Nutritional Evaluation of Whey Protein Concentrates and Their Fractions

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Abstract

Nutritional evaluation of whey protein concentrates and whey protein fractions was performed by Protein Efficiency Ratio and Net Protein Utilization assays as well as by calculations of chemical scores. The whey protein concentrates were industrially produced by gel filtration and ultrafiltration and were fractionated further by large-scale gel filtration. Nutritional values of the two concentrates were similar and high. Whey protein fractions containing α-lactalbumin had high Protein Efficiency Ratio and Net Protein Utilization values while fractions containing β-lactoglobulin had high Net Protein Utilization values but only moderate Protein Efficiency Ratio values. According to the calculated chemical scores, amino acid contents of the concentrates and of all the fractions were almost adequate to cover the needs of the human infant and more than adequate to cover those of young children. Utilization of the α-lactalbumin-rich fraction III in humanized breast milk substitutes is discussed.

Introduction

Casein, the dominating protein in cow's milk, is nutritionally inferior to whey proteins. However, as pointed out earlier (11), whey proteins are from cheesemaking in dilute solution with a high lactose content, which complicates their utilization as human food. Until recently, whey proteins have been commercially available as lactalbumin, a protein product with a high protein nutritive value (19) containing highly denatured whey proteins.

Recently, however, methods for large-scale production of undenatured whey protein concentrates have been developed (18). Such concentrates are of high nutritive value and can be valuable food ingredients (19). They can also be fractionated into their different protein components (11) which may be utilized in preparation of infant foods.

In this paper, nutritional evaluation of whey protein fractions obtained after gel filtration on Sephadex® G-75 of two whey protein concentrates prepared by gel filtration and ultrafiltration is described. The possible utilization of some of these fractions in breast milk substitutes is discussed. Preparation procedure and chemical evaluation of the fractions have been described previously (11).

Materials and Methods

Casein (Sheftene high nitrogen casein) was obtained from Sheffield Chemical, Norwich, New York, USA.

Gel-filtered and ultrafiltered whey protein concentrates (WPC) as well as whey protein fractions were obtained as described earlier (11). Also according to these results (11), fraction II contained mainly β-lactoglobulin and fraction III was rich in α-lactalbumin while fraction I represented a mixture of casein, bovine serum albumin, and possibly other proteins.

Protein Efficiency Ratio (PER) assay was according to a modification (10) of the method described by Campbell (3). Weaning male rats, 21 days old, were fed experimental diets containing 10% protein ad libitum for 4 wk. Food intakes and weight gains were recorded and PER values calculated after each week.

Net Protein Utilization (NPU) assay was according to a modification (7) of the method described by Miller and Bender (16). Weaning male rats, 28 days old, were fed experimental diets containing 9% protein ad libitum for 10 days, after which the nitrogen content of the rats was determined by carcass analysis.

Total nitrogen content was estimated as described earlier (11). For calculating the crude protein content the conversion factor was 6.25.

Amino acid compositions of the respective concentrates and fractions have been reported earlier (11).

For calculating the chemical score (A/T score), egg reference and human milk reference amino acid patterns 1973 (4), as well as the FAO provisional pattern 1973 (5), were used.

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TABLE 1. Nutritive values of gel-filtered and ultrafiltered whey protein concentrates (WPC) and the respective whey protein fractions.

<table>
<thead>
<tr>
<th></th>
<th>PER* (4 wk)</th>
<th>Significance of difference between means</th>
<th>NPU</th>
<th>No. of replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gel-filtered WPC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>4.01 ± .29</td>
<td></td>
<td>94.0 ± 2.2</td>
<td>2</td>
</tr>
<tr>
<td>fraction I</td>
<td>. . .</td>
<td></td>
<td>82.3 ± .4</td>
<td>2</td>
</tr>
<tr>
<td>fraction II</td>
<td>2.96 ± .21</td>
<td>x</td>
<td>86.7 ± 5.3</td>
<td>3</td>
</tr>
<tr>
<td>fraction III</td>
<td>4.16 ± .18</td>
<td>xxx</td>
<td>86.6 ± 1.6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Ultrafiltered WPC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>4.29 ± .20</td>
<td></td>
<td>84.8 ± 7.1</td>
<td>3</td>
</tr>
<tr>
<td>fraction I</td>
<td>. . .</td>
<td></td>
<td>74.9 ± 7.0</td>
<td>3</td>
</tr>
<tr>
<td>fraction II</td>
<td>3.52 ± .15</td>
<td>x</td>
<td>86.5 ± 6.6</td>
<td>3</td>
</tr>
<tr>
<td>fraction III</td>
<td>3.97 ± .20</td>
<td>x</td>
<td>90.1 ± 3.9</td>
<td>3</td>
</tr>
<tr>
<td>Casein</td>
<td>3.33 ± .30</td>
<td></td>
<td>78.9 ± 3.0</td>
<td>6</td>
</tr>
</tbody>
</table>

* Mean of 10 observations.

