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Yeast Single-Cell Protein Production Using Potato Processing Waste Water

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Four species of yeast, *Saccharomyces cerevisiae, Candida utilis, Saccharomycopsis fibuligera,* and *Schwanniomyces castellii* were evaluated for their ability to bioconvert potato processing waste water into microbial protein and the resulting single-cell proteins were evaluated as protein sources for rainbow trout, using *in vitro* analyses. The studies indicated that *Schwanniomyces castellii*, which utilizes starch dircetly and converts it into cell mass efficiently, was suitable for the bioconversion. In the single-stage continuous bioconversion, the yield *S. castellii* cell mass, which contained approximately 37% protein, was 77%, at dilution rate 0.25 h^{-1}. Reduction of total carbohydrate was 81%. During batch fermentations, cell mass yield was about 72% and total carbohydrate reduction was 81%. Among the yeasts tested, *S. castellii* possessed the most fragile cell wall and had a favorable amino acid profile for salmonid fish; protein score of 86% (Met). In an *in vitro* pepsin digestibility test 80% digestibility (23~38% above control) was observed when cells were pre-heated in a steam bath for 30 min. Results presented should be regarded as being preliminary in nature because they were derived from single experiments.

With increasing pressure on the world's agricultural and fishery resources from an expanding population, the search for new and abundant protein sources for human consumption has turned to single-cell proteins (SCP). Studies of the nutritive value of SCP in experimental and production animal species indicate SCP to be a source of protein comparable to casein, and having greater nutritive quality with added methionine (26). Accordingly, SCP may play an important role in meeting the world's demand for food and feed protein. One of the most worthwhile aspects of SCP production is that it can be produced from waste materials.

The State of Idaho produces approximately 4.5 million tons of potatoes per year which accounts for 32% of the total US production (8). About one half of the potato crop is processed into various food products. During potato processing, approximately 40% of the potato is lost to various waste-water streams (1). These waste-waters could be employed to produce valuable byproducts such as single-cell protein, and concurrently reduce the biological oxygen demand of the waste streams. Yeast SCP could potentially serve as a protein source for Idaho's rainbow trout aquaculture industry.

Much research investigating SCP, the use yeasts as SCP, and the use of starch-containing substrate for SCP production has been documented (1, 5, 13, 18, and 25). However, these researchers did not report SCP amino acid profiles. The yeast SCP amino acid profile was found in few (15, 21, and 27) but these SCP grown other than potato starch as the substrate.

Research investigating SCP as a trout feed protein supplement has been limited. However, several reports have demonstrated that yeast SCP may be an appropriate protein source for trout (4, 16 and 27). However; different yeast species and/or medium were used to produce the SCPs (i.e., Brewer's yeast or petro-yeast).

In this work, simulated potato blanching water was used as substrate for the production of various yeast SCPs. The cell yields, for each species of yeast based on consumed carbohydrate were compared. Crude protein, amino acid content and amino acid profile of the yeasts were determined. Yeast SCP bioconversions having potential for use as trout protein supplements were

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identified based upon amino acid profiles, estimated protein score for the trout, and calculated PER of the yeast SCPs.

MATERIALS AND METHODS

Yeast Strains

Non-amylolytic yeast strains employed for this experiment were *Saccharomyces cerevisiae* (Flocculent), ATCC 4097 and *Candida utilis*, NRRL Y-1084.

Amylolytic yeast strains empolyed for this experiment were *Saccharomycopsis fibuligera*, NRRL Y-1062 and *Schwanniomyces castellii*, ATCC 26077.

Media

Medium for starting culture was prepared 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% dextrose in tap water and sterilized by autoclaving at 121℃ for 15 min and adjusted pH at 4.8 or 5.5 with concentrated sulfuric acid.

A simulated potato blanching water served as the fermentation substrate. Fresh potatoes, 7.2 kg, were peeled and diced, mixed with 28 liter of tap water in a stainless steel vessel, and the mixture extracted by autoclaving at 121℃ for 30 min. While hot, the extract was filtered through Miracloth (Chicopee Mills, Inc., Milltown, NJ) and the pH adjusted to 4.8 or 5.5 with concentrated sulfuric acid. Thirty to 35 liter portions of the filtered extract were sterilized in 50 liter polypropylene carboys by autoclaving at 121℃ for 100 min.

