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Nutritional Profile of Food Yeast *Kluyveromyces fragilis* Biomass Grown on Whey

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Abstract

Biomass of food yeast *Kluyveromyces fragilis* (MTCC 188) grown on deproteinized whey supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3-acetic acid, had a crude protein content of 37%. The true protein content based on nitrogen fractionation procedure was 28.1%. Total nucleic acid content was 4.82%. This amount does not appear to be toxicologically offensive. Crude fiber, ash, and lipid content of *K. fragilis* dry cells were found to be 4.9%, 16%, and 7.8%, respectively. Essential fatty acids of both ω-3 and ω-6 series were found present in the fat of the yeast and represented 21.5% of the total fatty acids. All the essential amino acids were present in the proteins of *K. fragilis*; however, sulfur containing amino acids were found in lower amounts. Calculated protein scores indicate moderate biological value. B vitamins in the biomass were present as expected, but folic acid and pyridoxine were present in high concentration.

Index Entries: *Kluyveromyces fragilis*; whey; single-cell protein; food grade yeast; biomass; chemical score.

Introduction

Whey, the largest by-product of the dairy industry when discharged in the environment, causes not only environmental pollution but also loss of precious nutrients. It contains about 50% of the total solids of milk *(1)*. Worldwide production of whey is estimated to be around 115 million tons

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annually *(2)*. Biochemical oxygen demand of whey is very high (30,000–50,000 ppm), making its disposal a pollution problem. In order to reduce the pollution load of whey and to get some valuables, many researchers have treated it chemically and microbiologically to obtain different products having commercial importance *(3–10)*. We already reported the production of single-cell protein (SCP) from deproteinized whey via food grade yeast *Kluyveromyces fragilis (11,12)*. In this paper we describe the nutritive value of the biomass of *K. fragilis* grown on deproteinized whey.

Experimental Procedures

Microorganism

Kluyveromyces fragilis (MTCC 188) used in this study was obtained from The Institute of Microbial Technology, Chandigarh, India. The organism was maintained on agar slants containing 0.3% yeast extract, 1% peptone, 2% dextrose, and 1.5% agar and transferred at 30-day interval to maintain viability.

Whey

Fresh whey was obtained from local sweetmeat manufacturers and deproteinized by heating at 95°C for 30 min after adjusting lactose content to 4.6% and pH 4.6. The precipitated proteins were removed by centrifugation at 4°C in a Sorvall RC-5B refrigerated centrifuge at 5000 rpm for 15 min.

Chemicals

All chemicals used in this study were of analytical reagent or bacteriological grade and purchased from Sigma, USA, Oxoid, UK, and E. Merck, Germany.

Fermentation Medium

Fermentation medium contained deproteinized whey and 0.8% diammonium hydrogen phosphate. Ingredients were mixed after separately autoclaving at 121°C for 15 min. The pH of the medium was 5.8. Indole 3-acetic acid was dissolved in absolute alcohol and added to the medium to a final concentration of 10 ppm.

Inoculum and Fermentation

Flasks containing 100 mL of media each were inoculated with 1 mL of approximately 10^8 cells of *K. fragilis* grown previously in the same fermentation medium for 24 h. The flasks were incubated in a rotary action shaker (120 rpm) for 48 h at 37°C. At the end of the fermentation period, cells were separated from the medium by centrifugation in a Sorvall RC-5B refrigerated centrifuge at 5000 rpm for 20 min at 4°C. The cells were dried by lyophilization after washing twice with distilled water. Dried cells after weighing were kept at –20°C till use.

Fractionation of the Dried Cells

The cells were fractionated into cold TCA-soluble, alcohol-soluble, alcohol-ether-soluble, hot TCA-soluble, and alcohol-insoluble protein fractions according to the procedure used by Delany et al. *(13)*. DNA and RNA in hot TCA-soluble fraction were determined by diphenylamine method *(14)* and the procedure described by Gottlieb and Van Ettem *(15)*, respectively.

Nitrogen

Nitrogen contents of all the above five fractions and dried cells were determined by microkjeldahl procedure *(16)*. Protein was calculated from total nitrogen by multiplying with a conversion factor of 6.25.

Amino Acid

Amino acid analysis 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 except tryptophan, which was determined spectrophotometrically as described by Spies *(17)*.