PER = Protein Efficiency Ratio; NPU = Net Protein Utilization.

**Fig. 1.** Protein Efficiency Ratio (PER) values after 1 to 4 wk on diets containing gel-filtered and ultrafiltered whey protein concentrate (WPC) as well as fractions II and III from these two concentrates. Casein is shown as reference.
NUTRITIONAL EVALUATION OF WHEY PROTEINS

Statistical calculation of means and standard deviations were by conventional methods. Student's t-test was used to determine significances of differences between means, and significance is as described earlier (11).

Results

Table 1 shows the PER values after 4 wk of administration of gel-filtered and ultra-filtered whey protein concentrates as well as of fractions II and III, respectively, of these two concentrates. The PER value of casein is given for comparison. In Fig. 1, the PER values after 1 to 4 wk on the same diets are presented graphically.

Ultrafiltered WPC had a slightly higher PER value after 4 wk than gel-filtered WPC, the difference being almost significant (Table 1). However, the PER values of gel-filtered WPC were higher than those of ultra-filtered WPC during the 3 earlier wk of the PER assay (Fig. 1). Fraction III of gel-filtered WPC was better than fraction II of this concentrate, and the difference was highly significant after 4 wk (Table 1). This agrees with the PER results of fractions II and III from ultra-filtered WPC where, similarly, fraction III was better than fraction II after 4 wk (P < .001). From Fig. 1 these differences were even greater during the earlier weeks of the PER assay.

After 4 wk the PER value of fraction II from ultrafiltered WPC was superior (P < .001) to that of fraction II from gel-filtered WPC, but this superiority was less marked during the first weeks of the PER assay (Fig. 1). On the other hand, with respect to fraction III, its 4 wk PER value from ultrafiltered WPC was lower (.01 < P < .05) than that of fraction III from gel-filtered WPC (Table 1), and this inferiority was more pronounced during the first weeks of the PER assay (Fig. 1). The PER value of casein was 3.33 after 4 wk (Table 1).

The NPU values of gel-filtered and ultra-filtered WPC and of fractions I, II, and III from these two concentrates are in Table 1. Gel-filtered WPC had a higher NPU value than ultrafiltered WPC. Fractions II and III from gel-filtered WPC had the same NPU value, 87, while the NPU value of fraction III from ultrafiltered WPC was higher than that of fraction II from gel-filtered WPC, 90 as compared with 87. The NPU value of casein was 79. Some chemical nutritive values of ultrafiltered and gel-filtered WPC and of fractions I, II, and III from these concentrates are in Table 2.

Contents of essential amino acids were high in the two types of WPC and also in fractions II and III while fraction I had lower values. On calculation of chemical scores by the egg reference amino acid pattern (4), the two concentrates and all the fractions showed scores of about 70 while when the human milk amino acid reference pattern (4) was used, scores were about 90 to 100 in most cases. On using the FAO provisional pattern 1973 (5) as reference, chemical scores were above 100 for the two concentrates and for all the fractions.

Table 2. Chemical nutritive values of gel-filtered and ultrafiltered whey protein concentrates (WPC) and of fractions I, II, and III from these two concentrates. The contents of total essential amino acids are given in mg/g N, and chemical scores calculated with the use of egg and human milk amino acid reference patterns and the FAO provisional pattern are given together with the respective limiting amino acids.

<table>
<thead>
<tr>
<th></th>
<th>Total essential amino acids (mg/g N)</th>
<th>Chemical score calculated with the use of:</th>
<th>Limiting amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg ref.</td>
<td>Human milk ref.</td>
</tr>
<tr>
<td>Gel-filtered WPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>3630</td>
<td>75</td>
<td>95</td>
</tr>
<tr>
<td>fraction I</td>
<td>2925</td>
<td>77</td>
<td>104</td>
</tr>
<tr>
<td>fraction II</td>
<td>3855</td>
<td>75</td>
<td>98</td>
</tr>
<tr>
<td>fraction III</td>
<td>3765</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td>Ultrafiltered WPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>3610</td>
<td>71</td>
<td>92</td>
</tr>
<tr>
<td>fraction I</td>
<td>3110</td>
<td>64</td>
<td>87</td>
</tr>
<tr>
<td>fraction II</td>
<td>3770</td>
<td>73</td>
<td>94</td>
</tr>
<tr>
<td>fraction III</td>
<td>3630</td>
<td>72</td>
<td>92</td>
</tr>
</tbody>
</table>

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Phenylalanine and tyrosine were the limiting amino acids in gel-filtered and ultrafiltered whey protein concentrates as well as in fractions II and III from these concentrates. Fraction I, however, was limited by methionine and cysteine.

