

Submerged Yeast Fermentation of Cheese Whey for Protein Production and Nutritional Profile Analysis

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Abstract: In this study, ten whey samples collected from dairy industries in Rasht (Iran). Five lactose fermentative yeasts strains (designated A1 to A5) were isolated. Beta-galactosidase activity in the yeast strains showed that strain of *Kluyveromyces marxianus* designated as A2 had highest enzyme activity (up to 9012 EU/mL) and the most SCP production from whey with the yield of 12.68 g/L. Ammonium sulfate as nitrogen source had an increasing effect on biomass yield up to 30%. Crude fiber, lipids, carbohydrates and ash content of isolate dry cells were found to be 4.9, 7.23, 33.19 and 14.05%, respectively. The true protein content based on nitrogen fractionation procedure was 29.25%. The yeast biomass recovering by ultrafiltration reduced the total COD to 96.26% of its initial value in the raw whey.

Key words: COD, *Kluyveromyces marxianus*, protein, SCP, whey, yeast

INTRODUCTION

Cheese whey is a greenish-yellow liquid product from the cheese-making process. Because of its high organic content with high BOD, whey dumped directly to the environment is causing serious contamination problems (Cristiani-Urbina *et al.*, 2000; Ghaly and Kamal, 2004). To overcome these complications intense recent research attempts try to deal with the problem focusing on the development of technologies that employ whey as raw material to produce feeds or chemicals of added value (Koutinas *et al.*, 2007). The use of whey for the production of yeast biomass have the advantages that it is a simple treatment process, and the final discharge of the whey is facilitated since the pollutant load is significantly reduced and the whey lactose is converted into yeast biomass. Indeed, high production rates and protein yields as well as ease of production control makes SCP more attractive as a protein source compared with conventional plant and animal sources. Since the major constituent of the whey is lactose, the selected organism must be able to readily metabolize lactose SCP could be produced from whey, with employing of yeasts from different species including *Kluyveromyces*, *Candida*, and *Trichosporon*, which are normally capable of metabolizing lactose (Mansour *et al.*, 1993; Ghaly *et al.*, 2003). The most important characteristic of these single-celled organisms is their high protein content, ranging from about 40 to 80% of their dry weight on a crude protein basis. Also, their protein tends to be of high

quality, more closely resembling animal protein than plant protein, and is generally readily available nutritionally (Paul *et al.*, 2002; Schultz *et al.*, 2006).

In this study, a number of lactose fermentative yeasts strains were isolated and identified. The ability of the strains for consumption of lactose and COD removal was evaluated. The aims of this study were to investigate the effectiveness of batch submerged fermentation of the yeast in cheese whey for the production of SCP and product qualitative and quantitative analysis.

MATERIALS AND METHODS

Cheese whey was obtained from the dairy producing factories in Rasht. Some characteristics of the cheese whey used in this study are presented in Table 1.

Sampling and Isolation of yeast strains: Ten samples containing whey, yogurt and cheese were collected from Rasht dairy producing centers in Rasht. The samples collected in sterile 200 mL bottles and brought to the laboratory in a cooler box. For enrichment of yeast samples, 5 mL (or 5 g) of samples were inoculated in 50 mL of Yeast-Malt extract Broth (YMB) medium with the following composition: 5 g/L peptone, 3 g/L malt extract, 3 g/L yeast extract, 10 g/L glucose and 0.1 g/L chloramphenicol, and incubated at 34°C for 24 h with constant shaking of 180 rpm. After incubation, samples were taken and microscopically observed for existence. The media YMA containing 1.5-2% agar were used for

Table 1: Some characteristics of the raw cheese whey used in the study

Characteristics	Measured value
Specific gravity (g/mL)	1.028
Lactose (%)	4.900
Protein (%)	0.900
Fat (%)	0.050
pH	5.700

yeast strains isolation. Colonies with distinct morphological differences were selected and purified by streaking on agar plates with YM medium at 34°C and then slants stored at 4°C.

Identification of yeast strains: To isolate the lactose fermentative yeast strains capable of, the isolated yeasts were cultured in Durham tubes containing 2% (w/v) solution of lactose. Then the positive yeast strains for lactose fermentation were identified using the standard taxonomic key (Kurtzman and Fell, 1998). The identification based on different chemical tests including the fermentation of sugars, liquid assimilation of carbon compounds, liquid assimilation of nitrogen compounds, growth at 37 and 40°C, growth in 50% glucose and urease.

