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# Effects of Nutrient Supplements on Simultaneous Fermentation of Nisin and Lactic Acid from Cull Potatoes

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## Abstract

The feasibility of using cull potatoes as substrate for the simultaneous production of nisin, a natural food preservative, and lactic acid, a raw material for biopolymer production, was studied. Cull potatoes are potato tubers unacceptable for food processing because of size or damage caused by bruising or disease. Although cull potatoes are enriched in various nutrients including starch, minerals, and proteins, they alone still cannot provide enough essential nutrients for the growth and metabolism of *Lactococcus lactis* subsp. *lactis* (ATCC 11454). Stimulation of bacterial growth, nisin biosynthesis, as well as lactic acid production was observed when additional nutrients such as yeast extract, peptone from meat, peptone from soy (PS), corn steep solid (CSS), and distillers' dried grains with solubles were provided. Considering the cost and availability, PS and CSS were selected as nutrient supplements for nisin and lactic acid coproduction. The conditions for nisin biosynthesis and lactic acid coproduction by *L. lactis* subsp. *lactis* in a cull potato-based medium were subsequently optimized using a statistically based experimental design.

**Index Entries**: Nisin; lactic acid; cull potato; fermentation; optimization.

## Introduction

Potatoes are an important agricultural commodity in the United States, especially in the Pacific Northwest. In 2002, 1.28 million acres of potatoes were grown and 46.32 billion pounds of potatoes were produced in the United States *(1)*. Among those potatoes harvested, about 8% were graded as cull potatoes, which include undersized tubers; bruised, damaged, and deformed tubers; and tubers with low specific gravities/total solids, hollow heart, internal discoloration, or disease *(1)*. Cull potatoes bring little value to potato growers; the price of cull potatoes is currently less than $10/t when sold as livestock feed, but it costs farmers about

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$65/t to grow them. Therefore, technologies for producing value-added products from cull potatoes that can provide returns and benefits to growers are needed. Cull potatoes are an ideal substrate for microorganisms, because they contain various essential nutrients such as starch, protein, vitamins, and minerals to support the growth of microbes *(2,3)*. Therefore, using cull potatoes as feedstock for the production of value-added products such as nisin and lactic acid via fermentation provides an alternative utilization for cull potatoes.

Nisin is a small peptide with significant antimicrobial activity against almost all Gram-positive food-borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, and psychrotrophic enterotoxigenic *Bacillus cereus (4–7)*. Owing to its strong antimicrobial properties, nisin has been accepted as a safe and natural preservative worldwide and has been widely applied in the food industry *(8)*. The Food and Drug Administration views nisin derived from *Lactococcus lactis* subsp. *lactis* to be a generally recognized as safe substance for use as an antimicrobial agent *(9)*, and, therefore, direct addition of nisin to various types of foods, such as cheese, margarine, flavored milk, and canned foods, is permitted *(10)*. In addition, nisin is being considered for use in health and cosmetic products *(11)*.

Using cull potatoes as feedstock for ethanol *(3)* and lactic acid *(12)* production has been extensively studied; however, production of nisin from cull potatoes has not been reported. In the current industrial process, nisin is manufactured by fermentation using *L. lactis* subsp. *lactis* as a production strain on a milk-based medium. The biosynthesis of nisin is coupled with the growth of lactic acid bacteria and the formation of a significant amount of lactic acid *(7)*. Lactic acid is an important chemical for food processing but can also be used as a raw material in the production of the biodegradable polymer poly(lactic) acid *(13)*. Unfortunately, lactic acid is not recovered in the current nisin process. Our recent work *(14)* demonstrated that simultaneous production of lactic acid and nisin by *L. lactis* subsp. *lactis* using a cheese whey-based medium is possible, because the optimal conditions for the production of these two products are almost the same.

In the present work, the feasibility of using cull potatoes alone as substrate for nisin and lactic acid coproduction was evaluated. The effects of different nutrient supplements—yeast extract (YE), peptone from meat (PM), peptone from soy (PS), corn steep solid (CSS), and distillers' dried grains with solubles (DDGS)—on bacterial growth, nisin biosynthesis, and lactic acid formation were studied. The most effective and economical nutrient supplements were identified and then optimized using response surface methodology (RSM) *(14,15)*. The feasibility of developing a cost-effective process for simultaneous production of nisin and lactic acid from cull potatoes is also discussed.