Ash and Minerals

Ash content was determined by the procedure described in AOAC *(16)* using 5 g of the dried cells. The metal ions were determined by atomic absorption spectrophotometer (Perkin Elmer model 2380) using air-acetylene flame and specific hollow cathode lamp after refluxing 2 mg of the sample with conc. HNO_3 and 70% $HClO_4$ (1:1) for 30 min.

Fat and Fatty Acid

Fat content of the dried cells was determined by Soxhlet extraction procedure *(16)* using petroleum ether (60–80°C) as solvent. Identification and estimation of individual fatty acids were done by gas-liquid chromatography (Hewlett-Packard model 5730, USA) after hydrolysis and conversion into methyl esters *(18)*.

Crude Fiber

Fiber content of the dried biomass was determined *(16)* by removing fat from it as described above and then by boiling consecutively with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solution. The residue was washed thrice with water followed by ethyl alcohol and dried to a constant weight. The value was corrected for mineral contents of the sample.

Sugar

Dried biomass was hydrolyzed with 2 *M* trifluroacetic acid at 120°C for 2 h in a sealed tube *(19)*. The identification and estimation of the monosaccharides were done by gas-liquid chromatography (Hewlett-

Packard, model 5730, USA) after converting the liberated monomers into their alditol acetates by acetic acid and pyridine (1:1).

Vitamins

Thiamine, riboflavin, niacin, and pyridoxine were determined chemically according to Fujiwara and Matsui *(20)*, Scott et al. *(21)*, Melnick and Field *(22)*, and Scudi *(23)*, respectively.

D-Pantothenic acid *(24)*, folic acid *(25)*, and cyanocobalamin *(26)* were determined microbiologically using sensitive strains of *Lactobacillus arabinosus* ATCC 8014, *Streptococcus faecalis* ATCC 8043, and *Lactobacillus leichmannii* ATCC 7830 respectively.

Results and Discussion

Table 1 presents the general composition of *Kluyveromyces fragilis* biomass. Crude protein content of the biomass of the present strain of *K. fragilis* is similar to those reported by El-Samragy et al. *(27)* for *Saccharomyces fragilis*, *Candida tropicalis* ATCC 20401, or *Kluyveromyces marxianus* ATCC 28244 and *Kluyveromyces fragilis* PC 8002 by Shay and Wegner *(4)*.

Details of the nitrogen distribution in different fractions of *K. fragilis* cells are shown in Table 2. 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 76% of total nitrogen and this value is in well agreement with that reported by Delany et al. *(13)* for *S. fragilis* (75%) and "Bel" yeast (69%). The true protein content (alcohol insoluble N × 6.25) of the experimental yeast strain is 28.1% as compared to 31.9% for "Bel" yeast *(13)*. The cold TCA-soluble nitrogen fraction of the strain containing mainly free amino acids and peptones comprised 15.2% of the total cell nitrogen, whereas the same for *S. fragilis* and "Bel" yeast was a little less, being 12.8% and 13.0%, respectively *(13)*.

The combined nucleic acid content of *K. fragilis* was found to be 4.82% (Table 2) of total weight of the cells. This value is lower than the same reported earlier *(13)* for *S. fragilis* (5.7%) and "Bel" yeast (6.3%). Nucleic acid content often becomes the "limiting factor" for human consumption of yeast single-cell protein. In this respect, the low nucleic acid level in *K. fragilis* biomass is very significant, because as high as 6–11% of nucleic acid on a dry weight basis of yeast was reported previously by Bressani *(28)*. High nucleic acid intake may cause gout due to over production of uric acid crystals in joints or the formation of stone in the urinary tract. Edozien et al. *(29)* has shown that 2 g SCP nucleic acid per day is probably the safe limit for human consumption. On the basis of a maximum daily intake of

Table 1
General Composition
of *K. fragilis* Dried Cells Grown on Whey

Composition	g/100 g
Crude protein	37.0
Ash	16.0
Crude fiber	4.9
Fat	7.8
Carbohydrates (by difference)	34.3

Table 2
Nitrogen Distribution in the Cells of *K. fragilis*

Composition	Dry weight of cells (%)
Total nitrogen	5.92
Cold TCA soluble nitrogen	0.90
Alcohol-soluble nitrogen	0.18
Alcohol-ether-soluble nitrogen	0.09
Alcohol-insoluble nitrogen	4.50
RNA	4.25
DNA	0.57
Total nucleic acids	4.82

2 g SCP nucleic acid per day, *K. fragilis* biomass could be included in the diet up to 42 g per day without any difficulty of metabolizing nucleic acids. However, nucleic acid content can be reduced by a heat-shock incubation process for degrading RNA by yeast cell ribonuclease or by an alkaline extraction and acid coagulation process *(30,31)*.

