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HYDROLYSATES FROM AGRO-INDUSTRIAL PLANT WASTE AS POTENTIAL SUBSTITUTES FOR FETAL BOVINE SERUM IN CULTURED MEAT

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ABSTRACT

The primary challenge in large-scale cultured meat production is the replacement of fetal bovine serum (FBS), an expensive and animal-derived component in the cell culture medium. This study investigated the utilization of plant-based agro-industrial wastes as a beneficial alternative to fetal bovine serum based on their nutritional and bioactive properties. To achieve this, soybean and peanut meals were processed to obtain hydrolysates with a high content of low molecular weight peptides and free amino acids. The results confirmed the presence of essential nutritional factors and important bioactive properties, which were further emphasized in the animal cell growth and viability tests. The hydrolysates exhibited protein content similar to fetal bovine serum, and the distribution of peptide molecular weight indicated a high degree of hydrolysis. The findings suggest that the use of protein hydrolysates from plant wastes may be a low-cost and sustainable approach to supplementing the culture medium in cultured meat production.

Keywords: Soybean meal 1. Peanut meal 2. Hydrolysates 3. Cultured meat 4. Fetal bovine serum 5.

1 INTRODUCTION

Cultured meat has emerged as an innovative technology and a potential alternative to supplement meat supply and reduce the environmental impacts of conventional production.[1,2] Despite the various challenges still affecting the development of this technology on a commercial scale, the use of fetal bovine serum (FBS) as a supplement in the culture medium is considered the primary obstacle due to the significant burden it imposes on the sustainability and economic aspects of the process. Fetal bovine serum (FBS) is the primary supplement in animal cell culture media, providing various essential nutrients.[3]

Therefore, the development of low-cost routes for obtaining animal-derived component-free alternatives becomes imperative, which can complement the culture medium.[1,4] Studies suggest that hydrolysates derived from plant sources may meet the demands for non-essential amino acids required for cell culture while reducing cell biosynthetic burden.[3] Furthermore, the utilization of agro-industrial wastes from vegetal sources for obtaining these nutrients may promote more ethical practices in production and favor circular economy principles.[1] Among plant wastes with high protein content and widely available, soybean meal and peanut meal deserve special mention. Currently, the utilization of these wastes is primarily limited to use as animal feed or fertilizers.[5] In this context, this study conducted a thorough analysis of hydrolysate composition, providing evidence suggesting a potentially promising new route that aligns with some of the United Nations Sustainable Development Goals (SDGs) for producing independent nutrients derived from agro-industrial plant wastes for cell culture in the cultured meat processing.

2 MATERIAL & METHODS

The experiments were conducted in a 100 mL jacketed batch reactor using the pH-stat method with Alcalase™ 2.4 L. A suspension derived from the dried residual matrix from protein extraction (residue:water ratio of 1:10 w/v) was heated to 50°C and pH 8.0 for 5 hours. The enzyme-to-substrate ratio of Alcalase™ 2.4 L was 3.5% for hydrolyzing the residual matrix of soybean meal and 5.0% for the residual matrix of peanut meal.[6]

The protein content of the hydrolysates was quantified based on the total nitrogen content using the classical Kjeldahl method, employing a conversion factor of 6.25 for soybean meal, 5.46 for peanut meal, and the standard factor of 6.25 for FBS.[7,8] The free amino acids were extracted using a 0.1 M acidic solution (HCl) and shaken for 30 minutes. A portion of the filtrate was derivatized using reverse-phase column chromatography (Phenomenex C18) in high-performance liquid chromatography (HPLC, SHIMADZU® 116).[8,9] For size exclusion chromatography (SEC) analysis, sample solutions of the hydrolysates were prepared in water (0.1% TFA) at a concentration of 1 mg/mL. The solutions were separated using a BioSep SEC-S3000 column (5 µm, 600 x 7.8 mm, Phenomenex) on a Shimadzu HPLC system (Prominence UFLC model, Kyoto, Japan), with absorbance monitored at 214 nm. Proteomic sequencing was conducted through an untargeted proteomic analysis utilizing injection into capillary liquid chromatography coupled to mass spectrometry (LC-MS/MS), and data processing of filtered hydrolysate samples. The samples

underwent pre-treated with ZipTip C18. Protein identification within the protein extract samples was performed using a Proteome ID from the specific UniProt database (Swiss-Prot/TrEMBL) (*Glycine max* (Soybean) (*Glycine hispida*) (cv. Williams 82)) and (*Arachis hypogaea* (Peanut)) reviewed.

The obtained results were assessed and compared against analyses conducted for a specific batch of FBS from Nutricell (Campinas - SP, Brazil) (Brazilian Ministry of Agriculture registration: nº 9244/2006). Studies of the cytotoxicity and the effect of various concentrations of hydrolysates with different concentrations of FBS were investigated on the growth rate of L929 cells. Cell viability was determined using the Alamar Blue assay (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. In the cytotoxicity assay, doxorubicin (doxorubicin hydrochloride, Laboratory IMA S.A.I.C., Buenos Aires, Argentina) was used as a positive control. In assessing the effects of the hydrolysates, the relative cell growth rate was determined by comparing the results with cells cultured in DMEM medium supplemented with 10% FBS, serving as the control group with a relative growth rate of 1. One-way analysis of variance and Newman-Keuls multiple comparison tests were employed to determine the statistical significance of group comparisons in cytotoxicity and cell viability assays. Results were considered statistically significant when $p < 0.05$.

