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Fermentation as a Strategy for Bio-Transforming Waste into Resources: Lactic Acid Production from Agri-Food Residues

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Abstract: Lactic acid (LA) obtained by fermentation of carbohydrates is well-known and widely used in the food sector. This process is as an alternative to the chemical synthesis and ensures several advantages especially in terms of environmental sustainability. In particularly, the opportunity to use agro-food residues as fermentable raw materials could improve the overall process sustainability, without considering the indisputable advantages in terms of waste reduction and residual biomass valorization, in a bio- and circular economy perspective. This research deals with the study and development of the fermentation processes of various waste biomasses from the agro-food industries, including milk whey (MW), ricotta cheese whey (RCW), pear processing residues (PPR), potato pomace (PP), tomato pomace (PT), in order to obtain an experimental protocol applicable to the production of LA. *Lactobacillus casei* DSM 20011 (ATCC 393), a homofermentative L(+)-LA producing bacterium has been used, starting from small-scale tests to verify of the microorganism to grow in complex medium with different carbon sources and the possible presence of potentially toxic substances for microbial growth. Yields from $27.0 \pm 0.3\%$ to $46.0 \pm 0.7\%$ have been obtained. Then, a scaling-up was performed in a 1 L batch fermenter, using a mixed medium of RCW and PPR in different ratio. The best LA yield was 78.3% with a volumetric productivity of 1.12 g/L·h in less than 60 h.

Keywords: lactic acid; PLA; *Lactobacillus casei*; agri-food waste; circular economy

1. Introduction

Agri-food residues refer to organic waste that results from the processing and packaging of animal and/or plant products by the agri-food industry [1]. The Food and Agriculture Organization (FAO) estimates that about one-third of food worldwide produced for human nutrition is yearly lost or wasted [2]. Considering the entire supply chain, including agricultural production, for the EU-27 food waste can be quantified in about 129 million tonnes per year, with about 25% accounted for the sole postharvest and manufacturing stages [3]. Such a large quantity poses the question of high costs of management for collection and transportation, as well as the need of adequate treatment facilities. In fact, the Directive (EU) 2018/850 requires to reduce to 10% waste disposal by landfilling and prohibits landfilling for waste that can be recovered in line with the waste hierarchy [4]. In particular, the separate collection of bio-waste and the enhancement of its valorization rates are still a key issue in EU [5]. This ensure that economically valuable waste materials will be reinjected into the EU economy and prevent detrimental consequences for human health and the environment [6].

However, more studies are needed to foster the switch to more sustainable agri-food system and to promote the adoption of circular economy concepts [7]. As it is well known, from a circular economy perspective, bio-waste can become a secondary resource to be valorized in the construction of a new generation of bio-based building blocks [8]. In this

regard, a key-molecule is 2-hydroxypropanoic acid—better known as lactic acid (LA). Despite its late discovery at the end of the 1700s, this molecule occurs in almost every living organism and it plays an essential role in the anaerobic energy metabolism of billions of life forms [9]. In recent decades, LA has received increasing attention, eventually being identified as one of the highest potential *platform chemicals*, a short group molecules that are envisioned to bridge nature's abundant polysaccharide feedstock to the production of added-value chemicals and intermediate building blocks [10]. LA has been already used in the food industry as an emulsifying, acidulant, buffering, and preserving agent [11]. Due to its antibacterial and ink-erasure properties, it has also been used in the textile, detergent, and paper industries [12] and over the past few years it has received raising attention as monomer for biodegradable plastics such as polylactic acid (PLA) [13] or as a precursor of acrylic acid (AA) [14]. Currently, LA is starting to win also new market niches related to high value added products, in the pharmaceutical and cosmetic sectors [15].

Lactic acid can be synthetized by chemical synthesis and microbial fermentation. The first results in the production of racemic mixture of LA and involves the production of intermediates that derive from petrochemical sources, leading to environmental negative consequences [16]. On the other hand, over 90% of the current commercial production of LA is performed by bacteria through anaerobic fermentation of sugars [17]. Today, glucose and sucrose are the main starting resources. Other possible feedstocks are easily hydrolysable poly- or disaccharides derived from corn syrups, molasses, beet extracts, whey, and all kinds of starches [10].

In this context, the use of agriculture and agri-food waste represent an interesting alternative to replace the refined and costly feedstock and the bulk use of such materials will help to solve many environmental hazard [18]. In addition to milk whey and molasses, which are traditionally used in LA fermentation [19], great attention has been focused on cassava bagasse [20], sugar cane bagasse [21], sugar beet pulp [22], coffee husk and pulp [23], apple pomace [24], oil-cakes [25], and wheat/rice bran [26].