The essential amino acid composition of *K. fragilis* biomass (Table 3) shows that lysine, leucine, valine, and threonine were present in higher concentration in comparison to others while methionine content was poor. Similar trends were also reported by El-Samragy et al. *(27)* for different strains of yeast. The low concentration of methionine and sulfur containing amino acids in SCP produced by yeast have been reported earlier *(4)*.

Among nonessential amino acids glutamic acid (10.34 g/16 g N) and aspartic acid (7.47) were found in higher amounts followed by alanine (6.86), serine (3.98), and glycine (3.90). Proline (3.28), tyrosine (2.85), histidine (1.98), and cystine (0.29) were present in smaller amounts.

The evaluation of proteins and protein diets for nutrition can be accomplished more accurately by feeding experiments carried out under the conditions in which they are supposed to be consumed. In vitro evaluation of protein is suitable in predicting the quality of protein with reasonable accuracy. These methods may be used for the preliminary characterization of nutritional quality of microbial proteins even agreeing that the exact quality could only be judged by biological experiments.

Table 3
Essential Amino Acid Profile of *K. fragilis* Biomass

Essential amino acids	g Amino acid/16 g N
Threonine	4.45
Valine	5.02
Methionine	0.38
Isoleucine	3.82
Leucine	5.47
Phenylalanine	3.98
Lysine	6.91
Histidine	1.98
Arginine	4.30
Tryptophan	1.07

The concept of chemical or protein "scores" is based entirely on the amino acids required for growth; it is obvious that these "scores" can only be used as a partial measure of biological value of proteins. The nutritive value of *K. fragilis* protein was calculated according to Kuhnau *(32)*, using human milk as reference standard. The "total value," "pure value," and "supplementary value" of *K. fragilis* SCP are 72.7, 72.0, and 0.7, respectively, indicating moderate biological value of the protein and excess presence of some amino acids over the reference pattern.

Another important method of in vitro evaluation of the nutritive value of protein is the calculation of essential amino acid index according to the proposal of Oser *(33)*, which was subsequently modified by Mitchell *(34)*. The modified essential amino acid index (MEAAI) for *K. fragilis* protein was calculated to be 78.07, which was high. It is claimed that MEAAI is useful in predicting the maximum potential biological value of a protein and providing a logical basis for the mutual supplementation of proteins for the improvement of diets. The biological value and nutritional index were also calculated based on FAO reference protein *(35)* and the values were 73.40 and 28.90, respectively. Due to limitation of sulfur amino acids and of leucine, the biological value of the present protein is low compared to that of animal origin, although it contains some essential amino acids in considerable amounts. So the protein from this strain will not be suitable for nutrition when used alone; however, it may be used for supplementation and fortification of diets with other amino acids complementing this inadequacy.

Ash content of the dried cells of *K. fragilis* has been found to be 16% (Table 1). This amount is higher than that reported for SCP produced by yeast *(27)*, which usually varies from 7.6–10.4%. One of the reasons for high ash content may be due to higher mineral content in whey permeate. Shay and Wegner *(36)* also reported high ash content of *K. fragilis* when grown on concentrated whey permeate and the value can be reduced substantially by addition of small portions of inexpensive fermentable sugars in the

Table 4
Major and Minor Minerals
in *K. fragilis* Biomass

Major minerals	%
Potassium	1.30
Phosphorous	2.60
Calcium	2.10
Magnesium	0.12
Iron	0.04
Aluminum	0.05
Sodium	1.89
Minor minerals	**ppm**
Manganese	10
Copper	30
Zinc	180
Molybdenum	20
Nickel	30

media. Higher mineral content of SCP may be beneficial to some special animal feed or human food application depending on the nutritional and flavor requirements.

Concentration of individual elements in SCP materials varies from species to species *(27)*. Phosphorus, calcium, potassium, and sodium were found to be the major part of biomass produced by *K. fragilis* as shown in Table 4. This profile fits well with the findings of El-Samragy et al. *(27)* for other strains of yeast.

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. fragilis* cells has been found to be 4.9%, which is higher than that obtained by Shay and Wegner *(4)* for *Torula* yeast grown on sucrose.