Fermentation: A 2 L stirred tank bioreactor (INFORS, Switzerland) with a working volume of 1 L was employed in this experiment. 900 ml of sterilized cheese whey medium was transferred to the bioreactor which was then inoculated with 100 mL of microbial suspension. The bioreactor was maintained in conditions: 34°C, pH = 5, aeration 1 vvm, and 400 rpm. After inoculation the bioreactor was set to work for 28 h and samples were drawn every 2 h.

To eliminate whey's proteins, it was clarified by heating at 100°C for 15 min after adjusting the pH to 4.6 (isoelectric pH). After settling of the whey's proteins, the supernatant was ultrafiltered and the greenish yellow liquid was sterilized at 115°C for 10 min.

We also studied effect of nitrogen supplementation on SCP production with applying ammonium sulfate as the nitrogen source. For this purpose, the limpid liquid was supplemented with 0.5% (w/v) ammonium sulfate.

Analytical: The cell dry weight was determined as previous work (Anvari and Khayati, 2009). A certain volume of the culture broth centrifuged at 5000 rpm for 15 min, after washing the biomass with Ringer serum, it was precipitated for a second time before measuring dry weight, followed by drying of the cells at 105±1°C for 2-3h. In addition, the optical density of the culture was measured at 620 nm in a spectrophotometer (Bioscience, Ultrospec 2100 pro (UV/Vis) photometer). ONPG (O-Nitrophenyl-β-D-galactopyranoside) assay was applied for calculation of β-galactosidase (Nahvi and Moeni, 2004).

Nitrogen contents of dried cells were determined by microkjeldahl procedure (AOAC, 1985). Protein was calculated from total nitrogen by multiplying with a conversion factor of 6.25. Fat was extracted with a mixture of chloroform-methanol (1:1, v/v) according to AOAC (1985) method. The fat content in the extract was estimated gravimetrically after evaporation of the solvent at 100°C. The phenol-sulfuric acid method (Chaplin, 1994) was used to determine the carbohydrate content.

The amino acid composition assay of the SCP from whey was performed by hydrolyzing the dried cells with 6 N HCl at 110°C for 22 h and analyzing the hydrolysates by LKB Alpha plus amino acid analyzer. Ash content was determined by the procedure described in AOAC (1985) 5 g of the dried cells.

The supernatant was used for lactose and COD (chemical oxygen demand) measurement. The lactose concentration was determined using sugar analyzer (YSI Model 27, Yellow Springs, OH). Chemical oxygen demand was determined according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA, 1995).

Cell growth rate and enumeration of yeast cell population during growth and fermentation were observed with pour plate counts on potato dextrose agar (Difco). Serial dilutions were made in 0.1% phosphate buffer, and plates were incubated at 34°C and counted after 24 h. The specific growth rate (μ) was determined in batch culture during logarithmic growth by the formula:

$$\mu = \frac{\ln(N_2/N_1)}{(t_2 - t_1)}$$

where N_1 = number of yeast cells at t_1 , N_2 = number of yeast cells at t_2 and $t_2 - t_1$ = cultivation time in hours.

All experiments were repeated at least three times in order to acquire high accuracy. This procedure gave consistent and reproducible results.

All of experiments were done in Sciences Faculty Laboratory of Islamic Azad University in 2009-2010.

RESULTS AND DISCUSSION

In this study, 14 different yeast strains were isolated by using Yeast-Malt extract Broth (YMB) medium and Yeast-Malt extract Agar (YMA). The isolates were examined for lactose fermentation ability. Among them, 5 strains (A1-A5) were found capable of lactose fermentation (Table 2). These strains were identified by morphological and physiological properties using the standard taxonomic key as outlined and several chemical tests including fermentation of different sugars, liquid assimilation of carbon and nitrogen compounds, growth at 37 and 40°C, growth in 50% glucose, and their urase activity (Kurtzman and Fell, 1998; Gadaga *et al.*, 2000).

