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Physicochemical and protein profile of goat whey powder

Vivian dos Santos Pinheiro^{1,2} Mônica Volino Gonçalves de Souza¹ Gustavo Vieira de Oliveira^{1,3} Maria Lúcia Guerra Monteiro² Adriano Aquino² Carlos Adam Conte-Junior² Thiago Silveira Alvares^{1,3*}

¹Grupo de Pesquisa em Nutrição e Metabolismo do Exercício, Instituto de Alimentação e Nutrição, Centro Multidisciplinar Universidade Federal do Rio de Janeiro (UFRJ), 27979-000, Macaé, RJ, Brasil. E-mail: alvares@macae.ufrj.br. *Corresponding author. ²Programa de Pós-graduação em Ciência de Alimentos, Instituto de Química, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro,

³Programa de Pós-graduação em Produtos Bioativos e Biociências, Centro Multidisciplinar Universidade Federal do Rio de Janeiro (UFRJ), Macaé, RJ, Brasil.

ABSTRACT: The cheese after processing, generates whey, which is a residue that has potential benefit to human health. However, there are still few studies that seek to evaluate the composition and potential benefits of goat milk whey, even though it is a promising dairy source. Despite this, the large-scale generation of this waste can favor the environmental impact when improperly disposed of. Given this, the use of all content of dairy production can reverse this impact and increase the income of the dairy industry. Thus, this research prepared a powdered product based on goat's milk whey from the spray drying technique and evaluate its physicochemical composition by chemical analytical methods and protein profile through polyacrylamide gel electrophoresis. The elaborated product had a higher carbohydrate content than protein, and the identification of protein fractions showed that the most expressive bands represented beta-lactoglobulin and alpha-lactoalbumin. Therefore, the elaborated product has the potential to generate novel products for human consumption and with potential health benefits. **Key words**: goat whey powder, spray-drying, protein profile, electrophoresis (SDS-PAGE), environmental impact.

Perfil físico-químico e proteico do soro de leite de cabra

RESUMO: O queijo após processado gera o soro de leite, sendo este um resíduo que possui potencial benefício à saúde humana. No entanto, ainda são poucos os estudos que buscam avaliar a composição e os potenciais benefícios do soro de leite de cabra, mesmo esta sendo uma fonte láctea promissora. Apesar disso, a geração em larga escala deste resíduo pode favorecer o impacto ambiental quando descartado inadequadamente. Em vista disso, a utilização de todo conteúdo gerado na produção de lácteos pode reverter este impacto e aumentar o rendimento da indústria de laticínios. Diante disso, é importante buscar o desenvolvimento de novos produtos para favorecer a redução do impacto gerado. Assim, este trabalho teve como objetivo elaborar um produto em pó, à base de soro de leite de cabra a partir da secagem pela técnica de *spray drying* e avaliar sua composição físico-química por métodos químicos analíticos e perfil proteico através da eletroforese em gel de poliacrilamida. O produto elaborado apresentou maior teor de carboidratos do que proteína, e a identificação das frações proteicas mostrou que as bandas mais expressivas representavam beta-lactoglobulina e alfa-lactoalbumina. Portanto, o produto elaborado apresenta potencial para geração de novos produtos para consumo humano e com prováveis benefícios à saúde.

Palavras-chave: soro de leite de cabra em pó, spray-drying, perfil de proteínas, eletroforese (SDS-PAGE), impacto ambiental.

INTRODUCTION

Whey is a residue generated during cheese processing that presents a high nutritional value for human health due to its nutritional composition (essential amino acids, carbohydrates, and lipids). In addition, protein profile is one of the most significant contributors to the valorization of the use of milk from several species of mammals, such as bovine, sheep, and goat, being represented by the highest fraction of alpha-lactoalbumin (α -LA) (KHAN et al., 2019) due to its beneficial effects on human health, including antihypertensive, antihrombotic, antioxidant, among others (CAMPOS et al., 2022).

