

# Valorization of Potato-processing Residues for the Production of Lactic Acid

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Every year large amounts of residues accumulate from potato processing. The aim of this study is to develop a process to valorize these residues as feedstock for lactic acid fermentation in a way that is suitable for industrial production. An enzymatic hydrolysate obtained from potato residues (46 % w/w dry mass starch) was used as a substrate in batch and fed-batch fermentations with *Lactobacillus casei*. Supplementation of yeast extract (3 g L<sup>-1</sup>) was required to improve productivities. Despite the inhibitory effect exerted by phenolic compounds in the medium, reasonably high lactic acid concentrations (102 g L<sup>-1</sup>) and yields (92 %) were obtained, with 92 % (L-isomer) optical purity. It was clearly demonstrated that potato residues are suitable as feedstock for large-scale production of technical grade lactic acid and that the developed process is promising for large-scale biorefinery applications.

*Key words:*

biorefinery, enzymatic hydrolysis, lactic acid fermentation, *Lactobacillus casei*, potato residues

## Introduction

Losses along the food production chain are of great concern to the food industry as well as to the consumers, raising both economic and ethical issues. The worldwide production of potatoes in 2013 was  $3.68 \cdot 10^{11}$  kg<sup>1</sup>. Two-thirds of the entire production is utilized for human nutrition and is consumed as fresh or processed product<sup>2</sup>. About 10–15 % of the total amount of roots and tubers entering industrial food production are lost during processing and packaging<sup>3</sup>. This corresponds to a worldwide loss of  $2.30 \cdot 10^{10}$  kg potatoes from the food processing industry every year<sup>4</sup>.

Losses of potatoes at the processing level occur due to sorting, washing, peeling, size reduction or during process interruptions or accidental spillages<sup>3</sup>, resulting in residual streams with a high organic load. Such residues mainly consist of potato peels and, to a lesser extent, of pulp and slurry. The typical water content of potato peel residues is 77–85 % w/w<sup>5,6</sup> and the composition on a dry mass (DM) basis is as follows: 47–52 % w/w starch, 8–16 % w/w protein, ca. 7 % w/w ash, up to 1 % w/w soluble sugars, 0.5–2.6 % w/w fat, while the rest is fibers<sup>6,7</sup>.

An overview on current practices and potential applications of potato residues is available in the lit-

erature<sup>5,8</sup>. It is possible to use these materials as animal feed, however, the nutritive value is limited, and they undergo rapid microbial spoilage. Thus, in many cases, the residues are treated as waste. Because of the high water content, incineration is not appropriate, and therefore, biological treatment processes, such as composting and anaerobic digestion, are more suitable.

An alternative use for this kind of residue is as feedstock for industrial fermentation processes. The valorization of potato residues as starch-rich feedstock for the production of lactic acid (LA) within an integrated biorefinery represents an appealing option. The market for LA is constantly growing and will exceed  $10^9$  kg year<sup>-1</sup> by 2020<sup>9</sup>. Therefore, the effective production capacity must be extended rapidly. Currently, this appears to be challenging regarding the availability and costs of raw materials<sup>9</sup>. Ninety-five percent of the LA on the market is produced by microbial fermentation from cane or beet sugar or hydrolyzed cornstarch<sup>10</sup>. However, social and political concerns promote the development of sustainable production processes that do not interfere with the food supply chain<sup>11</sup>.

The major advantage of potato residues as an alternative substrate for LA production is the high starch content. In addition, potatoes also contain proteins, vitamins and minerals<sup>12,13</sup> that can supply at least part of the nutrients required for the microbial fermentation. For applications requiring high purity LA (e.g. polymer-grade), residues are not a

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suitable resource. Therefore, the LA obtained from potato residues should rather be used for technical applications, like textile dyeing and finishing, leather softening, descaling and cleaning in the oil industry<sup>14</sup>. Furthermore, there is a demand for the derived lactate esters, which can be utilized as solvents and cleaning agents in the paint and ink industry or in microelectronics. The market volume of lactate esters in 2013 was  $6.82 \cdot 10^8$  kg<sup>15</sup>. Food and cosmetic applications are also possible, provided that a chiral purity of 95–97 % L-LA can be guaranteed.

