Composition and Nutritive Value of Yeast Biomass and Yeast Protein Concentrates

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Summary Yeast biomass (Saccharomyces sp.) produced in local breweries as a by-product was utilized in this study. Percent proximate composition, amino acid composition, and protein nutritive value were determined for the yeast cell biomass (YC), a sodium perchlorate extracted and isoelectrically precipitated protein concentrate (P-PC), and a sodium trimetaphosphate treated extract followed by isoelectrical precipitation (TMP-PC). Protein concentrates averaged 75% protein as compared to 48.5% in the yeast biomass. Precipitation of the protein in the presence of either sodium perchlorate or sodium trimetaphosphate was reduced to 71% and 51% of the cell RNA content, respectively. Protein nutritive value was 70% of casein when measured by the protein efficiency ratio (PER), and over 90% of casein when net protein utilization (NPUa) was the criteria of evaluation.

Key Words yeast composition, biomass, protein concentrate, nutritive value

Microorganisms, particularly yeast, have been extensively used as food or food components (1, 2). They are an excellent source of protein, vitamins, particularly the B complex, some essential minerals and dietary fiber. Yeasts have been traditionally used in fermentation processes, bakeries, and as food flavouring and enrichment ingredients in the forms of yeast extract and autolysate.

The nutrient content of yeast cells depends mainly on the cell metabolic phase, and the protein nutritive value depends on the essential amino acid composition and bioavailability (1).

In Brazil, yeast biomass is produced in large quantity as a by-product of beer and ethanol manufacture. It is underutilized and constitutes a serious environmental pollution problem.

Two main factors have been cited in literature (3) as interfering with the protein

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as well as with the utilization of other yeast nutrients; they are the thick cell walls, which offer resistance to enzyme digestion (4), and the high content of nucleic acids, particularly RNA (5, 6). Several methods have been employed to obtain yeast derivatives with lower nucleic acid content (7–9).

The majority of work reported in literature used in vitro procedures to evaluate functional and nutritive properties of the protein concentrates recovered from yeast (10, 11).

The objective of this research work was to compare the overall composition and protein nutritive value of the whole yeast biomass (after the cell walls had been mechanically ruptured) with yeast protein concentrates obtained by extraction of the protein and precipitation at the isoelectric pH, after treatment with sodium trimetaphosphate. Protein nutritional evaluation was done in vivo with rats and compared with a recently described procedure (12, 13) of evaluation in vitro.

MATERIALS AND METHODS

Preparation of test materials

Raw material: Yeast biomass (Saccharomyces sp.) was obtained from local breweries, and prior to use, it was submitted to a debittering treatment.

Debittering procedure: Aqueous suspensions of yeast cells (50–60% w/v), as obtained from the factories, were washed twice with a 10% sodium carbonate solution (1:1 v/v) to raise the pH to 8.0–8.5. After each washing the suspension was centrifuged and the cells resuspended again. Final washings were performed with distilled water to eliminate salt excess.

Rupturing of cell walls: The cell walls were ruptured mechanically in a Dynomill, essentially according to the procedures suggested by Hedenskog and Mogren (14). After several trials, the following conditions were established: glass spheres of 0.6–1.0 mm diameter to fill 70% of the Dynomill chamber; a 40% yeast cell aqueous suspension (w/v) was mixed (1:1 v/v) with a 10% sodium carbonate solution; a feed rate of 80 mL/min; mill velocity at 2,400 rpm. The temperature of the biomass in the chamber was maintained at around 15°C with a cooling (water/ethyleneglycol) circulating mixture. The efficiency of rupturing was monitored by the proportion of biomass nitrogen going into the solution (supernatant) after centrifugation. Around 95% of the total nitrogen was recovered in the supernatant.

Preparation of protein concentrates. The procedures used for the preparation of sodium perchlorate extracted protein concentrate (P-PC) and sodium trimetaphosphate treated concentrate (TMP-PC) are illustrated in Fig. 1.

