Ultrasonics Sonochemistry 90 (2022) 106171

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Scaling up continuous ultrasound-assisted extractor for plant extracts by using spinach leaves as a test material

Jussi Tamminen, Janne Holappa, Dmitry Vladimirovich Gradov *, Tuomas Koiranen

School of Engineering Science, LUT University, Lappeenranta Campus, Yliopistonkatu 34, 53850 Lappeenranta, Finland

ARTICLE INFO

Keywords:
Ultrasound-assisted extraction
Process intensification
Scale-up
Natural ingredients
Extraction of chlorophyll
Extraction of carotenoids

ABSTRACT

The ultrasound-assisted extraction (UAE) process of chlorophylls (*a*, *b*) and carotenoids in aqueous ethanol solutions from spinach leaves was upscaled from a batch laboratory reactor to a continuous modular flow-cell of pilot scale. The extraction in the laboratory scale was organized in a loop reactor, where pulp was circulated between a stirred vessel and the ultrasound reactor. The pilot scale extraction was made in a novel continuous tubular flow-cell reactor. The analysis of the experimental data proved that the ultrasound application provided a better extraction yield. In the laboratory scale, the application of ultrasound (24 kHz and 2500 W/L) showed the 2.6-fold higher maximum extraction yield compared to non-sonicated conventional solvent extraction. In the pilot scale, the effect was less significant (1.9-fold), due to smaller ultrasound power density (25 kHz and 1500 W/L). The scale-up of the UAE was based on equal extraction yield at both scales. The scale-up revealed that 2.5-fold higher volume-specific ultrasound power is required in the pilot scale to reach the yield obtained in the laboratory scale reactor.

1. Introduction

Special interest in novel extraction technology UAE (Ultrasound-assisted extraction) has been paid because of enabling heat sensitive compounds processing, significant improvements in product yields, and short processing times can be obtained [1,2]. The UAE mechanisms promoting solvent extraction are cavitation effect, acoustic streaming and production of high local turbulence and shear forces [3]. The turbulence production has positive effect in solid–liquid mass transfer from particles to bulk solvent. The cavitating gas bubbles break cell tissues by generating microjets towards cell surfaces and thus abrading the surface [4].

High interest in UAE application can be found from annual publication data in scientific journals collected by Tiwari [4], and Chatel [5]. For example, during 2005–2014, the number of the related scientific publications has increased from 40 to 250 per year. However, this data includes mainly reporting of laboratory scale results. Most of those laboratory set-ups were batch reactors.

Materials such as leaves, fir-needle, grass blade, herblets, hops, petals, and other plant parts can be grouped in a separate class by the specific properties, which originate from the structure and orientation of fibers. Such properties are material matrix strength, shape flexibility, apparent viscosity of pulp etc. Therefore, leafy materials require special treatment during the processing stages of disintegration, pulp transport, ultrasound-assisted extraction, and solid–liquid separation. During the last two decades, the studies on ultrasound-assisted extraction from leafy and grassy materials were reported by Paniwnyk et al. [6], Shotipruk et al., [7], Albu et al. [8], Xia et al. [9], Jacques et al. [10], Petigny [11], Muñiz-Márquez et al. [12], Lee et al. [13], and Altemimi et al. [14,15]. Some researchers dried and crushed leaves before UAE. Such pretreatment produces fine particles due to improved disintegration of the dried material. However, this comes at the expense of some thermolabile chemical compounds that can be destroyed or degraded during high temperature treatment. The increased contact area between solid particles and solvent can indeed increase the extraction yield if the recovered ingredient is not affected by such pretreatment. Other researchers used wet disintegration that can be tricky with fibrous leafy materials. With suitable disintegration equipment one can benefit from material swelling effect due to reduced matrix strength.

The scale-up of sonochemical process into continuous or large-scale process is one of the crucial challenges for industrialization of UAE technology, as stated by Chatel [5]. Still, few industrial or pilot processing plants have been reported in literature [11,16,17]. In all the cases, continuous UAE were implemented using ultrasound probes

* Corresponding author.
E-mail address: dmitry.vladimirovich.gradov@gmail.com (D. Vladimirovich Gradov).

https://doi.org/10.1016/j.ultsonch.2022.106171
Received 15 February 2022; Received in revised form 11 September 2022; Accepted 16 September 2022
Available online 22 September 2022

inside flow tubes, and ultrasound devices were installed after crushing or milling units to intensify extraction. Using a commercial 1000 W ultrasound probe, Petigny et al. [11] reached 21.8 % extraction yield increase from boldo leaves after 30 min residence time at 36 °C. Preece et al. [17] reported about 4 % increase in protein concentration after ultrasound application in soybeans relative to non-sonicated samples, whereas residence times were below 200 s using 2000 W nominal ultrasound power. Clodoveo et al. [16] reported 30 % increase in carotenoids and nearly 50 % extraction increase in vitamin E derivatives from olives. Ultrasound processing time was 180–240 s, when using 4000 W nominal power in transducers.

