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Production of Plant Protein Concentrate and Yeast Biomass from Radish Greens

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Abstract

Radish green juice was used as a dual source for the production of plant protein precipitate and *Candida utilis* biomass. Precipitates ranging from 10.0 to 16.5g were obtained from a liter of radish green juice by heating at 80 ~ 100°C for 1 to 10 min or by modification of the pH of radish green juice. Crude protein content of the precipitate was between 25 and 38%. The residue remaining after protein precipitation was used in turn for the cultivation of the yeast, *C. utilis*, in order to produce yeast biomass. *C. utilis* grew well in radish green residual juice and completed growth within 24 hr at 30°C and 200rpm in shake flask experiments. Maximum dry cell weight obtainable from a liter of radish green residual juice was 19.5g, when the yeast was grown on the juice residue diluted 3 times or more with water to make sugar content be equal to or less than about 1.0%. Supplementation of 3-fold diluted radish green residual juice with yeast extract and $(NH_4)SO_4$ enhanced yeast biomass production and cell protein content significantly. Total high protein material obtainable from a liter of radish green juice was 33.0g.

Key words : radish green juice, *Candida utilis* biomass

INTRODUCTION

High quality protein sources such as microbial proteins(single cell protein ; SCP), fish protein concentrate and leaf protein concentrate have been developed to alleviate protein shortages facing mankind.

The use of fresh herbage as a raw material for the production of protein concentrates has received considerable attention over the past 200 years[1]. Plants such as alfalfa and ryegrass have been processed to obtain the juice, a liquid suspension containing soluble protein, nonprotein matter and chloroplasts[2]. Upon treatment with heat, acid, alkali or natural microorganisms the juice yielded a precipitable material of high protein and low fiber content.

There have also been world-wide efforts to produce SCP from agricultural by-products or food process wastes. Whey and molasses have been proven to be economically feasible in some countries in the world[3]. However, it has been unsuccessful in Korea to find an indigenous and readily usable carbon source available in quantities for the purpose of SCP production.

Radish green is a vegetable by-product not used efficiently and contains about 3.0% protein, 10.6% non-fibrous sugars, vitamins and minerals[4]. In a previous report we found that Chinese cabbage juice was an excellent substrate for the production of *C. utilis* biomass[5]. Therefore, it was assumed that residue from radish green juice after protein extraction would support good microbial growth, since the vegetable is readily fermented by lactic acid bacteria which are known to be nutritionally fastidious. Especially significant is the sugar content of

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radish greens, because sugar serves as an important nutrient for microbial growth.

In the present study, we examined the potential of radish greens, which is a renewable resource and can be produced in great quantities as an agricultural by-product in Korea, as a novel resource for the production of plant protein precipitate and yeast biomass by growing *C. utilis* in the residue after protein precipitation from radish green juice.

MATERIALS AND METHODS

Radish greens

Radish greens were obtained from the Karack Agriculture and Marine Market during summer and fall of 1991.

Preparation of radish green juice

Washed radish greens were cut into approximately 5 cm long pieces and pureed in a Waring blender. They were then strained through two layers of cheese-cloth.

Heat precipitation of proteins from radish green juice

Forty ml aliquots of radish green juice in 2.5 x 20 cm glass test tubes were heated to 80, 90 and 100 °C in a preheated water bath at 1 min intervals for 10 min and cooled by running tap water. Precipitates were obtained by centrifuging the heated juice at 1100 x g (Hanil Centrifuge Co.) for 10 min and washed once with cooled distilled water. Washed precipitates were dried at 105° C for 16hr, before measuring the dry weight of heat-coagulable precipitates.

pH adjustment of radish green juice to obtain precipitates

The pH of 40ml aliquots of radish green juice in 100ml glass beakers were modified to 3.5, 4.0, 4.5, and 5.0 with 0.1N-HCl, 0.1N-H_2SO_4, 0.1N-lactic acid and 0.1N-acetic acid and to 8.5, 9.0, 9.5, 10.0 and 10.5 with 0.1N-NaOH and NH_4OH. The pH-adjusted radish green juices were allowed to stand for 24hr at ambient temperature. The measurement of dry weight of precipitates was the same as the method used for heat-coagulable precipitates.

