CHEMICAL COMPOSITION OF A MIXTURE OF SINGLE-CELL PROTEIN OBTAINED FROM *Kluyveromyces fragilis* AND WHEY PROTEINS

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SUMMARY

A mixture of *Kluyveromyces fragilis* biomass and coagulated whey proteins was obtained by fermentation of whole whey. This product had a chemical composition similar to that of washed products reported in the literature with high-crude protein and low-ash contents. The product had a high content of sulphur-containing amino acids and tryptophan, which are usually limiting in yeast biomass. Lysine content was inexplicably lower than the expected value, being the limiting amino acid in this case. The chemical score of the protein was 91%. From the biomas-whey proteins product a protein isolate could be recovered with a yield of 80%. The protein content of the isolate was 75%, and the nucleic acids were reduced by 90.8%. The cell-wall debris were also considerably reduced.

INTRODUCTION

Whey and whey permeate have been used as substrate for the production of single-cell protein (SCP) from several microorganisms, the most important being *kluyveromyces fragilis*. Commercial processes have been developed in several countries (1, 2). The earlier research works were performed utilizing whole whey (3-7), but since whey protein concentrates (WPC) have gained commercial

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importance, *K. fragilis* biomass production has been developed mainly with ultrafiltrate permeate in order to increase the economic attractiveness of the process (8). SCP production from whey permeate has demonstrated to be economically feasible only in large-scale plants which can afford capital investments for WPC and biomass production, due to the low protein content in whey, and the low value of SCP (6-11)

Recently, the utilization of whole whey for the production of SCP has been proposed as an alternative for whey disposal of small cheese factories (10, 11). Additionally, in order to increase the economic attractiveness of the process of whole whey, the simultaneous production of valuable by-products such as pectinase (12, 13) and alcohol (14) have been reported.

SCP from *K. fragilis* has been used as feed and it is also accepted as human food (1, 15). Nevertheless, the nucleic acids have to be removed for human consumption; otherwise, the ingestion of significant amonts could lead to the accumulation of uric acid, producing gout and kidney stones (16). Cell wall also has to be removed due to its low digestibility and toxicological problems (17). *K. fragilis* protein is deficient in sulphur-containing amino acids and tryptophan (18-20). Due to the fact that whey proteins are rich in this kind of amino acids, the mixture of these proteins should increase the nutritional value of SCP.

The recovery of a protein concentrate from the fermentation of whole whey with *K. fragilis*, free of nucleic acids and cell wall, is dealt with in the present paper.

MATERIAL AND METHODS

Microorganism

Kluyveromyces fragilis NRRL-Y-1109 was used and it was maintained on potato-dextrose agar at 4°C.

Culture Medium

This was prepared from spray-dried whey suspended in distilled and deionized water (65g/lt) together with the following supplementary nutrients: (NH) SO 5 g/lt, K HPO 5 g/lt, and yeast extract 1 g/lt. The pH of the medium was adjusted to 5.0 with H SO. Medium was autoclaved for 15 min at 121°C.

Culture Conditions

A bench-scale fermenter (New Brunswick Micro Ferm) with 14 lt jar was used. Culture conditions were: volume of medium 11 lt, 30°C, air flow 1 vvm and agitation speed 400 rpm; 0.1 ml of sterile silicon antifoam were added. Fermentations were carried out during 18 hr.

Biomass Recovery

A mixture of biomass and coagulated whey proteins was recovered after fermentation by centrifugation of culture medium (3,200 g, 20 min, 4°C).

Amino Acid Analysis

After acid hydrolysis (5N HCl, 100°C, 16 hr) was performed in a Beckman analyzer Model 116 with anionic exchange column. Tryptophan was then analyzed according to Hernández and Bates (21).

Chemical Analyses

All of them —moisture by drying on vacuum oven, ash, crude protein by the Kjeldhal technique and fat content by the Golfish technique— were performed according to the AOAC (22). Carbohydrates were calculated by difference.

Cellular Disruption

Protein recovery was performed according to Vananuvat and Kinsella (23) using NaOH (0.4 g/100ml), 1 hr of extraction and precipitating with acid (pH 4.2, 60°C). In every case 0.5 g of biomass-whey proteins were treated; the suspension was taken up to a final volume of 50 ml, and protein was determined in the whole suspension. This suspension was centrifuged (5,000 g, 15 min) and protein was determined in the supernatant. In both cases, protein determination was done by the Kjeldhal method (22).

Nucleic Acids Determination

This was performed according to the Schmidt and Thannhauser procedure (24).

