Conversion of Potato Industry Waste into Fodder Yeast Biomass

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In this study, we evaluate potato pulp waste as a potential raw material for obtaining yeast biomass. A portion of the carbohydrates in the potato pulp waste can thereby be converted into more valuable protein. The potato pulp waste was analyzed in terms of protein and ash content, dry mass, simple sugars, and starch content. Two kinds of hydrolysis were performed (thermo-acidic and enzymatic) to produce media for cultivating Candida guilliermondii and Pichia stipitis. The hydrolysates and post-cultivation leachates were analyzed by High Performance Liquid Chromatography (HPLC). The highest biomass yield after 48 h (39.3%) was noted for Candida guilliermondii yeast grown on enzymatic hydrolysate-based medium. Our results prove that potato waste pulp is a promising raw material for the production of yeast single-cell protein (SCP).

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Abstract: In this study, we evaluate potato pulp waste as a potential raw material for obtaining yeast biomass. A portion of the carbohydrates in the potato pulp waste can thereby be converted into more valuable protein. The potato pulp waste was analyzed in terms of protein and ash content, dry mass, simple sugars, and starch content. Two kinds of hydrolysis were performed (thermo-acidic and enzymatic) to produce media for cultivating *Candida guilliermondii* and *Pichia stipitis*. The hydrolysates and post-cultivation leachates were analyzed by High Performance Liquid Chromatography (HPLC). The highest biomass yield after 48 h (39.3%) was noted for *Candida guilliermondii* yeast grown on enzymatic hydrolysate-based medium. Our results prove that potato waste pulp is a promising raw material for the production of yeast single-cell protein (SCP).

Keywords: fodder yeast; SCP; potato waste pulp; *guillermondii*; *stipitis*

1. Introduction

In the context of rapid population growth and insufficient food supplies, there is interest in developing alternative protein sources which could help improve the quality of life in developing countries. There are almost ten times the number of food-producing farm animals as there are people in the world. Around 70 billion animals are killed each year for food [1]. The amount of meat consumed is increasing each year and, with it, the demand for animal protein [2,3]. In order to supply that demand and to lower the cost of meat production, industrial waste can be converted into animal feed. Animal fodder may be derived from plants (e.g., straw, grains, potato, or beet pulp) or animal-based (milled by-products, fish, and fish-processing residues). It may also be synthesized, such as amino acids or microbial single-cell protein (SCP). Fodder for animals should be easily digestible, with high nutritional value in terms of protein content, fiber, fat, sugars, starch, mineral compounds, vitamins, and energy. On the other hand, there are economic considerations connected with the costs of production, energy consumption, and storage [4]. A food source of the future should provide complex nutrition from the minimum of resources, in terms of land, time, and production costs. Single-cell protein offers a promising solution. Numerous species of microorganisms can be used to produce SCP, including many species of yeasts, bacteria, and algae. The common feature of these microorganisms is their short generation time, which leads to a rapid increase in mass. These microorganisms also show high potential for genetic modification, which means their growth can be optimized. The process of

producing yeast-derived SCP is based on culturing selected strains on inexpensive raw materials, the vast majority of which originate from food-processing industries [5]. The yeast cells are then washed, inactivated, disrupted, and sometimes processed further to obtain digestible and valuable protein [6,7].

