Microwave-assisted conversion of novel biomass materials into levulinic acid

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Abstract

Levulinic acid is considered as one the most important platform chemicals. It is currently produced mainly from lignocellulosic biomasses. However, there are also other abundant biomass materials, which could be used as raw materials for levulinic acid production. In this work levulinic acid was produced from two novel biomasses in the presence of Brønsted (H_2SO_4) and Lewis acid ($CrCl_3 \cdot 6H_2O$ or $AlCl_3 \cdot 6H_2O$) catalysts. The studied materials were carbohydrate-rich potato peel waste and sporocarps of the fungus *Cortinarius armillatus*. Reaction conditions, *i.e.* time, temperature, H_2SO_4 and Lewis acid concentrations, were studied by utilizing full 2^4-factorial experimental designs. Microwave irradiation was used as the heating method. Based on the results the reaction temperature and the H_2SO_4 concentration had the greatest impact on the yield of levulinic acid. The highest yield obtained in this study from potato peel waste was 49% with 180ºC for reaction temperature, 15 min for reaction time and 0.5 and 0.0075 M for the concentrations of H_2SO_4 and $CrCl_3$, respectively. When *Cortinarius armillatus* was used as the raw material the highest yield was 62% with 180ºC for reaction temperature, 40 min for reaction time and 0.5 and 0.0075 M for the concentrations of H_2SO_4 and $CrCl_3$, respectively.

Keywords: Biomass; Cortinarius armillatus; Levulinic acid; Microwave; Potato peel waste

Abbreviations: PW, peel waste; LA, levulinic acid; S, sporocarbs

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1. Introduction

Most of the industrial chemicals are currently being prepared from fossil resources. However, the increase in the fossil fuel price as well as the depletion of the resources is driving forward the search for new and alternative renewable feedstocks in the production of renewable platform chemicals, which could replace the petroleum-based ones. [1] One of the most important platform chemicals is levulinic acid (LA) [2, 3]. Due to its chemical structure with ketone carbonyl and carboxylic functional groups, LA can be converted into various other important chemicals and hence be used as a raw material for e.g. resins, plasticizers, textiles, animal feed, coatings, antifreeze, fuel additives, polymer precursors, herbicides, pharmaceuticals and flavor substances. Since new applications of LA are being constantly explored, the demand for it is expected to grow. [4]

Presently the research is mainly concentrated on the production of LA from lignocellulosic biomass [5-8]. However, there are other abundant, novel carbohydrate-rich materials, which could be utilized as well. Such materials include potato peel waste (PW) and sporocarbs (S) of the fungus *Cortinarius armillatus*. PW is generated annually considerable amounts by food processing industry. One peeling factory can process up to 1000 tons of potato per year and depending on the peeling process the amount of waste is 15-40% of the amount of processed potatoes. Regarding the sporocarbs of edible macrofungi, it has been estimated that up to 15000 DW tons of them are produced annually in the forests of Northern Finland [9] and the production of non-edible macrofungi even exceeds that. *C. armillatus* is one of the most productive non-food macrofungi in Northern Finland with a long term average sporocarp yield of 0.3 kg DW/ha in all forest types, while maximum yield is reported to be 4.7 kg DW/ha in all forest types [10].

In this work, we have studied the conversion of PW and sporocarps of *Cortinarius armillatus* into LA. Both studied materials are cheap and have few other usages. In order to accelerate the conversion reactions microwave irradiation was used as the heating method. It has been found in previous studies with cellulose that besides accelerating the conversion reactions microwave heating also enhances the product selectivity [11, 12]. There is also a significant, up to 85-fold, energy saving involved in the microwave-assisted processes [13]. The effects of the reaction conditions on the yield of LA were studied in detail by utilizing experimental design. To our knowledge neither PW nor sporocarps from *Cortinarius armillatus* has been used as the raw material in LA production.