There are different processes for biotechnological production of LA. Having viscous and heterogeneous waste as substrate, batch fermentation is the most common approach to produce LA, because it permit the complete substrate depletion, but also continuous flow processes has been developed in order to try overcoming the typical disadvantages of discontinuous methods, such as long lag phase with low productivities and high capital costs, acidification of the medium, and substrate inhibition [27].

LA can be produced by several microorganisms, including wild-type and engineered LA-producing bacteria or genetically-modified yeasts. These organisms can be divided into four main groups, namely, lactic acid bacteria (LAB), *Bacillus* strains, *Escherichia coli*, and *Corynebacterium glutamicum* [28]. LAB are the most common bacteria for the production of LA at industrial scale. Most LAB, including Lactobacilli, are considered to be safe for industrial lactic acid production because they have had a long history of industrial-scale production without adverse health effects on either consumers or production workers. Commercially important LAB strains, such as *Lactobacillus* strains, i.e., *L. delbrueckii spp. bulgaricus, L. casei and L. helveticus*, have been particularly useful due to their ability to convert numerous mono- (both hexose and pentose) and di- saccharides, and high tolerance to broad interval of temperature (ranging from 20 to 55 °C) and pH (they can survive at pH 5 and lower) [29]. Depending on the metabolic pathways, they can produce L- or D-lactic acid (by homo-, hetero-, or mixed-acid fermentation) [30].

The potential *L. casei* and *L. farciminis* for LA fermentation of in microaerophilic conditions and mild sterility using pear pomace and ricotta cheese whey (RCW) as single substrate has been evaluated in a previous work [31].

In the present work, the production of optically pure L-LA by a strain of homofermentative L. casei from agri-food waste, without any other nutrients integration and without medium sterilization, has been studied. Organic waste, including milk whey, ricotta cheese whey, pear processing residues, potato pomace, tomato pomace, have been chosen based on the local availability [32], and tested as single and mixed substrates. Microaerophilic batch

fermentations have been carried out at small-test level (working volume, 100 mL) in order to verify the absence of toxic compounds in waste, and then scaled-up in a 1 L fermenter (working volume, 700 mL) where the fermentation conditions were preliminary optimized. Agri-food waste comprises a complex mixture of residues that can have different composition depending on regional and climatic factors. Additionally, agri-food waste is an ideal substrate for proliferation of LAB, and natural growth of LAB can occur within a wide range of conditions. Nevertheless, this endemic production of LA could become, in some cases, detrimental because of the indigenous production of a racemic mixtures, whereas in some application as the production of PLA, a very high optical purity is demanded. Moreover, horticultural and fruit waste are usually available for short period of the year, corresponding to the months of their harvesting and/or processing. In particular, they are accessible from the late spring to the early fall, differently from wheys that are constantly supplied during all the year. In particular, potato is available from June to July, tomato from late July to early September and pear from September to October.

This research lays the ground for the opportunity of exploit agri-food residues through fermentation to LA, hypothesizing a continuous process where the waste would be progressively added as carbon source, depending on the seasonal availability reducing as much as possible the cost of the process.

2. Materials and Methods

2.1. Microorganism and Culture Conditions

Lactobacillus casei DSM 20011 (ATCC 393), a homofermentative L(+) lactic acid producing bacterium, belongs to the collection of microorganisms of the Life Sciences and Biotechnology Department of the University of Ferrara and was purchased from the DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH) company.

The master cell bank is maintained at -20 °C in cryovials in a standard De Man, Rogosa and Sharpe (MRS) medium (1 mL) mixed with glycerol (0.5 mL) as crioprotective agent. The standard MRS medium (Fluka Analytical, Bucks, switzerland) contained glucose 20 g/L, bacteriological peptone 10 g/L, meat extract 8 g/L, yeast extract 4 g/L, $CH_3COONa \cdot 3H_2O$ 5 g/L, K_2HPO_4 2 g/L, ammonium citrate tribasic 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, $MnSO_4 \cdot 4H_2O$ 0.05 g/L [33]. The working cell bank has been stored at 4 °C in MRS-agar slants for six months and used for seed cultures. The optimal growth conditions were set up in a SKI 4 incubator (ARGO LAB, Modena, Italy) for 24 h, at pH 7.0, 30 °C under gentle stirring (130 rpm) in Erlenmeyer flasks under microaerophilic conditions maintained by the presence of a head-space of the flasks.