Research studies on the use of potato residues as feedstock for LA fermentation have been published recently<sup>16–21</sup>. They address the production of LA with different microbial strains and at different working conditions. In particular, Liang *et al.*<sup>16–17</sup> describe the production of LA with a mixed undefined microbial inoculum in batch and sequencing batch culture. The study conducted by Bilanovic *et al.*<sup>18</sup> evaluates the benefits of using potato residues as an alternative carbon source for LA production with *Lactobacillus* spp.. In another work, up to 10 g L<sup>-1</sup> LA were produced from sweet potato residues with *Lactobacillus* spp. after enzymatic hydrolysis<sup>19</sup>. Finally, direct fermentation of unhydrolysed potato residues with *Rhizopus oryzae* in immobilized form<sup>20</sup> and *Geobacillus stearothermophilus*<sup>21</sup> has been described. However, the LA concentrations reached in the abovementioned studies are limited to 10–60 g L<sup>-1</sup>, which is not comparable with conventional production processes. The approach for this study is to develop a process that reaches titers higher than 100 g L<sup>-1</sup> and yields higher than 90 %, as crucial requirements for process economy<sup>14</sup>.

The aim of this study was the valorization of potato residues as feedstock for the fermentative production of LA with *Lactobacillus casei*. The characterization of potato residues, the set-up of a suitable process to use them as a substrate to produce technical grade LA in accordance with the current industrial and economic requirements, and finally, the evaluation of the effectiveness and/or potential limitations of such a process were investigated.

## Materials and methods

### Potato residues: Physical pretreatment and characterization

Potato residues were obtained from two potato-processing facilities in Navarra (Spain) that together produce  $2.00 \cdot 10^6$  kg year<sup>-1</sup> of solid residues – a viscous mash of puree and peels. In order to preserve the product from degradation during transportation and long-term storage, the material was

dried in an industrial drum dryer to decrease the water content from  $86.7 \pm 1.4$  % to  $13.0 \pm 1.4$  %. The particle size was 4–15 mm in diameter.

### Enzymatic hydrolysis of starch

The enzymatic hydrolysis of starch in the potato residues was performed in a 5-L stainless steel vessel (id 190 mm), equipped with an overhead stirrer (model RW 20, IKA®-Werke GmbH & Co KG, Germany) and a Teflon lid to avoid evaporation losses. Two Rushton impellers were mounted on the stirrer. A hotplate and a temperature sensor were installed for temperature regulation. For liquefaction, boiling water was added to the dry biomass in order to have a dry solid content in the mash of 20–26 % w/w. Liquefaction was run at 70 °C and pH 6. The pH-value was adjusted from an initial value of  $4.0 \pm 0.1$  (due to the presence of LA in the residues) to  $6.0 \pm 0.2$  by the addition of 10 M NaOH. An amount of 2 g kg<sup>-1</sup> dry solids of  $\alpha$ -amylase BAN 240 L (Novozymes, Denmark;  $\geq 250$  units g<sup>-1</sup>) was added to the slurry together with CaCl<sub>2</sub> (0.5 g L<sup>-1</sup>) to generate maltodextrins of differing chain lengths and oligosaccharides. After 2-h reaction time, the pH-value was adjusted to  $4.3 \pm 0.2$  with 10 M HCl. This was done to provide optimal pH conditions for saccharification and, at the same time, to enhance the deactivation of BAN 240 L.  $\alpha$ -Amylase was deactivated at  $88 \pm 3$  °C for 15 min. Once the temperature in the mash reached 60 °C, saccharification was initiated by the addition of 1.2 mL kg<sup>-1</sup> dry starch of glucoamylase AMG 300 L (Novozymes, Denmark;  $\geq 300$  units mL<sup>-1</sup>) to generate glucose units from dextrins and oligosaccharides. After 20 h, the mash was collected, weighed, and centrifuged (30 min, 20 °C, 16000 g; centrifuge: J2-MI, rotor JA-10, Beckman, USA). The glucose-rich liquid fraction was used as a substrate for LA fermentations – after appropriate dilution with water – and was freshly prepared for each experiment.

### Chemicals

MRS broth (de Man, Rogosa, Sharpe) and corn steep liquor (CSL) were purchased from Sigma-Aldrich (Austria). Yeast extract (YE) type CMRT was obtained from Ohly GmbH (Germany), and glucose (Meritose) from Tereos Syral (France).

