciences and Engineering Research Council (NSERC), RPGPIN-2018-06296 and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), OAF-2019-100435

Acknowledgements

The authors would like to thank Robert Nicol, from the Bio-Industrial Process Research Centre (BPRC) at Lambton College, Sarnia, ON for providing samples, recommendations and expertise as well as Dr. Jianqun Wang from Carleton University, Ottawa, ON for providing technical assistance with scanning electron light microscopy. The authors would also like to acknowledge the Natural Sciences and Engineering Research Council (NSERC), RPGPIN-2018-06296 and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), OAF-2019-100435 for providing financial support.

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