2.2. Food Waste

Five batches of waste were collected from local agri-food industries: pear processing residues (PPR) (a mixture of unripe, overripe or damaged pears not suitable for market), tomato pomace (TP) (peels and seeds), potato pomace (PP) (damaged or broken tubers and peels), riches in carbohydrates [34], milk whey (MW), and ricotta cheese whey (RCW), which are the main by-products in cheese production processes, rich in proteins [35].

Waste were manually homogenized, divided into 250 g aliquots and stored at -20 °C until further usage or analysis. Before the fermentation, they were submitted to different pre-treatments, depending on their physical characteristics, as shown in Table 1.

Table 1. Pretreatments on waste biomass before fermentation.

Biomass	Pretreatments
Pear processing residues (PPR)	Grinding
Tomato pomace (TP)	Grinding and hydrolysis
Potato pomace (PP)	Grinding and hydrolysis
Milk whey (MW)	None
Ricotta cheese whey (RCW)	None

Pear-processing residues, potato and tomato pomace were grinded using a professional laboratory blender (KLARSTEIN, Berlin, Germany). Potato and tomato pomace were then submitted to hydrolysis treatment. PT and PP cannot be processed by fermentation without a preliminary hydrolysis pretreatment to obtain fermentable monomers (glucose and galacturonic acid, respectively). Hydrolysis was performed by treating 100 g of the waste with 250 mL of 1M HCl at a temperature of about 80 °C for 1 h in a 500 mL flask equipped with a condenser. All biomasses were sterilized in an autoclave before being fermented, and the pH adjusted to 7 with 0.15M NaOH.

2.3. Inoculum of Lactobacillus Casei

A loopful of *L. casei* was inoculated in 50 mL Erlenmeyer flasks containing 10 mL of sterile MRS medium and placed in an incubator at 130 rpm, 30 °C for 24 h. In the case of small-scale tests (100 mL), the suspension obtained is entirely used as an inoculum, while for the tests in the fermenter (700 mL), the suspension is used as a pre-inoculum for 70 mL of fresh MRS medium, which, grown under the same conditions, it is used as an inoculum.

2.4. Small-Scale Tests (Working Volume, 100 mL)

Fermentations were performed using 100 mL of waste in 250 mL flasks and inoculated with 10 mL of inoculum, prepared as previously described. The flasks were then placed in an incubator at 30 °C, pH 7 and 130 rpm. Samples were withdrawal (1 mL) under sterile conditions at the beginning of the fermentation (T_0) and at the end of the process, after 72 h (T_{72}). Each sample was centrifuged and the supernatant analyzed to quantify carbohydrates depletion and lactic acid production, comparing with blank samples. Blank samples derived from biomasses without inoculum and submitted to the same fermentation conditions. Fermentations have been carried out with single biomasses and in mixture, as reported in Table 2.

Table 2. Substrates used for fermentation, as single biomasses or mixtures of waste.

Substrates for Fermentation Process	Waste				
	MW (%)	RCW (%)	PPR (%)	PP (%)	TP (%)
MW	100				
RCW		100			
PPR pretreated			100		
PP pretreated				100	
TP pretreated					100
PP pretreated + TP pretreated				50	50
MW + PPR pretreated	50		50		
MW + PPR pretreated	30		70		
MW + PPR pretreated	70		30		
RCW + PPR pretreated		50	50		
RCW + PPR pretreated		30	70		
RCW + PPR pretreated		70	30		
RCW + PP pretreated		50		50	
RCW + PP pretreated + TP pretreated		60		20	20

2.5. 1 L-Scale Batch Fermentation (Working Volume, 700 mL)

Batch fermentations were carried out in a thermoregulated autoclavable Minifors^{TM} bioreactor (INFORS, Basel, Switzerland) of 700 mL of working volume (1 L of overall capacity), equipped with probes for pH, temperature and O_2 concentration monitoring (METTLER TOLEDO, Columbus, OH, USA). pH value was automatically maintained at 7.0 by adding 1N NaOH solution. The inoculum in MRS medium (70 mL, 10% *v/v*) was incubated at 130 rpm for 24 h on a shaking thermostatic incubator before the addition to the fermenter. Temperature and pH of inoculum were initially set up at 30 °C or 37 °C and pH 7.0. Before inoculation, the fermenter was filled with the pretreated waste biomasses.

The culture was maintained at 50 rpm by a mechanical stirrer. An airflow of 0.5 L/min was fluxed on the head space of the fermenter, as needed to maintain a slight overpressure. Before each fermentation, the fermenter was sanitized under steam stripping conditions (100 °C for 30 min). Batch processes were followed for a maximum of 144 h, samples collected after the first 6 h and then every 24 h. All experiments and analyses were repeated in triplicate and averaged.