The scale-up of sono-reactors is challenging due to the complicated physical nature of high frequency wave propagation in multiphase media and acoustically induced cavitation. Mapping homogeneous ultrasound intensity, providing sufficient macromixing and residence time are crucial for effective design of UAE apparatuses. Nevertheless, the research on sonoreactors scale-up is ongoing and Gogate et al. [18] have summarized several concepts of the sonicated reactors. However, the proposed UAE reactors are yet to be proven in practice. Due to high content of chlorophyll, spinach leaves were used in extraction efficiency evaluation of different methods in several studies [19,20].

Positive effect of natural chlorophyll on human health was widely recognized that boomed commercial interest towards chlorophyll extracts [21–23] as bioactive nutrition consumed in liquid and tablets forms. Natural chlorophyll was experimentally proven to inhibit the heme-induced colonic cytotoxicity and epithelial cell turnover unlike water soluble chlorophyllin which is often used as model compound to mimic chlorophyll in the nutrition related studies [24]. Being derived from chlorophyll, a sodium copper salt chlorophyllin in its turn showed antigenotoxic, antioxidant, and anticancer effects *in vitro* and *in vivo* studies [25]. Antiaging effect of copper chlorophyllin complex for epidermis was proven by McCook et al. [26] and explained via the mechanism of hyaluronidase inhibition preserving hyaluronic acid in skin. Water soluble chlorophyllin, encapsulated in poly (lactide-*co*-glycolide) nano particles delays lung cancer progression in mice that was induced by sodium arsenite and benzo[*a*]pyrene mixture [27].

In the current research, a scale-up study on UAE extraction of natural extracts from spinach leaves is presented. The target of the work is to develop a continuous pilot scale set-up from a batch lab scale loop-reactor. The major factors affecting the UAE extraction efficiency of chlorophylls and carotenoids from spinach are screened and analyzed using design of experiments and statistical analysis.

2. Material and methods

2.1. Experimental set-up

Experimental set-ups in both laboratory and pilot scale consist of feed tank, feed pump and ultrasound reactor (See Fig. 1). The volumes and other parameters of the laboratory and pilot scale reactors and experiments are shown in Table 1. The ultrasound reactors were made from stainless steel. The laboratory scale ultrasound reactor was a Hielscher flow cell FC22K, while a custom flow reactor was used in pilot scale. The ultrasound generators were Hielscher UP400St, and Weber Ultrasonics MG 2000 SD25 in laboratory and pilot scales, respectively. The sonotrodes were Hielscher S24d22L and Weber Sonopush. The pilot scale UAE reactor consisted of an 18 mm tube and a sonotrode installed into a casing. The reactor has earlier been utilized in ultrasound-assisted crystallization. See Ezeanowi et al. [28] for further details.

Table 1
The parameters describing reactors and experiments.

Parameter	Unit	Laboratory	Pilot
V_r	L	0.032	1
P	W	0, 20, 30, 40	0, 1500
P/V	W/L	0, 625, 940, 1250	0, 1500
V_{feed}	L	0.1	3
d_T	cm	4.5	16
Q	L/min	0.2	0.06
t_s	min	14.4	17.2
n_{circ}	–	90	0
f	kHz	24	25
V_{FT}	L	0.03	3
N_{FT}	rpm	800	500

The feed pumps were peristaltic pumps equipped with a food grade tubing. In the laboratory scale, the Masterflex L/S pump with Easyload II head was used, while in pilot scale a Flowrox NPP-D15 pump was utilized. The tubing in Masterflex pump were made from Tygon, while Flowrox pump had a nitrile butadiene rubber (NBR) tubing.

The agitation in the feed tanks was made using a four bladed turbine and Heidolph mixer motors. In laboratory scale, the impeller ($d = 2$ cm) was made from steel and blades were straight. In pilot scale, the pitched-blade turbine ($d = 10$ cm, blade angle 45°) was made from PTFE. The feed tanks and the ultrasound reactors were thermostated for temperature control. The Lauda thermostats were used for cooling of the UAE reactors and the feed tanks. The cooling fluid was water in laboratory scale and 80 % aqueous glycerol in pilot scale set-up.

2.2. The operational modes of the reactors

The residence time in pilot scale was chosen to be 17 min and it was operated as a one-pass reactor. The laboratory scale reactor had a volume of 32 mL and similar residence time was not practical to achieve using one-pass. The resulting feed flow rate would be too low (~2 mL/min) for pumping the feed containing solids, as the particles in the feed flow would settle and eventually block the flow. To minimize settling in the laboratory scale reactor, feed was circulated between the feed tank and the UAE reactor at higher flow rate (200 mL/min). The resulting residence time of the pulp in the UAE reactor was too short, for a few seconds, which was not enough for efficient ultrasound-assisted extraction. Therefore, by circulating the reactor outflow stream back into the feed tank (See Fig. 1a), the active residence time under ultrasound exposure (t_s) was increased. The sonication time for the laboratory scale reactor is then $t_s = (V_r/Q)n_{\mathrm{circ}}$, where V_r/Q is the residence time of pulp in the ultrasonic reactor. Number of circulation (n_{circ}) equals to $n_{\mathrm{circ}} = t_{exp}/(V_{tot}/Q)$, where V_{tot}/Q is the time for a single circulation through the laboratory set-up and t_{exp} is the length of a single experiment (here 45 min). Thus, $t_s = V_r/V_{tot} \times t_{exp}$. The resulting