Discussion

The nutritive values of the two whey protein concentrates were high and similar as indicated by the PER and NPU values as well as by their high contents of essential amino acids. However, ultrafiltered WPC had the highest PER value while gel-filtered WPC had the highest NPU value. Likewise, PER and NPU results differ in fractions II and III of the two concentrates. Fraction III from ultrafiltered and gel-filtered WPC has PER values well above those of fraction II from the same concentrates while the NPU values either show no or little difference.

This discrepancy between the PER and NPU results of fractions II and III is difficult to explain. It might be due partly to the fact that PER measures only the growth-promoting capacity of the test protein while NPU measures the adequacy of the test protein for growth as well as for maintenance (13).

However, nutritive values of both fractions II and III are high. Fraction III also would likely have a high nutritive value for human beings since its PER and NPU values as well as its contents of essential amino acids are high.

Fraction II seems of lower nutritive value than fraction III by their PER values, but whether this is valid for the human being cannot be stated from this study although the high contents of essential amino acids in fraction II also indicate a high nutritive value.

According to the chemical scores, all the concentrates and fractions are deficient in one or several amino acids as compared with egg protein since when egg is used as reference, the chemical score is about 70 in all diets tested. However, calculation using the human milk reference pattern gives higher scoring values, indicating that the essential amino acid contents of all the diets are almost adequate to cover the needs of the human infant. Chemical scores above 100 were still higher when the FAO provisional pattern 1973 was the reference; thus, the two concentrates and all the fractions should be adequate for young children since this FAO pattern is formulated to cover the amino acid requirements of all ages except infants (6).

The high content of essential amino acids in the whey protein concentrates is of interest since such concentrates could be utilized to improve the nutritional value of cereal proteins and other proteins of low quality (7, 8, 10). Whey protein fractions, on the other hand, might find application in the preparation of infant and dietetic foods, in which cases a high nutritive value is important. For example, Forsum and Hambraeus recently suggested the use of a whey protein fraction in the dietary treatment of phenylketonuria (9). Utilization of a whey protein fraction in an infant formula is discussed below.

In Sweden, so called humanized infant formulas intended as substitutes for human milk are commercially produced. In such formulas the ratio between casein and whey protein is adjusted to simulate the composition of human milk. Casein constitutes only 40% (15) of the protein in human milk while the corresponding figure in cow's milk is 80% (12); thus, human milk contains relatively more whey proteins than does cow's milk. This humanization is accomplished by the addition of dried whey to dried skim milk. However, the main bovine whey protein β-lactoglobulin is not in human milk (1) where α-lactalbumin probably is the main whey protein (14, 17). Thus, if α-lactalbumin could be used instead of whole whey in the preparation of breast milk substitutes, even more humanized products would be obtained.

This could be accomplished as shown in Fig. 2 where the amino acid composition of an hypothetical breast milk substitute is in percent of the amino acid composition of human milk. In this breast milk substitute, 40% of the nitrogen is derived from bovine casein, 40% from fraction III from ultrafiltered whey protein concentrate (which is rich in α-lactalbumin), and 20% from nonprotein nitrogen. This mixture was chosen since human milk contains approximately 40% casein, 40% whey protein, and 20% nonprotein nitrogen. In this figure the amino acid composition of a commercial Swedish breast milk substitute (2) is also given for comparison. Amino acid composition of the above protein mixture is more similar to that of human milk than is the commercial breast milk substitute.

Acknowledgements

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References

(1) Bell, K., and H. A. McKenzie. 1964. β-lac-
Fig. 2. The amino acid composition of a protein mixture consisting of 40% casein, 40% fraction III from ultrafiltered whey protein concentrate (WPC), and 20% nonprotein nitrogen, and of a commercial breast milk substitute, shown in percent of the amino acid contents of human milk.

(18) O'Sullivan, A. C. 1972. Whey processing by reverse osmosis, ultrafiltration and gel fil-