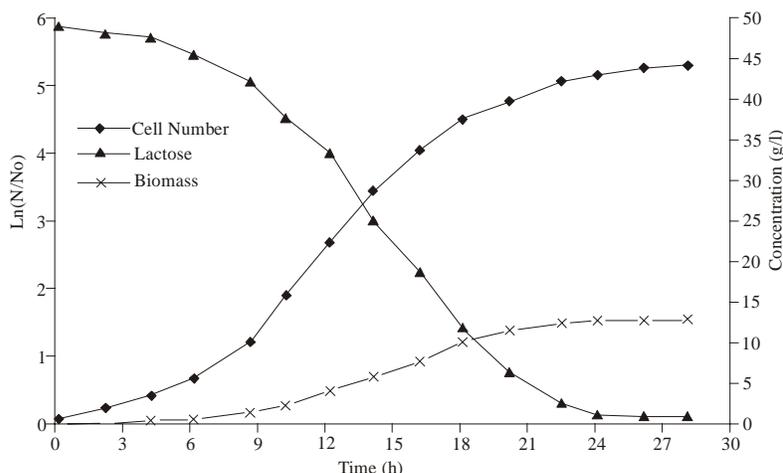


Fig. 1: Lactose consumption during the growth of *K. marxianus* on chess whey

The enzyme lactase (β -galactosidase) hydrolyzes lactose into glucose and galactose which are more soluble, and sweeter than lactose. This enzyme is produced by many microorganisms that use lactose as energy source (In and Jin, 1998). Enzyme activity of beta-galactosidase in yeast strains was measured (Table 2). Among 5 yeast strains, the A2 strain (*Kluyveromyces marxianus*) was found to have the highest enzyme activity, 9012 EU/mL. Also the A4 strain (*Kluyveromyces lactis*) was showed high enzyme activity, 6396 EU/mL.

In this study, the fermentor was operated under batch condition for approximately 28 h. The pH of the medium was maintained at 5, which is the optimum range for the growth of the many yeast strains as reported by several authors (Ghaly *et al.*, 2003; Ghaly and Kamal, 2004; Schultz *et al.*, 2006). The Single Cell Protein (SCP) production by the isolated yeast strains was studied (Table 3). In whey medium without any supplementation, the A2 (*K. marxianus*) and A1 (*K. lactis*) strains with 12.68 and 12.43 g/L dry biomass yield had, respectively, the most SCP production (Table 3). The results showed that there was a direct relationship between lactase activity and biomass production in cheese whey. Also among all isolated yeast the membranes of genus *Kluyveromyces* in compare to genus *saccharomyces* showed more enzyme activity that confirms the same studies (Rech *et al.*, 1999; Santos *et al.*, 1998). Although *K. marxianus* was chosen for the further experiments.

Supplementation of the media with 5 g/L of ammonium sulfate resulted in a significant increase in biomass yield. Our results showed an increasing yield up to 30% for *K. marxianus*.

Biomass and lactose concentration results obtained during the batch production of single cell protein are presented in Fig. 1. During the exponential growth phase, the biomass concentration increased exponentially with cultivation time and lactose concentration depleted

Table 2: The yeast isolated from diary product in the city of Rasht

Samples	No. isolated	Yeasts that fermented lactose
1	1, 2	2 (A1)*
2	3	-
3	4	-
4	5, 6, 7	5 (A2), 7 (A3)
5	-	-
6	8, 9	8 (A4)
7	-	-
8	10	-
9	11, 12	-
10	13, 14	14 (A5)

* Conventional name of yeast

Table 3: ONPG assay of β -galactosidase and the amount of SCP production in yeast strains

Isolated no.	Yeast species	Enzyme activity (EU/mL)*	Biomass (g/L)
A1	<i>K. lactis</i>	6266	12.43
A2	<i>K. marxianus</i>	9012	12.68
A3	<i>S. fragilis</i>	3403	9.62
A4	<i>K. lactis</i>	6396	11.98
A5	<i>S. cerevisiae</i>	2798	10.30

*: The β -galactosidase activity was measured using ONPG and calculated as follows: Units = $1000 \times OD_{420} / \text{volume (1 mL)} \times \text{time (min)} \times OD_{600}$ (Nahvi and Moeini, 2004).

rapidly. The lactose was reduced about 98.2% after 28 h. Specific growth rate were determined graphically as shown in Fig. 1. The specific growth rate was obtained from logarithmic phase of this curve as 0.23 per h.