Preparation of radish green residual juice for yeast cultivation

Radish green juice prepared as above was boiled at 100° C for 10 min to coagulate the heat-coagulable materials in order to prevent them from precipitating upon sterilization. The boiled and cooled radish green juice was centrifuged at 1100 x g for 10min and filtered through filter paper (Whatman # 41) to remove the heat-coagulated precipitates and other suspending solids. Clear juice so prepared was named as radish green residual juice and stored at -3° C until used.

Before each experiment, the frozen radish green residual juice was thawed at ambient temperature, and was used as it was, or aliquots were diluted to yield desired sugar contents. Nutrients such as glucose (2.0%), ammonium sulfate (0.2%), yeast extract (0.2%) and peptone (0.2%) were added separately to 3-fold diluted radish green residual juice before sterilization to test the supplementary effect of each of the nutrients on the growth of *C. utilis* and on the production of protein by the yeast.

Microorganism and growth conditions

Candida utilis ATCC 42416 obtained from the Korea Federation of Culture Collection, Seoul, was maintained on yeast extract (0.3%) -malt extract (0.3%) -peptone (0.5%) -glucose (2.0%) -agar (1.5%) slants. Inoculum was prepared by shake flask culture at 30° C and 200rpm (Low Temperature Shaking Incubator KSI-200L, Korea Instruments Co.) in media similar to those used in the fermentation studies.

Growth of the yeast

Radish green residual juice (undiluted or diluted , nutrient supplemented or unsupplemented) was dispensed into a 500ml flask in 100ml amounts and sterilized. The flasks were inoculated with 1ml of the inoculum grown for 24hr in appropriate media and shaken at 200rpm (Low Temperature Shaking Incubator) at 30° C.

Analyses

Absorbance at 620nm (Spectronic 20D, Milton-Roy Co., Rochester, N.Y.) was employed to measure the growth of the yeast. For dry cell weight measurements, aliquots of the growth medium were sampled at 4hr intervals and centrifuged at 1100 X g for 10min. Yeast cells washed once with cooled distilled water were transferred to a preweighed weighing pan and dried at 105° C for 16hr. pH was measured at 4 hr intervals using a pH meter (Dongwoo Medical Co.). Total sugar and soluble solids contents were measured by anthrone method[6] and by a refractometer (Atago Hand Refractometer, Atago Co., Japan), respectively. Crude protein contents of radish green juice precipitates and dried yeast cells were obtained by multiplying 6.25 to nitrogen contents measured by microKjeldahl technique[7].

RESULTS AND DISCUSSION

Precipitation of radish green protein by heat

The amount of heat-coagulated precipitate did not vary much, when one kind of radish green juice (6° Brix with 3.8% total sugar content) was heated at 80, 90 and 100° C, ranging from 10.0 to 12.5g/l of radish green juice, regardless of the length of heating time (Table 1). However, a somewhat higher amount of precipitate was observed when the juice was heated at 100° C.

Heat-coagulated precipitates from juices of 15 different kinds of radish greens ranged from 10.3 to 16.5g/l (average = 13.5g/l), and the crude protein contents of the dried precipitates were between 21% and 34% (average =27.0%) when the juices were heated at 100° C for 10 min (Fig. 1). The differences in the amount of precipitate and in the crude protein contents could be due to the differences in radish varieties, the cultural conditions or the post-harvest conditions. It was found that 13.5g/l of heat coagulable precipitate with 30% crude protein content can be obtained from radish green juices which otherwise are mostly wasted as an agricultural by-product.