2.6. Analytical Methods

Moisture has been determined using the thermogravimetric method [36], protein using the Kieldhal method [37] and ash content by means of the gravimetric assays [38]. Total sugars content has been determined by means of the phenol—sulfuric acid method [39], that is the most reliable method among all the quantitative assays for total carbohydrates estimation. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow-brown-colored product with phenol and has absorption maximum at 490 nm VIS spectroscopy.

Sugars (glucose, fructose, galactose, lactose) and sorbitol were analyzed by HPLC (Jasco, Easton, MD, USA) equipped with a refractive index detector (Jasco, Oklahoma City, OK, USA) and UV detector (Jasco, Oklahoma City, OK, USA) and Rezex ROA-Organic Acid H+ (8%), 300 × 7.8 mm (Phenomenex, Torrance, CA, USA).

Isocratic elution was carried out at 60 °C with 0.6 mL/min of 0.01 M H_2SO_4. Before the HPLC analysis, samples were centrifuged (6720 RCF; 10 min), placed at 80 °C for 10 min to eliminate possible interferences due to microbial enzymes and filtered using cellulose acetate filters (porosity 0.2 μm).

Yield of LA per gram of sugars (Y_{LA}) was calculated by dividing the LA concentration by the total fermentable carbohydrates concentrations present in the starting material. Yield of LA per gram of food waste (Y_{FW}) was calculated by dividing LA present in the reactor by dry matter of the respective waste. Since LA formation is growth-associated, productivity of batch cultures was calculated with LA titer after 8 h.

3. Results

3.1. Small-Scale Fermentations of L. casei (Working Volume, 100 mL)

The chemical composition of the residues is reported in Table 3. Biomasses characterization is referred to their effective composition just before being fermented, in order to consider the effect of possible material losses during pretreatments and organic molecules degradation (especially sugars and N-based molecules) during autoclaving caused by the well-known Maillard reaction.

Table 3. Chemical composition of the residues and waste used in this study.

	PPR	RCW	MW	TP	PP
Moisture (% *w/v*)	11.4 ± 0.7	93.9 ± 0.8	94.0 ± 1.2	15.1 ± 1.3	71.1 ± 0.9
Total carbohydrates (TC)(g/L)	80.3 ± 2.3	85.3 ± 2.2	51.4 ± 1.0	33.3 ± 0.5	68.7 ± 1.3
Protein (N 6.25) (g/L)	3.1	6.1	8.0	19.3	8.0
Ash (g/L)	9.0 ± 0.7	1.2 ± 0.0	7.0 ± 0.6	3.9 ± 0.2	6.3 ± 0.4

Any organic acids, including LA, have been measured at detectable level in samples. Total carbohydrates (TC) comprise different type of sugars, which typically characterized the agri-food residues.

The homo fermentative L(+)-enantiomer producing bacterium *L. casei* DSM 20011 (ATCC 393) has been used for LA production. In general, homo fermentative organisms have the conversion efficiency of 1 mol glucose to 2 mol LA [40].

In order to evaluate the capacity of *L. casei* to grow and produce LA on the agri-food used in this study, preliminary tests have been carried out on single substrate. Then, small-scale fermentations have been set up, mixing different residues. In particular, high-moisture waste as MW and RCW have been added to the others pretreated wastes. In

the case of PPR only grinding and homogenization were necessary, because it contains readily fermentable sugars, as fructose. In this study, HCl-hydrolysis has been carried out, even though in the case of industrial application the use of enzymes has been strongly recommended [41]. Table 4 shows the total sugar concentrations present in the different matrices, the respective concentration of lactic acid obtained after 72 h of fermentation *with L. casei* and the consequent percentage yield factor Y_{LA}.

Table 4. Small-scale lactic acid fermentations (100 mL).

