

# Elemental (Macro- and Microelements) and Amino Acid Profile of Milk Proteins Commercialized in Brazil and Their Nutritional Value

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**Abstract** Whey protein concentrate (WPC, 35 and 80% of protein), hydrolysate (WPH), isolate (WPI) and casein were evaluated regarding to essential and non-essential aminoacids and mineral content. WPC35, WPC80 and WPH were the products with the highest concentrations of macroelements (Ca, K, Mg, Na and P) reaching contents between 46.9 and 83.8% of Dietary Reference Intakes (DRIs) for calcium. For microelements (Cu, Fe, Mn and Zn) higher amounts were observed for casein with Zn and Fe content of 40.1 and 93.0% of the DRIs, respectively. For WPI values lower than 3.2% of the DRIs of Ca, Cu, Fe, K, Mg, Mn and Zn were observed, but WPI was a source of sulfur-containing and branched-chain aminoacids, lysine, threonine and total essential aminoacids whereas casein could be considered source of aromatic aminoacids with contents that reached the DRIs values. The sodium content (up to 59.3% of DRIs) was high for all whey proteins.

**Keywords:** essential and non-essential amino acids, macro- and microelements, composition, dairy products

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## 1. Introduction

The high nutritional value of milk proteins is widely recognized, and dairy products contribute significantly to daily protein intake from the diet [1]. The whey fraction of milk contains a great variety of proteins, and it can be considered the principal by-product of cheese and casein manufacture. Taking into account the presence of proteins of high nutritional value and the versatile functional properties of whey, the intensive use of this by-product by the food and pharmaceutical industries is expected [2]. However, the problem associated with whey utilization is that a very large volume of whey is produced worldwide each year, which contains only dilute concentrations of these valuable proteins. Therefore, whey protein concentrates (WPC), whey protein isolates (WPI) and the enzymatic hydrolysates of milk proteins (WPH) are manufactured industrially and have found extensive use in a wide range of foodstuffs, such as infant formulas, dietary supplements, and clinical and sports diet formulations [3,4].

The composition of milk could be affected by the environmental and nutritional conditions to which an animal is conditioned, as well as by post-milking handling, transportation and processing, which could affect the

composition of milk proteins. However, whey protein preparations are also largely affected by the method used to process them [2,4]. Membrane-separation processes are now industrially applied in the manufacture of ordinary whey powder [5], and WPC with a protein content between 30 and 80% can be obtained commercially [4]. Gel filtration and ion-exchange chromatography techniques can be employed in the manufacture of whey protein isolates (WPI) with a protein content that could reach 90-95% [4]. Fractions of whey protein can also be obtained by nanofiltration, which allows for the selective separation of salts and ions from whey and has made it possible to manufacture industrially demineralized whey protein [2,4]. It is important to mention that during processing modification of the chemical and nutritional properties of whey proteins could be observed, with denaturation of proteins caused by unfolding or aggregation processes (reversible or irreversible) [6]. The denaturation of whey proteins is often observed in heat treatments (influenced mainly by heating/cooling rates and also hold temperatures), but pH and protein concentration could also influence the extent of this process [6,7]. Therefore, depending on the origin of the milk used and the manufacturing process applied, variations in whey protein composition can be expected. However, the literature offers little information regarding the comparison of different whey preparations commercialized as ingredients for food manufacture. Thus,

in this study, several chemical analyses were performed (proximate composition, mineral and amino acids determinations) to evaluate WPC (35 and 80% of protein), WPH and WPI commercially available in Brazil. Casein was also evaluated for comparison of results. A principal component analysis (PCA) was applied to demonstrate the similarities and differences among milk proteins. The nutritional value of each milk protein is discussed, particularly in relation to the presence of macro- and micromineral elements.

## 2. Material and Methods

### 2.1. Samples

Whey protein samples were supplied by Doremus Alimentos, Guarulhos, SP, Brazil with labeled protein contents of 35 and 80% for whey protein concentrate (WPC35 and WPC80, respectively), 80% for WPH and 90% for WPI. Commercial casein (Vetec Química Fina Ltda, Rio de Janeiro, RJ, Brazil) was used in this study.

### 2.2. Instrumentation

An oven (model 400/2ND, Nova Ética, Vargem Grande Paulista, SP, Brazil) was used for moisture determination and for drying samples before the determination of amino acids and mineral elements. Samples were weighed on an analytical balance (model AY 220, max. 220 g, 0.1 mg of resolution, Shimadzu, Kyoto, Japan). A microwave oven (Multiwave 3000 microwave sample preparation system, Anton Paar, Graz, Austria) equipped with eight high-pressure quartz vessels (internal volume of 80 mL, maximum operational temperature and pressure of 280°C and 80 bar, respectively) was used in the experiments. Elements were determined using an inductively coupled plasma optical emission spectrometer (ICP OES, Optima 4300 DV, PerkinElmer, Shelton, USA) with an axial view configuration. A concentric nebulizer and cyclonic spray chamber were used. Argon (99.996%, White Martins, São Paulo, Brazil) was used for plasma generation, nebulization and as an auxiliary gas. A high-performance liquid chromatography instrument (HPLC, Shimadzu, Tokyo, Japan) equipped with a photodiode array (SPD-20A) and a Luna C-18 column (100 Å, 5 µm, 250 × 4.6 mm, 00G-4252-EQ, Phenomenex, Torrance, CA) heated to 50°C in an oven (CTO-20A) was used for total amino acid determination.