### Microorganism

The bacterial strain used in this study was initially obtained from a dairy. The species was confirmed as *Lactobacillus casei* by sequencing of 16S rDNA performed by LGC Genomics (Teddington, UK) and by BLAST similarity search analysis. We selected this strain because of its good LA production abilities and its specificity for L-LA. *L. ca-*

*sei* cultures grown in MRS broth for 14–16 h were preserved in 1-mL aliquots (10 % v/v glycerol) at  $-80^{\circ}\text{C}$ .

### Analytical methods

The total starch in the dry biomass and the amylose fraction were determined with enzymatic assay kits (K-TSTA 07/11 and K-AMYL 06/15, respectively, Megazyme, Ireland). Total Kjeldahl nitrogen (TKN) and  $\text{NH}_4\text{-N}$  in the potato residues were determined with an AutoKjeldahl Unit K-370 (Buechi, Switzerland). The crude protein content was estimated from the TKN, using a conversion factor of 6.25. Low MW water-soluble organic

Table 1a – Dry mass composition of potato residues (water content:  $86.7 \pm 0.3$  g per 100 g)

	(g/100 g) DM
Starch	$45.77 \pm 3.48$
of which amylose	$22.99 \pm 1.29$
Protein	$9.36 \pm 0.18$
$\text{NH}_4\text{-N}$	$0.25 \pm 0.03$
Lignin	$5.15 \pm 0.71$
Ash	$5.70 \pm 0.88$
Low MW water-soluble organic compounds	
Lactic acid	$5.01 \pm 1.60$
Ethanol	$0.69 \pm 0.01$
Succinic acid	$0.32 \pm 0.04$
Maltose	$0.26 \pm 0.05$
Fructose	$0.08 \pm 0.01$
Acetic acid	$0.07 \pm 0.02$
Glucose	$0.03 \pm 0.02$

Table 1b – Mineral composition of the potato residues as determined with ICP-OES

Minerals	(mg/100 g) DM
K	$2368.28 \pm 89.09$
P	$298.81 \pm 0.09$
Ca	$141.68 \pm 44.60$
Mg	$104.21 \pm 7.95$
Na	$62.99 \pm 5.37$
Fe	$13.76 \pm 1.28$
Al	$9.04 \pm 1.09$
Zn	$3.44 \pm 0.27$
Cu	$0.82 \pm 0.06$
Mn	$0.80 \pm 0.08$

compounds were determined by incubating 1 g of finely milled dry potato residues in 50 mL of bi-distilled water for 30 min at  $60^{\circ}\text{C}$ , and analyzed via HPLC. Inductively Coupled Plasma optical emission spectrometry (ICP-OES) analyses were performed in an ULTIMA 2 device (HORIBA Jobin Yvon GmbH, Germany) to determine the concentration of trace elements in the potato residues (Table 1b). Phenolic compounds were determined with the Folin-Ciocalteu phenol reagent<sup>22</sup>. Determination of acid insoluble lignin was performed via gravimetric analysis as described in Sluiter *et al.*<sup>23</sup>

Sugars and organic acids were determined via HPLC with a refractive index detector (Agilent Series 1100). An ion exclusion column ION 300 (Transgenomic) was used at  $45^{\circ}\text{C}$  with 0.005 M  $\text{H}_2\text{SO}_4$  at a flow rate of  $0.325\text{ mL min}^{-1}$  as mobile phase. The L- and D- optical isomers of LA were quantified by using an enzymatic assay kit (K-DLATE 06/08, Megazyme, Ireland).

All analyses were performed in duplicate.

### Fermentations in shake flasks

Preliminary lab-scale fermentations were run in 100-mL shake flasks (Schott Duran, Germany) with a working volume of 50 mL. The liquid potato residue hydrolysate obtained after centrifugation was used as a source of glucose ( $70\text{ g L}^{-1}$ ) after dilution with water. A multilevel factorial design ( $2^3$ ) with two factors (CSL, YE) defined on three levels (concentrations) was developed (Table 2). The aim was to study the effect of CSL and YE as N-rich supplements on LA production. In addition, two controls with  $70\text{ g L}^{-1}$  refined glucose instead of the hydrolysate were included: one in MRS medium, the other in YE  $6\text{ g L}^{-1}$  – CSL 5 % v/v. All treatments were run in triplicate; results are expressed as mean values and relative standard deviations of the LA production after 48 h (response). Differences between the treatments were evaluated with analysis of variance (ANOVA) and multiple range tests at the 95 % confidence level.