Analytical procedures. Moisture, ash, and total protein contents were determined by procedures described in the AOAC (15). Total carbohydrate was determined by the method of Dubois et al (16) and total lipids by the procedure of Blight and Dyer (17). Nucleic acid (RNA) was determined by the method of Hebert et al (18). Amino acid composition, except tryptophan, was determined in
Fig. 1. Flow diagram of the production of yeast protein concentrates (TMP-PC and P-PC). STMP, sodium trimetaphosphate.

an acid hydrolysate (6 N HCl, 110°C, 22 h) using an autoanalyser with a cation exchange column and ninhydrin reaction for quantification. Tryptophan was determined in an alkaline hydrolysate (4 N LiOH, 110°C, 24 h) by the same analytical procedure. Available lysine was determined after reaction with trinitrobenzene sulfonic acid (TNBS) by the procedure of Kakade and Liener (19). In vitro digestibility was determined by the method of Akeson and Stahman (20).

Protein phosphorylation. The degree of protein phosphorylation (DF) was determined as the ratio of the quantity of pyrophosphate produced during the reaction of trimetaphosphate to the quantity released theoretically on the basis of the serine content in the protein, under the complete substitution of OH groups. The pyrophosphate quantities were determined indirectly by complexometric
titration of zinc ions using a 0.01 EDTA solution with erichromate black as the indicator (21).

**Biological assays.** Protein nutritive values were determined by two different assays: nitrogen balance and protein efficiency ratio (PER). Weanling rats of the Wistar strain, specific pathogen-free (SPF) were used.

Diets were prepared containing 10% protein from the different yeast preparations and a 10% casein reference diet. All the diets contained, in addition, 8% lipids (soybean oil), and vitamin and mineral mixtures according to AIN-93G-MX and AIN-93-VX, respectively (22). Cellulose powder (5%) was added as fiber, except in the whole yeast biomass. Choline bitartarate and t-butylhydroquinone were also added to all diets. Carbohydrate (3:1 corn starch and sucrose) was added to complete 100%.

Groups of 7 rats were fed ad libitum and the laboratory conditions were kept at $21 \pm 2^\circ C$ under 12h light and 12h dark. The duration of the experiments was 28d for PER and a seven-day nitrogen balance test was done in the same experiment during the second week of diet administration.

**Statistical analysis.** A statistical analysis system (SANEST) was employed for the analysis of variance, and the differences between means were established by the Tukey test (23) at a 5% probability level.

### RESULTS

**Proximate percent composition**

The proximate percent composition of the yeast biomass (YC), a protein concentrate obtained by extraction with sodium perchlorate solution and precipitated at pH 4.2 (P-PC), and a protein concentrate obtained by treatment of the extract with sodium trimetaphosphate following precipitation at the isoelectric pH (TMP-PC) are shown in Table 1.

The main changes in the composition of the protein concentrates, compared to the whole yeast biomass were as follows: an increase in protein concentration from 48.5% in the biomass to 76% (P-PC) and 74.2% (TMP-PC); a substantial decrease in RNA, from 7.5% in the biomass to 2.2% (P-PC) and 3.7% (TMP-PC); there was also a substantial reduction in total carbohydrate, from 33% in YC to 8.8% (P-PC) and 8.3% (TMP-PC), probably as a consequence of the removal of cell wall which accounts for 30% of the total carbohydrate.

**Amino acid composition**

The amino acid profiles of yeast biomass (YC), the perchlorate protein concentrate (P-PC), and the sodium trimetaphosphate treated concentrate are shown in Table 2. By comparing with the FAO/WHO/UNU essential amino acids reference profile (24), it becomes apparent that the yeast proteins contain all the essential amino acids in excess of the reference, with the only exception being the sulfur-containing amino acids which appear as marginally limited in the yeast protein.
Table 1. Percent composition of yeast biomass and yeast protein concentrates (Saccharomyces sp.).