### 2.3. Reagents and Standards

Distilled, deionized water was purified (Milli-Q, 18.2 MΩ cm, Millipore, Billerica, MA, USA) before use. Analytical grade nitric acid (Merck, Darmstadt, Germany) was used to prepare samples and standards for element determination. A multi-element stock solution (SCP33 MS, SCP Science, Quebec, Canada) was used to prepare reference solutions for ICP OES determination. Argon (99.996%, White Martins-Praxair, São Paulo, SP, Brazil) was used for plasma generation and nebulization in ICP OES determination and as an auxiliary gas. For amino acid determination, dl-2-aminobutyric acid was used as an internal standard (Sigma-Aldrich Corp., St Louis, MO, United States). The solvents used for the mobile phase of

HPLC determination were of chromatography grade, and all other reagents were of analytical grade.

### 2.4. Proximate Composition Determination

The evaluation of proximate composition was performed using the methods described by the AOAC (Association of Official Analytical Chemists) [8]. Moisture was determined by loss on drying in an oven at 105°C; ash content was determined at 550°C, and protein content ( $N \times 6.38$ ) was determined by the micro-Kjeldahl procedure. Total lipid content was determined using 3.5 g of sample and chloroform, methanol and water (10, 20 and 8 mL, respectively) as solvents, as described previously [9]. Carbohydrates were calculated by the difference method. All determinations were performed in triplicate.

### 2.5. Microwave-assisted Digestion and ICP OES Determination Of Elements

Samples were digested according to a procedure described previously [10,11]. Diluted nitric acid (6 mL of 3 mol L<sup>-1</sup> HNO<sub>3</sub>) was used for the digestion of 400 mg of samples placed in high-pressure quartz vessels. After closing and capping the rotor, the vessels were pressurized with 7.5 bar of oxygen and placed inside the oven. The microwave-heating program was initiated by applying a power of (i) 1000 W with a ramp of 5 min, (ii) 1000 W for 10 min, and (iii) 0 W for 20 min (cooling step). After digestion, the pressure in each vessel was carefully released. The digests were transferred to 50-mL polypropylene vials and diluted up to the mark with water. After digestion, all vessels were cleaned with 6 mL of concentrated HNO<sub>3</sub> in the microwave oven at 1000 W for 10 min and 0 W for 20 min for cooling. The ICP OES was calibrated using analytical solutions of concentrations ranging from 1.0 to 100 µg L<sup>-1</sup> that were prepared in 0.7 mol L<sup>-1</sup> HNO<sub>3</sub> by appropriate dilution of the multi-element stock solution. The plasma operating conditions and selected wavelengths were used as recommended by the instrument manufacturer and according to previous work [12]. Glass and quartz material were soaked in 1.4 mol L<sup>-1</sup> HNO<sub>3</sub> for 24 h and further washed with water before use.

### 2.6. Determination of Amino Acids

Amino acid determination was performed as previously described by [13]. For total amino acid determination, preliminary hydrolysis was performed using 6 M HCl (24 h, 110 °C). After centrifugation, the supernatant was filtered through a 0.22-µm membrane, and neutralization was performed (with a 4:4:2 solution of 0.2 N trihydrate sodium acetate, methanol and triethylamine). A 40-µL aliquot was derivatized with phenylisothiocyanate for further injection of 20 µL into the liquid chromatograph. An internal standard (dl-2-aminobutyric acid acid) was added before derivatization.

### 2.7. Statistical Analysis

The obtained data were statistically analyzed by analysis of variance (ANOVA) and Tukey's test ( $p < 0.05$ ) using the Statistica 7.0 software (Tulsa, USA, 2004). Additionally, an exploratory analysis of the data via PCA was performed to evaluate the correlation between the

variables and the possible groupings among the samples in the Pirouette 3.11 statistical program (Woodinville, USA, 2003). The data were autoscaled so that each variable could contribute the same weight in the analysis.

### 3. Results and Discussion

#### 3.1. Proximate Composition

Casein and WPI presented the highest protein contents (82.78 and 85.82%, respectively) but the lowest contents of lipids, carbohydrates and ashes (Table 1). The moisture

content of casein was the highest (11.12%), whereas other milk proteins presented values between 4.73 and 8.10%. Casein and WPI showed the lowest total lipids contents (1.69 and 1.15%, respectively), and values between 3.48 and 5.02% were observed for WPC35, WPC80 and WPH. It is important to note that the presence of lipids could affect the functional properties of whey and promotes the development of oxidation reactions, which impart off-flavors [14]; therefore, methods for reducing the fat content in whey protein products have been developed [15]. Hence, this parameter could be considered important for the determination of the quality of whey protein.