Each group of replicates was inoculated with 5 mL of a separately prepared 14-h-old preculture of *Lactobacillus casei* IFA 32 ( $3.4 \pm 0.3 \cdot 10^9$  cells per mL, OD  $2.6 \pm 0.3$ ) grown in MRS broth. As a buffering agent, 3.3 g of  $\text{CaCO}_3$  were added to the medium to neutralize the LA formed throughout the fermentation and keep the pH-value between 5 and 6. Cultures were incubated for 144 h at  $37^{\circ}\text{C}$  and 90 rpm in an Infors HT Multitron rotary shaker.

### Fermentations in bioreactors

Batch and fed-batch fermentations were run in parallel in DASGIP 1-L bioreactors (DASGIP Information and Process Technology GmbH, Germa-

Table 2 – Production of LA at 48 h ( $n=3$ ) in shake flasks from the potato residue hydrolysate, at different CSL-YE concentrations. Initial LA concentrations in the medium were subtracted. Treatments with different indicators (a–e) have significantly different means ( $p > 0.05$ ). In all treatments: final  $63.02 \pm 2.68 \text{ g L}^{-1}$  LA reached within 144 h of fermentation ( $70 \text{ g L}^{-1}$  initial glucose).

Run	Factors		Response
	CSL (% v/v)	YE ( $\text{g L}^{-1}$ )	LA at 48 h ( $\text{g L}^{-1}$ )
1	0.0	0.0	$27.87^a \pm 0.55$
2	0.0	3.0	$43.17^c \pm 1.63$
3	0.0	6.0	$44.50^c \pm 1.12$
4	2.5	0.0	$38.98^b \pm 0.80$
5	2.5	3.0	$45.16^c \pm 1.38$
6	2.5	6.0	$44.44^c \pm 1.92$
7	5.0	0.0	$39.22^b \pm 0.80$
8	5.0	3.0	$43.07^c \pm 0.99$
9	5.0	6.0	$43.56^c \pm 0.83$
10	Control 1*		$61.35^e \pm 1.86$
11	Control 2**		$54.20^d \pm 0.38$

\*Control 1: MRS medium, refined glucose.

\*\*Control 2: CSL 5.0 % v/v – YE  $6.0 \text{ g L}^{-1}$ , refined glucose.

ny). Agitation (100 rpm) was provided by two six-bladed Rushton turbine impellers (od 46 mm). The initial glucose concentration was  $136.9 \pm 0.8 \text{ g L}^{-1}$  for batch fermentations (tot 540 mL). In the fed-batch mode instead, this value was limited to  $78.0 \pm 1.6 \text{ g L}^{-1}$  by dilution with water (tot 200 mL); the rest of the sugar was provided in two subsequent additions of hydrolysate (120 + 220 mL) as soon as the glucose concentration in the culture dropped to  $20 \text{ g L}^{-1}$ , to bring it again to  $80 \text{ g L}^{-1}$ . In the end, working volumes and total glucose were comparable in the two set-ups (Table 3).

Additional batch fermentations with a higher working volume (3 L) were carried out in a Biostat ED 5-L fermenter (Braun Biotech International GmbH, Germany). A flat-six-bladed-disc-turbine impeller (od 70 mm) was mounted on the stirrer shaft and the agitation speed was set to 150 rpm.

In all cases, the liquid potato residue hydrolysate – used as a source of glucose – and YE were sterilized separately at  $121 \text{ }^\circ\text{C}$  for 20 min before be-

ing mixed in the reactor. A 10 % v/v 16 h-old pre-culture was used as the inoculum. Throughout the process, temperature was regulated to  $37 \text{ }^\circ\text{C}$  and pH-value to 5.5 with the automatic addition of 10 M NaOH. Dissolved oxygen (DO) in the culture was monitored but not regulated during the process. DO levels dropped to zero during the exponential growth phase of *L. casei*, which was sufficient to ensure anaerobic conditions in the culture. All experiments were performed in duplicate; results are expressed as mean values and relative standard deviations.

## Results and discussion

### Characterization of the potato residues

The composition of the potato residues is reported in Table 1a-b. Values are expressed on a DM base and are relative to two batches, one collected in 2012, and the other in 2013. The data presented here are in good agreement with those available in the literature on potato-peel residues<sup>6,7</sup>.