For non-amylolytic yeasts, the medium prepared as above, but with the following modifications; after filtering the medium was treated for 30 min with α-amylase (4 ml of Taka-Therm, 170,000 mwu/g, Miles Laboratories, Inc., Elkhart, IN., per 100 liter medium). After α-amylase treatment, the medium was cooled to 60℃ and adjusted pH to 4.25 with concentrated sulfuric acid, treated for 60 min with glucoamylase (6 ml of Diazyme, 100 DU/ml, Miles Laboratories, per 100 liter of medium), and sterilized as above.

Fermentation Procedure

A starting culture was initially grown in complex medium at 30℃ with shaking at 200 rpm for 18 hours (overnight). The culture was transferred to a flask of potato extract medium at pH 5.5 and incubated for 8~10 hours. The resulting culture served as the fermentor inoculum at 0.05% to 0.1% of fermentor working volume.

Batch fermentations were performed in an 80 liter fermentor (Lab-Line Bioengineering, LTD., Melrose Park, IL). The fermentation parameters were: pH 4.8 or 5.5, oxygen above 50% saturation, and 30℃. Continuous fermentations were performed in Microferm fermentors (New Brunswick Scientific Co., Edison, NJ). Culture growth was monitored and a 25 ml sample was collected at four hour intervals and assayed for turbidity, cell mass, carbohydrate, and enzyme activity.

The turbidity was measured using Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., Inc., New York, NY) equipped with green filter (spectral range; 500~570 nm). The assay blank was uninoculated medium.

The cell mass was collected from two 10 ml of samples via filtration through pre-weighed 0.8 μm membrane filters (Metricel, Gelman Sciences, Inc., Ann Arbor, MI). The filtered membranes were dried in an oven at 65℃ for two days, cooled in a desiccator and weighed. The filtrate from the cell mass determination was used for measuring pH, carbohydrate content, and amylase activity.

Carbohydrate Assay

Total carbohydrate (TC) assays were performed based on the method of Pirt and Whelan (20) and Chaplin (6).

Amylase Assay

The amylase enzyme activity was measured as described by Lemmel *et al.* (13) and Admassu *et al.* (1) and enzyme unit was employed as the later's.

Protein and Amino Acid Analysis

During fermentation, cells were collected in a 20 liter container (Nalgene) which was held in a refrigerated (2~4℃) harvest cart. Cells were separated from the fermentation broth by centrifugation using a Cepa semicontinuous centrifuge (Carl Padberg, Lahr/Boden, Germany), or by a lab-scale ultrafiltration system (model DC 10L/DC 10LA Amicon, Danvers, MA) equipped with a H5MP01-43 cartridge (0.1 μm cut off). After separation from the fermentation broth, the cells were frozen, dried by lyophilization, and stored at −70℃ until assayed.

To treat test samples for digestibility assays, centrifuged cells were placed in a tray, an equal volume of water was added, the pH adjusted to 9.5 with 3.5 N NaOH, and treated in steam bath (90℃) for 30 min according to a modified method of Freeman *et al.* (10), and dried by lyophilization. For other treatments, cells were oven dried at 75℃ for 40 hours or exposed to microwave heating for 7 min and oven dried at 75℃ for 40 hours. The latter two treatments were at pH around 5.5 with no alkali added.

For crude protein and amino acid determinations, the lyophilized SCP products were ground to fine powder and then assayed. Crude protein was determined by the micro-Kjeldahl method as described by AOAC (3).

Amino acid assays were performed by high performance liquid chromatography (HPLC) using a Hewlett-Packard (Avondale, PA) HP 1090 chromatograph equipped with an ODS 3 μm (60×4.6 mm) column according to the methods of Shuster (23) with minor modifications

such as the solvent elutions.

Tryptophan assay was conducted as described by Delhaye and Landry (7). Cysteine, cystine and methionine in the sample were assayed as described by Moore (17).

Additional amino acid analyses were performed by Bioanalytical Laboratory at Washington State University, Pullman, WA. Where a Beckman 121 Amino Acid Analyzer (Beckman Instruments, Fullerton, CA) was employed.