**Table 1. Proximate composition of casein and whey proteins (mean  $\pm$  standard deviation, n=3).**

| Parameters (%) | Casein                        | WPC35                         | WPC80                         | WPH                           | WPI                           |
|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Moisture       | 11.12 $\pm$ 0.02 <sup>a</sup> | 4.73 $\pm$ 0.02 <sup>es</sup> | 8.10 $\pm$ 0.08 <sup>b</sup>  | 7.31 $\pm$ 0.07 <sup>c</sup>  | 6.73 $\pm$ 0.03 <sup>d</sup>  |
| Ashes          | 1.91 $\pm$ 0.13 <sup>e</sup>  | 7.14 $\pm$ 0.03 <sup>a</sup>  | 2.87 $\pm$ 0.05 <sup>c</sup>  | 5.00 $\pm$ 0.02 <sup>b</sup>  | 2.39 $\pm$ 0.02 <sup>d</sup>  |
| Protein        | 82.78 $\pm$ 0.33 <sup>b</sup> | 33.80 $\pm$ 0.10 <sup>d</sup> | 72.89 $\pm$ 0.75 <sup>c</sup> | 74.21 $\pm$ 0.30 <sup>e</sup> | 85.82 $\pm$ 0.30 <sup>a</sup> |
| Total lipids   | 1.70 $\pm$ 0.11 <sup>d</sup>  | 3.48 $\pm$ 0.09 <sup>e</sup>  | 4.34 $\pm$ 0.44 <sup>b</sup>  | 5.02 $\pm$ 0.08 <sup>a</sup>  | 1.15 $\pm$ 0.11 <sup>d</sup>  |
| Carbohydrates  | 2.49 $\pm$ 0.35 <sup>e</sup>  | 50.85 $\pm$ 0.14 <sup>a</sup> | 11.80 $\pm$ 0.87 <sup>b</sup> | 8.46 $\pm$ 0.32 <sup>c</sup>  | 3.91 $\pm$ 0.32 <sup>d</sup>  |

Same letters in the same column indicate that the data do not differ statistically among them (Tukey's test;  $p \leq 0.05$ ).

The highest ash contents were observed for WPC35 and WPH, while for casein and WPI, lowest values were observed. The ash content could be directly related to the amount of Ca, K, Mg, Na and P presented in each sample (Table 2). By combining the concentrations of these elements and considering a ratio relative to the concentrations observed for WPC35 (the sample that presented the highest concentrations of these elements) for each sample, values of 0.28, 0.43, 0.71 and 0.27 for casein,

WPC80, WPH and WPI, respectively, could be observed. Similar ratios were observed for the ash content by making the same comparison with WPC35 (the sample that presented the highest ash content) (0.27, 0.40, 0.70 and 0.33 for casein, WPC35, WPC80, WPH and WPI, respectively), demonstrating that the same proportion is maintained. Therefore, the ash content appears to be correlated to the amount of Ca, K, Mg, Na and P of milk proteins evaluated.