RESULTS

The chemical composition of the mixture of *K. fragilis* biomass and coagulated whey proteins as it was recovered from the culture medium and the composition of the proteins isolate are reported in Table 1. Results are given as g/100g of dry product; those informed by other authors are also included for comparison purposes. Table 1 also shows the nucleic acids content and nucleic acids to protein ratio before and after protein extraction.

According to protein determinations done before and after centrifugation of the protein suspension treated with NaOH, the yield of the alkaline extraction to obtain the proteins isolate was 80% of crude protein.

The total amino acids content of the biomass-whey proteins is

TABLE 1

CHEMICAL COMPOSITION OF BIOMASS-WHEY PROTEINS AS RECOVERED FROM THE CULTURE MEDIUM, AND AFTER PROTEIN ISOLATION

(Data from *K. fragilis* biomass reported by other authors are also shown. Data are given as g/100 g of dry product)

	Biomass/ whey protein	Proteins isolated	a	c	f	g
Protein (N x 6.25)	50.8	75	52.3	58.9	—	—
Fat	3.9	19	8.9	10.6	—	—
Ash	5.5	5	8.0	5.9	—	—
Carbohydrates*	39.8	1	30.8	24.6	—	—
Nucleic acids	8.7	0.8	5.7	—	8.6	5.7
Nucleic acids/ crude protein	0.17	0.01	0.11	—	0.15	0.07

* Calculated by difference.
a Biomass produced on whey permeate (19).
c Washed biomass with coagulated whey proteins (7).
f Biomass produced on whey permeate in batch culture (26).
g Protein isolated from biomass grown on whey permeate (23).

shown in Table 2. Other reports found in the literature are also displayed. From these data, essential amino acids scores were calculated, and results are displayed on Table 3. The score of other proteins is also shown. In every case the reference protein was the FAO/WHO 1973 pattern (25). Due to the fact that Vananuvat and Kinsella (23) reported a loss in tryptophan using the same technique for protein isolation used here, the content of this amino acid was analyzed in the protein isolate obtained; the result was 1.53 g per 100 g of protein. The score for this amino acid in this case was 153.

DISCUSSION

The chemical composition of the biomass-whey proteins product obtained is analogous to other similar products obtained by other authors (Table 1). Crude protein is around 50% of the composition. Fat content was lower in this work than data shown on Table 1 for other authors; however, other investigators notified fat contents between 1 to 3% for *K. fragilis* biomass and biomass with whey products (14, 29). The product recovered from the culture medium reported herein was similarly obtained to that reported by Amundson (7); both of them are a mixture of *K. fragilis* biomass and coagulated whey proteins. Amund-

TABLE 2

AMINO ACIDS COMPOSITION OF THE MIXTURE OF BIOMASS AND WHEY PROTEINS RECOVERED FROM CULTURE MEDIUM

(Data from other authors are also reported, and units are expressed as g/100 g protein)

	Biomass/ whey protein	a	b	c	d	e	f	g
Isoleucine	5.5	4.8	6.0	6.1	5.1	6.0	4.3	3.8
Leucine	10.6	8.1	9.6	11.0	—	9.6	7.2	8.5
Lysine	5.0	8.0	11.1	10.4	11.1	10.2	8.5	8.3
Methionine	2.3	1.5	1.6	2.0	1.6	1.2	1.5	1.4
Phenylalanine	4.6	4.2	5.1	4.4	5.1	5.4	3.6	3.6
Threonine	4.3	5.3	6.5	7.5	5.6	6.5	4.1	4.5
Tryptophan	2.2	1.7	—	2.3	—	—	0.9	0.4
Valine	6.7	5.6	7.8	7.4	5.7	7.8	5.6	5.1
Histidine	1.6	2.0	4.0	3.0	4.0	2.0	4.0	0.7
Cysteine	6.3	1.7	—	3.8	—	—	1.9	1.2
Tyrosine	4.0	3.9	3.4	4.1	4.6	3.4	4.5	—
Aspartic acid	11.2	9.4	—	—	10.4	11.2	9.6	11.5
Serine	4.1	4.7	7.0	6.8	5.2	7.0	4.7	5.8
Glutamic acid	15.4	13.8	—	—	15.2	13.3	16.5	11.6
Proline	4.5	4.2	—	—	—	—	3.7	3.7
Glycine	4.4	3.7	—	—	4.2	4.6	5.0	4.6
Alanine	6.6	5.8	—	—	7.2	8.2	6.4	6.3
Arginine	3.0	4.9	7.4	5.7	7.4	7.1	5.6	4.3

a *K. fragilis* NRRL-Y-1109 grown on whey permeate (19).
b *K. fragilis* biomass only (7).
c Biomass with coagulated whey proteins (7).
d *K. fragilis* NRRL-Y-1109 grown on whole whey (27).
e Protein isolated from *K. fragilis* NRRL-Y-1109 biomass (27).
f Biomass grown on whey permeate (18).
g Protein isolated from biomass grown on whey permeate (18).