<table>
<thead>
<tr>
<th>Components*</th>
<th>YC</th>
<th>P-PC</th>
<th>TMP-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein¹ (%)</td>
<td>48.51</td>
<td>76.00</td>
<td>74.16</td>
</tr>
<tr>
<td>RNA (%)</td>
<td>7.52</td>
<td>2.20</td>
<td>3.67</td>
</tr>
<tr>
<td>Total lipids (%)</td>
<td>3.44</td>
<td>6.30</td>
<td>3.99</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>8.33</td>
<td>6.10</td>
<td>9.81</td>
</tr>
<tr>
<td>Total carbohydrate (%)</td>
<td>32.86</td>
<td>8.78</td>
<td>8.30</td>
</tr>
<tr>
<td>Total fiber</td>
<td>12.19</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>9.59</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>2.60</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Percentages on a dry basis; ¹ protein was calculated using the N factor 5.8, 6.2 and 6.05 for YC, P-PC and TMP-PC, respectively, after discounting the sample nucleic acid N; YC, yeast cells (biomass); P-PC, perclorate protein concentrate; TMP-PC, trimeta- phosphate protein concentrate; ND, not determined.

Table 2. Amino acid composition for the yeast biomass and protein concentrates.

<table>
<thead>
<tr>
<th>Amino acids (g/100 g protein)</th>
<th>YC</th>
<th>P-PC</th>
<th>TMP-PC</th>
<th>FAO/WHO/UNU reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.13</td>
<td>8.78</td>
<td>8.64</td>
<td>5.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.84</td>
<td>8.62</td>
<td>7.47</td>
<td>6.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.64</td>
<td>5.09</td>
<td>4.99</td>
<td>2.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>6.16</td>
<td>4.07</td>
<td>4.41</td>
<td>3.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.10</td>
<td>1.39</td>
<td>1.15</td>
<td>1.1</td>
</tr>
<tr>
<td>Valine</td>
<td>6.20</td>
<td>5.91</td>
<td>6.12</td>
<td>3.5</td>
</tr>
<tr>
<td>Methionine + Cystine</td>
<td>2.84</td>
<td>2.30</td>
<td>2.48</td>
<td>2.5</td>
</tr>
<tr>
<td>Phenylalanine + Tyrosine</td>
<td>9.98</td>
<td>8.79</td>
<td>8.43</td>
<td>6.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.06</td>
<td>2.77</td>
<td>2.69</td>
<td>1.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.50</td>
<td>1.82</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.30</td>
<td>4.96</td>
<td>4.64</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>13.15</td>
<td>9.88</td>
<td>9.74</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>11.98</td>
<td>11.66</td>
<td>11.07</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>6.13</td>
<td>3.68</td>
<td>3.86</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>4.45</td>
<td>3.16</td>
<td>3.44</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>7.07</td>
<td>5.55</td>
<td>6.02</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>4.93</td>
<td>4.14</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>4.11</td>
<td>4.96</td>
<td>4.92</td>
<td></td>
</tr>
</tbody>
</table>

YC, broken yeast cell biomass; P-PC, perclorate protein concentrate; TMP-PC, trimetaphosphate protein concentrate.
concentrates, but not in the whole biomass. It should be noticed that the lysine and threonine concentrations are fairly high, which makes this source of protein suitable for correcting cereal protein amino acid imbalance.

**In vitro indexes of protein quality**

*In vitro* protein digestibility, available lysine, amino acid chemical score, and the protein digestibility-corrected amino acid scoring (12) are shown in Table 3.

*In vitro* protein digestibility was lower (83%) for the whole biomass (YC) and quite high (91.7 and 89.4%) for the P-PC and TMP-PC concentrates, respectively. Available lysine was highest (7 g/100 g protein) for the TMP-PC concentrate, and lowest (5 g/100 g protein) for the concentrate extracted with perchlorate (P-PC).