**Table 2. Mineral content of milk proteins (mean  $\pm$  standard deviation,  $\mu\text{g g}^{-1}$ , n=3).**

| Elements | Casein                         | WPC35                          | WPC80                         | WPH                            | WPI                            |
|----------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|
| Al       | 8.3 $\pm$ 0.4 <sup>a</sup>     | < 1.5 <sup>*c</sup>            | < 1.5 <sup>*c</sup>           | < 1.5 <sup>*c</sup>            | 3.9 $\pm$ 0.1 <sup>b</sup>     |
| B        | 1.47 $\pm$ 0.14 <sup>b</sup>   | 7.72 $\pm$ 0.33 <sup>a</sup>   | 0.91 $\pm$ 0.17 <sup>b</sup>  | 1.67 $\pm$ 0.60 <sup>b</sup>   | 1.69 $\pm$ 0.24 <sup>b</sup>   |
| Ba       | 0.485 $\pm$ 0.029 <sup>c</sup> | 0.365 $\pm$ 0.006 <sup>d</sup> | 1.01 $\pm$ 0.03 <sup>a</sup>  | 0.786 $\pm$ 0.009 <sup>b</sup> | < 0.10 <sup>*e</sup>           |
| Ca       | 1697 $\pm$ 15 <sup>d</sup>     | 8377 $\pm$ 145 <sup>a</sup>    | 5252 $\pm$ 386 <sup>b</sup>   | 4693 $\pm$ 227 <sup>c</sup>    | 200 $\pm$ 9 <sup>e</sup>       |
| Cu       | 0.956 $\pm$ 0.015 <sup>a</sup> | 0.215 $\pm$ 0.004 <sup>c</sup> | 0.76 $\pm$ 0.06 <sup>b</sup>  | 0.998 $\pm$ 0.031 <sup>a</sup> | 0.285 $\pm$ 0.041 <sup>c</sup> |
| Fe       | 74.4 $\pm$ 6.6 <sup>a</sup>    | 4.14 $\pm$ 0.31 <sup>bc</sup>  | 8.77 $\pm$ 0.75 <sup>bc</sup> | 12.5 $\pm$ 0.2 <sup>b</sup>    | 1.99 $\pm$ 0.22 <sup>c</sup>   |
| K        | 395 $\pm$ 4 <sup>d</sup>       | 14023 $\pm$ 285 <sup>a</sup>   | 4456 $\pm$ 363 <sup>c</sup>   | 11819 $\pm$ 526 <sup>b</sup>   | 516 $\pm$ 26 <sup>d</sup>      |
| Mg       | 132 $\pm$ 2 <sup>c</sup>       | 1128 $\pm$ 17 <sup>a</sup>     | 703 $\pm$ 59 <sup>b</sup>     | 665 $\pm$ 31 <sup>b</sup>      | 20 $\pm$ 2 <sup>d</sup>        |
| Mn       | 1.04 $\pm$ 0.07 <sup>a</sup>   | < 0.05 <sup>*d</sup>           | 0.12 $\pm$ 0.00 <sup>c</sup>  | 0.550 $\pm$ 0.018 <sup>b</sup> | < 0.05 <sup>*d</sup>           |
| Na       | 342 $\pm$ 12 <sup>e</sup>      | 6422 $\pm$ 340 <sup>b</sup>    | 1748 $\pm$ 169 <sup>d</sup>   | 5277 $\pm$ 226 <sup>c</sup>    | 7719 $\pm$ 374 <sup>a</sup>    |
| P        | 7754 $\pm$ 146 <sup>a</sup>    | 6757 $\pm$ 84 <sup>b</sup>     | 3597 $\pm$ 268 <sup>c</sup>   | 3428 $\pm$ 126 <sup>c</sup>    | 1496 $\pm$ 138 <sup>d</sup>    |
| S        | 6491 $\pm$ 160 <sup>b</sup>    | 4810 $\pm$ 94 <sup>b</sup>     | 9914 $\pm$ 749 <sup>a</sup>   | 9632 $\pm$ 337 <sup>a</sup>    | 10772 $\pm$ 1341 <sup>a</sup>  |
| Sr       | 2.07 $\pm$ 0.05 <sup>d</sup>   | 8.68 $\pm$ 0.32 <sup>a</sup>   | 5.11 $\pm$ 0.03 <sup>b</sup>  | 3.17 $\pm$ 0.08 <sup>c</sup>   | < 0.01 <sup>*e</sup>           |
| Zn       | 44.1 $\pm$ 0.9 <sup>a</sup>    | 3.42 $\pm$ 0.01 <sup>c</sup>   | 8.38 $\pm$ 0.33 <sup>b</sup>  | 7.19 $\pm$ 0.42 <sup>b</sup>   | < 1.0 <sup>*d</sup>            |

Same letters in the same column indicate that the data do not differ statistically among them (Tukey's test;  $p \leq 0.05$ ).

\* limit of quantification.

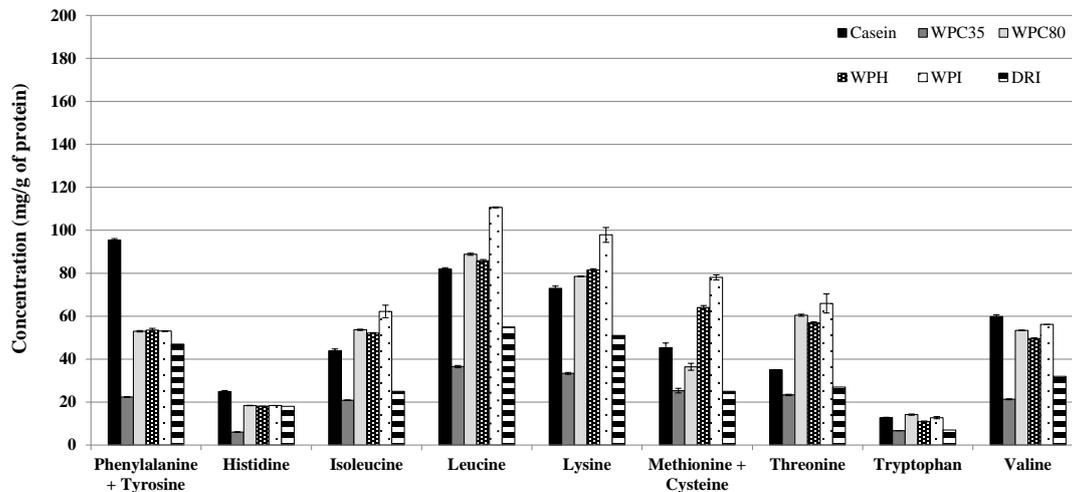
#### 3.2. Mineral Elements

Mineral salts affect the functionality and the value of whey products, and their determination could be used as an important quality parameter. The elements observed in the highest concentration for the evaluated milk proteins were Ca, K, Mg, Na, P and S. As previously mentioned, these macroelements, with the exception of sulfur, could be directly related to the ash content of samples. The total sulfur content could be related only to the total amount of