Precipitation of proteins by pH adjustment

The pH of radish green juice was adjusted with acids and alkalis to obtain acid- or alkali-coagulable proteins as precipitates. As in the case of heat precipitation, there was only a slight difference in the amount of precipitates obtainable by pH adjustment. pH adjustment by lactic acid showed the highest amount of precipitation among those acid-

Table 1. Precipitation of heat-coagulated materials (g/l) during heating the juice from a single kind of radish green at 80, 90 and 100° C for 1 to 10 min

Temperature (° C)	Heating time (min)									
	1	2	3	4	5	6	7	8	9	10
80	10.8	10.5	11.0	10.5	10.3	12.0	13.0	12.0	11.5	12.3
90	11.3	10.5	11.0	10.0	10.5	10.8	11.5	10.0	12.3	10.5
100	11.5	12.3	12.3	11.5	12.5	11.5	11.0	12.5	10.3	12.3

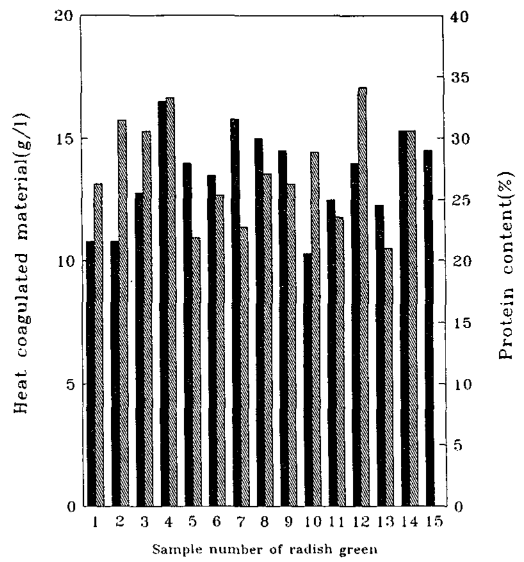

Fig. 1. Heat-coagulated precipitates of radish green juices and their protein contents.

■ : Heat-coagulated material, ▨ : Protein contents.

treated samples, regardless of pH values (Table 2). In the case of the radish juices with pH adjusted to the alkaline side, the amount of precipitate was higher when pH was adjusted with sodium hydroxide than with ammonium hydroxide (Table 2).

Growth of *C. utilis* on radish green residual juice

C. utilis was grown on undiluted radish green residual juice and on YMPGB for the purpose of comparison (Fig. 2). The yeast grew equally well on both of the media. This result was the same as the previous report that *C. utilis* had a lag phase when it was grown in undiluted Chinese radish juice but not in YMPGB[5]. Maximum absorbances attained were comparable between the two media[5]. In this study the final absorbances were even higher when *C. utilis* was grown in radish green residual juice than in YMPGB (Fig. 2). This result apparently implies that the radish green residual juice was a good substrate for the growth of *C. utilis*.

Table 2. Precipitation of proteins (g/l) from radish green juice by pH adjustment with acids and alkalis

pH	Acids				Alkalis	
	HCl	H_2SO_4	Acetic acid	Lactic acid	NH_4OH	NaOH
3.5	12.0	10.3	10.0	12.0		
4.0	11.3	10.5	10.8	12.0		
4.5	10.3	10.0	10.8	11.8		
5.0	10.3	10.0	11.0	11.8		
8.5					10.0	11.3
9.0					11.0	11.8
9.5					10.3	12.5
10.0					10.8	12.8
10.5					10.6	13.0

Fig. 3 shows the growth curves of *C. utilis* on undiluted and 2-, 5- and 8- fold diluted radish green residual juice. Yeast growth on undilted and 2- to 10-fold diluted radish green residual juice are as in Table 3. From these results it was found that radish green residual juice contains growth inhibitory substance(s) against *C. utilis* like cabbage juice[5] and alfalfa residual juice[8]. There was no difference in growth rate in the diluted radish green residual juice, regardless of the extent of dilution, as long as radish green residual juice was diluted up to 7 times (Fig. 3).