Total sulfur-containing amino acids (Met+Cys) were marginally limited in relation to the FAO/WHO/UNU reference. The chemical scores were 0.92, 0.99 and 1.14 for P-PC, TMP-PC and YC, respectively. The protein digestibility-corrected amino acid scoring (PDCAAS) varied from 82 to 90%, being highest (90%) for TMP-PC and lowest (82%) for P-PC.

**Nitrogen balance and PER**

An *in vivo* evaluation of the yeast protein nutritive value was performed by nitrogen balance and PER determination.

Table 4 shows the results of the assays, including data from the casein control diet. Comparing with the casein diet, the nitrogen retention (NB) was statistically identical for the whole yeast (YC) and inferior (*p*≤0.05) for the two protein concentrates, P-PC and TMP-PC. Urine N was significantly higher for YC, intermediate for the yeast protein concentrates, and lowest for casein. Fecal N was identical for casein and TMP-PC but statistically higher (*p*<0.05) for P-PC and YC. The intake of N did not differ for casein, P-PC or YC, but it was significantly lower for TMP-PC.

Apparent protein digestibility (Da) was identical for casein and TMP-PC, but

<table>
<thead>
<tr>
<th>Determination</th>
<th>YC</th>
<th>P-PC</th>
<th>TMP-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestibility (%)</td>
<td>83.06</td>
<td>91.72</td>
<td>89.45</td>
</tr>
<tr>
<td>Available lysine (g/100 g protein)</td>
<td>6.39</td>
<td>5.06</td>
<td>7.07</td>
</tr>
<tr>
<td>Amino acid scores&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.14</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>PDCAAS&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>86.00</td>
<td>82.00</td>
<td>90.00</td>
</tr>
</tbody>
</table>

<sup>1</sup> Based on the most limiting amino acid by comparison with FAO/WHO/UNU reference standard (24) for children 2 to 5 years of age; <sup>2</sup> Protein digestibility-corrected amino acid scoring (12).
Table 4. Protein nutritional evaluation in vivo of yeast biomass (YC) and two protein concentrates (P-PC, TMP-PC) prepared by different procedures.

<table>
<thead>
<tr>
<th>Index of evaluation</th>
<th>YC</th>
<th>TMP-PC</th>
<th>P-PC</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen retention (g)</td>
<td>1.62 ± 0.19a</td>
<td>1.38 ± 0.11b</td>
<td>1.47 ± 0.11b</td>
<td>1.81 ± 0.21a</td>
</tr>
<tr>
<td>Apparent protein digestibility (%)</td>
<td>85.36 ± 1.36b</td>
<td>90.86 ± 1.18a</td>
<td>89.13 ± 1.37a</td>
<td>92.55 ± 1.10a</td>
</tr>
<tr>
<td>Apparent protein biological value (%)</td>
<td>87.99 ± 4.79a</td>
<td>86.26 ± 3.59a</td>
<td>88.64 ± 6.43a</td>
<td>91.05 ± 3.04a</td>
</tr>
<tr>
<td>Apparent net protein utilization (%)</td>
<td>75.10 ± 3.55c</td>
<td>78.43 ± 2.51b</td>
<td>79.01 ± 5.95b</td>
<td>84.27 ± 3.00a</td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>2.16 ± 0.16b</td>
<td>2.26 ± 0.11b</td>
<td>2.06 ± 0.14c</td>
<td>3.16 ± 0.24a</td>
</tr>
</tbody>
</table>

1 Mean value (24 h) for seven rats; a,b,c values with different superscript letters indicate statistically different values (p ≤ 0.05); YC, yeast cell biomass; P-PC, protein concentrate obtained with sodium perchlorate; TMP-PC, protein concentrate phosphorylated with sodium trimetaphosphate; CAS, casein used for comparison.

significantly lower (p ≤ 0.05) for P-PC and YC. YC protein digestibility was the lowest. Protein apparent biological value (BVa) was identical for all treatments. NPUa was identical for casein and P-PC, but lower (p ≤ 0.05) for YC and TMP-PC. PER was significantly higher for casein in relation to all three yeast preparations. Among the yeast preparations, PER was identical for YC and TMP-PC but significantly lower for P-PC.