On a fresh mass basis (100 g), potato residues before drying consisted of  $86.7 \pm 0.3 \text{ g}$  water,  $6.1 \pm 0.5 \text{ g}$  starch,  $1.3 \pm 0.0 \text{ g}$  proteins, and  $0.7 \pm 0.2 \text{ g}$  LA. An amount of  $62.3 \pm 1.1 \%$  of LA was present as D-LA, and the pH-value was  $4.0 \pm 0.1$ . In comparison to the residues, fresh potatoes consist of  $62.7\text{--}87.0 \text{ g}$  water,  $9.1\text{--}22.6 \text{ g}$  starch, and  $0.9\text{--}4.2 \text{ g}$  proteins per  $100 \text{ g}^{12}$ .

The composition of potato residues may vary from one batch to another, depending on factors like potato variety, processing, and storage conditions (duration, temperature, humidity, etc.) before drying and/or use. The relatively high water content is an important factor affecting transportation costs, as well as the microbiological stability of the residues over time<sup>5</sup>. Due to the activity of indigenous microbial species, considerable amounts of LA can already be formed during storage (Table 1a).

Because they are collected after processing, potato residues contain less starch than the fresh crop. Nevertheless, the starch content is still considerable, which – together with the amounts of proteins, ammonium and minerals – makes potato residues an excellent substrate for fermentations.

Table 3 – Comparison of lactic acid fermentations on the potato hydrolysate as a C-source with  $3 \text{ g L}^{-1}$  YE

Reactor	Mode	Culture volume (mL)	LA yield (%)	Productivity ( $\text{g L}^{-1} \text{ h}^{-1}$ )	Optical purity (% L-LA)	Reference
DASGIP	batch	540	$75.15 \pm 0.17$	$0.56 \pm 0.01$	$90.12 \pm 0.01$	Figure 2
DASGIP	fed-batch	540*	$67.16 \pm 0.34$	$0.53 \pm 0.01$	$89.35 \pm 0.19$	Figure 3
Biostat	batch	3000	$92.21 \pm 0.15$	$1.44 \pm 0.00^{**}$	$92.37 \pm 0.13$	Figure 4

\*Final volume

\*\*Productivity at 62 h.

## Enzymatic hydrolysis of the potato residues

Potato residues were treated enzymatically to obtain a glucose-rich hydrolysate from starch (Figure 1). This approach was preferred to acidic treatments in order to limit the formation of undesired inhibitory by-products like hydroxymethylfurfural<sup>24</sup>.

Unlike refined starch used in traditional industrial processes, the starch fraction in the potato residues constitutes scarcely 50 % of the DM. The rest of the solids, however, contributes to the viscosity of the slurry. Still, it is necessary to operate at a high solids load to reach high glucose concentrations in the hydrolysate. At the conditions described earlier, the highest possible solids load was 26 % w/w, resulting in a maximum final glucose concentration of 200 g L<sup>-1</sup>.

It was decided to insert a centrifugation step before fermentation in order to avoid complications due to the solids in the bioreactor. After centrifugation, 51 % w/w of the total mash was recovered as a liquid fraction. The discarded solids (32 % w/w DM) also contained a considerable amount of glucose, and therefore, the total recovery of sugar in the liquid was limited to 63.5 ± 1.7 %. In an integrated biorefinery approach, the solid fraction may still be used for biogas production, composting or as animal feed, especially if mixed with the microbial biomass as a protein source<sup>25</sup> and dried.

## Fermentations in shake flasks

The performance of the potato residue hydrolysate as a substrate for LA fermentation was evaluat-

ed in multilevel factorial design tests in shake flasks. The aim was to determine how well the liquid hydrolysate works as a sole C- and N-source for *L. casei*, and if additional nutrients were necessary, e.g., to increase the productivity. LA-producing microbial strains are usually supplemented with YE as an effective (but expensive) source of amino acids and essential vitamins<sup>13</sup>. YE and CSL were selected as N-rich nutrients<sup>26</sup> to be tested. The results are presented in Table 2.

The LA concentration after 48 h was chosen as the response, since differences in LA production among the different treatments were most evident at this stage (Table 2). Five homogeneous groups with significantly different means ( $p > 0.05$ ) were identified. The highest LA concentrations at 48 h were obtained with controls 1 (MRS) and 2 (CSL 5 % v/v – YE 6 g L<sup>-1</sup>), where refined glucose was used as a C-source instead of the hydrolysate. These results suggest that the hydrolysate may contain substances that inhibit the growth of LA bacteria.