Waste	Ratio	Total Fermentable Carbohydrates (g/L)	Lactic Acid (g/L)	Y_{LA} (%)
MW	100	51.4 ± 1.0	15.3 ± 0.9	30.4 ± 1.7%
RCW	100	85.3 ± 2.2	34.0 ± 0.3	40 ± 2.2%
PPR pretreated	100	80.3 ± 2.3	29.6 ± 2.3	36.8 ± 2.5%
TP pretreated	100	33.3 ± 0.5	8.91 ± 0.2	27.0 ± 0.3%
PP pretreated	100	68.7 ± 1.3	20.2 ± 1.5	30.6 ± 2.1%
MW + PPR pretreated	50:50	65.8 ± 3.3	28.6 ± 1.5	44.0 ± 1.3%
MW + PPR pretreated	30:70	71.7 ± 2.7	25.6 ± 0.6	36 ± 3.2%
MW + PPR pretreated	70:30	59.7 ± 1.4	24.8 ± 2.2	41.5 ± 1.6%
RCW + PPR pretreated	50:50	82.4 ± 1.4	29.9 ± 0.6	35.4 ± 0.8%
RCW + PPR pretreated	30:70	81.5 ± 2.3	25.4 ± 1.9	31,2 ± 1.4%
RCW + PPR pretreated	70:30	84.3 ± 1.6	38.8 ± 0.4	46.0 ± 0.7%
RCW + PP	50:50	59.4 ± 1.7	24.6 ± 0.6	41.4 ± 1.8%
RCW + PP + TP	60:20:20	71.6 ± 0.5	21.5 ± 1.3	29.8 ± 0.7%

As it can be seen, the yields were generally low, due to the fact that in small-scale tests pH cannot be regulated and spontaneously tends to reach acidic values jeopardizing the biomass growth. In fact, the aim of these tests principally was to exclude the presence of toxic substances that could have been inhibited microbial growth.

Small-scale preliminary trials have shown that the potential as a substrate for lactic fermentation is promising. The different mixing percentages of MW/RCW and PPR also had the purpose of evaluating the "workability" of the final fermentation medium. As the percentage of MW/RCW increased, the medium appeared less dense and the fibrous parts of the plant matrix more diluted. With the same yield obtained, the mixture containing RCW and pear (70:30) was considered the best result for the following experiments. Mixtures between RCW and PP, and RCW, PP, and PT have shown that even the addition of these matrices generates a medium that may be suitable for the production of lactic acid.

3.2. 1 L-Scale Batch Fermentation

Based on the results obtained from the small-scale tests, a scaling-up to 1 L has been carried out, in order to control the operating conditions such as pH and oxygen concentration that could not be monitored in small-scale tests and improve the yields. In these tests, the consumption of the various carbon sources has been monitored, together with the production of LA at different time scales. As mentioned above, the most promising waste mixtures was RCW + PPR at a ratio of 70:30. As a comparison, an in order to understand the separate contribution of the two matrices, a fermentation with the sole RCW has been carried out.

3.2.1. 1 L-Scale Batch Fermentations with RCW at Different Temperatures

In the literature, examples of lactic acid production using continuous [28] and fed-batch [42] fermentations are reported but the batch system is still the simplest system that guarantees high fermentation yields. For improving the yield in LA, batch fermentations have been set up at two different temperatures, 30 °C and 37 °C, respectively. Based on HPLC results on RCW, about 96% of the total carbohydrates in RCW is represented by lactose (82.4 ± 1.4 g/L), 3.5% by galactose (3.0 ± 0.3 g/L) and about 0.5% of glucose (0.4 ± 0.0 g/L).

In Figure 1 the results deriving from the fermentation of RCW at 30 °C and 37 °C are reported.
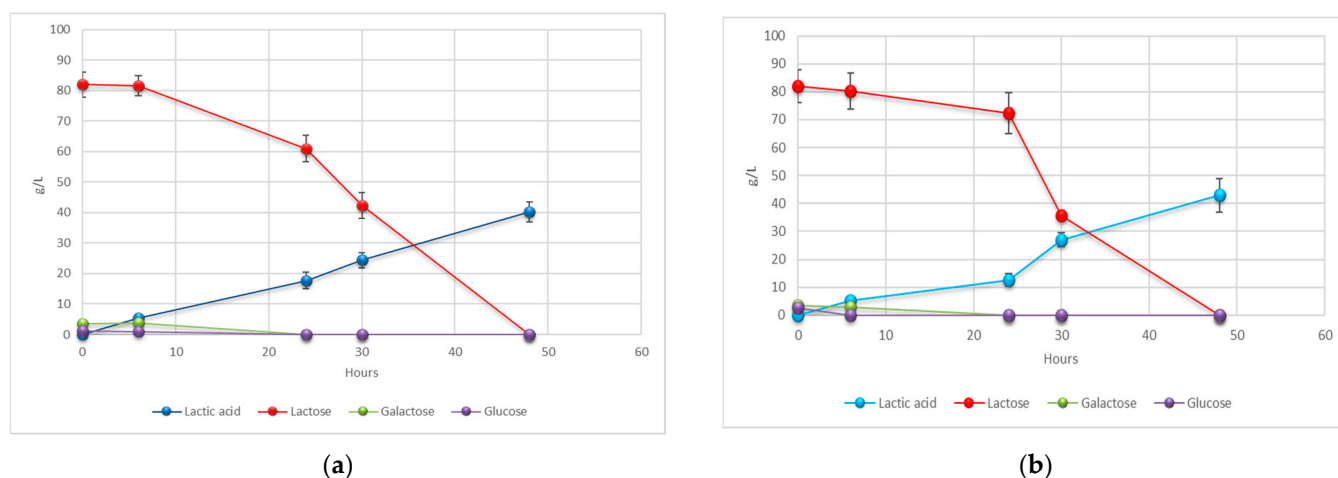
Figure 1. Trend in the concentration of sugars and LA in fermentations with RCW: (**a**) 30 °C; (**b**) 37 °C.