TABLE 3

ESSENTIAL AMINO ACIDS SCORE OF BIOMASS-WHEY PROTEINS OBTAINED FROM THE CULTURE MEDIUM

(Data from other sources are also shown. The score was calculated with respect to the FAO/WHO 1973 pattern (25), according to the following equation: (g AA/g AA in pattern) x 100)

	Biomass/ whey protein	a	b	c	d	f	g	Whey protein h
Isoleucine	138	120	150	153	128	108	95	190
Leucine	151	116	137	157	—	103	121	169
Lysine	91	145	202	189	202	155	151	205
Met + Cys	246	91	—	166	—	97	74	149
Phe + Tyr	143	135	142	142	162	135	—	117
Threonine	108	133	163	188	140	103	113	210
Tryptophan	220	170	—	230	—	90	40	240
Valine	134	112	156	148	114	112	102	144
Total	143	124	—	164	—	117	—	169

a *K. fragilis* NRRL-Y-1109 grown on whey permeate (19).
b Biomass only (7).
c Biomass with coagulated whey proteins (7).
d *K. fragilis* NRRL-Y-1109 grown on whole whey (27).
f Biomass grown on whey permeate (18).
g Protein isolated from biomass grown on whey permeate (18).
h After Hambraeus (28).

son informed a great difference in composition between washed and unwashed products; for instance, ash content decreased 69% and protein increased 110% after washing his yeast-whey product. Amundson's data —shown in Table 1— correspond to the washed product; in spite of the fact that biomass/whey proteins product obtained in this work was not washed, its chemical composition is closer to the washed product reported by Amundson than to the unwashed one. Similar conclusions are obtained when comparing this product with the washed and unwashed products (Amber Nutrex and Amber YFS, respectively) cited by Bernstein, Tzeng and Sisson (14); the former had a crude protein content of 45-55% and an ash content of 6-10% (with a 3-4% of moisture), while the unwashed product had 35-50% of crude protein and 12-20% of ash (with 3-4% moisture). Therefore, it is not necessary to wash the product obtained here in order to eliminate undesirable compounds; the ash content was low and the other compounds were found in similar concentrations to washed products reported by other authors.

The composition of the proteins isolated from the biomass-whey proteins product showed a considerable increase in crude protein. Carbohydrates were practically eliminated by the process, and as the cell wall of the yeast is composed in a high proportion of these compounds (30), it can be assumed that the cell wall was efficiently removed. Considering that the cell wall contains aminosugars (30) and the nucleic acids content in biomass is quite high, the proportion of non-protein nitrogen accounting for the crude protein value should be high. Once the cell-wall debris and nucleic acids are removed, the protein value in the isolate should be closer to a true protein value. Data obtained from nucleic acids content in the present work are similar to those informed by other authors. Additionally to data shown on Table 1, Vananuvat and Kinsella (31) reported a 10% content of nucleic acids in biomass grown on whey permeate in continuous culture, while Castillo and de Sánchez (32) notified 9.3% for biomass grown on deproteinized whey in batch culture. The relative content of fat increased significantly in the isolate, an increase that should be due to a relative decrease in the other constituents, including protein, during the process.

In spite of the fact that the technique for protein extraction with nucleic acids reduction used here was the same as that reported by Vananuvat and Kinsella (see Table 1), it can be seen that it was more efficient in this case; the treatment reduced nucleic acids by 90.8% in the present paper, while their data show 33.7% only. The reason for this difference is unknown. In their case, the yield for protein recovery was equal to the one reported herein (23).

In regard to the amino acids content (Table 2), the general composition of the biomass-whey proteins was very similar to other analogous compounds informed in the literature. Table 2 depicts data reported for other authors for the same strain used by us, grown on whey permeate (a) and whole whey (d). Data from *K. fragilis* biomass only and biomass-coagulated whey proteins mixture reported by Amundson for an unspecified strain are also included (b and c). Comparisons between biomass of an unspecified strain and its protein isolate obtained with the same technique used here (f and g), and with a different technique of isolation in the same strain used in this work (d and e) are also given in Table 2.