sulfur-containing amino acids, such as cysteine (determined as cystine) and methionine (Figure 1). By considering the total amount of sulfur-containing amino acids to be the sum of the total cysteine and methionine concentrations determined by HPLC as well as the estimate based on the data regarding the total sulfur content determined by ICP OES (considering the same proportion between cysteine and methionine observed by HPLC), the results obtained by ICP OES presented a level of agreement between 85 and 102% with respect to the

HPLC values obtained for casein, WPH and WPI. Thus, the total sulfur content of these samples obtained by ICP OES could be associated with the samples' concentration of sulfur-containing amino acids. However, for the WPC35 and WPC80 samples, the levels of agreement

observed were 127 and 167%, respectively, which could indicate that other sulfur-containing amino acids or other sulfur species (e.g., sulfates) might be concentrated together during the manufacture of whey protein concentrates.



**Figure 1.** Concentration of total essential amino acids (EAA) in different milk proteins and respective Dietary Reference Intakes (DRIs)

It is well known that the mineral content of milk is not constant but varies according to several different factors, such as stage of lactation, feed, and genetic variance [16], and thus, the concentration of elements could change during the production of different milk proteins. In this work, the samples used were not produced from the same raw material, and the processes applied were different for each milk protein. Nevertheless, even when considering these factors, important differences among the samples evaluated could be established. In general, WPC35, WPC80 and WPH were the products with the highest concentrations of macroelements (Ca, K, Mg, Na and P), as shown in Table 2. With the exception of the P and Na content of casein and WPI, respectively, the concentrations of macroelements were lower for these products. However, the highest concentration of microelements (Cu, Fe, Mn and Zn) was observed for casein.

Casein showed a high content of phosphorus that could be related to phosphoserine-containing peptides found in the composition of  $\alpha_{s1}$ -casein,  $\alpha_{s2}$ -casein and  $\beta$ -casein. These peptides have a high concentration of negative charges and could be related to the efficient binding of microelements by casein [16]. For iron, the concentration in casein was at least six times higher than that observed

in the other milk proteins studied. For zinc, similar behavior was observed, with its concentration in casein at least five times as high. In contrast to casein, WPI presented the lowest concentration of phosphorus and was the poorest milk protein with respect to both macro- and microelement content. However, sodium was the only element whose concentration was observed to be higher in WPI than in the other milk proteins. For WPC35, a high concentration of macroelements (Ca, K, Mg, Na and P) was observed, and the concentration of these elements was always higher than that of WPC80. On the other hand, the microelement content (Cu, Fe, Mn and Zn) was always higher in WPC80 than in WPC35. Therefore, the mineral content of milk protein could be affected by protein type as well as by corresponding manufacturing process. In addition, the effects of these factor appear to vary for macro- and microelements.

Despite the importance of Table 2 in comparing the differences among milk proteins, there is no way to directly evaluate the nutritional significance of elements observed in these samples because the requirements are different for each element. Therefore, to provide this information, the data were compared to the Dietary Reference Intakes (DRIs) [17] of the elements considering the ingestion of 100 g of each sample per day (Table 3).

**Table 3.** Concentration of elements in 100 g of each milk protein and their Dietary Reference Intakes (DRIs).

| Elements      | DRIs (per day) | Casein | WPC35  | WPC80 | WPH   | WPI    |
|---------------|----------------|--------|--------|-------|-------|--------|
| Ca (mg)       | 1000*          | 170    | 838    | 525   | 469   | 20.0   |
| Cu ( $\mu$ g) | 900*           | 95.6   | 21.5   | 76.0  | 99.8  | 28.5   |
| Fe (mg)       | 8*             | 7.44   | 0.414  | 0.877 | 1.25  | 0.199  |
| K (g)         | 4.7**          | 0.040  | 1.40   | 0.446 | 1.18  | 0.052  |
| Mg (mg)       | 400-420*       | 13.2   | 113    | 70.3  | 66.5  | 2.00   |
| Mn (mg)       | 2.3**          | 0.104  | <0.005 | 0.012 | 0.055 | <0.005 |
| Na (g)        | 1.3-1.5**      | 0.034  | 0.642  | 0.175 | 0.528 | 0.772  |
| P (mg)        | 700*           | 775    | 676    | 360   | 343   | 150    |
| Zn (mg)       | 11*            | 4.41   | 0.342  | 0.838 | 0.719 | <0.100 |

\*Recommended Dietary Allowances (RDAs)

\*\*Adequate Intakes (AIs).