Cell Fragility Test and *In Vitro* Digestibility

Cell fragility tests were conducted by sonicating a 15 ml portion of cells in the exponential growth phase, for 0.5, 1, 2, 4, 8, and 16 min with Biosonik III (Bronwill Scientific. Rochester, NY) at an intensity level 80. Plate counts, expressed as colony forming unit (CFU), were conducted using the complex agar medium incubated at 30℃ for 24 to 36 hours. Direct microscopic counts of intact yeast cells in serial decimal dilutions of samples were performed with a hematocytometer at 400 X magnificiation.

Pepsin digestibility tests were conducted by treating 1.0 g of dried cell samples suspended in 200 ml of 0.075 N HCl with 10 mg pepsin (3,500 units/mg) and incubating at 37℃ for 0, 1, 2, 4, 8, 12, and 16 hours in a Bioflo fermentation jar (New Brunswick Scientific. Edison, NJ) with 100 rpm agitation. At the end of each digestion period, the mixture was adjusted to pH 8.0 to stop pepsin activity and the cell mass recovered by centrifugation at 9,000×g for 30 min. The cell mass residue was lyophilized and weighed and the crude protein content was determined by micro Kjeldahl assay.

Calculation Method of PER

Alsmeyer *et al.* (2) developed three equations for predicting the PER of a protein source. The third equation was employed as follows:

$$PER = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr)$$

Computing the DC-PER followed the AOAC (3) method. For trout feed protein quality calculations, the National Research Council (NRC) amino acid requirements for the trout were substituted into the equation and the casein PER used was 1.97 instead of 2.5 based on the findings of Atack and Matty (4).

$$\% NRC = [(g\ aa/16\ g\ N)/NRC\ std] \times \% \text{ digestibility}$$

where NRC standard for trout is assumed to be: Arg=6.0, His=1.8, Lys=5.0, Met+Cys=4.0, Thr=2.2, Ile=2.2, Leu=3.9, Val=3.2, Phe+Tyr=5.1, Trp=0.5.

RESULTS AND DISCUSSION

Fermentation and Medium

Simulated potato blanching water contained total carbohydrate content of 6.74± 0.74 g/l, reducing sugar content of 0.47± 0.07 g/l, and starch content of 6.26± 0.81 g/l. For fermentations with non-amylolytic yeasts, the medium was treated with α-amylase and glucoamylase which resulted in a total carbohydrate content 6.5 g/l; similar to the total carbohydrate content of unhydrolyzed simulated potato blanching water. Reducing sugar content of the enzyme treated medium increased to 4.61 g/l. Starch content of the medium was 1.89 g/l indicating that the starch hydrolysis was incomplete or that limit dextran were present in the hydrolyzed medium.

Cell mass of each organism at different dilution rates was expressed as yield (g dry yeast cell produced per g consumed carbohydrate). The *S. fibuligera* yield increased with increasing dilution rate, however, the *S. castellii* yield decreased at dilution rates of 0.2 h^{-1} and 0.3 h^{-1} when compared to the yield at 0.1 h^{-1} but increased at 0.4 h^{-1}. The *S. cerevisiae* yield decreased slowly with increasing dilution rate. At a near optimal operation level, D=0.2 h^{-1}, the yields for *S. cerevisiae*, *S. Fibuligera* and *S. castellii*, were 1.15, 1.01, and 0.84, respectively (Fig. 1).

Total carbohydrate consumption (reduction) was approximately 60% at all dilution rates evaluated (Fig. 2). With the amylolytic yeasts, decreased carbohydrate reduction was observed with increasing dilution rate. With *S. fibuligera*, the carbohydrate reduction rate rapidly decreased with increasing dilution rate. At dilution rate D= 0.2 h^{-1}, the carbohydrate reductions for *S. cerevisiae*, *S. fibuligera* and *S. castellii*, were 64, 57, and 75% respectively.