The growth curves for the rats fed the various diets for 28 d are shown in Fig. 2. The ranking with regard to rat growth after 28 d on the diets was casein first, followed by whole yeast biomass (YC), then TMP-PC and last P-PC.

**DISCUSSION**

According to Kinsella (7), in the intact yeast cell, soluble cytoplasmic proteins, ribosomal proteins and cell wall and/or membrane-bound proteins account for approximately 40, 40, and 20%, respectively, of the total protein. During cellular disruption some of the cytoplasmic proteins become complexed with nucleic acids which, together with the ribosomal protein, precipitate in the isoelectric pH (~4.2) as a nucleoprotein complex. Some 50–60% of the protein precipitates as nucleoprotein, containing 15–20% nucleic acid.

Chemical dissociating agents have been used in an attempt to isolate protein from yeast biomass with lower nucleic acid contents. In this research work, NaClO₄ (a chaotropic anion) at 0.5 M was effective in reducing the RNA concentration in the yeast biomass from 7.5% to 2.2% in the isoelectric precipitate. Similar results
Fig. 2. Growth curves of weanling rats (groups of 7 rats) on diets containing 10% protein from casein (●); sodium trimetaphosphate treated protein concentrate (□); sodium perchlorate extracted protein concentrate (▲), and brewer's yeast broken cell biomass (×).

were obtained and reported by Damodaran and Kinsella (25, 26). These authors concluded that the chaotropic anions act by weakening the hydrogen-bonded structure of water, altering the interfacial properties in protein association and reducing the RNA content of the isolated proteins.

Phosphorylation has also been used for the purpose of lowering the RNA content of precipitated yeast protein. The phosphorylation of yeast nucleoprotein epsilon-amino groups using phosphorous oxychloride (POCl₃) at pH 9.0 progressively reduced nucleic acid, and a 90% reduction in the RNA content of isoelectrically precipitated protein was obtained when 30% of the available epsilon-amino groups was phosphorylated (27, 28).

Protein phosphorylation has also been accomplished using sodium trimetaphosphate (STMP) on soy protein (29) and yeast protein isolate (10).

According to Sung et al (29), there is evidence that phosphoesterification of hydroxyl amino acids and phosphoramidation of lysine may take place when STMP is reacted with a protein. Specifically, the primary hydroxyl group of serine residues in soy protein is preferred to the secondary hydroxyl group of threonine residues,

J Nutr Sci Vitaminol
reacting irreversibly with STMP under alkaline conditions, and resulting in the formation of stable O-phosphoserine and an equivalent amount of pyrophosphate. The epsilon-amino group of lysine residues may react more efficiently with STMP in alkali to produce an acid labile ε-N-lysinosotriphosphoramidate. If the reaction mixture is acidified to below pH 5.0, the phosphoramidic bonds are cleaved reversibly.

Conflicting data have been reported (30, 31) as to whether or not STMP can covalently bind to proteins. Matheis (31) failed to detect any covalently bound phosphate when soybean protein and lysozyme were treated with STMP as described by Sung et al (29).

Several advantages have been demonstrated by treating food protein with sodium trimetaphosphate, such as: the reaction is not destructive to lysine; it improves protein functional properties; and sodium trimetaphosphate has been recognized as a generally recognized as safe (GRAS) substance by the United States Food and Drug Administration (FDA).

Whole yeast biomass contain a fairly high fiber content (~12%) on a dry basis (Table 1). Almost 10% of the total is soluble fiber composed mainly of α-glycans, mannans and glycoproteins (1). These fiber components may have important functions as food ingredients and their physiological importance should be better explored.

The amino acid profiles of the whole yeast biomass and of the two protein concentrates derived from it (Table 2) are adequate to meet the essential amino acid requirements of 2 to 5 year-old children according to the FAO/WHO/UNU reference standard (24). The whole yeast biomass (YC) showed an excess of all essential amino acids when compared to the reference standard (24). The two protein concentrates P-PC and TMP-PC exhibited only a marginal deficiency of the total sulfur-containing amino acids.