2. Materials and methods

2.1 Reagents

Potato peel waste, produced by abrasion peeling process, was provided by Tervakankaan Peruna Oy, Finland. PW was dried (105ºC until constant weight) and ground into fine powder before use. The original water content of PW was 80%. All reactions were done using the same batch of PW. The *Cortinarius armillatus* sporocarps were collected in 2007 in a subarctic forest with mountain birch and Scots pine at Lapland Research Station, Kevo, Finland, dried and ground into fine powder with a homogenizer. Other reagents, *i.e.* sulfuric acid (95-97%, Merck), $AlCl_3 \cdot 6H_2O$ (99%, Alfa Aesar) or $CrCl_3 \cdot 6H_2O$ (98%, Alfa Aesar) were used as received from the suppliers.

2.2 Conversion of potato peel waste or sporocarps into levulinic acid in microwave reactor

In a typical experiment *C. armillatus* sporocarps or PW (0.5 g) was weighted into a microwave reactor vessel (size 2-5 ml) equipped with a magnetic stirring bar. $AlCl_3 \cdot 6H_2O$ or $CrCl_3 \cdot 6H_2O$ catalyst (the amount corresponding to the final concentration of 0.0023-0.03 M, Tables 1 and 3) and 5 ml of aqueous H_2SO_4 (0.1-0.5 M, Tables 1 and 3) were added. The amount of Lewis acid catalyst as well as the concentration of H_2SO_4 was based on the experimental design. The mixture was heated in the microwave reactor (Biotage Initiator with a single-mode microwave unit), which maximum power output was controlled by setting its value to 90 W. Reaction temperature and time were based on the experimental design (140-180ºC and 15-64 min, respectively, Tables 1 and 3). After the reaction, a sample (1 ml) was taken from the reaction mixture, filtered with a syringe filter and analysed with HPLC-PDA.
The energy input for the conversion reactions was calculated with equation 1:

Energy input (kJ) = [power output of the reactor (J/s) x reaction time (s)] x 1000 (1)

2.3 HPLC analysis

The LA concentration of the samples taken after each reaction was analyzed with high-performance liquid chromatography (HPLC; Waters 2695 Separation module) fitted with a Atlantis dC18 (5µm, 4.6x150 mm) column and a photodiode array (PDA) detector (Waters 996). Water:methanol (90:10) mixture with 0.1% (v/v) of TFA was used as the mobile phase with a flow rate of 1 ml/min. The injection volume was 2 µl. The column temperature was kept constant at 30ºC and the calibration was performed using LA analytical standard (Sigma Aldrich). The UV detection was done at 267 nm for LA.
The yield of LA was calculated with equation 2:

Yield (%) = [concentration of LA in the sample/theoretical maximum concentration of LA in the sample] x 100% (2)

The theoretical maximum yield of LA was calculated from the total carbohydrate content of the starting material, which, according to the supplier, was 80% for the dry PW. No data on the carbohydrate content of *C. armillatus* sporocarps is available but the carbohydrate content of 48.6% DW has been reported for the sporocarps of mixed *Cortinarius* species [14]. However, a high yearly variation occurs in the general sporocarp production, and the composition of the main constituents of sporocarps varies. This can be partially due to varying methodology and conversion factors used, typically overestimating protein and underestimating carbohydrate contents [15]. In a review on 11 macrofungal species in Agaricales studied, maximum carbohydrate content of 75% DW for the sporocarps was measured [15]. In another European study on 6 species of Agaricales [16] maximum carbohydrate content of 71.2% DW was measured. In order not to overestimate the LA yield, the total carbohydrate content of 80% was used for the sporocarbs in this study.