The cell mass productivity (g cells/g carbohydrate consumed/h) plots (Fig. 3) showed that *S. cerevisiae* reached maximum productivity at dilution rate D=0.2 h^{-1}. Ho-

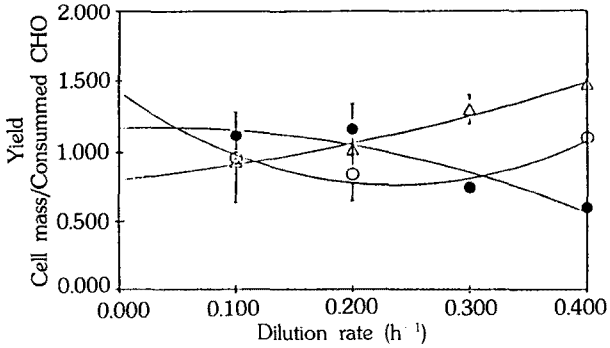

Fig. 1. Single-stage fermentation yield of cell mass.
○ *S. castellii*, ● *S. cerevisiae*, △ *S. fibuligera*

wever, the cell production rates of *S. fibuligera* and *S. castellii* increased with increasing dilution rate indicating that maximum productivity for these yeasts will probably be otained at dilution rates greater than D=0.4 h^{-1}. The productivity of amylolytic yeasts were similar, but the *S. castellii* productivity was 10 to 20% greater than that of *S. fibuligera*. At dilution rates of D=0.1 h^{-1}, and D=0.4 h^{-1}, 10 and 20% differences in productivity, respectively, were observed.

The activity of amylase excreted by amylolytic yeasts was determined using cell free broth from each fermentation. *S. fibuligera* and *S. castellii* behaved similarly; however, *S. castellii* expressed two folds greater enzyme activity than did *S. fibuligera*, except dilution rate at 0.3 h^{-1} and 0.4 h^{-1} where were similar to each other (Fig. 4).

The above results indicate that *S. castellii* is a good prospect for continuous SCP production with potato waste water as a substrate. The organism utilized starch directly and exhibited good cell mass productivity and total carbohydrate reduction. The greater amylase activity of *S. castellii* supports the results of higher cell mass productivity and greater total carbohydrate reduction.

To potentially eliminate the need for centrifugation for cell recovery, a two-stage fermentation with the amylolytic yeast; *S. fibuligera* or *S. castellii*, with a flocculent strain of *S. cerevisiae* was examined. This research was out of expectation. The flocculent yeast grew slower than the amylolytic yeasts in the continuous fermentations. As a result, the amylolytic yeasts quickly dominated the fermentation to the point that *S. cerevisiae* cells, could not be detected/demonstrated.

The results for continuous fermentation for *S. castellii* at dilution rate 0.25 h^{-1}, with parameters of cell mass production, enzyme activity, and substrate consumption are as shown in Fig. 5. *S. castellii* converted carbohydrate into cell mass very effectively. The cell mass yield was 0.77 (g of dried cell per g of carbohydrate consumed) and total carbohydrate reduction was 81%. During the continuous fermentation, amylase activity varied between approximately 1.5 and 3.0 units/ml. However, cell mass production was relatively constant throughout the fermentation. This yield was greater than Boze *et al.* (5) reported for starch (Y=0.59) and glucose (Y=0.62) at concentrations of 10 g/l of medium. However, the yield was lower than Lemmel *et al.* (13) and Admassu *et al.* (1)

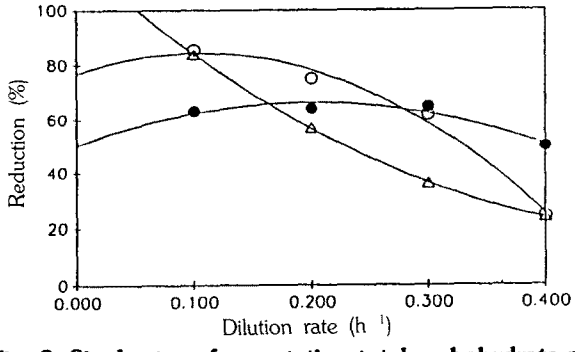

Fig. 2. Single-stage fermentation total carbohydrate reduction.
○ *S. castellii*, ● *S. cerevisiae*, △ *S. fibuligera*

