J. Agric. Sci., Tokyo Univ. Agric., 52 (3), 147–150 (2007)
東京農大農学集報，52（3），147–150（2007）

資　料
Research Data

Practical Liquefaction of Potato Pulp and Sugar-beet Pulp by Commercial Enzymes

By

Tatsuro MIYAJI*, Shuki FUJIMURA*, Tomoyuki NAKAGAWA**
and Ayumu TAKANO***

(Received May 17, 2007/Accepted September 4, 2007)

Summary : With the goal of effective utilization of potato pulp and sugar-beet pulp as foodstuffs, we developed a simple and practical method for partial liquefaction of the pulps through the use of commercial food-processing enzymes. To improve the liquefaction process, swelling of insoluble components and gelatinization of starch in the pulps were performed by autoclaving at 121℃ for 20 min prior to enzyme treatment. The liquefaction rates of the components in the autoclaved pulps were calculated from the dry weight of the residue obtained after enzymatic liquefaction. A screen of several commercial enzymes revealed that Pectinase PL, Cellulase A and Biozyme A (α-amylase preparation) were the most efficient enzymes for liquefaction. Using a combination of these three enzymes, the components in the autoclaved potato and sugar-beet pulps were digested with liquefaction rates of 89% and 59%, respectively.

Key words : potato pulp, beet pulp, liquefaction, food-processing enzyme

Potato pulp and sugar-beet pulp are by-products from the manufacture of potato starch and beet sugar, respectively, and are produced on a massive scale in Hokkaido, Japan. The two pulps contain many useful components. Potato pulp includes, as the percentage of dry matter : 15.3–17.0% pectin, 17.0–26.0% cellulose, 13.9–14.0% hemicellulose, 17.1–46.5% starch, 4.0–14.9% protein and 1.8–5.2% ash[1–4]. Sugar-beet pulp includes : 11.7–25.0% pectin, 18.7–24.0% cellulose, 12.8–36.4% hemicellulose, 5.8–17.3% sucrose, 5.1–8.0% protein, 1.8–5.6% lignin and 9.0–12.2% ash[1,5,6]. The use of these pulps, however, is limited to livestock feed and compost because they are insoluble in water, not fermentable by yeast, and perishable.

Studies on the bioconversion of agroindustrial by-products for useful purposes have revealed that, compared with direct fermentation, conversion with enzyme preparations has two merits : enzymatic liquefaction does not require costly equipments and prolonged reaction times, and it does not consume useful constituents. A number of studies of enzymatic digestion and saccharification have been carried out on potato pulp[1–3,7] and sugar-beet pulp[1,5,6]. However, very little screening for superior commercial enzymes has been performed. With the aim of maximizing the use of potato and sugar-beet pulps as food materials, we screened combinations of commercial enzymes for their effectiveness in liquefaction.

Raw potato pulp was provided from a potato starch factory at Koshimizu agricultural cooperative in Hokkaido, Japan and was freeze-dried immediately after sampling. Powdery sugar-beet pulp was a gift from Nippon beet sugar manufacturing Co., Ltd., Japan. Commercial food-processing enzymes, Pectinase PL "Amano" (a pectinase containing cellulase from *Aspergillus niger*), Cellulase A "Amano" 3 (an endo-β-glucanase containing exo-β-glucanase, β-glucosidase, xylanase and pectinase from *Aspergillus niger*), Cellulase T "Amano" 4 (an exo-β-glucanase containing endo-β-glucanase, β-glucosidase and xylanase from *Trichoderma viride*), Biozyme A (an α-amylase containing protease from *Aspergillus oryzae*), Biozyme F10SD (an α-amylase containing

* Department of Food Science and Technology, Faculty of Bioindustry, Tokyo University of Agriculture
** Department of Food Science and Technology, Faculty of Bioindustry, Tokyo University of Agriculture (Faculty of Applied Biological Sciences, Gifu University)
*** Hokkaido Sugar Co., Ltd.

reduced amounts of protease from *Aspergillus oryzae*), Amylase AD "Amano" 1 (an α-amylase from *Bacillus subtilis*), Gluczyme AF6 (a glucoamylase not containing transglucosidase from *Rhizopus niveus*), and Kleistase M8 (an endo-α-amylase from *Bacillus subtilis*) were a gift from Amano enzyme Inc., Japan. Pectinex Ultra SP-L (a polygalacturonase from *Aspergillus aculeatus*) and Celluclast 1.5 L (a cellulase from *Trichoderma reesei*) were donated by Novozymes, Denmark. Hemicellulase (H-2125) containing cellulase from *Aspergillus niger* was purchased from Sigma-aldrich Corporation, U.S.A.