It could be demonstrated that the potato residue hydrolysate can serve as a substrate for LA fermentation without further supplements. However, the conversion is significantly faster ( $p > 0.05$ ) if supplements like CSL and YE are added. In particular, YE showed a stronger positive effect than CSL: Glucose (70 g L<sup>-1</sup>) was completely consumed and converted to LA in 48–72 h, except for the treatments without YE (1, 4 and 7), where the fermentation process lasted longer. The results show that a concentration of 3 g L<sup>-1</sup> of YE in the hydrolysate is sufficient to boost the production of LA by *L. casei*, and higher amounts of YE or the addition of CSL will not improve the process. Based on these find-

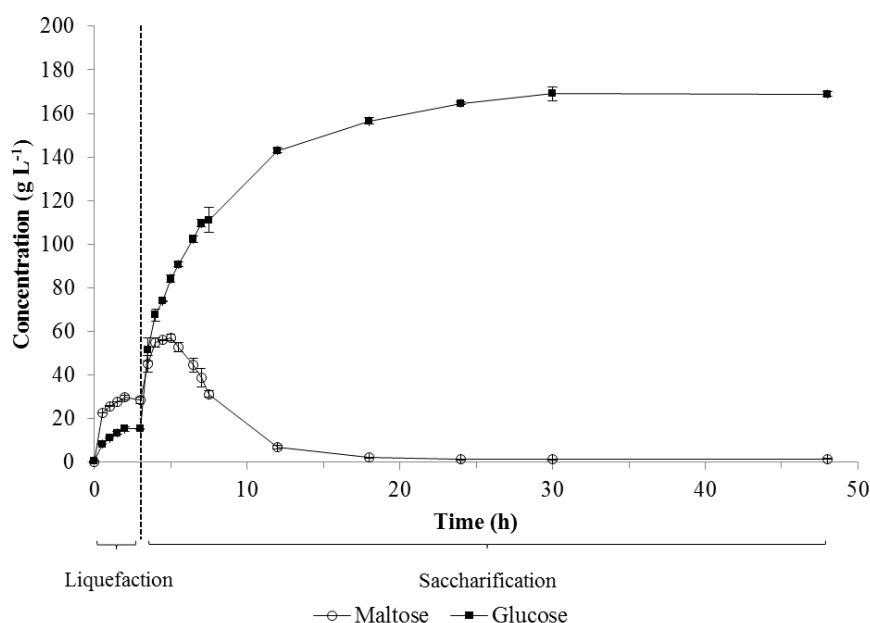


Fig. 1 – Time-course profile of the enzymatic hydrolysis of potato residues (20 % w/w solids)

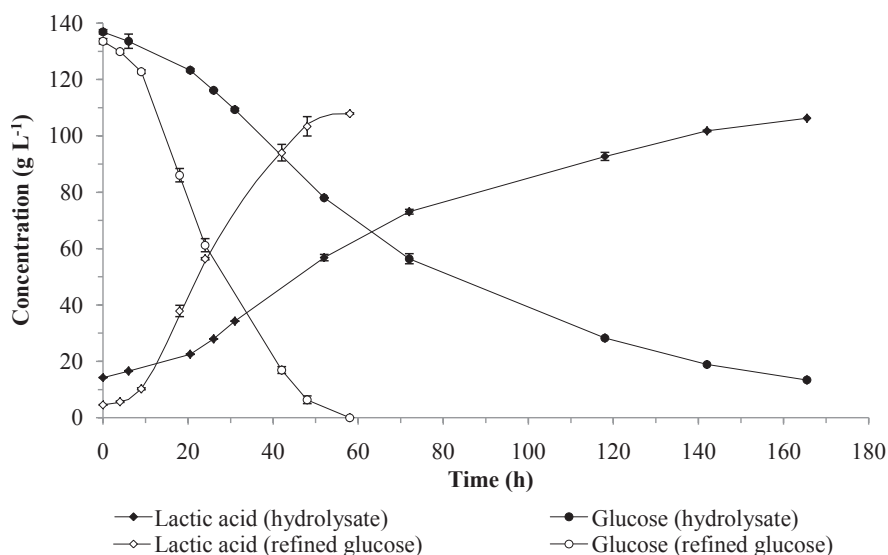


Fig. 2 – Time-course profile of LA batch fermentation (37 °C, pH 5.5) on:  
 – Potato residue hydrolysate as a C-source and 3 g L<sup>-1</sup> YE (black bullets)  
 – CSL-YE medium and refined glucose as a C-source (white bullets)

ings, it was decided to supplement 3 g L<sup>-1</sup> of YE to the liquid potato residue hydrolysate for further experiments in lab-scale bioreactors.