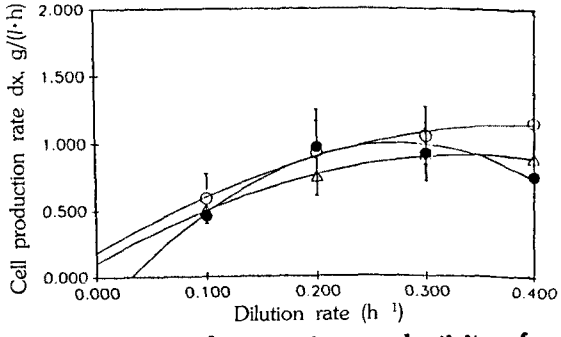

Fig. 3. Single-stage fermentation productivity of cell mass.
○ *S. castellii*, ● *S. cerevisiae*, △ *S. fibuligera*

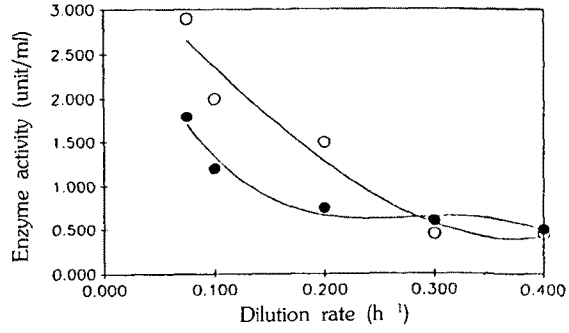

Fig. 4. Single-stage fermentation enzyme activity.
○ *S. castellii*, ● *S. fibuligera*

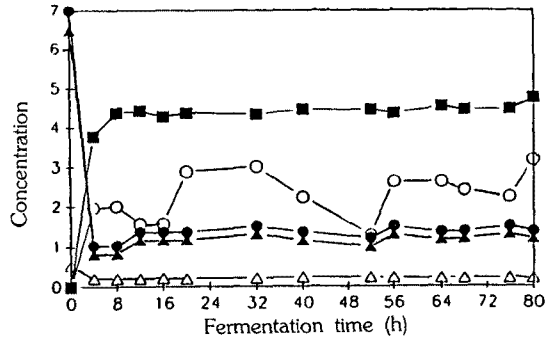

Fig. 5. Single-stage continuous fermenentation with *S. castellii* dilution rate (D)=0.25 h^{-1}.
○—○ Enzyme activity (unit/ml)
●—● Total carbohydrate (g/l)
△—△ Reducing sugar (g/l)
▲—▲ Starch (g/l)
■—■ Cell mass (g/l)

reported; 0.84 and 0.95, respectively, with the *S. fibuligera* and *C. utilis* continuous bioconversion. Results from the large-scale, batch fermentations of *S. castellii* using a Lab-line 80 liter fermentor, are presented in Fig. 6. the cell mass yield was 0.72, 5% less than with the continuous fermentation. However, total carbohydrate reduction was 81%. If the *S. castelllii* yield is calculated on the carbohydrate provided, the yield was 0.62 and 0.58 with continuous and batch fermentations, respectively. These yields were greater than the values reported by Touzi *et al.* (24), Y=0.48, with cassava flour at 15 g/l medium (fortified with ammonium sulfate, urea, potassium phosphate, and yeast extract) and greater than the yield Moresi *et al.* (18) reported, Y=0.432, for potato flour (0.6~3.1% w/v) substrate which was fortified with ammonium sulfate, potassium phosphate, and yeast extract.

Yeast SCP Amino Acid Analysis

The SCP crude protein contents were as followed; *S. cerevisiae* was highest 54%, *C. utilis* was 45%, similar to *S. castellii* which was 43% and *S. fibuligera* was lowest 35%.

The SCP protein scores based on the salmon (fingerling) amino acid requirements are shown in Table 1. *S. cerevisiae* showed the greatest score, 88%, followed by *S. castellii* at 86%. The scores for *C. utilis* and *S. fibuligera* were 81 and 63%, respectively. In all cases, limiting amino acid was methionine except for *S. cerevisiae* which was arginine. Yeast SCP are typically low in methionine (27). It appears that these SCP sources all would reuqire fortification with methionine and/or arginine for maximum utilization by salmonids.

The amino acid content of yeast SCP produced from different substrates are compared (not shown), which showed minor variations within amino acid and between substrate. Most of the amino acid assay values were obtained from ion-exchange chromatography using the auto-amino acid analyzer (Beckman 121 amino acid analyzer). HPLC assay results were used to determine the tryptophan content in some cases. It appears that the HPLC assay consistently gave higher estimates of tryptophane than were obtained with ion-exchange chromatography. In addition, HPLC data for other amino acids (not shown) also gave higher estimates than were obtained by ion-exchange chromatography.