The COD was used in this study as an indirect measurement of the soluble and insoluble organic matter in cheese whey. The difference between the initial and final values of the soluble COD is due to the consumption of the dissolved biodegradable organic matter (mostly lactose) by the yeast and final reduction in soluble COD was 88.49% of the original value which is higher than the reduction in the total COD of 42.98% of the original value. The 88.49% reduction in the soluble COD shows that the soluble organic material (lactose)

Table 4: The chemical composition of SCP from *K. marxianus* (A2) on a dry weight basis

Components	g/100 g
Crude protein	40.63
Ash	14.05
Crude fiber	4.90
Fat	7.23
Carbohydrates	33.19

Table 5: Essential amino acid composition of SCP produced by *K. marxianus* (A2) from whey and to the guideline of FAO*

Amino acid	SCP	FAO
Isoleucine	4.42	4.5
Leucine	6.33	7.0
Lysine	7.12	5.5
Methionine	1.95	3.0
Phenylalanine	3.19	6.0**
Threonine	3.79	4.0
Valine	4.98	5.0
Tyrosine	2.68	6.0**

*: Amino acid concentration are expressed as grams of amino acid per 16 g of N; **: Value reported is the sum of values for phenylalanine + tyrosine

was converted to insoluble material (yeast cells). Reduction in soluble COD of about 85-95% of the initial values was reported in deferent studies (Ghaly and Kamal, 2004; Schultz *et al.*, 2006). Therefore, a biomass recovery for use as animal feed supplements and in human food is necessary for further pollution potential reduction before the ultimate disposal of the spent medium. In this study, the ultrafiltration of the spent culture reduced in an effluent having total COD reductions of 96.26%.

The composition of single cell protein obtained from the aerobic fermentation of cheese whey by *K. marxianus* is shown in Table 4. The crude protein content of the dry cells was about 40.63% (w/w). The protein content of SCP material is calculated from total nitrogen by multiplying with a conversion factor of 6.25. Besides protein, total nitrogen includes nitrogen bound to nucleic acids, nucleotides, free nitrogen bases, and also cell membrane amino sugars. These are usually devoid of nutritional value. The alcohol-insoluble nitrogen fraction is termed as true protein nitrogen fraction, which may account for 64-70% of total nitrogen of microbial cells. The protein nitrogen of the experimental strain is 72% of total nitrogen, which was higher than the value reported for similar products (Paul *et al.*, 2002). The true protein content (alcohol insoluble N \times 6.25) of the experimental yeast strain is 29.2%.

In Table 5, the essential amino acid composition of the SCP from *K. marxianus* is compared with that of the Food and Agriculture Organization (FAO) reference protein (FAO, 1970). The results showed that the concentration of essential amino acids in SCP from *K. marxianus* was equal to or greater than those in the FAO reference protein, suggesting that *K. marxianus* is a good protein source for human adults. Most SCP produced to date is deficient in methionine (Paul *et al.*, 2002; Schultz

et al., 2006). SCP from *K. marxianus* examined in this study was also low in methionine, compared with the amount in FAO reference protein.

Ash content of the dried cells of *K. marxianus* has been found to be 14.05% (Table 5), which was lower than the value reported for similar products (Paul *et al.*, 2002). Fiber consists largely of cellulose or other indigestible cell wall polymers. Although fiber is indigestible, it plays a significant nutritional role, for example, it helps clean and maintain the proper motility of the intestinal tract. The crude fiber content of *K. marxianus* cells has been found to be 4.90%, which is agreement with that reported by Paul *et al.* (2002). Carbohydrate content of *K. marxianus* cultivated on whey medium was found to be 33.19% (Table 5). Fat content of the biomass was found to be 7.23% (Table 5) which is higher than that obtained by Shay and Wegner (1986) for *Torula* yeast and *K. fragilis*.

CONCLUSION

We isolated and identified five lactose positive yeasts from cheese whey. The SCP production was studied in the batch conditions. The most SCP yield was obtained from A₂ isolate (*Kluyveromyces marxianus*) with yield up to 30%. The total COD reduction in our experiment was notably 88.49% of original value. The SCP components evaluation showed the protein nitrogen of the best experimental strain was 72% total nitrogen (higher than the similar works). Also it's a good source from the point of view amino acid composition and fat content with compared FAO standards.

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