The production of dairy products from goat's milk has been increasing because of its better digestibility and for being an alternative to the consumption of cow's milk products that have a high allergenic potential in the population (MEDEIROS et al., 2018). This contributed to a large-scale increase in whey produced and discarded in the environment, generating around 9 liters of whey for every 1 kilogram of cheese produced. Therefore, wasted whey becomes an important polluting source, mainly of water resources, determining a serious environmental problem when it is not properly treated before being discarded (TRINDADE et al., 2019).

In this sense, a strategy to reduce the possible environmental impact and still use a food

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source that induces a positive effect on human health would be the application of this by-product in the generation of novel food products from the use of drying technique, such as spray drying, for the development of fortified foods, and/or in the production of goat whey protein concentrate, expanding the market for products for the human population. However, o pool of data showing the nutritional characteristics of goat whey protein are still insufficient. For example, determination of goat whey protein profile should be interesting since whey protein products are widely consumed worldwide for those that desire to increase protein and amino acids supplementation. Thus, this study evaluated the physicochemical and protein profile of spray-driedbased product from goat's milk whey.

MATERIALS AND METHODS

Goat milk whey powder preparation

The goat whey of the breed of Saanen was used in the present study being obtained from a local cheese production farm (Capril Rancho Grande, Nova Friburgo, Rio de Janeiro, Brazil) in the period of June to July 2021. Whey was produced from the production of feta cheese with the addition of calcium chloride, mesophilic cultures (R-704, Christian Hansen®) commercial rennet. After the collection, the whey was filtered first in organza to eliminate suspended solids and achieved greater homogeneity for the subsequent spray-dryer drying step. This process was carried out by a mini spray dryer (Model B-290, Büchi), having a 1.0 mm standard diameter nozzle and an evaporation capacity of 1.0 L/h, and the drying conditions were: the inlet and outlet temperatures were 160°C and 57°C, respectively, a feed rate of 30%, and airflow of 70%. The powder of goat whey was stored in a freezer (-80°C) until performing the analysis.

Proximate composition

The moisture, protein, lipid, and ash contents were determined following the Association of Official Analytical Chemists (AOAC, 2012). The moisture was determined by drying the sample in an empty dish at 100-102°C until constant weight (AOAC method 950.46B). The protein content was estimated using the Kjeldahl method (N×6.38; AOAC method 955.04), and the ash content was obtained after sample incineration in a muffle furnace at 550°C (AOAC method 920.153). The total lipid content was extracted with petroleum ether as organic solvent using a Soxhlet extractor (AOAC method 991.36). The amount of carbohydrate was determined

by equation % carbohydrates = 100% - (% moisture + % protein + % ash + % lipid) (MERRILL & WATT, 1973). All analyses were performed in triplicate.

Protein fractions analysis

A sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to visualize the protein profile in the profile in the goat whey powder (GWP). The samples were prepared according to the method described by ALMEIDA et al. (2016), with some modifications. The stacking gel and resolution gels were 4% and 12% of acrylamide, respectively. The gel was run (200 V, 30 mA for approximately 70 min) after full polymerization and application of 20 µL of the sample (5 mg/mL of protein) in each well. After electrophoresis, gels were stained with Coomassie Blue solution G-250 for 24 h and then detained with a solution of 20% acetic acid, 20% methanol, and 60% distilled water until the background was completely clean. To estimate band density, the detained gels were photographed, and the data were evaluated by TotalLab Quant® software. The apparent molecular weights were calculated using electrophoresis protein standard (Precision Plus Protein Dual Color Standards, Bio-Rad). All analyses were performed in triplicate.

Bacteriological analysis

The bacteriological analysis included aerobic heterotrophic mesophyll bacteria (AHMB), *Staphylococcus aureus*, thermotolerant coliforms at 45°C, and *Salmonella* spp. (APHA, 2001 and CABRAL et al., 2017). These analyses were carried out to evaluate the processing conditions such as heat treatment and hygienic practices during the preparation of whey protein, ensuring safe final products based on standard limits of the national agency (ANVISA, 2019). All analyses were performed in triplicate.

Statistical analysis

Results were evaluated by descriptive statistics using measures of central tendency. The sample units were individually evaluated using the physicochemical and microbiological reference values for whey powder established by normative instruction n^o 80 of the Ministry of Agriculture, Livestock, and Supply (MAPA, 2020).