The chemical composition of yeast depends on the medium on which it was cultured. The protein content can range from 35% to over 60% of dry matter (DM). The biomass also contains some fats (0.5–8%), carbohydrates (18–43%), and minerals (4–10% of DM). Yeast cells contain significant amounts of lysine but are low in sulfur amino acids. Not only is yeast-derived fodder rich in B vitamins, but micro- and macro-elements can also be also found, including P, Na, Cu, Mn, Zn, and Fe. Yeasts are used as food for pigs and poultry, as well as less often for cattle. Their energy values when consumed by pigs and poultry are around 12 MJ EM/kg and 11 MJ EM/kg, respectively [8]. Of the hundreds of yeast strains available for animal feed preparation, *Candida guilliermondii* and *Pichia stiptis* are among the best known. *Scheffersomyces (Pichia) stipitis* is recognized for its ability to ferment xylose to ethanol, L-lactic acid, and other products from hemicellulose. As *P. stipitis* naturally ferments xylose, its genes were targeted to create a recombinant strain of *S. cerevisiae*, which is able to utilize xylose as a substrate in ethanol production [9]. *Pichia stipitis* cells usually have spherical or oval shapes and occur in very short chains or as single cells. Under aerobic conditions, these cells are able to form well-branched pseudohyphae. Apart from xylose, they can also ferment glucose, maltose, and trehalose [10–12]. Found in nature all over the world, the yeast strain *Candida guilliermondii* has been isolated from insects, flowers, fruits, and some food products. *Candida guilliermondii* is considered an opportunistic pathogen in humans and animals. *Candida guilliermondii* cells are oval or elongated and can occur in pairs or chains consisting of a few cells. This strain is capable of fermenting glucose, sucrose, raffinose, and trehalose. It is known for its ability to produce vitamin B2 (riboflavin) and a sweetener called xylitol [12].

One of the by-products of the starch industry is potato pulp, which is produced as a residue when starch is washed from potatoes using cold water. Potato pulp contains starch, cellulose, hemicelluloses, pectin, and a small amount of protein. [4,13] Although this waste can be used for many applications, including as a base for ethanol, phenolic acid, or α-amylase production, potato pulp is mostly used as a feed for livestock animals (especially for cattle). Such utilization of potato pulp as animal feed is by no means an agricultural novelty. Potato pulp is reported to have been used as a livestock feed since the 1940s. Potato pulp waste is also more widely used in composite feed than other ingredients, such as barley. However, due to its low content of protein and vitamins, it is considered to be of poor nutritional value for livestock animals. In this study, we evaluate potato pulp waste as a potential raw material for obtaining yeast biomass. A portion of the carbohydrates in the potato pulp waste, which is inexpensive and available in bulk, can thereby be converted into more valuable protein. Using potato pulp waste to produce yeast SCP may be a cost-efficient way to improve its nutritional features.

2. Materials and Methods

2.1. Microorganisms and Materials

Pure yeast cultures of *P. stipitis* LOCK 0049 (PS) and *C. guilliermondii* ATCC 6260 (CG) from LOCK 105 (Culture Collection of the Institute of Fermentation Technology and Microbiology), Lodz, Poland, were used in the experiments. Potato waste pulp was supplied by Przedsiębiorstwo Przemysłu Ziemniaczanego in Trzemesznie, Poland, and stored in a freezer at −20 °C. Three enzyme preparations were used. Termamyl S.C. (Novozymes) is an enzyme preparation containing alpha-amylase (1,4-alpha-D-glucan glucanohydrolase (EC 3.2.1.1)). SAN Extra L (Novozymes) is a preparation containing glucoamylase and acid-alpha-amylase. The main component of SAN Extra is an amyloglucosidase that hydrolyses 1,4- as well as 1,6-alpha-linkages in starch and dextrins. SAN Extra also contains acid alpha-amylase (AFA), which hydrolyses the 1,4-alpha-glucosidic linkages in amylose and amylopectin. Cellic CTec2 (Novozymes) is a blend of cellulases, β-glucosidases, and hemicellulose. It specifically catalyzes the hydrolysis of β-1,4 glucosidic bonds in cellulose.

YPD medium, consisting of yeast extract (10 g/L), peptone (10 g/L), and glucose (20 g/L), was stabilized to pH 5 ± 0.2. Then, 150 mL of the medium was poured into 1 L round flat-bottomed flasks, and sterilized in an autoclave at 121 °C for 30 min. This medium was used for inoculum cultivation.