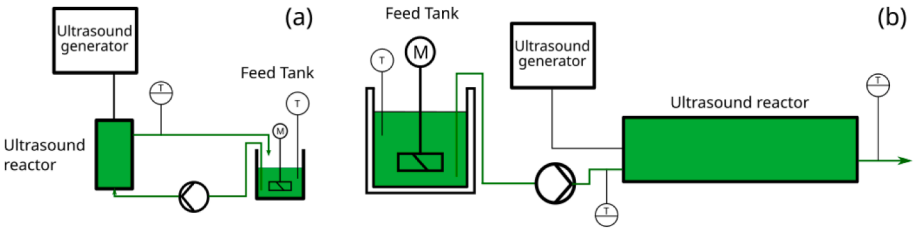

Fig. 1. Experimental set-up of ultrasound-assisted extraction in laboratory scale (a) and pilot scale (b). The ultrasound reactors and the feed tanks are jacketed for temperature control.

sonication time (t_s) in the laboratory and the pilot scale UAE reactors were close.

2.3. Preparation of the feed

Spinach (Spinacia oleracea) leaves, used as a raw material in the extraction experiments, originated from Finland Italy, Spain, and Sweden, and, and were purchased from local grocery stores in Lappeenranta, Finland. In order not to store the spinach leaves for long periods, the raw material for the experiments was purchased in batches. This resulted in variation of spinach origin. In addition, as pilot scale experiments were made during several weeks, seasonal variation had an effect on the available spinach leaf age, i.e., harvesting time. Part of the spinach batches were so-called "baby leaf spinach", which was harvested younger than other part. This led to variation in the feed chlorophyll content, as smaller, younger spinach leaves contain more chlorophylls (See Drews [29]).

The spinach leaves were rinsed to remove any remaining dirt, after which the leaves were cut manually to 1–2 cm pieces. Spinach was then processed twice using a centrifugal juicer (Wilfa Squeezy JEB-800S), once with manual screw press and twice with shredder (Wilfa Essential MC3B-400S) for removal of some liquid and decreased particle size. The spinach was disintegrated at the same day prior the laboratory scale experiment, with typical delay, i.e., raw age was about 2–3 h. Pilot scale experiments required larger amount of raw material, and preparation took longer and was typically made on previous day. The disintegrated spinach was stored under refrigeration in vacuum-sealed plastic bags. No added chemicals were used, as the aim was to do preprocessing and extraction in as short time as possible.

2.4. Analyses

The sample in the laboratory scale experiments were taken from the feed tank after the experiment. The sampling points in the pilot scale experiments were in the feed tank and in the UAE reactor outlet flow. The samples containing spinach pulp were taken at preset times. The extract samples, still containing solid raw material particles, were filtered through a 1.5 mm sieve, which separated large particles. Smaller particles were removed by centrifugation of the sample for 10 min at 4000 rpm and the liquid phase was then separated by pipetting from the remaining solids. To minimize extractive degradation, the samples were stored away from heat and direct sunlight. The analysis was made within the same day.

The concentrations of chlorophylls *a* and *b* in addition to total carotenoids were determined by using UV/VIS spectrophotometer (Agilent 8454). The samples were diluted with analysis grade acetone using dilution factor of 5, so that acetone concentration was 80 % (v/v) in the diluted sample. The chlorophyll *a*, chlorophyll *b* and total carotenoids content were calculated by using Eqs. (1)–(3) [23]. Any remaining sample haziness was taken into account with background correction, which was made using single reference wavelength (750 nm). The resolution of the spectrophotometer was 1 nm. The effect of the resolution and the ethanol presence in the samples were assumed to be negligible.

The chlorophyll *a* (c_a), chlorophyll *b* (c_b) and total carotenoid (c_c) concentrations in the diluted sample were calculated from the following equations for 80 % (v/v) acetone solutions [30].

$$c_a = (12.25A_{663nm} - 2.79A_{647nm})mg/L \quad (1)$$

$$c_b = (21.50A_{647nm} - 5.10A_{663nm})mg/L \quad (2)$$

$$c_c = ((1000A_{470nm} - 1.82c_a - 85.02c_b)/198)mg/L \quad (3)$$

where A_{663nm}, A_{647nm} and A_{470nm} refer to the absorbance measured at the wavelength specified in the subscript. The concentrations in original sample were then calculated by multiplication with dilution factor (i.e. 5).

2.5. Extraction procedure in laboratory scale

The ultrasound reactor and the feed tank were filled with solvent (aqueous ethanol) at the beginning of experiment. The circulation pump and the feed tank mixing had been started before the disintegrated raw material was added into the feed tank. The total pulp volume was 100 mL. The ultrasound was activated for 45 min, after which a sample was taken for analysis. Similar tests but without ultrasound were made for comparison.

The feed variation was taken into account by making the reference (maceration) point for each spinach batch. Spinach and 20 % (v/v) ethanol solution were mixed for 4 h at room temperature (R/S = 0.1, V_{tot} = 50 mL) using magnetic stirrer. The relative extraction efficiency or yield (c^*) was then calculated:

$$c^* = \frac{c}{c_m}, \quad (4)$$

where c is the concentration from laboratory scale UAE experiment and c_m is the concentration from the maceration experiment. Raw material batch was same in both extractions.