## Materials and Methods

### Microorganisms, Media, and Cultivation

L. *lactis* subsp. *lactis* (ATCC 11454) was the nisin-producing microorganism used. *Micrococcus luteus* (ATCC 9341) was used as an indicating microorganism in the bioassay of nisin concentrations.

The compositions of media used for the growth of the microorganisms were as follows: Medium I, used for seed culture of *L. lactis* (pH 7.0), contained 5 g/L of glucose, 5 g/L of polypeptone, and 5 g/L of YE. Medium II, used for bioassay of nisin (pH 7.0), contained 10 g/L of glucose, 5 g/L of polypeptone, 5 g/L of YE, and 5 g/L of NaCl. Medium III, used for the main fermentation, was potato hydrolysate containing 50 g/L of glucose and a predetermined amount of other nutrients shown in the experimental designs (Table 1 and Table 2). Thirty grams/liter of $CaCO_3$ powder was added to medium III in order to maintain a stable pH during fermentation. Medium IV, the control medium to quantify the performance of the fermentation medium (medium III), was the optimal medium for nisin and lactic acid coproduction that we obtained in our former work *(14)*. Its composition was 50 g/L of whey, 12 g/L of YE, 5 g/L of polypeptone, 0.6 g/L of $KH_2PO_4$, 0.6 g/L of $MgSO_4$, 1 g/L of Tween-80, and 30 g/L of $CaCO_3$. Medium V, used for measuring the biomass of the bacteria by the colony-counting method, contained 10 g/L of glucose, 5 g/L of polypeptone, 5 g/L of YE, 5 g/L of NaCl, and 10 g/L of agar.

The method for preparing potato hydrolysate was as follows: Fresh potatoes were cut into $1 \leftrightarrow 1 \leftrightarrow 1$ $cm^3$ and then were homogenized using a blender. Next, 10% NaOH solution was applied to adjust the pH of the potato mash to 6.0 prior to the addition of α-amylase. The mixture was then heated to 100°C and maintained for 2 h. When the temperature of the potato mash decreased to 65°C, the pH of the mash was adjusted to 4.0 and α-glycosidase was added. After maintaining the temperature for 16 h, the potato hydrolysate was obtained when the solid in the mash was removed via filtration.

Seed culture of *L. lactis* was conducted in 125-mL Erlenmeyer flasks placed on an orbital shaker at 160 rpm and 30°C for 8 h. Main fermentations were performed in 250-mL Erlenmeyer flasks containing 100 mL of medium III and 5 mL of the seed medium.

### Analysis

Nisin concentration was measured using a bioassay procedure based on the method of Shimizu et al. *(16)*. A high-performance anion-exchange chromatography method *(17)* was used for lactic acid analysis. Reducing sugar content in potato hydrolysate was measured using the 3,5-dinitrosalicylic acid colorimetric method *(18)*. The biomass of *L. lactis* in the fermentation broth was quantitively analyzed by $OD_{600}$ or by the colony-counting method.

Table 1
CCD of Factors in Coded Levels with Nisin, Biomass, and Lactic Acid Concentration as Response

| Run | Type | CSS | PS | Nisin (mg/L) | Biomass ($10^9$ CFU/mL) | Lactic acid (g/L) |
|---|---|---|---|---|---|---|
| 1 | Center | 0 | 0 | 85.1 | 4.73 | 19.8 |
| 2 | Center | 0 | 0 | 85.3 | 4.75 | 19.6 |
| 3 | Center | 0 | 0 | 85.0 | 4.70 | 20.1 |
| 4 | Center | 0 | 0 | 84.5 | 4.69 | 19.9 |
| 5 | Center | 0 | 0 | 84.9 | 4.72 | 19.7 |
| 6 | Axial | 0 | +1.41 | 88.2 | 4.90 | 20.8 |
| 7 | Axial | –1.41 | 0 | 81.7 | 4.54 | 18.8 |
| 8 | Axial | +1.41 | 0 | 87.2 | 4.80 | 20.2 |
| 9 | Axial | 0 | –1.41 | 70.9 | 3.95 | 18.3 |
| 10 | Fact | –1 | +1 | 88.5 | 4.90 | 20.1 |
| 11 | Fact | +1 | –1 | 76.4 | 4.30 | 19.1 |
| 12 | Fact | –1 | –1 | 68.6 | 3.80 | 17.2 |
| 13 | Fact | +1 | +1 | 89.8 | 5.00 | 21.0 |