2.2. Potato Waste Pulp Conversion into Cultivation Medium

Samples of 200 g potato pulp were added to 2 L beakers. Two types of hydrolysis were performed. For acid hydrolysis, 800 mL of 5% (*w/v*) sulfuric acid was added to the potato pulp, and the mixture was incubated at 100 °C for 40 min. For enzymatic hydrolysis, 200 g of potato pulp was mixed with 800 mL of water, and the mixture was preheated to 100 °C. Next, 0.05 mL of Termamyl was added. Once the temperature dropped to around 55 °C, 0.1 mL of San Extra and 0.1 mL of Cellic CTec 2 [15FPU] were added to the mixture. Subsequently, the pH of the hydrolysates were adjusted with 30% (*w/v*) NaOH to a value in the range of 4.8–5.2. Enzymatic hydrolysis was carried out at 55 °C for 48 h. The hydrolysates were then centrifuged (15 min, 8000RCF), filtered (0.45 mm), and enriched with $(NH_4)_2HPO_4$ and $MgSO_4x7H_2O$ at doses of 0.2 g/L or 0.06 g/L of the medium, respectively.

2.3. Yeast Cultivation

In a laminar flow cabinet, a suspension of the pure yeast culture, stored at −70 °C and thawed at room temperature before use, was added to flasks containing YPD medium. The flasks were then placed in a thermostatic room at 35 °C and incubated for 48 h on a reciprocal shaker. The biomass was next separated by centrifugation (15 min, 4500 RCF), rinsed twice with distilled water, and suspended in water at 1/5 of the initial volume. Next, 1L flasks containing 150 mL of the hydrolysate-derived medium were inoculated with 2 g of DM from the yeast strain and shaken. The cultures of yeast SCP were kept for 48 h at 35 °C.

2.4. Other Assays and Statistical Analysis

The carbohydrate content, both in the media and in the postcultivation effluents, was determined by HPLC according to the methodology described by Dziekońska-Kubczak et al. [14]. Starch content was measured according Faithfull et al. [15]. The dry mass content of the potato pulp samples was assayed as described by Pielech-Przybylska et al. [16]. Total minerals (ash) were assayed gravimetrically after sample mineralization at 600 °C. Crude protein was assayed using the Kjeldahl method, described in [17]. Biomass growth was also determined by the gravimetric method. Biomass yield was expressed as a percentage of the total sugars present in the initial hydrolysate. All assays were carried out at least three times. Each cultivation option was performed as eight parallel replications. Statistical analysis (analysis of variance, determination of SD, and Student's *t*-test at a significance level $\alpha = 0.05$) was carried out using the Origin 7.5 computer program.

3. Results and Discussion

3.1. Composition of Potato Waste Pulp

To determine the composition of the potato pulp and evaluate its suitability for fodder yeast growth, its dry mass, crude protein, reducing sugars, starch, and ash content were analyzed. The dry matter content of the sample was 31.2 ± 0.3%, which is slightly different from the results reported by Obidziński, who found the dry mass content of potato pulp to be around 13% [18]. This difference may be a result of the use of partial compressing, which is a more efficient form of waste pulp dehydration, whereby more water is removed than in the standard process of dehydration by filtration. A review of the literature [19–22] shows that the dry matter content in potato peel waste varies widely, from 12.4% [20] up to 84% [22], or even 92.1% [21]. Although in the latter two studies, dried potato peels were probably used.

The crude protein content of the analyzed potato waste pulp was equal to 4.5 ± 0.14% DM. The assayed protein content value is comparable to that reported by Meyer (4% of DM) [23]. According

to other research, the content of protein may range from 4.1 to 8% of dry mass [19,21,24]. A much higher protein content, at 12.4% of DM, was obtained by Yamada [20]. These variations may be the result of the cultivation conditions, the varieties of potatoes used, and the storage parameters.