RESULTS AND DISCUSSION

Proximate composition

The evaluation of the physicochemical composition of the powdered goat's milk whey is shown in table 1. The proximate composition of the Table 1 - Proximate composition of the whey powder of goat.

Carbohydrate (%)	67.40 ± 0.54	
Protein (%)	14.20 ± 0.18	
Ash (%)	8.52 ± 0.01	
Lipid (%)	7.51 ± 0.23	
Moisture (%)	2.37 ± 0.18	

Data are expressed as means of triplicates (\pm standard deviation).

powder showed the highest carbohydrate content (67.40%), followed by the protein (14.20%), ash (8.52%), lipid (7.51%) and moisture (2.37%). These data are in accordance with the established criteria in the normative instruction, which describe the minimum and maximum requirements for using the product for human consumption (MAPA, 2020).

The composition of whey can be influenced by the type of milk (cow, sheep, buffalo, ovine, goat) (GOMES, et al. 2013), as well as, the breed of the animal can contribute to a variation in composition. For instance, DA COSTA et al. (2014) observed differences protein composition of two goat breeds, the Alpine (3.60g/100 g) and the Saanen (3.15g/100 g). In the present study, it was observed a greater protein concentration (14.20 g/100g) in the GWP compared to goat milk in the study of DA COSTA et al. (2014). However, it is a bit difficult to make comparison between the findings observed in these studies since GWP is expected to exhibit the most part of protein present in milk.

Regarding ash content, it was observed that GWP exhibited 8.52%, which is agreement with the recommendation of the legislation, which establishes the maximum value of 9.5% ash in a powdered product (MAPA, 2020), demonstrating an adequate amount of mineral. Moreover, this result is in agreement with the reported in the studies of GOMES et al. (2013) and PALATNIK et al. (2015) that observed that goat milk presents 0.30g and 0.55g, respectively. Due to this content, goat milk represents a rich source of minerals, can be compared to bovine, buffalo, and sheep milk, containing mainly mineral salts such as potassium, sodium, calcium and magnesium (KHAN et al., 2019).

The content of lipids in the GWP (7.51%) showed that the product still has a considerable amount of this constituent after drying. This data is in agreement with the reported in the study by DA SILVEIRA et al. (2015) which produced a goat beverage that presented 0.60g of fat in composition, as also in the study by GOMES et al. (2013) when comparing the bovine

and goat whey (0.62g and 0.48g, respectively). In comparison to bovine milk, goat milk has smaller fat globules that contribute to the better digestibility of the product. Furthermore, the fatty acid profile present in this food favors of the use this by-product in human health (CAMPOS et al., 2022).

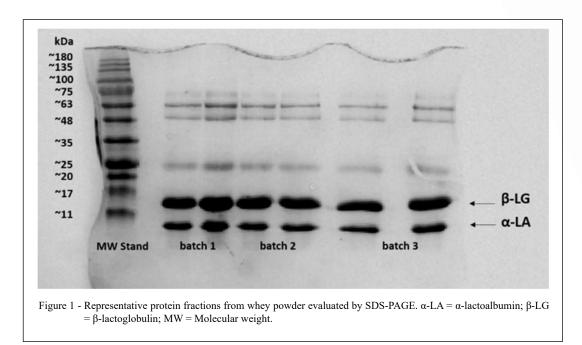
As expected, GWP exhibited a lower moisture content (2.37%), which is very low compared to goat milk. For instance, DA COSTA et al. (2014) showed that goat milk produced by Alpine and Saanen breeds presented 90.93 and 88.39 g/100 g. The higher moisture content in milk is an important factor that reduce the shelf life of this products (TORO-SIERRA et al., 2013). The spray drying process is a technology wildly applied to increase the shelf life of food products since the moisture content is reduce by hot air. Therefore, powder produced presents lower water activity, which is a limiting factor to microbial growth. Thus, the possibility of deterioration of the product is reduced (TORO-SIERRA et al., 2013).