The fermentations were carried out in both cases for 48 h until the complete consumption of the available sugars. Regarding LA production, 40.2 g/L were obtained in fermentations carried out at 30 °C with a yield Y_{LA} = 47.3% on total sugars and a volumetric productivity Q_p = 0.98 g/L·h. In the case of fermentations carried out at 37 °C, an amount of LA equal to 43 g/L was obtained, corresponding to a fermentation yield Y_{LA} = 50.6% on total sugars and a volumetric productivity Q_p = 1.05 g/L·h.

Both graphs show the depletion of lactose, galactose and glucose over 48 h as the concentration of LA increases. As expected, LA is production is mostly attributable to the consumption of lactose, but *L. casei* uses also glucose and galactose as carbon source [43]. The effect of temperature seems to be the occurring of a prolonged lag phase at 30 °C. At 37 °C, lactose concentration does not decrease significantly until the complete depletion of glucose and galactose. The higher yield of LA at 37 °C is coherent with results previously obtained [44] at the same pH value and with an inoculum age of 24 h, as in our study. Nevertheless, considering the very slight difference in both yield and productivity between the two temperatures tested, we decided to use 30 °C as reference temperature because of the advantages in terms of process costs to maintain higher temperature. In fact, 30 °C can be considered as room temperature in summer in Italy and any equipment has to be provided to heat the fermenter.

3.2.2. 1 L-Scale Batch Fermentations with RCW + PPT

These fermentations were carried out in order to scale up the most promising matrix in the production of lactic acid from the small-scale trials. The RCW + PPR pretreated (ratio 70:30) has shown a fermentation yield of about 46% in small-scale tests.

Scaling up to the fermenter scale, different kind of pretreatments have been investigated, in order to reduce as much as possible, the process costs, as mentioned above. In particular, a comparison between grinded PPT and grinded and the supernatant of grinded and centrifuged PPR has been attempt. The supernatant would contain soluble sugars and nutrients, and by removing the fibrous part, the culture medium appears less dense and viscous. The results of this test may also be important for the purpose of recovering LA from the fermentation broth and for the setup of the downstream protocol. Considering the fact that centrifugation reduces the mass by approximately 50%, the amount of PPR has been appropriately increased to 90:10, in order to maintain the same total sugars content of the two batches. Table 5 shows the sugar concentrations of the respective starting mixtures with RCW + PPR in both cases. It is worthwhile noting that, even if the total sugars concentrations are almost identically in the two cases, the sugars speciation is different, due

to the more percentage of RCW in the 90:10 ratio fermentation, which enriches the medium of lactose. Fructose and galactose have been reported as a sum because in the HPLC elution conditions this two isomers cannot be differentiated, having the same retention time. Sorbitol, as well as fructose, derived from PPR, being both among the principal components of soluble organic matter of pears.

Table 5. Sugar composition of the mixtures of RCW + PPR (70:30) and RCW + centrifuged PPR (90:10).

	Total Sugars (g/L)	Lactose (g/L)	Fructose/Galactose (g/L)	Glucose (g/L)	Sorbitol (g/L)
RCW + PPR (70:30)	84.0 ± 4.2	57.0 ± 2.5	12.2 ± 2.1	6.2 ± 0.3	8.0 ± 1.2
RCW + centrifuged PPR (90:10)	84.5 ± 5.9	74.0 ± 3.5	4.7 ± 0.6	4.6 ± 1.4	3.9 ± 0.2