2.4 Experimental design

The full 2^4-factorial design was chosen as the experimental design in order to study the effect of the reaction conditions on the conversion of sporocarbs and PW into LA. The factors (time, temperature, H_2SO_4 and Lewis acid concentration) and their levels used in the experiments are given in table 1. The factorial design consisted of four factors with two levels (high and low), including three centre points. The levels for the factors were chosen based on the literature [17, 18] and some preliminary experiments. They were also selected to be moderate but different enough from each other. Nineteen experiments were carried out including three replications determined at the centre point of the design in order to obtain the estimate for the experiment uncertainty. All experiments were carried out in a random order and LA yield (%) was used as the response. Once the yields were gained the data was fitted using the multiple linear regression method in MODDE 9.1 (Umetrics) computer software. During modelling the data points, which were considered as outliers (set 1, exp. 3, Table 2), were excluded to improve the model. The decision was based on the normal probability plot of residuals. The statistical validation was determined using the ANOVA test at a 95% confidence level.

Table 1. The factors and their levels used in the full 2^4-factorial designs.

Design method with factors	Low	Centre	High
Full 2^4-factorial design			
Reaction time (min)	15	37.5	60
Reaction temperature (ºC)	140	160	180
H_2SO_4 concentration (mol/l)	0.1	0.3	0.5
Lewis acid concentration (mol/l)			
$CrCl_3 \cdot 6H_2O$ $AlCl_3 \cdot 6H_2O$	0.0075	0.0188	0.03

Based on the results from the factorial experiments some additional reactions (8 reactions) were performed (Table 3). In these reactions the reaction temperature was set for 180ºC and the concentration of H_2SO_4 for 0.5 M.

3. Results and discussion

3.1 Full 2^4-factorial designs

In this study dried and ground potato peel waste and *Cortinarius armillatus* sporocarps were used in the production of levulinic acid. The full 2^4-factorial experimental design was first used to study the effect of various factors on the yield of LA, which was used as the response in the design (Table 2).

Table 2. Reaction conditions used in the 2^4-factorial design experiments for the peel waste (PW) and sporocarbs (S), and the yield (%) of LA.

Exp.	t (min)	T (°C)	H_2SO_4 (M)	$CrCl_3$ or $AlCl_3$ (M)	Set 1	Set 2	Set 3
					LA (%, PW, Cr)	LA (%, S, Cr)	LA (%, S, Al)
1	15	140	0.1	0.0075	8	6	4
2	60	140	0.1	0.0075	15	9	7
3	15	180	0.1	0.0075	~~29~~	27	23
4	60	180	0.1	0.0075	40	37	36
5	15	140	0.5	0.0075	30	29	31
6	60	140	0.5	0.0075	45	33	35
7	15	180	0.5	0.0075	49	42	46
8	60	180	0.5	0.0075	45	46	53
9	15	140	0.1	0.03	6	7	7
10	60	140	0.1	0.03	15	10	12
11	15	180	0.1	0.03	41	19	30
12	60	180	0.1	0.03	39	32	36
13	15	140	0.5	0.03	27	24	22
14	60	140	0.5	0.03	41	32	32
15	15	180	0.5	0.03	44	39	43
16	60	180	0.5	0.03	41	39	48
17	37.5	160	0.3	0.0188	45	26	27
18	37.5	160	0.3	0.0188	46	28	28
19	37.5	160	0.3	0.0188	44	26	28

According to the analysed data, time, temperature and the concentration of H_2SO_4 had a statistically significant effect on the LA yield in all sets. This can also be seen from the data presented in table 2 and Supplementary material (Tables S1, S3 and S5). The LA yields generally increase, when time, temperature and H_2SO_4 concentration increase (see also for figures S7-S9). The concentration of Lewis acid catalyst ($CrCl_3$) had a statistically significant negative effect on the LA yield, when sporocarb powder was used as the starting material. On the other hand, no effect was found when $CrCl_3$ and $AlCl_3$ catalyst were used for PW or sporocarb powder in sets 1 and 3, respectively. All sets contained an interaction term between temperature and H_2SO_4 concentration. In addition, there was an interaction term between time and temperature as well as a square term of time in the model of set 1 and an interaction between H_2SO_4 concentration and $AlCl_3$ catalyst in the model of set 3 (see for Supplementary material, Tables S1-S6).