The amino acid scores and the protein digestibility-corrected amino acid scoring (PDCAAS) calculated according to Henley and Kuster (12) are compared in Table 3. Assuming the score of 1.0 for casein (12), the scores for yeast protein are nearly equal to casein. The PDCAAS, calculated by multiplying the limiting amino acid scores by digestibility, in vivo, ranged from 82% for P-PC to 90% for TMP-PC, a fairly high index of protein utilization.

According to the Young and Pellet tentative adult requirement for essential amino acids (13), the essential amino acid composition of yeast protein seems to be adequate for both children and adults.

In vitro protein digestibility and available lysine also appear in Table 3. Digestibility was very high for P-PC and TMP-PC, but considerably lower for YC. The lower digestibility for the whole yeast protein as compared to the protein concentrates is suggestive of interference of cell wall components on the proteolytic action of the digestive enzymes.

Available lysine was highest (7.0 g/100 g protein) in TMP-PC, lowest (5.0 g/100 g protein) in P-PC and intermediate (6.4 g/100 g protein) in the whole yeast biomass.
The high availability of lysine in the phosphorylated protein may be a consequence of the reversible reaction of the sodium trimetaphosphate in changing from a strongly alkaline to a slightly acidic condition. On the other hand, the low availability of lysine in P-PC may result from the alkaline conditions of extraction and/or the effect of a high concentration of residual sodium perchlorate on the protein.

The nutritional properties of yeast protein and casein as determined by two different procedures in vivo appear in Table 4. To the authors’ knowledge no in vivo nutritional evaluation of yeast protein concentrates treated with sodium perchlorate or sodium trimetaphosphate have been reported.

Protein digestibility was high for the two concentrates (P-PC and TMP-PC) and did not differ statistically ($p \leq 0.05$) from casein digestibility. The whole yeast biomass (YC) showed statistically inferior protein digestibility.

In terms of nitrogen utilization, the whole yeast biomass (YC) promoted the highest nitrogen retention (NB), which was statistically identical to casein. For the protein concentrates P-PC and TMP-PC, this index was statistically identical and inferior to casein and YC ($p \leq 0.05$). Net protein utilization (NPUa) was highest for caseins inferior for TMP-PC and P-PC but identical among themselves ($p \leq 0.05$), and significantly lower for YC. No statistical differences were detected ($p \leq 0.05$) in the BVA of all the protein sources tested.

All yeast protein preparations gave PER significantly inferior to casein. The lowest PER (2.06) was determined for P-PC, while YC and TMP-PC gave PER statistically identical but superior to P-PC and inferior to casein ($p \leq 0.05$).

The growth promoting capacity of the three yeast protein diets is compared with that of casein in Fig. 2. Casein showed the best results, followed by the whole yeast biomass (YC) and the protein concentrate TMP-PC. The lowest performance was observed for P-PC concentrate.

Taking the PER and NPUa as the most significant indexes of protein quality, and averaging them up for the three yeast preparations and comparing them with the casein values, one verifies that the NPUa for yeast protein preparation stands above 90% of that for casein. For the PER, the yeast protein values averaged about 70% of casein.

The data presented in this paper permit us to conclude: 1) Yeast biomass is an excellent source of protein both quantitatively and qualitatively; 2) Extraction of the yeast protein with 0.5M sodium perchlorate or treatment of the cell suspension or protein extract with 3% sodium trimetaphosphate under alkaline conditions were both effective in lowering the RNA content in the protein precipitate at isoelectric pH; 3) The various yeast preparations exhibited protein nutritive values in the range of 70 to 90% of casein, depending on the index used for evaluation; and 4) NPUa and PDCAAS values for the yeast protein came closer to casein values than the PER.

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Yeast Biomass and Yeast Protein Concentrates 611
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