Maximum dry cell weight attainable from unit

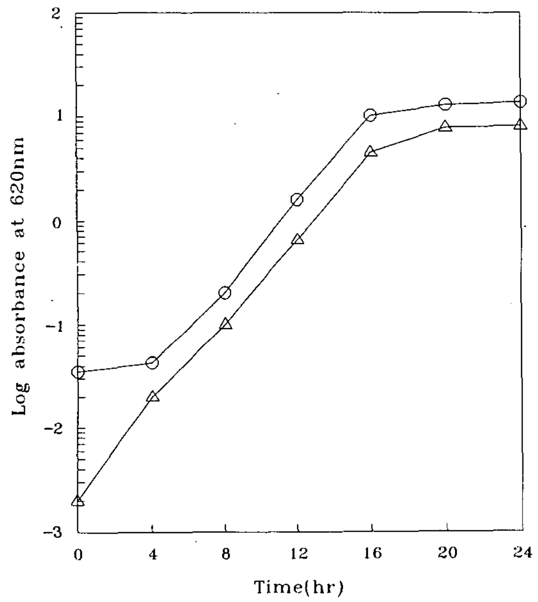

Fig. 2. Growth(OD_{620}) of *Candida utilis* on undiluted radish green residual juice and YMPC broth.
-○- : undiluted radish green residual juice, -△- : YMPG broth.

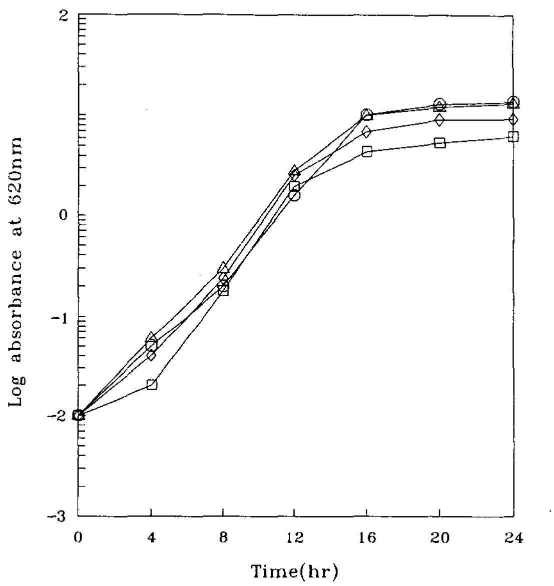

Fig. 3. Growth(OD_{620}) of *Candida utilis* on radish green residual juice of different strength.
-○- : undiluted, -△- : 2-fold, -◇- : 5-fold, -□- : 8-fold.

volume of radish green residual juice was accomplished when the yeast was grown on the residual juice diluted more than 3 times (Table 4) to make a total sugar content be equal to 1.0% or less. Most or all of the sugars in the radish green residual juice or diluted radish green residual juices were depleted in about 20hrs of cultivation (Fig. 3) regardless of dilution. From the Fig. 5, *C. utilis* completed its growth within 20hrs and pH decreased as the yeast grew. pH increased after the 20th hr, when the yeast almost completed its growth. This can be explained in that, during earlier phase of the cultivation, organic acids were produced from sugars as metabolic by-products, which in turn were used by *C. utilis* as carbon sources, as fermentable sugars were depleted. As radish green residual juice was diluted up to 5 times the cell yield was increased dramatically (Fig. 4). Further dilution did not improve either one

Table 3. Growth (OD_{620})* of *Candida utilis* on undiluted and 2-to 10-fold diluted radish green juice (DRGJ)

	Cultivation time (hr)						
	0	4	8	12	16	20	24
Undiluted juice (Control)	0.01	0.05	0.20	1.59	10.00	12.70	13.50
2X DRGJ	0.01	0.06	0.30	2.81	9.89	11.93	12.98
3X DRGJ	0.01	0.05	0.33	2.73	9.21	10.87	11.34
4X DRGJ	0.01	0.03	0.20	2.66	7.31	9.10	9.56
5X DRGJ	0.01	0.04	0.24	2.54	6.84	8.95	9.08
6X DRGJ	0.01	0.02	0.23	2.55	5.93	6.97	7.16
7X DRGJ	0.01	0.03	0.24	2.53	5.42	6.27	6.89
8X DRGJ	0.01	0.02	0.18	1.96	4.36	5.29	6.10
9X DRGJ	0.01	0.01	0.16	1.73	3.69	4.87	5.34
10X DRGJ	0.01	0.02	0.15	1.34	3.63	4.55	5.05