Based on the obtained models from the factorial designs, it was concluded that the reaction temperature and the concentration of H_2SO_4 had the greatest impact on the yield of LA. PW seemed to react faster than sporocarbs since for PW the highest LA yield (49%, exp. 7, Table 2) was achieved in 15 min at 180ºC with H_2SO_4 concentration of 0.5 M and the concentration of the $CrCl_3$ catalyst 0.0075 M. For sporocarbs, the highest LA yield, 46% or 53%, with $CrCl_3$ or $AlCl_3$ catalyst, respectively, was achieved at 180ºC in 60 min with 0.5 M for H_2SO_4 concentration and 0.0075 M for the concentration of the catalyst (exp. 8, Table 2).

3.2 The effect of Lewis acid catalyst and the reaction time on levulinic acid yield

It was found intriguing that according to the factorial experiments the concentration of the Lewis acid catalyst did not have a positive effect on the yield of LA in any of the sets. In studies with glucose the Lewis acid catalysts have been found to improve the conversion of biomass into LA, since they catalyse the glucose to fructose isomerization step, which is necessary for the conversion reaction to occur [19, 1]. The exact route for the $CrCl_3$ catalysed isomerization of glucose to fructose is not known but based on the recent literature a plausible route is presented in Figure 1. [20, 21] Therefore the effect of the Lewis acid was studied in more detail with some additional reactions (Table 3). First, the abovementioned experiments 7 and 8 for PW and sporocarps, respectively, were repeated without the Lewis acid catalyst. For PW the LA yield was 38% and for sporocarps 25% (exps. 20 and 21, Table 3). The decreased yields indicated that the additional catalyst had some effect on the LA yield. Next the effect of the Lewis acid catalyst was studied with reduced amounts, 0.0012-0.0086 M (Table 3), of catalyst compared to the amounts used in the full factorial designs (0.0075-0.03 M, Table 2). The amount of the Lewis acid was reduced since in the full factorial designs higher LA yields were achieved with small amounts of the Lewis acid. Also the reaction time was varied since its effect on the LA yield was not as apparent in the full factorial designs as that of the reaction temperature or H_2SO_4 concentration. The studied reaction times were 40 and 50 min. Seeing that with PW the highest LA yield was reached already in 15 min in the factorial experiments, these additional reactions were only performed with sporocarbs. Also, the reactions were performed only with $CrCl_3$, since the results achieved for sporocarbs with $AlCl_3$ and $CrCl_3$ in the full factorial designs were quite similar. The LA yields as well as the reaction conditions for each additional experiment are presented in table 3.

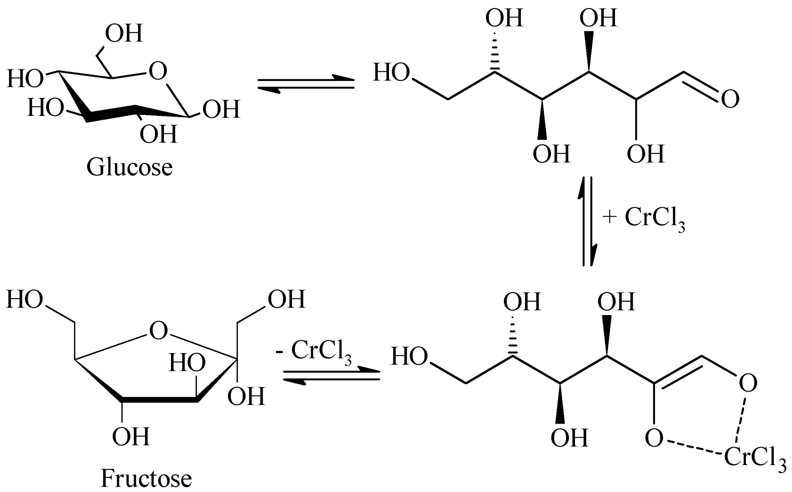

Figure 1. Proposed route for the $CrCl_3$ catalysed isomerization of glucose to fructose. [20, 21]

Table 3. Reaction conditions used in the additional reactions with peel waste and sporopcarbs and the yield (%) of LA. The reaction temperature and the sulfuric acid concentration were in all reaction 180ºC and 0.5 M, respectively.