The design of experiments (DoE) was made using Modde Pro v. 12.1 [29]. The D-optimal method was used in creation of the design. The design table is shown in Table A1 (in Supplementary Information). The factors in the design were ultrasound power (P/V = 0–2500 W/L), ethanol concentration (x = 10, 20 or 30 % (v/v)) and temperatures (T = 20, 30, or 40 °C). The comparative extraction efficiency calculated from Eq. (4) for chlorophyll *a* (c_a^*), chlorophyll *b* (c_b^*) and carotenoids (c_c^*) are the responses of laboratory scale DoE. R/S ratio was 0.1 in all experiments. The statistical modeling was made using a partial least square regression. The results are shown in Table 2.

2.6. Extraction procedure in pilot scale

The UAE reactor and the feed tank were pre-filled with the solvent and reactor temperature was equilibrated. The feed tank content was mixed, and disintegrated spinach was added into the tank. Mixing was continued for 10 min to ensure homogeneous composition, after which the mixture was pumped into the module. Ultrasound was activated as the pulp entered the UAE reactor. The residence time in the tubes between the feed tank and the ultrasound reactor was approximately 5 min. Nitrogen gas was used as a protective atmosphere in the feed tank during the experiment. The reactor and the feed tank were thermostated to set temperature before starting the experiment.

When color change was visually observed at the reactor outflow, the collection of the samples for the concentration analysis from the reactor outlet was started. To evaluate the extraction performance of the UAE reactor, the samples were also taken from the feed tank. The feed tank is a conventional maceration batch reactor. Temperatures were measured from the feed tank and the UAE reactor inlet and outlet. Concentrations from both the feed tank and the reactor outlet were measured as a function of time in Fig. 3). The relative extraction efficiency or yield in pilot scale is defined as (compare also to Eq. (4)):

$$c^* = \frac{c_{US}}{c_{FT}}, \quad (5)$$

where c_{US} is the average concentration measured from the pilot UAE reactor outlet and c_{FT} is the average concentration measured from the feed tank. Measured concentrations and average concentration determination for each extracted compound are illustrated in Fig. 3.

A full factorial DoE (See Table A2) was made for the pilot scale extraction tests using Modde Pro [24]. The variables were for ultrasound power (P = 0 or 1500 W), ethanol concentration (x = 15, 20, or 25 % (v/v)), temperature (T = 20, 30, or 40 °C). In order to conserve the

Table 2
The extraction of chlorophylls and carotenoids using the laboratory scale circulatory UAE reactor. The measured ultrasound power, temperatures at UAE reactor outlet and in the feed tank are time-averaged in each experiment. The extraction yields were calculated from Eq. (4).

N⁰	P/V W/L	T_{USout} °C	T_{FT} °C	c_a mg/L	$c_{a,m}$ mg/L	c_a^*-	c_b mg/L	$c_{b,m}$ mg/L	c_b^*-	c_c mg/L	$c_{c,m}$ mg/L	c_c^*-
1	0	22.0	20.1	21.1	30.0	0.7	8.4	12.3	0.7	6.9	10.0	0.7
2	2730	28.0	22.8	38.0	18.0	2.1	15.1	7.2	2.1	14.1	6.8	2.1
3	0	21.6	19.7	27.4	31.6	0.9	11.1	12.8	0.9	9.1	10.6	0.9
4	2630	26.7	21.4	48.1	18.7	2.6	19.0	7.5	2.5	16.6	6.9	2.4
5	1580	25.7	22.3	62.0	30.0	2.1	24.9	11.6	2.1	18.9	9.2	2.0
6	0	40.1	38.9	11.0	14.3	0.8	4.8	5.9	0.8	3.7	5.0	0.7
7	2060	41.8	38.0	32.9	14.3	2.3	14.1	5.9	2.4	11.8	5.0	2.3
8	0	40.3	38.6	6.2	14.3	0.4	2.8	5.9	0.5	2.6	5.0	0.5
9	2710	41.6	37.7	51.2	30.0	1.7	20.8	11.6	1.8	15.7	9.2	1.7
10	2750	40.8	36.6	40.5	19.0	2.1	16.6	8.4	2.0	15.1	8.1	1.9
11	2590	32.1	27.6	59.2	30.0	2.0	24.1	12.3	2.0	18.3	10.0	1.8
12	1560	28.9	26.2	27.2	19.0	1.4	11.3	8.4	1.3	10.4	8.1	1.3
13	0	31.0	29.8	17.1	18.7	0.9	7.2	7.5	0.9	6.5	6.9	0.9
14	2080	31.5	28.1	65.9	30.0	2.2	26.8	12.3	2.2	19.6	10.0	1.9
15	2110	33.3	29.7	61.4	30.0	2.1	24.9	11.6	2.1	18.6	9.2	2.0
16	2120	31.5	28.0	71.3	31.6	2.3	29.5	12.8	2.3	22.1	10.6	2.1

sonotrode, pilot scale ultrasound power (1530 W/L) was more limited compared to the laboratory scale (up to 2500 W/L). As the required solvent volumes were rather large, the ethanol concentration was limited to 25 % (v/v) in order to minimize ethanol consumption. The center point experiment was repeated four times (trials 2, 6, 7, and 8). Trials 12–14 (x = 20 % (v/v)) were tests with no ultrasound made at different temperatures. The raw age was also variable in DoE. Raw to solvent ratio (R/S) was 0.1 in all experiments. The responses in pilot scale DoE are extraction yields for chlorophyll a (c_a^*), chlorophyll b (c_b^*) and total carotenoids (c_c^*). The beneficial effect of ultrasound on the extraction process is underlined in the results shown in Table 3.