The content of simple sugars in the pulp was equal to 3.3 ± 0.11% of DM, while the starch content was equal to 42 ± 1.4% of DM. This value is higher ($p < 0.05$) than the values reported by Meyer F. et al., who found the content of starch in potato pulp waste to be 37% DM [5]. Lower values for starch content were presented by Atitallah [22] at 4.3% of DM, Yamada [20] at 7.7% of DM, and Gao [21] at 4.1% DM. At the other extreme, Arapoglou and co-workers [19] found the starch content in potato waste to be 52.14% of DM. Such differences in starch content may be the result of a number of independent factors, such as the potato variety, the conditions under which the potatoes were stored and cultivated, the processing and dehydration methods, and the storage conditions of the potato pulp waste. Raw material containing 42% starch seems to be a very promising raw material for conversion into assimilable sugars and then into yeast SCP. The total mineral content in the samples after thermal mineralization was equal to 5.7 ± 0.08% of DM. This is higher ($p < 0.05$) than the results reported by Obidziński (4.42% of ash content). [18] The differences in the compositions of various potato pulp samples may be ascribed to the same reasons as the differences in starch content, as well as to the fact that potato pulp waste is not a standardized product.

3.2. The Composition of Hydrolysates Obtained after Acid and Enzymatic Hydrolysis

The central aim of this research was to evaluate the possibility of converting potato pulp waste into fodder yeast biomass. Hydrolysis of the starch present in the raw material was performed according to the method described above. The total amount of the sugars present in the hydrolysates was evaluated by HPLC analysis. The results for both hydrolysates are presented in Table 1.

Table 1. Selected sugar content in potato waste pulp hydrolysates.

Compound	ACID Hydrolysate (AH)	ENZYME Hydrolysate (EH)
	g/L	
arabinose	0.43 ± 0.012	ND
xylose	2.65 ± 0.125	0.2 ± 0.012
maltotriose	1.22 ± 0.042	0.03 ± 0.002
maltose	1.35 ± 0.053	0.92 ± 0.033
glucose	19.32 ± 0.235	24.68 ± 0.271
SUM	24.97	25.95

ND—not detected using the HPLC technique.

Our results reveal that the enzymatic hydrolysate (EH) contained significantly ($p < 0.05$) more glucose than was present in the acid hydrolysate (AH). The total sugar content was at the same level, between 24.97 and 25.95 g/L, for both hydrolysates. Gao and co-workers [21] reported the presence of 114 g/L of reducing sugars after the hydrolysis of potato waste peels. The value reported by Arapoglou and co-workers was 18.5 g/L [19], but it should be emphasized that the concentration of reducing sugars released during hydrolysis depends on the raw material, the method of hydrolysis, and processing conditions, and may therefore vary widely. The content of arabinose and xylose was higher ($p < 0.05$) in AH than in EH. The same dependence was noted for maltotriose and maltose. The higher concentration of polymerized glucose, in the form of maltotriose and maltose, shows that the conditions of acid hydrolysis (acid concentration, time, and temperature) selected in the preliminary experiments (unpublished data) were too mild to achieve a better degree of hydrolysis. Comparing the glucose concentration with the initial starch content in the pulp revealed that approximately 73.7% of the starch was converted into glucose during acidic hydrolysis, and 94.2% of the starch was converted into glucose by enzymatic hydrolysis. The significantly ($p < 0.05$) higher concentrations of arabinose and xylose in AH were probably due to the more thorough decomposition of hemicellulose than

during EH. The sum of the sugars shows that both hydrolysates can be used as a medium for efficient yeast cultivation.

3.3. Fodder Yeast Cultivation in Hydrolysates

Hydrolysates prepared by thermo-acidic or enzymatic treatment were enriched with mineral salts and used for the cultivation of *Pichia stipitis* LOCK 0049 (PS) and *C. guilliermondii* ATCC 6260 (CG) at 35 °C for 48 h. The biomass content after cultivation is presented in Figure 1.
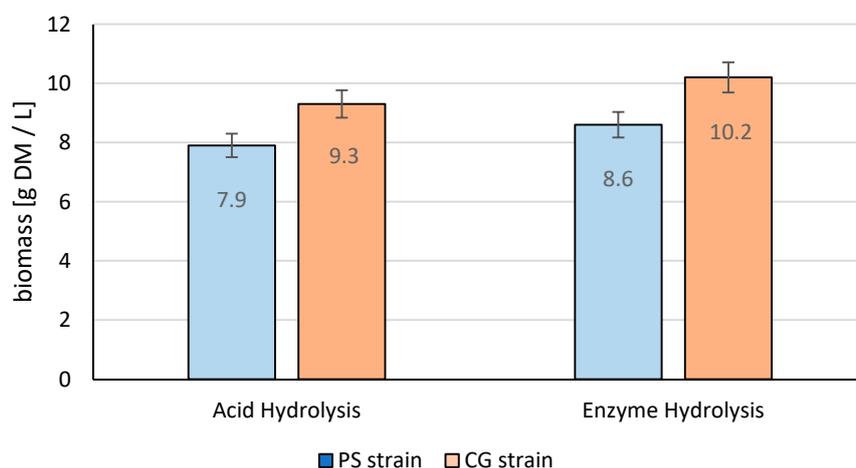