#### Batch and fed-batch lactic acid fermentation of potato residue hydrolysate

For the production of LA in bioreactors, a batch and a fed-batch configuration were compared. The aim was to develop a process that can reach high LA concentrations (more than 100 g L<sup>-1</sup>) and productivities. In the batch process, an initial glucose concentration of 140 g L<sup>-1</sup> was set, and 106 g L<sup>-1</sup> of LA were obtained after 166 h (Figure 2).

The fed-batch process started at an initial glucose concentration of 80 g L<sup>-1</sup> in order to avoid substrate inhibition<sup>27</sup>. Consequently, the production of LA started faster and with a higher productivity compared to the batch case. However, the productivity dropped notably after each addition of the hydrolysate: In the end, the overall productivity of the fed-batch process was reduced, and a lower concentration of LA (97 g L<sup>-1</sup>) was reached after 166 h, when the process was stopped (Figure 3).

In both configurations, there were residual unconverted sugars in the broth after almost one week of fermentation. This suggests that elevated hydrolysate concentrations and an increasing LA titer exert an inhibiting effect on the strain and delay further LA production. Previous experiments (data not shown) demonstrated that *L. casei* IFA 32 is able to tolerate glucose concentrations higher than 250 g L<sup>-1</sup> and produce LA up to 150 g L<sup>-1</sup> in MRS medium. Therefore, the sugar and LA titers alone do not explain the observed delay. Moreover, when

*L. casei* was tested with refined glucose as a C-source in 5 % v/v CSL-6 g L<sup>-1</sup> YE, the sugar was completely converted within 58 h (Figure 2). It is known that potatoes – and peels in particular – contain phenolic compounds like chlorogenic and caffeic acid<sup>12,28</sup>. The content of phenolic compounds in the fermentation broth was 4.40 ± 0.10 g L<sup>-1</sup> of vanillin equivalent. Studies on the effect of phenolic compounds on the growth of *L. plantarum* show that caffeic acid, at a concentration higher than 1 g L<sup>-1</sup>, inhibits growth and reduces bacterial counts<sup>29</sup>. It can be expected that the inhibitory effect exerted by phenolic compounds will also affect other cell functions, e.g. LA production. Hence, it was decided to reduce the hydrolysate concentration in the fermentation medium, thus lowering the glucose concentration and the phenolic content.

#### Batch lactic acid fermentations at a reduced hydrolysate concentration

It was demonstrated that diluting the hydrolysate from 140 g L<sup>-1</sup> glucose to 100 g L<sup>-1</sup> shortens the total duration of the batch process to 62 h (Figure 4). This can mainly be attributed to the dilution of the phenolic compounds (and other potential inhibitors). The chosen hydrolysate concentration still allows fulfilling the industrial production requirements (titer, yields). A final product concentration of 101.7 ± 0.3 g L<sup>-1</sup> was obtained, with yields (92 %, Figure 4, Table 3) comparable to those obtained on refined glucose as a C-source (91 %, Figure 2). However, a lower total productivity was observed (1.44 g L<sup>-1</sup> h<sup>-1</sup> instead of 1.78 g L<sup>-1</sup> h<sup>-1</sup>), which most likely results from the relatively high initial LA concentration and the presence of growth inhibitors