3. Results and discussion

3.1. Laboratory scale extraction

The results from the laboratory scale extraction are presented in the Table 2. The inspection of the data reveals that the use of ultrasound increases the extraction compared to the maceration, as expected (). Increasing ultrasound power further from the smallest value (P = 40 W) promotes the extraction, but the extraction increment is less significant. Extraction yield is about 2.5 (Trial 4, P/V = 2630 W/L, T = 27 °C) at maximum.

The spinach leaves are thin, and they are easily broken by ultrasound induced impact which was clearly demonstrated by Chemat et al. [3]. As the laboratory scale reactor tubing is transparent, the breakage of leaves and decrease of particle size could be visually observed during the first minutes of sonication. After this, particles were small enough (below 1 mm) that they could no longer be visually distinguished from the flow. When the particle size decreases, larger fraction of the cells has been broken and larger leaf surface area is exposed for solvent. This is presumed to be the mechanism for ultrasound enhanced extraction. Breakage of spinach leaves is already profound at the lowest ultrasound power that was tested and thus increasing the power gives only a small additional effect. The visual observation of the decrease in the particle size due to sonication is sufficient for the purposes of this work. The extraction yields of experiments without ultrasound are in range of 0.5–1.0 both in laboratory scale (Table 2) and the pilot scale experiments (Table 3). The extraction yield below one implies adsorption of extracted compounds to the solid material surface, i.e. back-extraction.

In addition to particle size decrease, the use of ultrasound leads to better micromixing, which enhances solvent extraction. The auxiliary effect of ultrasound exposure during solvent extraction on swelling ratio of vegetal tissues (35 % increase for mint leaves [31]) decreases the tissue shear strength of the sonicated materials. The reduced shear strength of material tissue facilitates the associated effects of cavitation such as tissue rupture, surface peeling, erosion, sonoporation, and permeabilization of cell walls which increase the efficiency of solvent extraction [32]. The effect of the studied variables on the extraction yield of the studied compounds (chlorophylls and carotenoids) are very

Table 3
The results of ultrasound-assisted extraction of chlorophylls (a and b) and carotenoids from spinach in the pilot scale equipment. The ultrasound power density P/V, temperature (T), ethanol concentration (x) and raw age (t_{raw}) were varied in experiments. The extraction yields (c^*) were calculated using Eq. (5). Average temperatures during experiment are shown.

N⁰	P/VW/L	T_{USout} °C	T_{FT} °C	x% (v/v)	t_{raw}, d	c_a mg/L	$c_{a,FT}$ mg/L	c_a^*-	c_b mg/L	$c_{b,FT}$ mg/L	c_b^*-	c_c mg/L	$c_{c,FT}$ mg/L	c_c^*-
1	1530	23.2	20.6	20	12	2.6	2.7	1.0	5.1	4.3	1.2	4.8	4.1	1.2
2	1530	30.7	27.6	20	18	2.9	2.3	1.3	1.6	1.3	1.2	1.7	1.5	1.1
3	1530	39,2	36.7	20	11	4.9	2.5	1.9	2.5	1.3	1.9	2.4	1.6	1.5
4	1530	40.3	36.7	15	7	6.9	5.1	1.4	3.4	2.4	1.4	2.8	2.3	1.2
5	1530	40.3	36.4	25	1	11.6	7.5	1.6	4.9	3.4	1.4	4.2	3.4	1.2
6	1530	32.1	28.6	20	2	14.0	8.3	1.7	6.0	3.8	1.6	5.1	3.5	1.5
7	1530	32	27.7	20	1	13.3	8.5	1.6	5.9	4.0	1.5	5.4	4.1	1.3
8	1530	32.1	27.9	20	1	20.0	12.6	1.6	7.8	5.1	1.5	6.1	4.2	1.5
9	1530	32.1	28	15	1	16.6	12.6	1.3	6.4	5.1	1.3	5.0	3.9	1.3
10	1530	31.6	28.1	25	1	15.4	9.3	1.7	6.1	4.0	1.5	5.4	3.9	1.4
11	1530	23.8	20	15	1	19.4	11.0	1.8	7.5	4.3	1.7	6.7	4.2	1.6
12	0	29.5	28.6	20	1	8.0	10.1	0.8	3.2	4.3	0.8	3.5	4.5	0.8
13	0	38.2	36.4	20	1	5.3	6.7	0.8	2.1	2.7	0.8	2.4	3.0	0.8
14	0	21.1	20	20	1	9.2	9.4	1.0	3.5	3.7	1.0	3.2	3.2	1.0
15	1530	24.2	20.4	25	1	15.3	9.8	1.6	6.2	4.1	1.5	6.3	4.4	1.4

similar (See Table 2).