Figure 1. *Pichia stipitis* (PS) and *Candida guilliermondii* (CG) biomass concentrations after 48 h of cultivation in acid hydrolysate (AH) or enzymatic hydrolysate (EH). Error bars show 5% deviations.

The highest biomass concentration (10.2 g DM/L) was observed for CG cultivated in EH. When the same strain was cultivated in acid hydrolysate, the biomass content at the end of the process was 9.3 DM /L. The lowest biomass at 7.9 g DM /L was present in the case of the PS strain when acid hydrolysate was used as a medium, whereas when the same strain was cultivated in enzymatic hydrolysis medium, 8.6 g DM/L was observed. With both kinds of media, the concentration of CG biomass was higher ($p < 0.05$) than the amount of PS. The differences between the biomass concentrations obtained for the same strain cultivated in different media were statistically insignificant ($p > 0.05$). Many factors affect the results for yeast biomass, including the strain, the cultivation conditions, and the presence of inhibitors, so comparison with data presented in the literature is difficult. However, our result for CD cultivated in enzymatic hydrolysate at 10.2 g DM/L is almost equal to the 10.1 g/L obtained by Gélinas and Barrette for the same *Candida* species [25].

The biomass yield, expressed as the percentage of sugars converted into dry matter, varied between 31.6% for PS cultivated in AH medium and 39.3% for CG after 48 h of bioconversion on EH medium. The yields observed for CG (37.2% for AH medium and 39.3% for EH medium) were higher ($p < 0.05$) than those obtained for PS species (31.6% and 33.1% for AH and EH media, respectively). According to Chmiel [26], under aerobic conditions the biomass yield of yeast may reach 43%, which is significantly higher ($p < 0.05$) than our results. However, it must be emphasized that, apart from biomass, other products of yeast metabolism are also formed and released to the medium, so some of the sugars are converted into short-chain fatty acid esters. Ethanol and glycerol may also form, especially if there is insufficient aeration.

HPLC Analysis of Post-Cultivation Effluents.

Although the concentration of biomass is the key parameter for determining bioconversion efficiency, a secondary parameter is the concentration of residual sugars and metabolism by-products, such as low molecular organic acids or ethanol. These can be treated as indirect indicators of the

conversion efficiency of sugars to SCP. The post-culture liquids were subjected to HPLC analysis and the results are shown in Table 2.

Table 2. Selected compounds in post-cultivation effluents.

Compound	*Pichia stipitis* (PS)		*Candida guilliermondii* (CG)	
	ACID Hydrolysate (AH)	ENZYME Hydrolysate (EH)	ACID Hydrolysate (AH)	ENZYME Hydrolysate (EH)
		g/L		
arabinose	0.42 ± 0.01	ND	0.01 ± 0.0008	ND
xylose	0.65 ± 0.025	0.12 ± 0.004	0.23 ± 0.01	0.11 ± 0.003
maltotriose	0.21 ± 0.0018	0.03 ± 0.002	0.05 ± 0.007	0.03 ± 0.004
maltose	ND	ND	ND	ND
glucose	ND	ND	ND	ND
succinic acid	0.08 ± 0.0023	0.19 ± 0.0022	0.06 ± 0.0018	0.14 ± 0.001
lactic acid	0.02 ± 0.001	0.07 ± 0.001	0.018 ± 0.001	0.2 ± 0.0052
glycerol	0.21 ± 0.002	0.28 ± 0.02	0.2 ± 0.01	0.22 ± 0.0047
formic acid	0.11 ± 0.003	0.17 ± 0.001	0.09 ± 0.005	0.14 ± 0.002
acetic acid	0.12 ± 0.0021	0.23 ± 0.0021	0.09 ± 0.004	0.18 ± 0.002
ethanol	1.12 ± 0.018	0.83 ± 0.026	0.57 ± 0.032	0.32 ± 0.028