Table 2
Coded and Actual Values of Factors in CCD

| Factor | Name | Unit | Axial (–1.41) | Low (–1) | Central (0) | High (+1) | Axial (+1.68) |
|---|---|---|---|---|---|---|---|
| A | CSS | g/L | 0 | 1.72 | 5.86 | 10 | 11.72 |
| B | PS | g/L | 0 | 1.72 | 5.86 | 10 | 11.72 |

### *Design of Experiments Using RSM*

RSM *(14,15)* was applied to determine the optical values of the most effective and economical nutrient supplements identified. A 13-run central composite design (CCD) was developed. Table 1 gives the matrix for the statistically based experimental design, and Table 2 presents the true values for these variables in the design. After the responses were obtained, they were subjected to multiple nonlinear regression and optimization using the software Design-Expert (V6.0; Stat-Ease, Minneapolis, MN).

## Results and Discussion

### *Cull Potato Hydrolysate Alone as Substrate for L. lactis*

Although cull potatoes are an undervalued agricultural commodity primarily used as a supplement to animal feed, the possibility exists for their value-added use because they contain nutrients such as starch, protein, vitamins, and minerals that are capable of supporting the growth

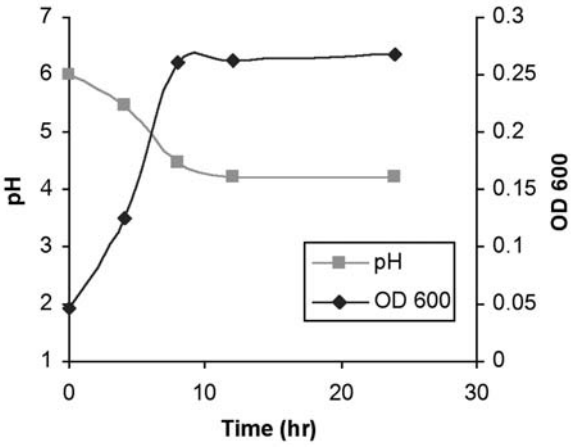


**Fig. 1.** Growth of *L. lactis* (ATCC 11454) on potato hydrolysate containing 50 g/L of reducing sugar.

of microbes via fermentation *(1,2)*. Considering this fact, we first explored the possibility of using cull potato hydrolysate alone as the substrate for the simultaneous production of nisin and lactic acid by *L. lactis.*

Figure 1 shows the growth of *L. lactis* and the pH profile of the fermentation broth when potato hydrolysate alone was applied as the substrate for the bacteria. One can easily draw the conclusion that the growth of the bacteria on potato hydrolysate alone was very poor, because the $OD_{600}$ reached only 0.28 after 24 h of incubation. Because $CaCO_3$ was not added in this experiment, fermentation pH dropped rapidly from 6.0 to 4.2 owing to the formation of lactic acid. Therefore, sufficient $CaCO_3$ was required to neutralize the lactic acid formed and to maintain the pH.

To quantify the performance of potato hydrolysate, the optimal medium for nisin and lactic acid coproduction obtained in an earlier study *(14)* was used as control. $CaCO_3$ was provided to maintain the fermentation pH around 5.5. The biomass, nisin biosynthesis, and lactic acid formation after 24 h of fermentation were compared. As shown in Fig. 2, the production of nisin and lactic acid on potato hydrolysate was only half that at the earlier optimized conditions. Therefore, it can be concluded that potato hydrolysate itself cannot provide enough essential nutrients for the growth and metabolism of *L. lactis*, because the nisin-producing strain *L. lactis* is a well-known nutritionally fastidious microorganism *(19)* requiring an abundance of nutrients for cell growth and metabolism.