Besides protein yeast biomass also contains some amount of lipids, carbohydrates and vitamins which also contribute to the overall nutritional value of this product. Fat content of the biomass was found to be 7.8% (Table 1) which is higher than that obtained by Shay and Wegner *(36)* for *Torula* yeast and *K. fragilis* PC 8002. The distribution of fatty acids in the fat is presented in Table 5. The percentage of saturated fatty acids was higher than of other yeasts. Linoleic acid, with two double bonds at Δ^9 and Δ^{12} and α-linolenic acid (C_{18}, $\Delta^{9,12,15}$) are essential fatty acids and cannot be synthesized by mammals. Once ingested in mammals linoleic and linolenic acids may be converted into certain other important polyunsaturated acids of ω-6 and ω-3 series, respectively. Unlike in other yeasts, linolenic acid was found to be present in much higher amounts than linoleic acid.

Table 5
Fatty Acid Distribution of Yeast Product

Fatty acid	Composition (% total)		
	K. fragilis MTCC 188	*Torula* yeast grown on sucrose[a]	*K. fragilis* PC 8002[b]
10 : 0 Capric	2.80	—	<0.7
12 : 0 Lauric	6.73	—	0.12
14 : 0 Myristic	19.29	—	1.15
16 : 0 Palmitic	22.03	14.2	25.0
17 : 0 Heptadecanoic	—	2.2	2.33
18 : 0 Stearic	9.61	1.0	2.13
20 : 0 Arachidic	18.35	—	<0.01
16 : 1 Palmitoleic	—	6.2	14.30
18 : 1 Oleic	—	20.3	12..60
18 : 2 Linoleic	6.93	49.0	38.80
18 : 3 Linolenic	14.22	5.6	2.51

[a]Ref. *4*.
[b]Ref. *36*.

Table 6
Sugar Composition
of *K. fragilis* Dried Cells

Monosaccharides	Content (%)
Arabinose	2.62
Mannose	44.19
Galactose	6.47
Glucose	46.30

Carbohydrate content of *K. fragilis* cultivated on whey medium was found to be 34.3% (Table 1). Table 6 shows high amounts of glucose and mannose in *K. fragilis*, since glucan and mannan are the main polysaccharide components of yeast cell wall. Glucan as food has beneficial effect for lowering the serum cholesterol, a risk for cardiovascular diseases *(37)*. Radioprotective effect of yeast has been reported by Chernysheva et al. *(38)*.

Table 7 shows that *K. fragilis* biomass obtained by fermentation on whey medium is a good source of B-group vitamins. Riboflavin and vitamin B_{12} contents of the cell were, however, poor. Except niacin and riboflavin, other B vitamin contents of the present strain are higher than *Torula* yeast and *K. fragilis* PC 8002 as shown in Table 8.

Recommended dietary allowance of folic acid, which is 0.4 mg, can be met by consuming only 2 g of the present yeast biomass. However, for thiamine and niacin much higher amounts of cells are required if consumed solely as source of these vitamins.

Table 7
Vitamin Content of *K. fragilis* Cells (mg/kg)

Vitamin	Content	Recommended daily dietary allowance for adults (mg)[a]
Thiamine	18.2	1.5
Riboflavin	6.75	1.7
Niacin	268.00	19.0
D-Pantothenic acid	200.00	—
Pyridoxine hydrochloride	169.00	2.2
Folic acid	200.00	0.4
B_{12}	0.01	0.003

[a]Ref. *39*.

Table 8
Comparison of B Vitamins of Different Yeast Cells

Vitamin content (mg/kg)	*K. fragilis* (MTCC 188)	*Torula* yeast grown on sucrose[a]	*Torula* yeast grown on ethanol[a]	*K. fragilis* PC 8002[b]
Thiamine	18.20	9.50	8.0	3.30
Riboflavin	6.75	44.10	45.0	71.00
Niacin	268.00	450.00	550.0	126.00
D-Pantothenic acid	200.00	189.00	94.00	74.00
Pyridoxin hydrochloride	169.00	79.10	83.00	22.00
Folic acid	200.00	21.50	4.00	30.00
B_{12}	0.01	0.01	0.004	0.008

[a]Ref. *4*.
[b]Ref. *36*.

It may be concluded that essential amino acid profile in the protein of *K. fragilis* biomass meets the requirements recommended by FAO *(35)*, except sulfur-containing amino acids. Relatively low nucleic acid content, presence of essential fatty acids—linoleic and linolenic acids and high amount of folic acid in the dried biomass of *K. fragilis*—are an added advantage for the fortification of diets low in nutritive value.

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