The yeast SCPs were gererally low in sulfur containing amino acids and histidine, and high in aspartic, glutamic acids and lysine. Total amino acid content of the amylolytic yeasts was lower than that for the non-amylolytic yeasts. Approximately 5 to 20% variation in total SCP amino acid content was observed within a yeast species.

Cell Fragility, Digestibility Test, and Nutritional Values

The *S. castellii* cell wall was the most fragile, followed by *C. utilis*. The most resitant yeast was the *S. cerevisiae* (flocculent) as shown Fig. 7. Direct microscopic count and viable cell count results were similar but the effect of sonication on the viable cell counts was more pronounced.

To determine the digestibility of the *S. castellii* SCP, an *in vitro* pepsin digestibility test was performed on *S. castellii* cells treated with and without heat. The results

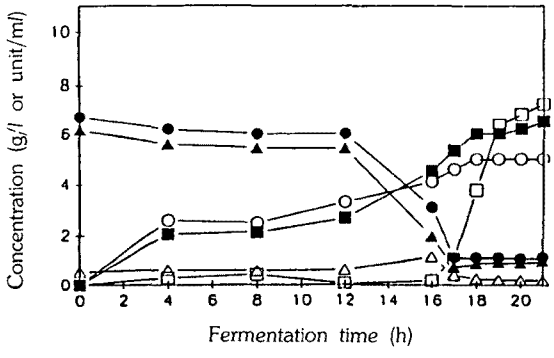

Fig. 6. Batch fermentation. 80 liter lab-line fermentator with *S. castellii*.

○−○ Turbidity (×10) ●−● Total CHO
△−△ Reducing sugar ▲−▲ Starch
□−□ Enz. activity ■−■ Cell mass

Table 1. Yeast SCP amino acid contents and it's protein score (%) for rainbow trout feed (g amino acid/100 g yeast protein)

Amino acid	Req. EAA*	*S. cerev*	*C. utilis*	*S. fibul*	*S. cast*
1. Arg.	6.0	5.26	5.91	5.17	7.69
2. His.	1.8	2.09	2.64	2.25	2.02
3. Ile.	2.2	4.97	5.57	5.42	5.16
4. Leu.	3.9	7.37	8.60	8.18	6.94
5. Lys.	5.0	8.53	6.81	7.11	8.47
6. Met.	4.0	1.72	1.64	1.62	1.59
7. Cys.	**	1.82	1.58	0.89	1.59
8. Phe.	5.1	4.37	4.51	4.38	3.98
9. Tyr.	***	3.02	3.54	3.49	2.85
10. Thr.	2.2	4.87	4.91	5.58	4.46
11. Trp.	0.5	2.71	3.67	3.57	5.27
12. Val.	3.2	5.63	6.15	5.87	5.57
Protein score (%)		88(Arg)	81(Met)	63(Met)	86(Met)

*Based on Essential Amino Acid (EAA) requirements referred from Nutrient Requirements of Trout, Salmon, and Catfish. (#11). 1973, National Academy of Sciences. Washington DC.
**Included in Methionine.
***Included in Phenylalanine.
Protein score (%): (g amino acid per 100 g yeast protein)/the specific essential amino acid requirement×100.

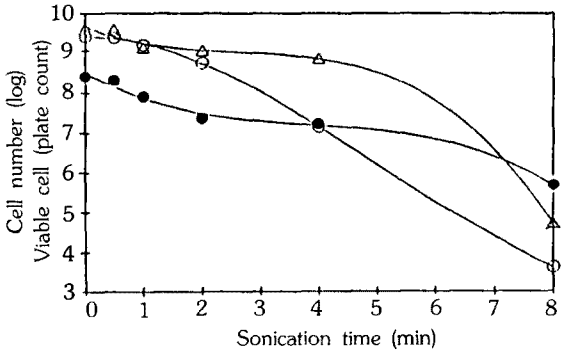

Fig. 7. Cell wall resistance against sonication. Log growth phase cell
○ *S. castellii*, ● *S. cerevisiae*, △ *C. utilis*