## *Effects of Nutrient Supplements on Nisin and Lactic Acid Production*

Because potato hydrolysate alone cannot support the growth and metabolism of *L. lactis*, additional nutrients were required for the simultaneous production of nisin and lactic acid. YE, PM, and PS, the most

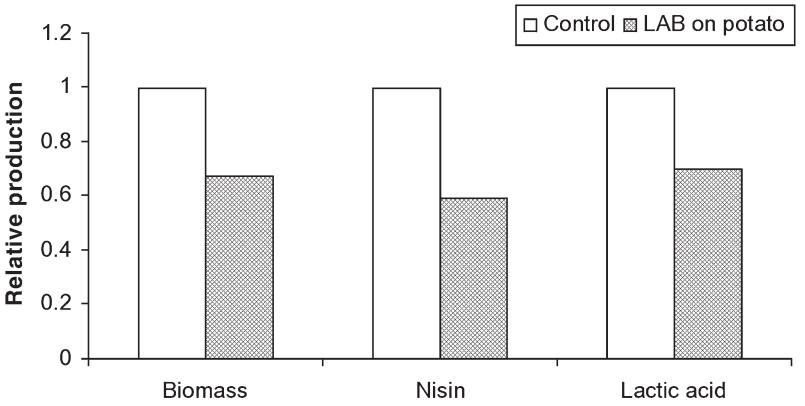


**Fig. 2.** Relative production of biomass, nisin, and lactic acid on potato hydrolysate containing 50 g/L of reducing sugar in contrast to that on control medium.

widely used organic nutrient supplements in fermentation studies, as well as DDGS and CSS, the two byproducts of corn biorefinery, were selected as the candidates for nutrient supplement. The stimulation effects of these nutrient supplements on cell growth, nisin formation, and lactic acid production were studied. Figure 3 compares the results.

YE and PM, as seen by the comparative yields with the control, are ideal sources of nutrient for nisin and lactic acid coproduction from cull potatoes. CSS also performed very well; the yield of nisin and lactic acid was >80% of the control. De Vuyst *(20)* found that the amino acids serine, threonine, and cysteine highly stimulated nisin production, indicating their precursor role during nisin biosynthesis. In the present work, the significant effect of these nutrient supplements supported these findings. Because CSS is an inexpensive nutrient, owing to its being an undervalued byproduct of the corn wet-milling process *(21)*, CSS was selected as the main nutrient supplement for this nisin and lactic acid coproduction process.

Figure 4 presents the stimulation effects of different combinations of the nutrient supplement candidates. The combination of CSS and PS showed the biggest improvement in nisin and lactic acid production, with the yield of nisin and lactic acid being the same as that of the control. Therefore, PS was chosen as the auxiliary nutrient supplement.

## *Optimization Using RSM*

In the following optimization step, the exact values of CSS and PS in the potato-based medium for nisin and/or lactic acid production were determined using a CCD (Table 1). Table 2 provides the coded and actual values of each variable. The fermentation medium (pH 6.5) was composed of potato hydrolysate containing 50 g/L of reducing sugar, 30 g/L of $CaCO_3$, and the predetermined amount of CSS and PS assigned by the

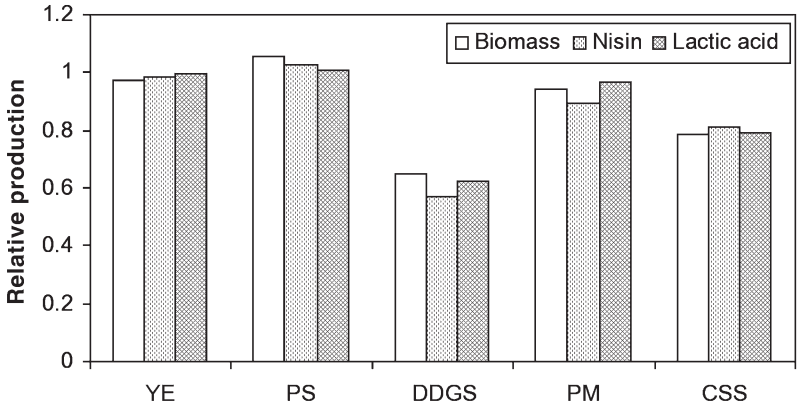


**Fig. 3.** Stimulation effects of different nutrient supplements on biomass, nisin, and lactic acid production on potato hydrolysate.