Protein profile

The protein profile observed in the SDS-PAGE demonstrated that the most expressive bands were located in the region with a molecular weight of around 25 and 11 kDa, representing the apparent molecular weight of β -LG and α -LA, respectively, as illustrated in figure 1. These data are in agreement with the previous study by DA COSTA et al. (2014), which evaluated the composition of proteins present in goat milk in different goat breeds, which showed expressed bands of approximately 20 kDa, and 14 kDa of β -LG and α -LA, respectively. In addition, MEDEIROS et al. (2018) also identified β -LG (~18 kDa) and α-LA (~14 kDa) in GWP. Moreover, CAMPOS et al. (2022) also reported 18.3 kDa and 14 kDa of β -LG and α -LA, respectively, present in the GWP. These findings are in line with milk from other sources, such as sheep (β -LG = 18 kDa and α -LA = 14 kDa) and camel (β -LG = not detected and α -LA = 13 kDa) (YASMIN et al., 2020). Another study also

Ciência Rural, v.54, n.1, 2024.

Pinheiro et al.



investigated the camel milk finding the α -LA (14.4 kDa); however, no detect β -LG (WANG et al., 2020).

The β -LG and α -LA are the major fractions of protein present in GWP. For example, GWP present approximately 45% and 15% of β-LG and α -LA, respectively (CAMPOS et al., 2022). These protein fractions are comparable to those reported in bovine milk whey (ALMEIDA et al., 2016) being the bovine milk whey widely consumed worldwide. In addition, when compared to bovine milk, these protein fractions present better digestibility, which is an important propriety that can contribute as an alternative for human consumption, given the allergenic potential bovine dairy products have, as well as the improvement in the absorption of its nutrient (ALMEIDA et al., 2015). Studies have shown that GWP presents biological activities benefiting human health, such as antithrombotic and antihypertensive, among others (KHAN et al., 2019; CAMPOS et al., 2022). However, there are still few studies investigated the biological activities of protein fractions of GWP ingestion in humans, given that the most part of studies are carried out in vitro or in animals (MANTHOU et al., 2014; MEDEIROS et al., 2018; VESKOUKIS et al., 2020).

Based on our findings of protein profile, the GWP could be used for the elaboration of isolated and/or concentrated whey protein through specific technologies to reduce lactose content and/or other components present in this by-product to enable their use and minimize the excess of whey existing after the production of cheeses (MEENA et al., 2017; ILCHENCO et al; 2018). This type of technology has been widely used for protein concentrate production in sources of bovine milk.

Bacteriological evaluation

The result of the microbiological evaluation is shown in table 2. All whey powder formulations were suitable for consumption in accordance with the standard microbiological limits recommended by the national agency (ANVISA, 2019), indicating that processing conditions (i.e., heat treatment and hygienic practices) were adopted during the elaboration of GWP formulations resulting in safe final products.

The goat whey powder formulations demonstrated presented satisfactory conditions as demonstrated by the counts of AHMB (1.33 log cfu/g), thermotolerant coliforms and Staphylococcus aureus (<1 log cfu/g) and the absence of Salmonella spp, which is in accordance with the established standard for the production of whey powder. According to the legislation, the maximum limits are 2 log cfu/g for AHMB and thermotolerant coliforms, 1 log cfu/g for Staphylococcus aureus, and absence for Salmonella spp. (MAPA, 2020).

CONCLUSION

The present study showed that goat whey protein powder present nutritional value based on

AHMB	1.33 log cfu/g
S. aureus	< 1 log cfu/g
Coliforms	< 1 log cfu/g
Salmonella	Absent

Data are expressed as means of triplicates.

AHMB: aerobic heterotrophic mesophyll bacteria.

its physicochemical and protein profile properties. This powder product could be available for population consumption given the beneficial effect of goat milk present when compared to bovine milk. Nonetheless, futures studies investigating the effect of supplementation of goat whey protein powder on human health parameter is warranted.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

AUTHORS' CONTRIBUTIONS

Conceptualization: VSP, MVGS and GVO. Data acquisition: VSP, MLGM, and AA. Design of methodology and data analysis: VSP, MLGM, and AA. VSP, MVGS, GVO, CACJ and TSA prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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