are shown in Table 2. Without heating, the protein was digested 57% with pepsin. However, cell protein of the control sample containing no pepsin gave a 42% digestibility value, probably due leakage of soluble protein through cell wall/membrane which was disrupted during freezing (14). Three different heat treatments produced similar results. Approximately 80% protein was digestible in the pepsin assay. In the absence of pepsin (the control), only 45% was digested/released. Heat treatment produced significantly different results when pepsin was added. However, heat treatment did not significantly affect digestibility/release of soluble protein when pepsin was omitted from the incubation mixture. Based on the results, cells heated at 75~90℃ for 20~30 min. at a pH of 9.5 would provide the best source of SCP for *in vivo* evaluation. However, if the fish feed is processed into a pellet form using a steam treatment, a heat treatment (resulting duplicate heat treatment) to enhance digestibility may not be necessary.

Hibino and Terashima (12) reported on fish digestive enzyme action on yeast cells. Chitinase activity increased approximately two fold in the stomach when trout were fed yeast SCP. Other enzymes, such as stomach pepsin, trypsin in the pyloric caeca and intestine, and amylase, β-1.3-glucanase and ribonuclease activities in the above mentioned three organs were detected in substantial amounts but stayed at the same level when trout were fed control diets or diets containing yeast SCP. Other fish tested such as eel, auy, and carp showed some differences, but they all had the enzymes necessary to digest yeast SCP. Therefore, yeast SCP could potentially be used as a feed ingredient for fish without any pre-treatment to improve digestion. However, as reported by Windell *et al.* (27), slight changes in the processing temperatures and application of freeze drying techniques may substantially increase the digestibility of these products. Their digestibility test results on brewer's yeast and brewer's single-cell protein were 76.1% and 53.3%, respectively. This range of results compares favorably with results obtained in this study.

Table 2. Results of *in vitro* pepsin digestibility test (◆) for *S. castellii* cells (Cell crude protein contents was 43.27%)

Cell Treatment	Lyophilized	Steam Bath* 90℃ for 30 min	Oven dry** 75℃ for 40 h	Microwave*** oven for 7 min
With pepsin (100 mg/200 ml):				
Residue (mg)	630.4	580.8	439.2	439.7
Protein (%)	29.4	15.0	15.9	19.4
Digested Protein[a] (%)	57.0	79.9	83.8	80.4
Without pepsin:				
Residue (mg)	769.9	746.4	697.5	664.6
Protein (%)	32.6	34.7	33.2	35.8
Digested Protein (%)	42.0	40.1	46.4	45.0

[a] Pepsin digestion: 1 g of dried cell sample in 200 ml of 0.075 N HCl without or with 10 mg of pepsin (3,500 units/mg) incubated at 37℃ for 12 h with 100 rpm agitation. pH was adjusted to stop pepsin actgivity and the cell mass recovered by centrifugation at 9,000×g. The residue was lyophilized and weighed, the crude protein content determined by micro-Kjeldahl method.
*Steam bath treatment; centrifuge harvested cells were placed in a tray and an equal volume of water was added, the pH adjusted to 9.5 with 3.5 N NaOH, and treated in a steam bath (90℃) for 30 min.
**Oven dry; centrifuge harvested cells were placed in a tray and dried in a forced air oven at 75℃ for 40 h.
***Centrifuge harvested cells were treated in amicrowave oven for 7 min and oven dried in a forced air oven at 75℃ for 40 h.

Evaluation of the biological value of SCP can expressed as the protein efficiency ratio (PER). The Table 3 listed several methods employed to estimate PER of yeast SCP for trout feed. The predicted PERs calculated as described by Alsmeyer *et al.* (2), for yeast SCP from *S. cerevisiae*, *C. utilis*, *S. fibuligera*, and *S. castellii*, were 2.27, 2.82, 2.45, and 2.26, respectively, and the value for casein was 2.0.