Exp.	Biomass	t (min)	$CrCl_3$ (M)	LA (%)
20	PW	15	0	38
21	S	60	0	25
22	S	50	0.0012	34
23	S	50	0.0049	37
24	S	50	0.0086	39
25	S	40	0.0023	41
26	S	40	0.0075	62
27	PW	40	0.0075	38

Based on the results the LA yield increased with the increasing amount of $CrCl_3$, when the reaction time was kept at 50 min (exps. 22-24, Table 3). This indicated that the Lewis acid concentration had some effect on the LA yield. In addition, the reaction time had some effect on the LA yield, i.e. with shorter reaction time, 40 min, the LA yield was higher (exps. 25 and 26, Table 3). This indicated that LA started to decompose with prolonged reactions times. The highest LA yield (62%, exp. 26, Table 3) in the study was achieved with the sporocarbs at 180ºC in 40 min with 0.5 M for the concentration of H_2SO_4 and 0.0075 M for the $CrCl_3$ catalyst. Finally, the reaction conditions, which were found optimal for sporocarbs were also used for PW conversion into LA (exp. 27, Table 3). However, with PW, the LA yield was only 38% when reaction time was increased into 40 min. The result verified that for PW the optimal reaction time is shorter than for sporocarbs (15 min, exp. 7, Table 2).

Overall, based on the results PW and *Cortinarius armillatus* sporocarbs, proved to be excellent starting materials for the LA production. Previously reported results for the LA yields include for example Weiqi and Shubin [19] with 54% LA yield from glucose at 170ºC in 4 h, Mukherjee and Dumont [22] with 55% LA yield from corn starch at 180ºC in 15 min, Jeong [18] with 25% LA yield from glucosamine at 188ºC in 49 min and Shen et al. [23] with 39% LA yield from cellulose in 2 h. It should be noted that in the abovementioned studies the starting materials were pure carbohydrates where as in this study biomass was used as received without prior separation of carbohydrates. Thus the LA yields achieved in this study are highly comparable with the yields reported in literature.

3.3 Microwave irradiation as the heating method

In this study the maximum power output of the microwave reactor was set to 90 W in order to inhibit the overheating of the reaction solutions and also to maintain similar heating conditions between individual reactions. However, the full 90 W of power was required only to reach the reaction temperature, which took 4-5 min, depending on the said temperature. Once the temperature was reached the power output of the reactor was quite constant, 40-50 W, corresponding to the reaction temperature of 140-180ºC, respectively. The energy input for each reaction was calculated with equation 1 and was found to vary from ca. 36 to 180 kJ. For reactions, which produced the highest yields in this study from sporocarbs or PW (exp. 27, Table 3 or exp. 7, Table 2, respectively) the energy input was ca. 120 or 45 kJ, respectively. Microwave irradiation is non-contact heating, due to which energy is not lost to the heating of the reaction vessel or e.g. an oil bath. Instead energy transfers directly to reacting compounds, which ensures rapid heating and low power consumption. [13]

4. Conclusions

In this study carbohydrate contents of potato peel waste and *Cortinarius armillatus* sporocarps were efficiently converted into LA with microwave irradiation as the heating method. Reaction conditions were studied by utilizing experimental design. Based on the results the reaction temperature and the H_2SO_4 concentration were found to have the greatest impact on the yield of levulinic acid. The Lewis acid concentration and reaction time had also some effect on the LA yield. The highest LA yield, 62% was achieved with fungal sporocarps as the raw material in 40 min at 180ºC, with H_2SO_4 concentration of 0.5

M and $CrCl_3$ concentration of 0.0075 M. Experiments regarding the use of other novel biomasses such as other fungal species are on-going.

Supplementary data

Supplementary data of this work can be found online.

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Conflict of interest

The authors declare that they have no conflict of interest.

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