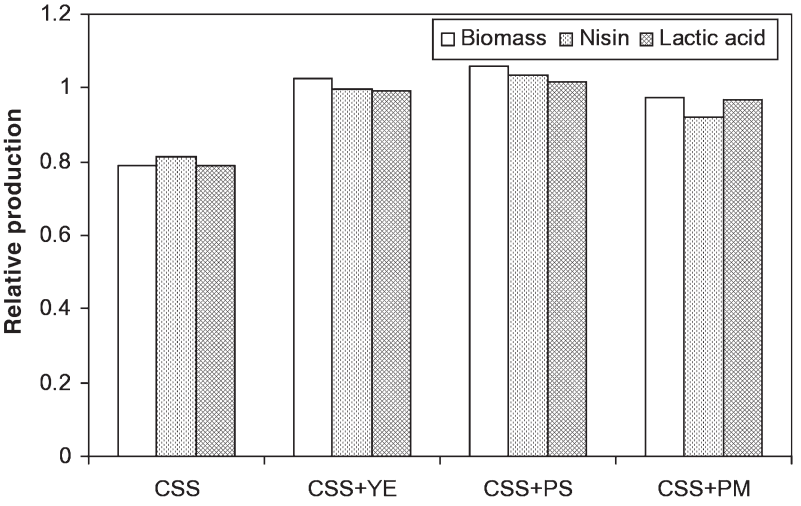


**Fig. 4.** Stimulation effects of different combinations of nutrient supplements on biomass, nisin, and lactic acid production on potato hydrolysate.

CCD. The content of nisin and lactic acid after 24 h of fermentation at 30°C were measured and are presented as responses in Table 1.

The responses of nisin, biomass, and lactic acid were analyzed using the same methodology as we did in an earlier study *(14)*. The optimal conditions for biomass, nisin biosynthesis, and lactic acid formation were obtained by numerical analysis of the response surface using Design-Expert software and are presented in Table 3. The solution to the maximal nisin biosynthesis was 7.88 g/L for CSS, and 11.32 g/L for PS. The solution to the maximal lactic acid production was 7.58 g/L for CSS, and 11.95 g/L for PS. For biomass, the optimal condition was 11.10 g/L of PS and 5.54 g/L of CSS. Finally, the solution to the simultaneous maximal production of nisin and lactic acid was 7.32 g/L of CSS and 11.52 g/L of PS.

Table 3
Optimal Conditions for Nisin and Lactic Acid Production by *L. lactis* (ATCC 114.54) Obtained from Responce Surface Analysis

| Criteria | | Optimal values | | Predicted results | | |
|---|---|---|---|---|---|---|
| Nisin | Lactic acid | CSS (g/L) | PS (g/L) | Biomass ($10^9$ CFU/mL) | Nisin (mg/L) | Lactic acid (g/L) |
| Maximum | — | 7.88 | 11.32 | 4.95 | 89.4 | 20.9 |
| — | Maximum | 7.58 | 11.95 | 4.94 | 88.9 | 21.1 |
| Maximum | Maximum | 7.32 | 11.52 | 4.95 | 89.2 | 21.0 |

The data in Table 3 reveal that the optimal conditions for nisin biosynthesis and lactic acid formation by *L. lactis* were almost the same, and that the predicted values of nisin and lactic acid under these three optimal conditions in Table 3 were not significantly different. The optimal conditions obtained from the statistically based experimental design were confirmed by a verification experiment conducted separately. The verification results showed 88.7 mg/L of nisin and 20.5 g/L of lactic acid being obtained under the conditions of 7.32 g/L of CSS and 11.52 g/L of PS. These results were very close to the predicted values of 89.2 mg/L of nisin and 21.0 g/L of lactic acid. In addition, the nisin result also agreed well with the "ceiling concentration" of nisin previously reported by Kim et al. *(22)*. Thus, simultaneous production of nisin and lactic acid is feasible.

In summary, we aimed at developing a fermentation process for the simultaneous production of lactic acid and nisin by *L. lactis* from cull potatoes. CSS and PS proved to be effective nutrient supplements for this process. The optimal values of these two nutrient sources were obtained using a statistically based experimental design. However, in terms of industrial application, this work is only the very first step necessary for bioprocess development. Further research on the improvement of nisin and lactic acid production such as the improvement of nisin and lactic acid concentration, yield, productivity, as well as the development of effective product recovery methods, is required.

## Acknowledgment

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