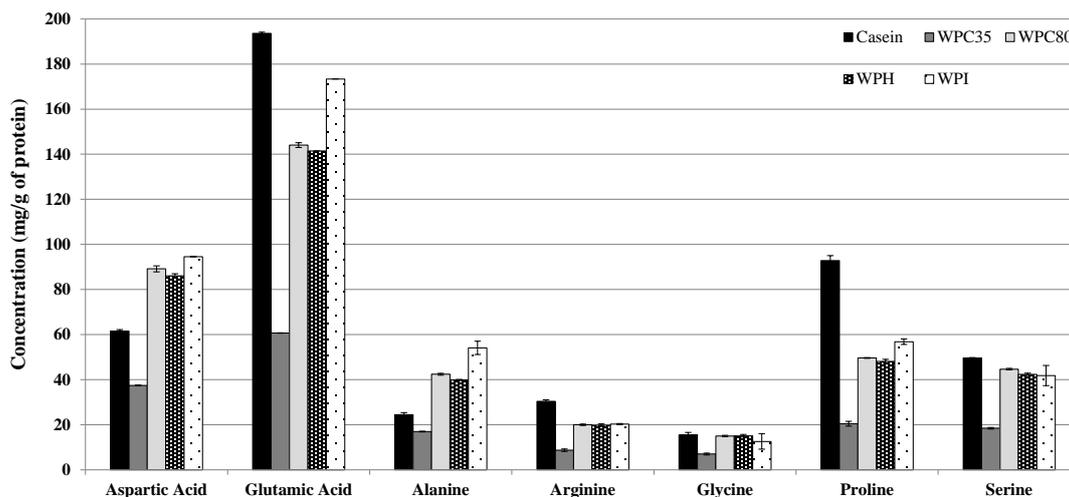
For mineral elements determined in WPI, only a reasonable content of P (21.4% of DRIs) and a high content of Na were observed, whereas values lower than 3.2% of the DRIs of Ca, Cu, Fe, K, Mg, Mn and Zn were observed. Thus, WPI could not be considered nutritionally important with respect to mineral content. The concentration of Na was observed to be high in all whey proteins, ranging from 11.7 (WPC80) to 59.3% (WPI) of the DRIs, and negligible in casein (lower than 2.6% of DRIs). All proteins presented a high content of P ranging from 21.4 (WPI) to 110.7% (casein) of the DRIs. Whey protein concentrates and hydrolysate showed high contents of calcium (between 46.9 and 83.8% of DRIs) and could be considered important calcium sources. Reasonable concentrations of Mg and K were observed in the WPC35 sample (26.9 and 30.6% of the DRIs of Mg and K, respectively). For microelements, only casein could be considered a source of Zn and Fe, with concentrations of 40.1 and 93.0% of the DRIs, respectively.

It is important to note that the content of whey salts, especially NaCl derived from the production of cheese, could cause a drop in the quality of whey protein, limiting its use as a food ingredient. Thus, to avoid changes in flavor and to allow for the use of whey proteins in products for low-sodium diets, several whey protein

demineralization processes are used [2,5]. However, a high sodium content was observed for all commercially available whey proteins evaluated, demonstrating that this element could not be efficiently separated in the manufacture process, and thus, casein was the milk protein with lowest concentration of this element.

### 3.3. Amino Acids

Previously dried samples were evaluated with respect to their total contents of essential and non-essential amino acids (EAA and NEAA, Figure 1 and Figure 2, respectively). The sum of the concentrations of the amino acids could be related to the total protein content of samples, with differences ranging from 1 to 9%. The content of NEAA was between 45.0 and 49.8% of the total amino acid value for all milk proteins evaluated, indicating a slight predominance of EAA. WPC35 clearly presented the lowest values of EAA and NEAA and was not used for the quantitative comparison of these amino acids. In contrast, WPI showed the highest total amount of EAA, with similar results obtained for WPH, casein and WPC80. Leucine and lysine were the EAA observed in the highest concentrations in milk proteins, whereas histidine and tryptophan were observed in the lowest concentrations.



**Figure 2.** Concentration of total non-essential amino acids (NEAA) in different milk proteins and respective Dietary Reference Intakes (DRIs)

WPI was rich in branched-chain EAA (leucine, isoleucine, and valine), with concentrations thereof ranging between 14.5 and 18.9%, higher than those observed in other milk proteins. These amino acids are believed to play a role as metabolic regulators in protein and glucose homeostasis and in lipid metabolism, and as such, the amino acids may play a role in weight control [18, 19]. With the exception of WPC35, all milk proteins presented concentrations higher than their respective DRIs, and concentrations 2.5, 2.0 and 1.8 times the DRIs values [17] were observed for isoleucine, leucine and valine, respectively, in WPI. Casein, WPC80 and WPH presented similar concentrations of these amino acids (from 1.5 to 2.1 times the DRIs values).