Discriminant computed-protein efficiency ratio (DC-PER), developed by Satterlee *et al.* (22) and adopted by Association of Official Analytical Chemists (AOAC) in 1984, was also used to estimate the protein quality. The DC-PER for each of the yeast was lower than the predicted PER, but same trend was observed. The non-amylolytic and amylolytic yeast were differentiated by DC-PERs of 2.7 and 1.9, respectively. The DC-PER for casein was 2.69 rather than 2.50, indicating that the

Table 3. Calculated and predicted protein efficiency ratio (PER) of yeast SCP

Protein Sources	*Sacchromyces cerevisiae*	*Candida utilis*	*Sacchromy. fibuligera*	*Schwannio. castellii*	Casein*
Digestibity (%)	89.97	90.09	85.36	80.31	88.25
Z-Value**	2.77	2.73	2.28	2.69	2.50
Group** for PER	4	4.	2	2	4
DC-PER**	2.70	2.69	1.96	1.86	2.69
For Salmonid[a]:					
Z-value	2.98	3.01	2.41	2.94	1.97
DC-PER (1)[b]	2.82	2.84	1.97	1.61	3.15
Predicted PER***	2.27	2.82	2.45	2.26	2.00

*Casein amino acid contents was taken from FAO: Nutritional Studies NO. 24. Food and Agricultural Organization of the United Nations. Rome, Italy (1970)

**Digestibility, Z-Value, Group and DC-PER were calculated by AOAC (1984) section 43.267.

***Predicted PER was calculated by Alsmeyer (1974) equation number 3.

[a]Calculated based on salmonid essential amino acid requriements referred from Nutrient Requirements of Trout, Salmon, and Catfish (#11). National Academy of Sciences. Wash. DC. 1973.

[b]DC-PER equation was delivered by discriminant group selection by the method.

DC-PER for casein was little inflated value. Trout feeding trials performed by Atack and Matty (4), established the actual PERs of petro-yeast and casein were 2.01 and 1.97, respectively. Considering this result and the essential amino acid requirements of salmonids, modified DC-PER calculation method was used to estimate the DC-PER for each yeast SCP. The DC-PER increased slightly except for *S. castellii* which decreased to 1.61. However, casein PER was 3.15 instead of 1.97 which indicated that this calculation greatly overestimated the PER for casein. This may indicate that the calculations developed by Satterlee *et al.* (22) for predicting protein quality in the rat probably can not be modified for salmonids. To rely upon these equations to estimate the PERs without trout feeding trials is not wise. However, if feeding trial results compare favorably to the predicted PER's the equations could be used for estimation of protein quality for the trout. In the light of this situation, the DC-PER may bee not applicable for estimating the biological quality yeast SCP for trout at present.

Selection of one organism for SCP production from potato processing waste streams based upon the assimilation of waste water carbohydrate, cell mass productivity, the complexity/simplicity of the bioconversion process, protein score and PER for the trout is difficult to assess. The data presented in this report, with the exception of the predicted PER and DC-PER, indicate that *S. castellii* may be the most appropriate organism for the production of SCP. Trout feeding trials to determine the relationship between predicted PER and DC-PER and the true PER, and to select the yeast most suitable for SCP production are necessary.

CONCLUSION

Schwanniomyces castellii appears to be a suitable microorganism for the production of SCP to serve as a source of protein for rainbow trout production using potato prcessing waste water as the substrate. *S. castellii* can utilize starch directly and convert starch into cell mass efficiently in either a single-stage continuous or batch bioconversion. The yield of cell mass was about 77%, at dilution rate 0.25 h^{-1}; the dilution rate where an appropriate balance between cell productivity and substrate utilization occurred. Reduction of total carbohydrate was 81%. During batch fermentation, cell mass yield was about 72%.

Considering the cell fragility test results and the amino acid requirements for trout, *S. castellii* possessed the most fragile cell wall and a favorable protein score, 86% (Met), among the yeast tested. *In vitro* pepsin digestibility tests demonstrated that digestibility was increased from 57% to 80% when cells were pre-heated, e.g., steamed for 30 minutes.

Biological value of yeast SCP expressed as DC-PER for the non-amylolytic yeasts, *S. cerevisiae* and *C. utilis*, was 2.82 and 2.84, and for amylolytic yeasts, *S. fibuligera* and *S. castellii*. was 1.97 and 1.61, respectively. Future trout feeding trials are required to determine the validity of the calculated PER for yeast SCP and to determine which organism is most appropriate as a protein source for trout aquaculture. The preliminary data presented in this report suggest that the most appropriate organism for SCP production may by *S. castellii*.

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