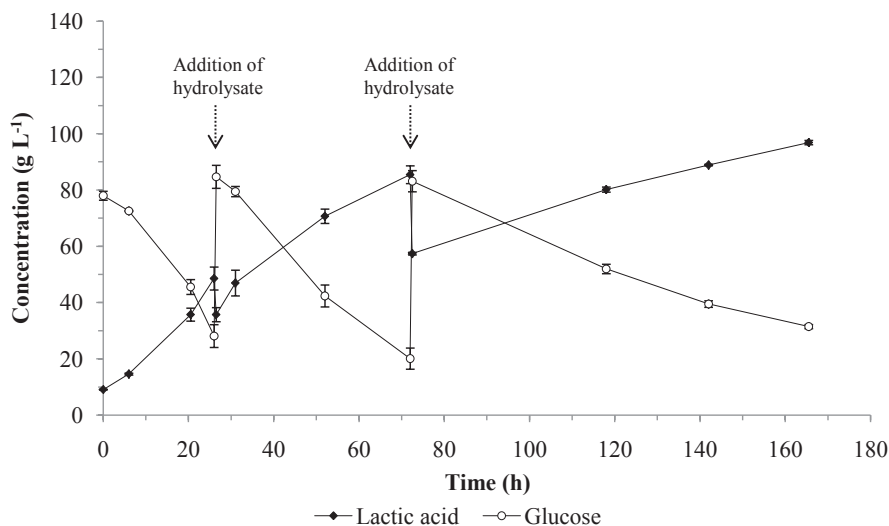


Fig. 3 – Time-course profile of LA fed-batch fermentation (37 °C, pH 5.5) on the potato residue hydrolysate as a C-source, with 3 g L<sup>-1</sup> YE

(e.g. phenolic compounds) in the potato residue hydrolysate. A practicable strategy to limit the inhibitory effect exerted by phenolic compounds is to treat the hydrolysate with laccase formulations for detoxification. For instance, oxidative coupling and oligomerization/polymerization of polyphenols catalyzed by laccases is already exploited in wine production for stabilization<sup>30</sup>. Another way to achieve higher productivities is to run simultaneous saccharification and fermentation, with the advantage of limiting substrate inhibition effects due to glucose accumulation<sup>31</sup>. This implies, however, that fermentations have to be carried out in the presence of solids, and at temperature and pH conditions suitable for both the LA-producing microorganism and the

amylolytic enzymes. The abovementioned strategies can be implemented to obtain even higher product concentrations and productivities, thus increasing the suitability of the process for industrial applications.

Due to the initial amount of LA already present in the potato residue hydrolysate ( $12.3 \pm 0.1$  g L<sup>-1</sup> with 53 % D-LA), the optical purity of the LA in the fermentation broth was 92 % L-LA. This is still practical for many non-polymer applications, while an optical purity of minimum 99 % is required for PLA production<sup>32–33</sup>, which makes refined substrates more convenient for the fermentation of polymer grade LA. Hence, it is expected that LA produced from potato residues will be suitable for technical applications after purification. According to the results obtained in this study, it is possible to produce 54 kg of technical grade LA (90 % w/w) from 10<sup>3</sup> kg of fresh potato residues.

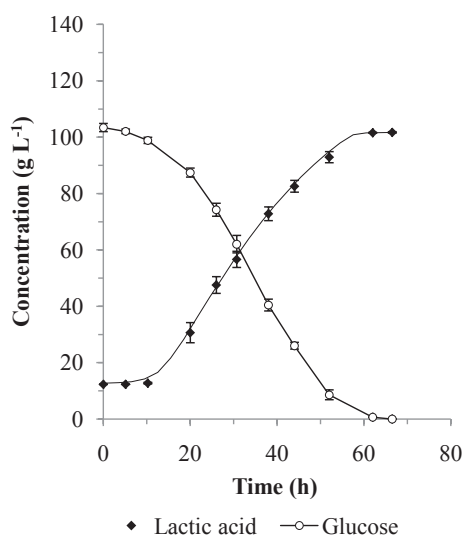


Fig. 4 – Time-course profile of LA fermentation on the potato residue hydrolysate as a C-source with 3 g L<sup>-1</sup> YE. In comparison to Figure 2, the medium has been diluted to limit the initial glucose concentration to 103 g L<sup>-1</sup>.

## Conclusion

Residues from potato processing are generated in large amounts, and are a suitable substrate for large-scale lactic acid production. They provide starch as a C-source and additional nutrients for microbial growth. Supplementing the enzymatic hydrolysate with yeast extract (3 g L<sup>-1</sup>) significantly increases LA productivities. Inhibitory effects of phenolic compounds on *Lactobacillus casei* were mitigated successfully by limiting glucose concentrations to 100 g L<sup>-1</sup>. The developed process meets the industrial requirements of reasonably high LA concentrations (101.7 g L<sup>-1</sup>) and yields (92 %). The lactic acid produced (92 % L-isomer) is of suitable quality for numerous technical applications.

## ACKNOWLEDGMENTS

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## Abbreviations

LA	– lactic acid
MRS	– de Man, Rogosa, Sharpe
CSL	– corn steep liquor
YE	– yeast extract
ICP-OES	– inductively coupled plasma optical emission spectrometry
DO	– dissolved oxygen
DM	– dry mass
id	– inside diameter
od	– outside diameter
tot	– total

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