Sonication at the studied power range increases liquid temperature in the ultrasound reactor (Table 2). The temperature in the feed tank was typically several degrees lower, due to cooling and heat losses. Cooling was necessary to maintain the set temperature in the set-up. As high specific ultrasound power was used, the temperature rise in the ultrasound reactor was not possible to avoid in the experiments.

The statistical analysis was made for studying the effects of ultrasound power, temperature, and ethanol concentration on extraction of chlorophylls and total carotenoids. The data is shown in Table 2. As temperature in setup was not constant, average of feed tank (T_{FT}) and module outlet temperatures (T_O) was used as model factor in analysis. Other factors were as stated in Table 2. The results from the statistical analysis are shown in Fig. 2 and A1–A4 (Supplementary information). Parameter values are shown in Fig. 2. The measured extraction yield data is predicted reasonably well by using the model (See Fig A2), which is also supported by the correlation coefficients (Table A3). The residuals for all responses are within two standard deviations (Fig A1). Two data points (No. 3 and 5) were excluded from the model as outliers.

Expectedly, the increase in ultrasound power promotes the extraction yield (Fig. 2). When the ultrasound power is further increased, the extraction yield approaches constant value, which is taken into account by the $(P/V)^2$ term in Eq. (6). The extraction yield reaches a constant value at high power, which is consistent with the particle size reaching minimum, stable, value. Then, the specific interfacial area and extraction yield will reach a maximum value. In principle, it is possible that the extraction capacity of the solvent (i.e., solubility) is saturated.

The statistical model equations with unscaled parameter values for laboratory scale UAE data are:

$$\begin{aligned} c_a^* &= 0.73536 + 0.00146\frac{P/V}{W/L} - 3.7\times10^{-7}\frac{(P/V)^2}{(W/L)^2},\ c_b^* \\ &= 0.75484 + 0.00159\frac{P/V}{W/L} - 4.2\times10^{-7}\frac{(P/V)^2}{(W/L)^2},\ c_c^* \\ &= 0.73536 + 0.00146\frac{P/V}{W/L} - 3.7\times10^{-7}\frac{(P/V)^2}{(W/L)^2}, \end{aligned} \quad (6)$$

where model factor is the volume-specific ultrasound power (P/V).

The initial statistical analysis showed that the ethanol concentration and temperature are not statistically significant parameters (See Fig. 2.). However, from solvent extraction point of view, both parameters are relevant. It can be thus concluded that the changes in temperature and ethanol concentration were not large enough to achieve statistically significant increment in the extraction yield. Compared to the optimal conditions for pomegranate peels, studied by Živković et al. [31] in wider ranges of the processing conditions, the influence of ethanol concentration and temperature becomes stronger at much lower solid-to-solvent content (1:50) though. It is clear that the severance of the processing conditions is related to material matrix breaking strength. Hence, ultrasound-assisted extraction from leavy materials requires less energy which can be used to increase solid-to-solvent ratio.

3.2. Pilot scale extraction

The inspection of the pilot scale extraction results presented in Table 3 shows that the maximum observed extraction yield was about 1.9 for chlorophylls (trial 3, $T = 40$ °C, $x = 20$ % (v/v)) and about 1.6 for total carotenoids (trial 11, $T = 20$ °C, $x = 15$ % (v/v)). The extraction yield was used in the comparison of the extraction data and illustration of the benefits gained by using sonication, as was case also in laboratory scale. The results of four center point experiments ($T = 30$ °C, $x = 20$ % (v/v)), indicate that the deviation in the results was noticeable, which is due to the variation in raw material origin and storage times.

The feed tank data in Fig. 3 illustrates the extraction kinetics during the maceration. About 1 h residence time is not enough for the maceration in feed tank to reach the equilibrium concentration, whereas the UAE reactor outlet concentration reaches the equilibrium in 15 min when the spinach pulp leaves the ultrasound module. Mixing conditions in the feed tank are mild, and it is presumed that the extraction proceeds mainly via swelling and subsequent breakage of spinach particles. This contrasts with UAE reactor, where ultrasound causes cavitation and more intense micromixing. Together these factors lead to more rapid particle breakage in the UAE reactor. The resulting particle surface area is larger, and extraction will be more rapid in ultrasound reactor. Thus, it can be expected that mechanism observed in laboratory scale set-up also applies here.

The statistical modeling of the pilot scale extraction results was made using Modde Pro [34]. The temperature in the setup was not constant and the average of the feed tank (T_{FT}) and the module outlet temperatures (T_{USout}) was used as model factor in the analysis also here. Other model factors were as well shown in Table 3. The measured data is