In Figure 2 is shown the trends of batch fermentations carried out with RCW + PPR (70:30) and RCW + centrifuged PPR (90:10), respectively.
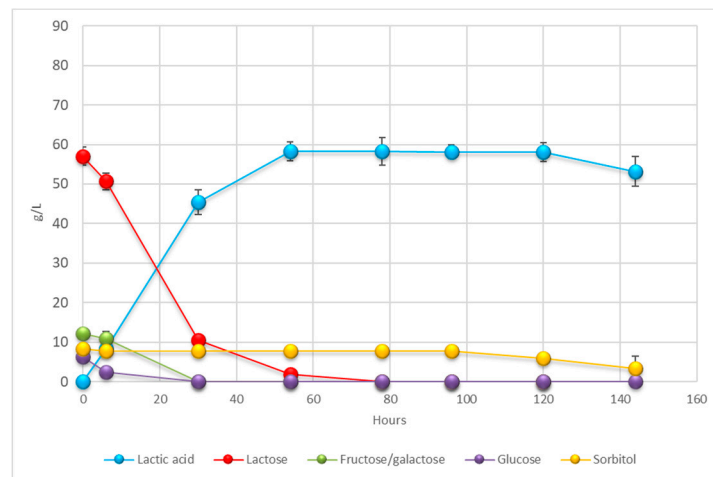
(**a**)
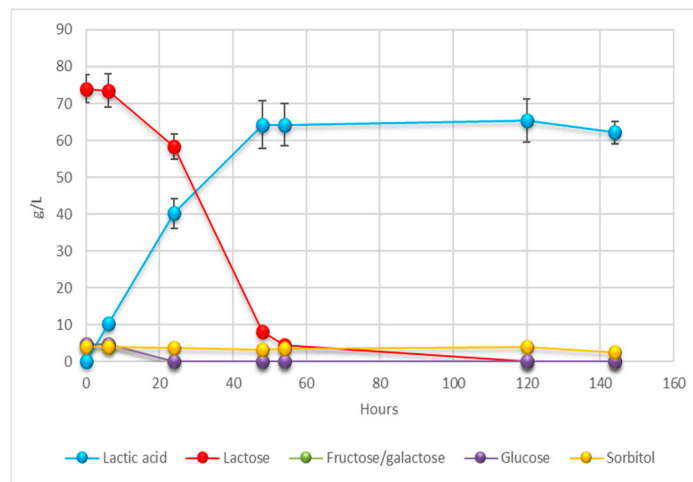
(**b**) [1]

Figure 2. Trends in the concentration of sugars and LA in fermentations with RCW + PPR (70:30) (**a**) and RCW + centrifuged PPR (90:10) (**b**), respectively. [1] the trend of fructose and galactose is covered by that of glucose.

In the RCW + PPR (70:30) batch fermentation, *L. casei* quickly begins to metabolize the available sugars, with a very short lag phase and in less than 60 h all sugars have been completely depleted, with a production of 59.2 g/L of LA. Otherwise, in the RCW + PPR

(90:10) batch fermentation the trend in sugars depletion is almost identical, except for a shot lag phase in the first 8 h. In the first 24 h, fructose, galactose, and glucose were completely metabolized and lactose significantly decreased. In the RCW + PPR (90:10) batch fermentation at 58 h there is the maximum production of 65.4 g/L of LA.

In both fermentations, sorbitol has maintained its concentration constant at the initial values during all the processes, with a slight decrease at the very end of the process, toghether with a decrease in LA concentration, probably due to the fact that, in absence of other carbon sources and with the depletion of other nutrients, *L. casei* has started to consume the residual carbon sources present. The ability of *L. casei* to utilize D-sorbitol as carbon source has been already reported [45]. After about 60 h of fermentation, the yields were Y_{LA} 77.2% and Y_{LA} 78.3% for RCW + PPR (70:30) and RCW + PPR (90:10), respectively.

As a result, the removal of the fibrous part of PPR does not seem to provide significant differences in the production yield. The greatest amount as absolute value of LA (65.4 g/L) derives from the greatest amount of fermentable sugars in the starting mixture (84.5 g/L). Moreover, being similar the process duration until the complete sugars depletion, the volumetric productivity is similar (Q_p = 1.05 g/L·h and Q_p = 1.12 g/L·h, respectively).

As a second important results, it seems that the addition of a centrifugation step in the upstream does not have significant effects on the fermentation. Taking into account the industrial costs deriving from the insertion of a centrifugation step upstream of fermentation, it is not believed that this entails an advantage in terms of production.