WPI was also a source of sulfur-containing EAA (methionine and cysteine - determined as cystine), as was WPH, though to a lower extent. These amino acids serve a critical role as anti-oxidants, as precursors to the potent intracellular anti-oxidant glutathione, and in one-carbon

metabolism [19, 20]. The amount of sulfur-containing EAA in casein and WPC80 was approximately two times lower than that in WPI and could not be explained solely by the total protein values (82.78, 72.89 and 85.82% for casein, WPC80 and WPI, respectively), demonstrating that the source of protein and/or the method of manufacture could affect the content of these amino acids. It is important to note that all milk proteins evaluated could be considered a source of sulfur EAA, because all concentrations were greater than the DRIs values, but the highest concentrations of these amino acids were observed in WPH and WPI, which reached 2.56 and 3.12 times the DRIs values, respectively.

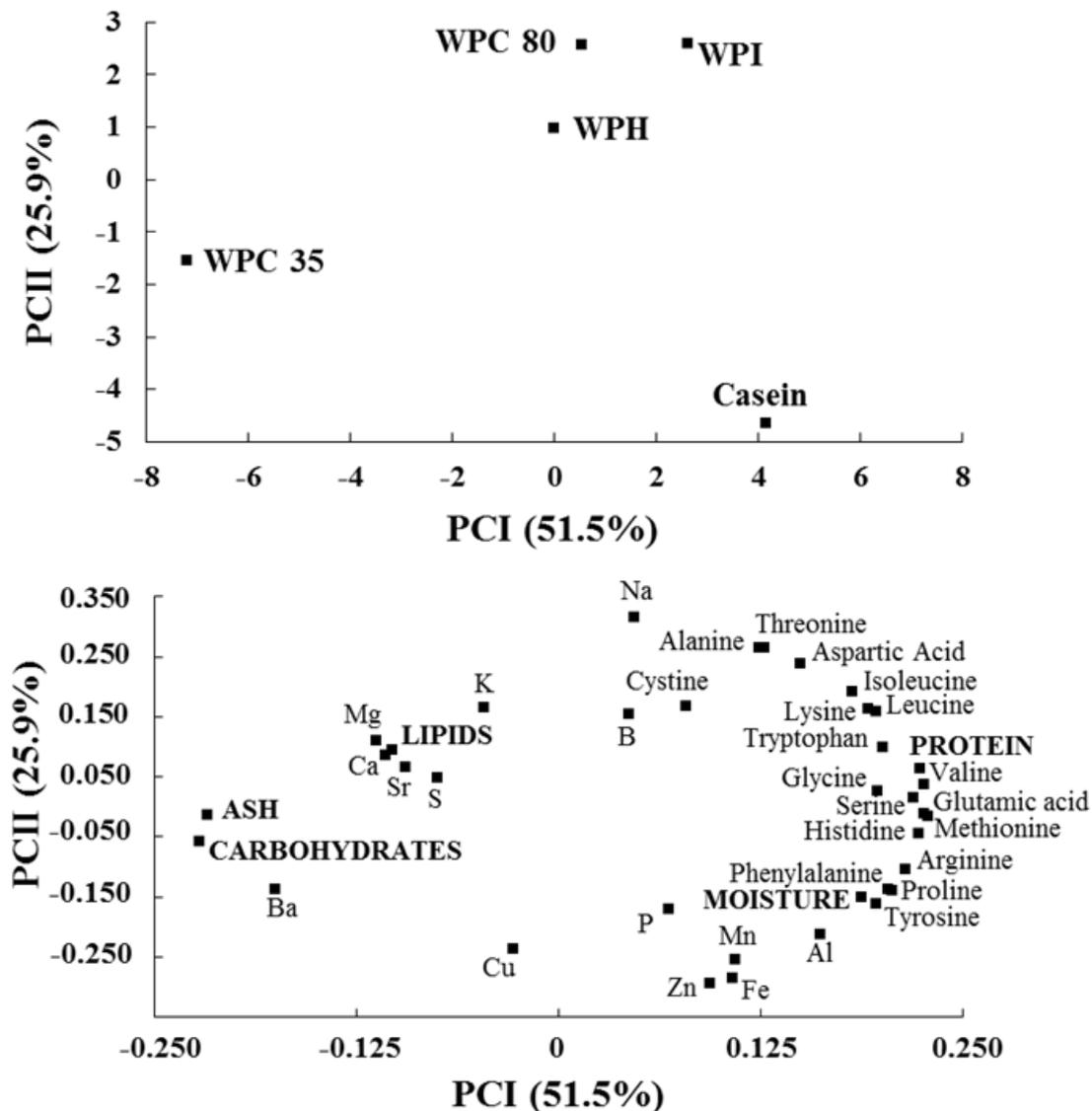
The concentration of aromatic essential amino acids (phenylalanine, tyrosine, histidine and tryptophan) was very similar among WPC80, WPH and WPI, with values slightly higher than the DRIs values, with the exception of tryptophan. Phenylalanine and tyrosine were observed in equal proportions in all milk proteins (approximately 50%