To improve the enzymatic liquefaction of insoluble components, the swelling and gelatinization of starch in the pulps were carried out as follows : 0.2 g of dried pulp was added to 9.8 m*l* of pure water in a test tube, and was autoclaved at 121℃ for 20 min. To the autoclaved pulp, 0.1 m*l* of the liquid enzyme or 0.5 m*l* of a 10% (w/v) solution of the solid enzyme was added to liquefy water-insoluble components in the pulp. The enzyme solutions were filtered (0.2 μm) in advance for sterilization and removal of insoluble stabilizer. Judging from the liquefaction ratios, the amount of enzyme added was in excess for the substrate in all of the reactions. The same procedure was followed for the combinations of enzymes. The enzyme reactions were carried out at 40℃ for 16 hr by shaking. The pH of the reaction mixtures was not optimized for the enzymes because the liquefied pulp was intended to be fit for human consumption.

The precipitate obtained by centrifugation (10,000×g, 20 min) of the liquefied pulp was vigorously washed 3 times with pure water. The dry weight of the precipitate was measured after drying on an aluminum tray in an air incubator at 105℃ overnight. In previous studies on liquefaction of the two pulps, the liquefaction efficiency has been determined by measuring the decrease of polysaccharides such as pectin, cellulose and starch. However, this method is unsuitable for unknown insoluble components in the pulps. We chose to assess the liquefaction rate simply and directly by calculating the ratio of the dry weight of each sample to that of a control in which an equal volume of sterilized water was substituted for the enzyme solution.

Table 1 shows the results of the screen for enzyme preparations capable of liquefying water-insoluble components in autoclaved pulps. Although the reported main components of the cell wall, such as pectin, cellulose and hemicellulose, in potato and sugar-beet pulps were very similar, the potato pulp was more readily liquefied by all of the enzymes. Pectinase PL "Amano" was superior to Pectinex Ultra SP-L for both potato and sugar-beet pulps. This was of interest because Pectinex Ultra SP-L has been reported to be more effective for the liquefaction of soybean milk residue (okara)[8]. Cellulase A "Amano" 3 liquefied the residue from potato pulp as effectively as Pectinase PL "Amano". When Biozyme A (α-amylase preparation) was applied to the potato pulp, the liquefaction rate was 21.4%. To detect starch remaining in this suspension, it was applied to an iodine-starch solution which contained 1% (w/v) iodine and 5% (w/v) potassium iodine. The iodine-starch reaction was negative indicating that starch in the suspension was completely degraded by Biozyme A. Based on these results, Pectinase PL "Amano", Cellulase A "Amano" 3 and Biozyme A were chosen for further examination.

Table 1 Screening for enzyme capable of liquefying insoluble components in autoclaved pulps

Enzymes	Liquefaction rate (%)	
	Potato pulp	Beet pulp
pectinase:		
Pectinase PL "Amano"	82.6±0.1*	49.4±5.9
Pectinex Ultra SP-L	68.3±1.6	47.0±0.4
cellulase:		
Cellulase A "Amano"3	80.0±8.5	27.2±2.2
Cellulase T "Amano"4	76.7±0	12.1±1.5
Celluclast 1.5L	73.6±1.3	26.6±1.2
α-amylase:		
Biozyme A	21.4±0.9	5.6±0.9
Biozyme F10SD	19.3±1.3	3.6±1.1
Amylase AD "Amano"1	8.5±4.0	2.5±0.5
Kleistase M8	8.3±1.8	2.3±0.2
glucoamylase:		
Gluczyme AF6	19.0±0.9	4.5±1.4
hemicellulase:		
Hemicelllase from Sigma-Aldrich	−	8.6±0.8

* mean and standard deviation (n=3)

Next, we improved liquefaction of the two kinds of autoclaved pulps by combining the selected enzymes. The combination with pectinase, cellulase and α-amylase showed the highest liquefaction rate : 89.4% and 58.6% in the potato and the sugar-beet pulps, respectively (Table 2). This result contrasted with a study by BELDMAN *et al.*[1] that reported opposite results by measuring reducing sugars from pulps. This was probably

Table 2 Liquefaction of insoluble components in autoclaved potato and beet pulps by a combination of selected enzymes

Combination of enzymes	Liquefaction rate (%)	
	Potato pulp	Beet pulp
pectinase, cellulase, α-amylase	89.4±0.6*	58.6±1.1
pectinase, cellulase	87.3±2.9	57.0±2.0
pectinase, α-amylase	82.1±0.9	55.9±1.1
cellulase, α-amylase	70.6±1.1	27.4±2.3

Pectinase: Pectinase PL "Amano", cellulase: Cellulase A "Amano" 3, α-amylase: Biozyme A
* mean and standard deviation (n=3)

due to the difference in assessment method for liquefaction and saccharification used in the two studies. Although no monosaccharides were detected in the autoclaved potato pulp, a large quantity of glucose and other unknown monosaccharides was detected in the solution liquefied with the three-enzyme combination (data not shown). The pH of the potato pulp suspension before autoclaving was 5.2 and 4.4, after autoclaving. After liquefaction with the three-enzyme combination, the pH was 3.3. The pH drop can be explained by the de-esterification of pectin. These data show that sufficient saccharification was also achieved during the liquefaction of the potato pulp.