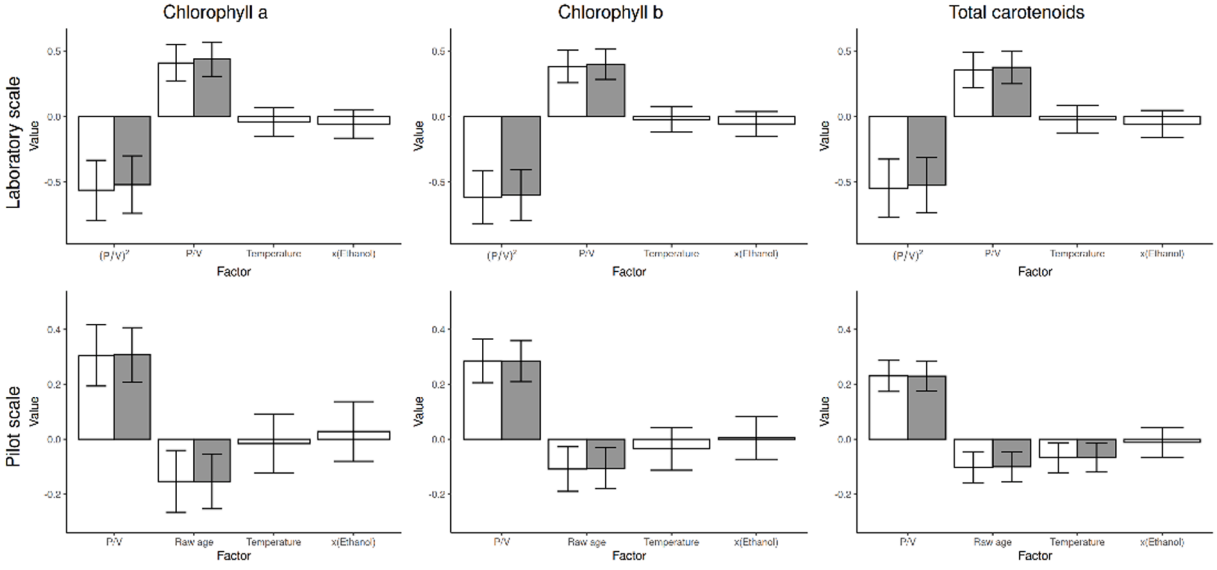

Fig. 2. The scaled and centered coefficient values of the statistical models for UAE from spinach of chlorophylls (*a*, *b*) and total carotenoids. The statistically significant factors (shown as gray bars) of DoE models at laboratory and pilot scale are shown. The determined values for all factors (White bars) prior removing insignificant factors from model are shown for comparison.

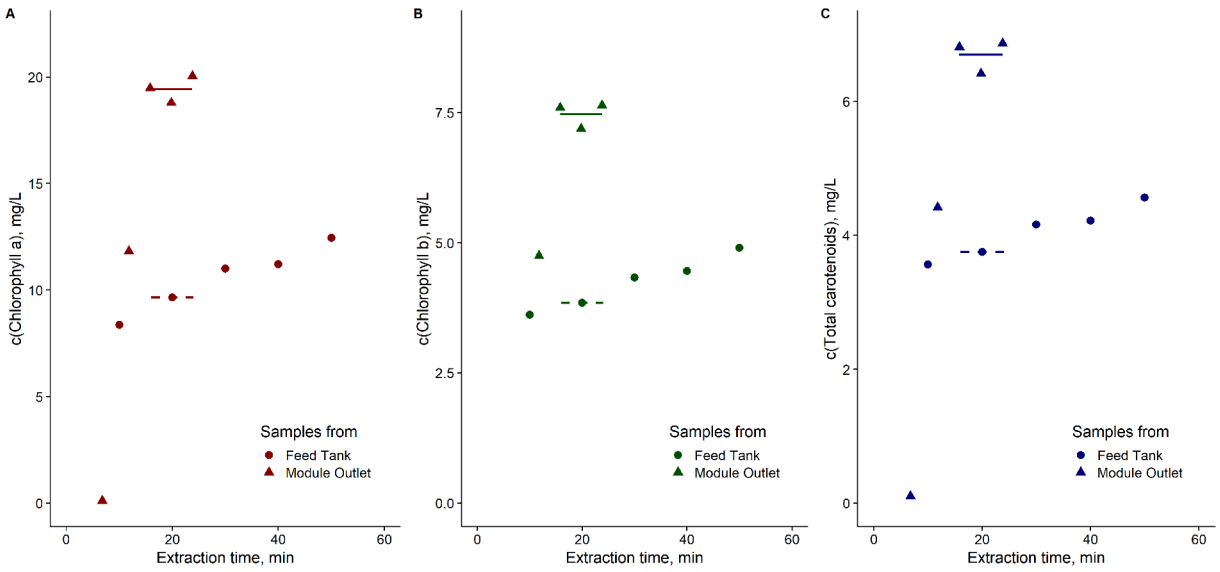

Fig. 3. Measured chlorophyll *a* (A), chlorophyll *b* (B) and total carotenoid concentrations (C) in UAE experiments (DoE experiment no 11 data is shown, compare Tabs. A2 and 3). Concentrations were measured from the feed tank and the UAE reactor outlet at different times. Lines indicate the average concentration in feed tank and ultrasound reactor outlet at selected timeframe (16–24 min. Nominal residence time of reactor system, including pump, tubes, and UAE reactor was 28 min. It was subtracted from the reactor outlet sampling times, in order to compare the feed tank and the UAE reactor outlet data. The corresponding reactor outlet sample times are in the range of 16–24 min. Note, that in this case only one feed tank sample, but three ultrasound reactor samples were taken within the selected timeframe.

predicted well by using the model (Fig A4). The residuals for all responses are within two standard deviations (Fig A3), and the normal probability plots for both scales are also similar. One DoE experiments (No. 3) was removed from the statistics as an outlier in the pilot scale data analysis.