of each one), and their highest concentration was observed in casein, which was twice the DRIs value [17]. For WPC35, WPC80, WPH and WPI, concentrations equivalent to approximately 47.7, 112.8, 113.7 and 112.9% of the DRIs values were observed for these amino acids, respectively. Casein also presented the highest concentration of histidine, which reached 1.4 times the DRIs value; thus, this milk protein could be considered a source of aromatic amino acids. Clearly, the use of casein should be carefully considered when preparing low-phenylalanine food, and WPC, WPH and WPI are better options for preparing food containing milk protein in this case.

Lysine and threonine were observed in high concentration in WPI, but all milk proteins (with exception of WPC35) exceeded the DRIs values for these amino acids. For lysine, a similar concentration was observed in casein, WPC80 and WPH. However, for threonine, casein presented a lower concentration than did WPC80 and WPH.

The NEAA were also evaluated, and their concentrations in milk proteins are shown in Figure 2. Casein and WPI presented the highest amounts of NEAA.

Glutamic acid was observed in high concentration in all milk proteins, followed by aspartic acid, with exception of casein, which presented a high content of proline. In contrast, arginine and glycine were the NEAA observed in the lowest concentration. The concentrations of aspartic acid, arginine, glycine, proline and serine were similar among WPC80, WPH and WPI. Casein showed the highest amounts of glutamic acid, arginine and proline. According to Wu et al., it is assumed that all NEAA are synthesized in sufficient amounts in the body to meet the requirements for maximal growth and health, but there has been no compelling experimental evidence to support this assumption [21]. Some NEAA (e.g., glutamine, glutamate, proline, glycine and arginine) are involved in the regulation of gene expression, cell signaling, antioxidative responses, neurotransmission, and immunity. Moreover, glutamate, glutamine and aspartate are major metabolic fuels that help the small intestine maintain its digestive function and protect its mucosal integrity [21]. Thus, NEAA should be taken into consideration in balanced diets to improve protein accretion, food efficiency and health, and milk proteins could be considered a good source of these compounds, particularly casein and WPI.



**Figure 3.** Principal component analysis of proximate, mineral and amino acid composition of the milk proteins. a - Score plots (samples), WPC35 - whey protein concentrate 35%, WPC80 - whey protein concentrate 80%, WPH - whey protein hydrolysate, WPI - whey protein isolate; b - weight plots (variables)

### 3.4. Exploratory Analysis

In this analysis, it was possible to extract relevant information regarding the correlation among different variables to characterize milk proteins. With respect to the first two principal components of PCA, 77.4% of the total variance of the data was obtained (Figure 3). WPC35 was singled out mainly due to its high contents of ash and carbohydrates and also due to its lowest content of proteins (and amino acids), as expected. However, the other whey proteins (WPC80, WPH and WPI) formed a group of proteins, distinct from casein, demonstrating the similarity among them. The high contents of phosphorus and microelements, as well as phenylalanine, tyrosine, arginine and proline, in casein were responsible for the differences observed in relation to the other whey proteins. The lowest amount of sodium observed in casein was also a parameter that was responsible for its distinction from whey proteins.

### 4. Conclusion

The mineral and amino acid profile of commercialized milk proteins presented important information regarding their nutritional importance. All proteins could be considered sources of both EAA and NEAA, with the exception of WPC35 due to its lower content of protein compared to that of other milk proteins. Casein could be considered a source of aromatic AAs and WPI a source of sulfur-containing and branched-chain AAs, lysine, threonine and total EAA. Microelements (mainly Fe and Zn) were observed in high concentration in casein. Macroelements (mainly Ca, Mg, K) were observed in high concentration in whey proteins (WPC35, WPC80 and WPH), however, WPI was the poorest protein in terms of mineral element content (macro- and microelements). The sodium content (up to 59.3% of DRIs) was determined to be high for all whey proteins and should be carefully considered in the elaboration of low-sodium products. Despite the limited number of products evaluated, the protein source and/or method of manufacture appears to be related to the differences observed in both the amino acid and mineral contents among milk proteins.

### Declaration of Interest Statement

This article does not contain any studies with human or animal subjects.

Sabrina Vieira da Silva declares that she has no conflict of interest.

Rochele Sogari Picolotto declares that she has no conflict of interest.

Roger Wagner declares that he has no conflict of interest.

Neila Silvia Pereira dos Santos Richards declares that she has no conflict of interest.

Roger Wagner declares that he has no conflict of interest.

Juliano Smanioto Barin declares that he has no conflict of interest.

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