Pulp by-products are abundant in Japan, and research into their effective uses is needed. Here we have presented an efficient method for the liquefaction of potato and sugar-beet pulps using an optimized combination of the enzymes. Potential uses of this procedure will be numerous. Fuel ethanol has been made from potato pulp via liquefaction with Pectinex and Cellclast, (pectinase and cellulase preparations), followed by alcohol fermentation with engineered bacteria[6]. Vinegar also has been made from potato pulp via saccharification with fungus, followed by alcohol fermentation with yeast and acetic acid fermentation with acetic acid bacteria[9]. In addition to the experiments described here, we tried to fermentatively produce ethanol and acetic acid from the potato pulp liquefied with the three-enzyme combination. Using alcohol fermentation with *Saccharomyces cerevisiae* and acetic acid fermentation with *Acetobacter tropicalis,* sufficient amounts of ethanol and acetic acid were formed at each step (data not shown). In conclusion, the three-enzyme combination identified in this study is efficient at liquefying both potato and sugar-beet pulps and has the potential to facilitate the production of alcohol, acetic acid, and foodstuffs from those pulps.

Acknowledgements

We are grateful to Ms. Mika Nagashima and Ms. Yuria Uno of the Department of Food Science and Technology, Tokyo University of Agriculture, for their technical assistant.

References

1) Beldman, G., Rombouts, F.M., Voragen, A.G.J. and Pilnik, W., (1984.) Application of cellulase and pectinase from fungal origin for the liquefaction and saccharification of biomass. *Enzyme Microb. Technol.,* **6**, 503–507.
2) Slominska, L. and Starogarzka, G., (1987.) Application of a multi-enzyme complex in the utilization of potato pulp. *Starch,* **39**, 121–125.
3) Nebesny, E., (1995.) Utilization of potato pulp for baking of bread. *Starch,* **47**, 36–39.
4) Mayer, F. and Hillebrandt, J.-O., (1997.) Potato pulp : microbiological characterization, physical modification, and application of this agricultural waste product. *Appl. Microbiol. Biotechnol.,* **48**, 435–440.
5) Hassanien, F., Makhzangy, A.EL and Ayyad, K., (1990.) Application of polysaccharide enzymes in beet pulp. *Nahrung,* **34**, 469–473.
6) Doran, J. and Foster, B., (2000.) Ethanol production from sugar beet pulp using engineered bacteria. *Int. Sugar Jnl.,* **102**, 336–340.
7) Liu, Q., (2002.) A study of enzymatic hydrolysis of starch in potato pulp. *J. Food Sci.,* **67**, 2113–2117.
8) Kasai, N., Murata, A., Inui, H. and Sakamoto, T., (2004.) Enzymatic high digestion of soybean milk residue (Okara). *J. Agric. Food Chem.,* **52**, 5709–5716.
9) Tamura, Y., Iwashita, A., Sasaki, S. and Ohhori, T., (2004.) The manufacture of vinegar made from potato pulp fermented with *Rhizopus oryzae* NBRC 4704. *J. Brew. Soc. Japan,* **99**, 202–207.

ポテトパルプとビートパルプの市販酵素剤による液化

宮地竜郎*・藤村朱喜*・中川智行**・鷹野アユム***

（平成 19 年 5 月 17 日受付/平成 19 年 9 月 4 日受理）

要約：ポテトパルプとビートパルプを食品素材として有効に利用する目的で，市販酵素剤を用いたパルプの簡便な液化を試みた。121℃，20 分間のオートクレイブ処理によりパルプの単細胞化とデンプンの α 化を行い，パルプ中の不溶性成分の液化率は液化後に得られた残渣の乾燥重量より求めた。各種酵素剤を用いたスクリーニングの結果，ペクチナーゼ PL，セルラーゼ A，ビオザイム A（α–アミラーゼ製剤）が二種のパルプの液化に効果的であった。三種類の酵素剤を組み合わせることで，オートクレイブ処理をしたホテトパルプとビートパルプ中の不溶性成分は，それぞれ 89% と 59% の液化率で消化された。

キーワード：ポテトパルプ，ビートパルプ，液化，食品加工用酵素

* 東京農業大学生物産業学部食品科学科
** 東京農業大学生物産業学部食品科学科（岐阜大学応用生物科学部食品生命科学課程）
*** 北海道糖業株式会社