*Absorbances are corrected for 0 time value

Table 4. Effects of nutrient addition to 3X diluted radish green residual juice (DRGJ) on the growth of *Candida utilis*, dry cell weight production and protein contents of dry cell mass

	Dry cell weight (g / l) from 3X DRGJ	Dry cell weight (g / l) from undiluted RGJ	Crude protein content (%)
Three-fold DRGJ (3X DRGJ)	4.4	13.2	25.4
3X DRGJ + glucose (2%)	4.0	12.0	24.1
3X DRGJ + peptone (0.2%)	4.8	14.4	31.5
3X DRGJ + yeast extract (0.2%)	5.2	15.6	35.4
3X DRGJ + $(NH_4)_2SO_4$ (0.2%)	5.2	15.6	45.1

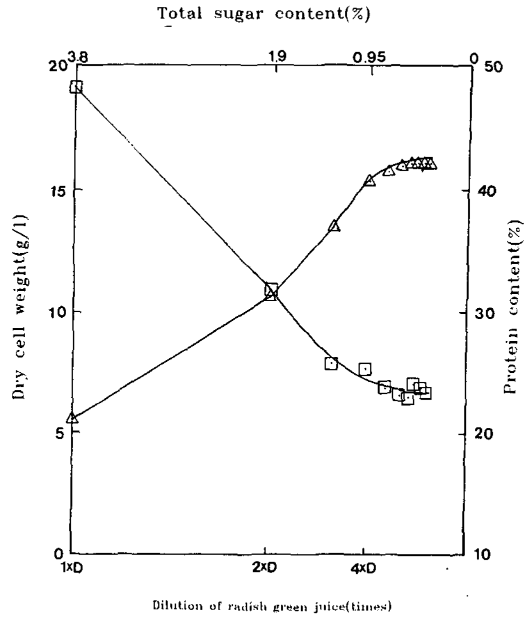

Fig. 4. Yeast cell production and crude protein content of dry biomass on diluted radish green juices.
-△- : dry cell weight, -□- : protein contents.

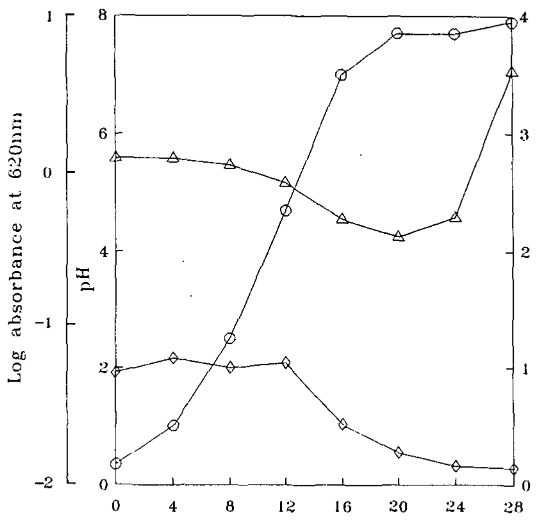

Fig. 5. Time-course of changes of pH, total sugar and cell growth on 3-fold dilluted radish green juice.
-△- : pH, -◇- : total sugar, -○- : absorbance.

of the fermentation parameters. From the view point of operational costs and efficiency of sugar utilization, diluting radish green residual juice of 3.0% total sugar content 3 times was found to be optimal for the maximum production of yeast biomass.

Effect of supplementary nutrients

Table 4 and Fig. 6 show the effects of various supplementary nutrients on the growth, the final dry cell weight and the protein content of the dried ceii of *C. utilis* grown on 3-fold diluted radish green juice. There was not much difference noted in the growth rate (Fig. 6), but there were many differences in the attainable final dry cell weight (Table 4). There was more or less a 20% difference in dry cell weight production when nutrients other than glucose were supplemented to 3-fold diluted radish green residual juice (Table 4).