4. Discussion

In this study, the capacity to be fermented by various waste biomass has been studied starting from small-scale tests, in order to evaluate the possible presence of toxic substances for *L.casei* during LA production, the general conditions of growth and the potential yield of LA production. Based on the analyses carried out and the results obtained, it could be evidenced the promising potential of the some agri-food waste, used as single or mixed sources. As reported in literature, for PPR, the prompt fermentable fructose represents more than 75% of the TC [32], whereas TP is constituted for about 22% by pectin (as galacturonic acid) [38] and PP of about 75% by starch [5]. In RCW and MW TC fraction is made up by the sole lactose [39]. The approach that involves the use of mixed waste biomass essentially derives from the need to set up an operational plan that overcomes the problem of the seasonal nature of waste and makes it possible to set up a production system that works throughout the year. In fact, RCW and MW do not derive from food-chain directly connected to agricultural production and are always available, unlike fruit and vegetable waste which have rather short periods of quantitative peaks, are then no longer available in all other months of the year and it is inconceivable to refrigerate and storage them for a long time.

Small-scale trials have shown that the LA production is promising, and that, as already highlighted, the agri-food waste considered in this study are suitable for fermentation by *L. casei* to obtain LA. In fact, they do not contain toxic substances for the growth and development of the microorganism, they contain a sufficient quantity of other nutrients (organic and inorganic nitrogen, mineral salts) to support microbial growth without adding any other nutrients, and do not require particular pre-treatments before fermentation.

Moreover, the yields in LA obtained in mixed fermentations were averagely similar or higher than yields of the single-substrates, confirming that *L. casei* is able to grow on different carbon sources. This is particularly important in view of the future perspective of a continuous fermentation processes where different residues and waste could be progressively integrated from time to time depending of the seasonal availability to a "base ground" of MW and RCW, making it possible to setup a production system that works throughout the year or at least during the entire agricultural season.

Preliminary tests carried out on 1 L scale fermenter have been realized with RCW rather than MW because at local level RCW is the very final waste of the dairy production supply chain, whereas MW could be currently used for ricotta cheese production and

it is not always available on market. Then, PPT was the first agri-food waste because it has presented the best performance at small scale, but it is only the first step in order to optimize the continuous process, as above mentioned.

Preliminary tests carried on batch fermentations (1 L-scale) have permitted to almost doubled the LA yield, controlling the fermentation parameters as pH, temperature and level of oxygen. The have been carried out on RCW in order to understand the fermentation potential of the base-medium. The purpose of the test was to verify whether the increase in temperature could have an effect on the speed of sugar consumption and on the production of lactic acid, decreasing fermentation times. Based on the results obtained, we concluded that there are no substantial differences either in terms of product yield or in process speed, between 30 °C and 37 °C. On the contrary, as the temperature increases, the management costs of the process would increase, having to use thermal energy to heat the fermenter. The increase in temperature was therefore not interesting for the purposes of the project.

PPR was used as a model biomass, together with RCW, testing the fermentation of the biomass as it is, only homogenized, and of the biomass previously centrifuged to remove the fibrous part. From the results obtained it emerged that the removal of the fibrous part of the pear was not decisive, as it did not give significant differences in the production yield if compared to the test in which the fibrous component is present. Taking into account the industrial costs deriving from the insertion of a centrifugation step upstream of fermentation, it is not believed that this entails an advantage in terms of production.

Overall, the LA yield obtained in fermenter, even if doubled with respect to small-scale tests, are still rather lower in comparison to the theoretical yield, but comparable to those obtained in other studies where LA is produced by different kind of waste biomass, as a yield of 52,4% reported by Ge et al. [46] from Jerusalem artichoke residues, or 81.4% from tamarind kernel [47] and 70% fermenting hydrolyzed corn stover [48]. In all those cases, and in the present research, the key aspect should not be the yield optimization but the possibility to valorize a waste and recovery a value-added product at the lowest costs as possible.

5. Conclusions

In conclusion, all the tests carried out have shown that the most satisfactory fermentations are certainly those carried out with the RCW matrix, individually and in the form of a mixture with pear pulp waste. RCW as such is a waste from the agro-industrial dairy industry, it is available all year round and having no supply problems linked to seasonality, it could be used as a fermentation base to be added to other agro-industrial waste present only in certain seasons. Furthermore, RCW does not currently have a defined position in terms of reuse, as whey does in the feed sector. In the future, its potential use as a fermentation substrate would allow a real enhancement as a by-product. It could then regain a second life and be reintroduced to the market for the production of LA. Further investigations will be necessary, integrating in the fermenter the other biomass and testing the best fermentation strategy which optimize the overall fermentation performance in terms of yield and productivity without burdening on process costs.

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