Lysine content of the product obtained is quite lower than any other reported; comparing data from biomass only with biomass-whey proteins reported by Amundson (b and c), it can be seen that lysine content diminished, although the decrease was not very high; therefore, that cannot be the explanation for such a low lysine content. A possible particularity of the strain is also unlikely due to the fact that data reported for the same one (a, d and e) do not show a low lysine content. At present this unusual low content of the amino acid is inexplicable.

Methionine content is low in *K. fragilis* biomass (19, 20); the content of this amino acid in the product obtained in our case is higher than that reported by other authors (Table 2). This result is probably due to the methionine content in whey proteins, a fact that can be concluded from Amundson's data (b and c). Similar conclusions can be obtained in regard to tryptophan and cysteine.

According to data given by Vananuvat and Kinsella (f and g in Table 2), the technique used here for protein isolation did not affect significantly the amino acid content (except hystidine). However, tryptophan —which is usually found in low concentration in these proteins— was reduced by the process; data obtained in our study showed that tryptophan content in the protein isolated from the biomass-whey proteins product diminished only 34%. The technique used for protein isolation by Wasserman based on TCA and ethanol extractions and precipitations (d and e) showed more significant changes in some amino acids content.

Comparing in Table 2 similar products from different strains (for instance a, b and f) and the same strain but grown and obtained in different conditions (the product obtained here, a and d), it seems that all these factors are critical to the amino acid composition, in spite of the fact that Vananuvat and Kinsella did not find significant differences in amino acids composition of a *K. fragilis* strain grown under different conditions (18).

Data in Table 3 show that lysine is the limiting amino acid in the biomass-whey proteins product obtained in this work, being the only one with a score lower than 100%. Nevertheless, according to this, the chemical score of the protein was 91% with respect to the 1973 FAO/WHO pattern. The highest total essential amino acids score in Table 3 is for whey proteins. The amino acids profile of *K. fragilis* biomass protein was improved when it was mixed with whey proteins, as can be seen in the data notified by Amundson (c). The product obtained here showed higher total essential amino acids score than products containing biomass only (a and f). Even the amino acids score of *K. fragilis* NRRL-Y-1109 without whey proteins is very high, as can be observed in a. The chemical score in this case was 91%. Delaney, Kennedy and Walley (19) reported a lower chemical score (58%) for this strain due to the fact that they used the older FAO/WHO pattern, based on whole egg, which has a higher essential amino acids content. In both cases, the limiting amino acids were the sulphur-containing ones. In every case where the protein came from biomass only (a, f, g), the score for these amino acids was lower than 100%, while biomass-whey proteins products showed higher values.

The tryptophan score for the proteins isolated in the present work was reduced 30% with respect to the biomass-whey proteins obtained from the culture medium. Data from Vananuvat and Kinsella (f and g) indicate a 56% reduction. In these authors' data, tryptophan is the limiting amino acid giving to the biomass protein a chemical score of 90% and to the protein isolate only 40%; therefore, such reduction is most critical in that case but is not in the product obtained in the present work.

CONCLUSIONS

The technique reported by Vananuvat and Kinsella for yeast protein isolation was successfully applied in the recovery of the protein from a mixture of biomass and coagulated whey proteins with a yield similar to that informed by them, but with a lower nucleic acids content. From the nutritional point of view, a mixture of biomass and whey proteins is more convenient than the biomass only, as can be seen from this product and others cited in the literature. Therefore, a mixture of proteins from whey and SCP with a good amino acids profile practically free from nucleic acids and cell wall debris, is suitable for human consumption, and can be obtained from the fermentation of whole whey.

RESUMEN

COMPOSICION QUIMICA DE UNA MEZCLA DE PROTEINA UNICELULAR OBTENIDA DE *Kluyveromyces fragilis* Y PROTEINAS DE SUERO DE LECHE

De la fermentación de suero de leche entera se obtuvo un producto consistente en una mezcla de biomasa de *Kluyveromyces fragilis* y proteínas coaguladas del suero. El producto tuvo una composición similar a la de productos lavados de que se informa en la literatura, con un alto contenido de proteína cruda y un bajo contenido de cenizas. Asimismo, acusó también un alto contenido de aminoácidos azufrados y de triptofano, los que usualmente son limitantes en la biomasa de levadura. El contenido de lisina fue inexplicablemente más bajo de lo esperado resultando ser el aminoácido limitante. La calificación química de la proteína fue 91%. Del producto de biomasa con proteínas de suero se obtuvo un concentrado proteínico con un rendimiento de 80%. El contenido de proteína del aislado fue de 75% y el contenido de ácidos nucléicos se redujo en 90.8%. Los restos de pared celular también se redujeron considerablemente.

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