Nitrogenous nutrients such as yeast extract and ammonium sulfate increased cell protein content by up to 18%. The enhancement of dry cell weight production and cell protein content by supplementing such nitrogenous nutrients as yeast extract and ammonium sulfate was significant compared to the amounts of nutrients supplemented.

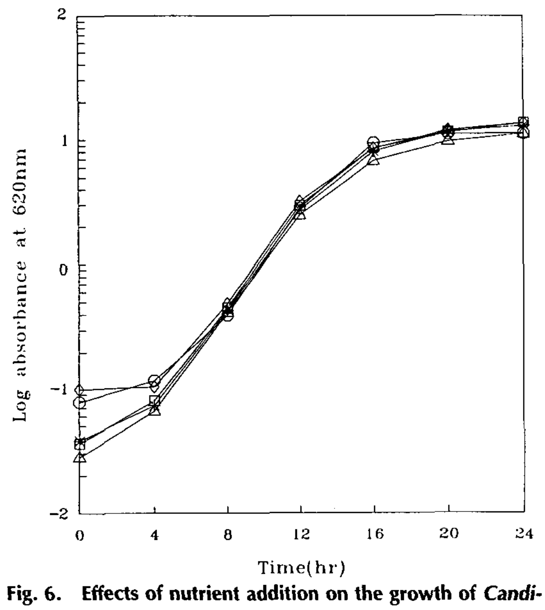

Fig. 6. Effects of nutrient addition on the growth of *Candida utilis*.
-○- : 3-fold dilution, -△- : glucose 2%, -◇- : peptone 0.2%, -□- : yeast extract 0.23%, - * - : $(NH_4)_2SO_4$ 0.2%

REFERENCES

1. Edwards, R. H., Miller, R. E., de Fremery, D., Knuckles, B. E., Bickoff, E. M. and Kohler, G. O. : Pilot plant production of an edible white fraction leaf protein concentrate from alfalfa. *J. Agric. Food Chem.*, **23**(4), 620(1975)
2. Pirie, N. W. : Leaf protein as a human food. *Science*, **152**, 1701(1966)
3. Smith, J. E. : *Biotechnology*, 2nd ed., Edward Arnold, London, p.14(1988)
4. Rural Development Administration : Food composition table. 3rd edition, Rural Nutrition Institute, p. 34 (1986)
5. Lee, N. S. and Kyung, K. H. : Single cell protein production from Chinese cabbage juice. *Korean J. Food Sci. Technol.*, **23**(5), 646(1991)
6. Morris, D. L. : Colorimetric determination of total carbohydrates. *Science*, **107**, 254(1948)
7. A.O.A.C. : *Official methods of analysis*, 14th ed., Association of Official Analytical Chemists, Washington, D.C., p.16(1984)
8. Okagbue, R. N. and Lewis, M. J. : Use of alfalfa residual juice as a substrate for propagation of red yeast *Phaffia rhodozyma*. *Appl. Microbiol. Biotechnol.*, **20**, 33(1984)

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무청즙액을 이용한 녹엽단백질과 효모균체의 생산

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요 약

무청즙액을 식물녹엽단백질의 생산과 효모의 균체생산을 위한 두가지 목적으로 이용하는 연구를 하였다. 무청즙액을 80~100°C로 가열하거나 pH를 조정하여 침전시켰을 때 침전물의 양은 모두 무청즙액 1리터당 10.0~16.5g(평균 13.5g)이었고 이 침전물의 조단백질 함량은 25~30%이었다. 단백질을 침전시켜 제거한 여액은 전당의 함량이 1.0%정도 되게 희석하여 효모 *Candida utilis*를 배양하였을 때 무청 여액원액 1리터당 최고 19.5g의 효모균체를 생산할 수 있었다. 따라서 무청즙액 1리터로부터 생산할 수 있는 고단백질자원은 33g이었다.