ND - not detected using HPLC.

The results of HPLC analysis reveal that the glucose and maltose concentrations in all the leachates were below the indication range. This shows that these two sugars were fully assimilated by both strains used in the experiments. Traces of maltotriose were found in each effluent, but the highest concentration, at 0.21 g/L, was observed in the AH/PS strain post-cultivation liquid. This suggests that not all maltotriose was assimilated by the strain of PS used. The arabinose contents in the leachates after PS cultivation in AH remained at the same levels as before inoculation. This may be a result of the properties of the PS strain used which, according to data presented by Kurtzman et al. [13], is unable to assimilate arabinose. According to the same authors, CG strains are able to assimilate arabinose, which is confirmed by our study. The concentration of arabinose in the AH post-cultivation effluent was 0.01 g/L ± 0.0008, which is significantly less than the 0.43 g/L for the initial acid hydrolysate. The starting concentrations of xylose in the hydrolysates were 2.65 and 0.32 g/L for AH and EH, respectively. The content of xylose after cultivation varied between 0.1 g/L for EH/CG and 0.65 g/L for AH/PS. This shows that xylose was assimilated by both strains, and supports the data presented by Kurtzman et al. [13].

The four short-chain fatty acids (formic, acetic, lactic, and succinic acid) present in the leachates were also examined. The lowest concentration for these acids (0.018 g /L) was observed for lactic acid after CG cultivation in AH medium. The highest level was found for acetic acid (0.23 g/L) in the case of PS/EH. The levels of low-carbon fatty acids in the EH post-cultivation media were usually significantly higher ($p < 0.05$) than those following cultivation in AH medium. For example, the concentration of succinic acid was 0.08 g/L ± 0.0023 after cultivation of PS in AH medium and 0.19 g/L ± 0.0022 ($p < 0.05$) when the same strain was cultivated in EH medium. This correlation may be explained by the hydrolysis time, which was much longer for enzyme treatment, and probably caused the development of microflora that produced additional amounts of short-chain organic acids. The ethanol and glycerol contents in the leachates were also assayed. While the glycerol content varied in a very narrow range, between 0.2 and 0.28 g/L for all combinations of strains and media, the ethanol content was significantly higher in the post-effluents of PS cultivated in AH and EH media (1.12 g /L ± 0.018 and 0.83 g/L ± 0.026, respectively) than in the leachates after CG cultivation (0.57 g/L ± 0.032 for AH medium and 0.32 g/L ± 0.028 for EH medium). This proves that PS can convert other sugars, not only xylose, into ethanol, which is also in agreement with the literature [27,28].

4. Summary and Conclusions

The aim of this research was to evaluate potato pulp waste as a raw material for obtaining fodder yeast biomass. Two yeast strains were compared, *Pichia stipitis* and *Candida guillermondii*, as well

as two methods of hydrolysis for preparing the cultivation media. Our results suggest that potato pulp waste, after efficient enzymatic processing, is a promising raw material for obtaining yeast SCP. Optimization of the method of hydrolysate preparation, as well as of the cultivation conditions and the strain used, is key to ensuring efficient biomass yield. Further research should consider enzymatic and thermo-acidic processing methods, since both can lead to efficient bioconversion of potato pulp waste into SCP. The local availability and low cost of potato pulp waste, as well as the relatively simple process of hydrolysate preparation, make conversion of potato pulp waste into yeast biomass a promising solution to help meet constantly rising demand for animal fodder.

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