The temperature was found to be statistically insignificant for the extraction of chlorophylls (*a*, *b*), similar to laboratory scale extraction. The total carotenoid concentrations UAE in pilot scale was an exception, as temperature had a statistically significant decreasing effect on the extraction. Ethanol concentration (x) did not have statistically significant effect on the extraction yield, as was case in laboratory scale. As temperature has a significant effect on the carotenoid extraction, it supports the assumption that increasing temperature and ethanol concentration beyond the range used in this work, they will have an observable effect on the extraction yield. The statistical model equations (Eq. (7)) for pilot scale data are:

$$\begin{aligned} c_a^* &= 0.88252 + 0.00047\frac{P/V}{W/L} - 0.02918\frac{t_{raw}}{d}, \quad c_b^* \\ &= 0.86005 + 0.00044\frac{P/V}{W/L} - 0.02005\frac{t_{raw}}{d}, \quad c_c^* \\ &= 1.19693 + 0.00035\frac{P/V}{W/L} - 0.01903\frac{t_{raw}}{d} - 0.01107\frac{T}{^\circ C}, \end{aligned} \tag{7}$$

The model factors are the specific ultrasound power (P/V) and the raw age (t_{raw}). The experimental and statistical modeling results of both scales were quite similar. This supports the expectation, that the extraction mechanism was indeed same in both set-ups.

3.3. Scale-up

The scale-up was made based on equal extraction yield c^* in both equipment. The ultrasound power of the laboratory scale device can be set to 625, 1250, 1875 or 2500 W/L at low power range (P = 20–80 W). The available ultrasound power range in pilot scale is 1000–2000 W/L. The experiments in laboratory scale were made in the ultrasound power range of 1550–2750 W/L. The extraction yield at 625 W/L was calculated using Eq. (6) assuming equal extraction yield in laboratory and pilot scales. The power which gives the same extraction yield at pilot scale was found from Eq. (7), by setting $t_{raw} = 0.125$ d in calculations. As the total carotenoids extraction in pilot scale was found to be affected by temperature, it was taken to be 20 °C in calculations. The results of scale-up are presented in Table A4. The ratio of pilot to laboratory scale ultrasound power is 2.0–2.7, though the value for each extracted compound is slightly different.

4. Conclusions

The ultrasound-assisted extraction (UAE) of chlorophylls *a*, and *b* and total carotenoids from spinach was studied here. The solvent was aqueous ethanol solution. The extraction in laboratory scale was made in a loop batch reactor, where pulp was circulated between a feed stirred tank and ultrasound reactor. The experiments in pilot scale were made using a continuous tubular reactor. The design of experiment was made for both laboratory and pilot scale experiments using Modde Pro commercial software.

The spinach was obtained batch-wise during experiments to avoid spoiling it. As a result, there was variation in spinach origin and leaf age. These factors led to the variation of chlorophyll and carotenoid content in the feed material. To take the variation into account, the maceration experiments were made from each spinach batch. The extraction yield was defined as a ratio of DoE experiment and the maceration results. The extraction yield was increased as ultrasound power grew.

The increment in the ultrasound power (P/V) led to a better extraction yield in both scales. The increase was higher in laboratory scale compared to pilot scale reactor. When the extraction was made without ultrasound, the extraction yield was below unity in both scales. This implies the adsorption of the extracted compounds back onto the solid material surfaces, i.e., back-extraction.

Unexpectedly, volumetric specific ultrasound power (P/V) has a large effect on the extraction in both scales. The observed extraction yields were 2.6 and 1.9 at maximum in the laboratory and pilot scale reactors, respectively. As the extraction mechanism is anticipated to be same in both scales, the differences are presumed to be due to the differences in geometries of ultrasound reactors, in setup constructions and in operational modes of setups. The initial analysis revealed that temperature and ethanol concentration were statistically insignificant. It is emphasized here, that these variables are not irrelevant to extraction,

but statistical insignificance is caused by the selection of too narrow range for these variables. For example, the effect of ethanol concentration is expected to be more prominent when concentrations closer to 100 % are used in extraction.

The scale-up of chlorophyll and carotenoid extraction from spinach was made based on equal extraction yield in both scales. The specific ultrasound power of 625 W/L ($P = 20$ W) was selected for laboratory scale. Using the statistical model built for the laboratory scale data, the extraction yield was calculated. Next, the pilot scale ultrasound power giving same extraction yield was determined from pilot scale statistical model. It was found out, that the total ultrasound power needs to be 2.5-fold higher in pilot scale module, in order to reach equal extraction yield to laboratory scale. The experimentally proven high yield achieved in short time creates great potential for the tested novel UAE continuous module in food and dietary supplement industry. [33].

CRediT authorship contribution statement

Jussi Tamminen: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Janne Holappa:** Investigation, Data curation, Writing – original draft. **Dmitry Vladimirovich Gradov:** Project administration, Supervision, Conceptualization, Writing – original draft, Writing – review & editing. **Tuomas Koiranen:** Supervision, Methodology, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

Financial support for this work was received from Business Finland (TUTLI project: “NATINREC - Multiproduct factory for Superefficient natural ingredient recovery”). Authors gratefully acknowledge this contribution.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106171.

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