3 RESULTS & DISCUSSION

The protein hydrolysates were characterized for protein content and compared with FBS. The soybean meal hydrolysate (SH) was quantified to have a protein content of 69.43 ± 0.04%, while the peanut meal hydrolysate contained 54.23 ± 0.14%, and the FBS contained 69.80 ± 0.03%. While the protein hydrolysates exhibit a protein content similar to that of FBS, it's crucial to consider the difference in protein compositions. Whereas FBS proteins primarily consist of high molecular weight proteins, the hydrolysates contain a protein content distributed in low molecular weight peptides. To investigate the peptide distribution in the hydrolysates, analyses of size exclusion chromatography (SEC) and proteomic sequencing of the hydrolysates were conducted. The resulting peptides were then categorized into molecular weight ranges, as illustrated in Figure 1.

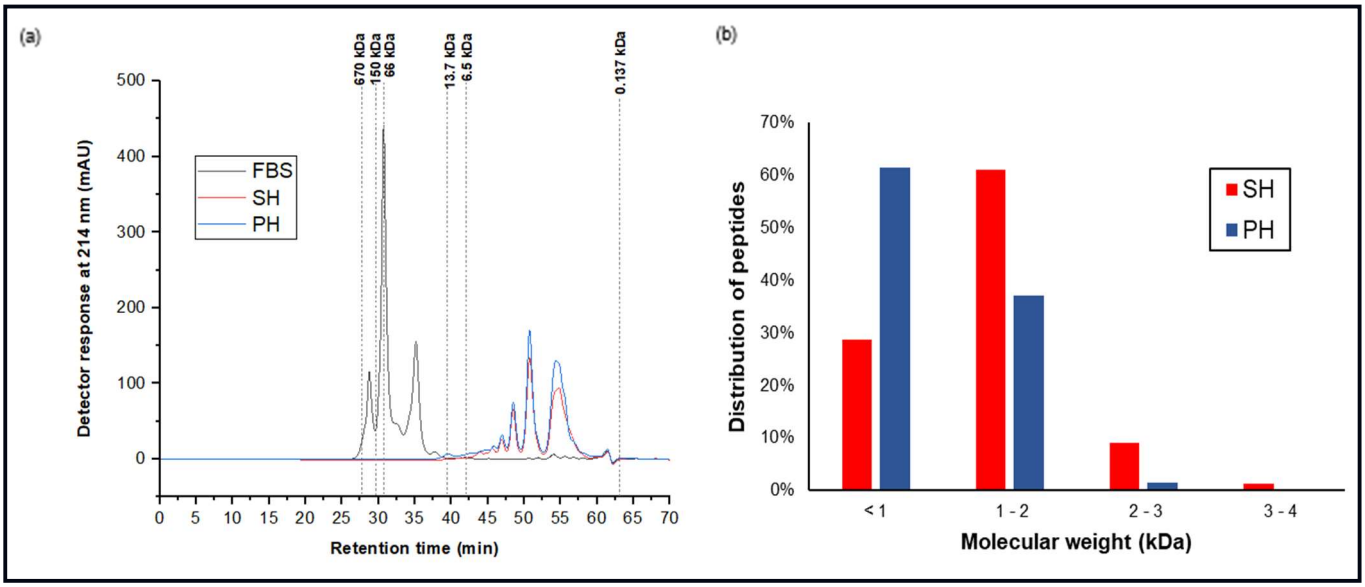

Figure 1 Size exclusion chromatograms of protein hydrolysates from soybean meal (SH), peanut meal (PH), and fetal bovine serum (FBS) (a). Molecular weight distribution of soybean meal hydrolysate (SH) and peanut meal hydrolysate (PH) (b).

The findings depicted in Figure 1 can be deemed favorable, as several studies suggest that low molecular weight peptides exhibit positive nutritional effects and distinct biological activities in animal cell growth.[4] Thus, the peptide sequences identified through proteomic sequencing were compared with information from the BIOPEP - UWM virtual database. This comparison indicated a sequence with potential anticancer and anti-obesity action and another sequence with alpha-glucosidase inhibitory activity in the soybean meal hydrolysate. For the peanut flour hydrolysate, two peptide sequences with antibacterial activity were identified.

The quantification of free amino acids found in the hydrolysates and their comparison with the profile of free amino acids in FBS was also conducted. The presence of free amino acids is crucial for the production of inputs intended for animal cell culture media, as their absence constitutes a limiting factor in animal cell growth.[1,3,4] The content of free amino acids in the hydrolysates exceeds that found in the FBS, according to the data in Figure 2. However, the role of free amino acids in animal cell growth should be evaluated beyond their nutritional functionality for the application of hydrolysates as inputs for animal cell culture media.

To this end, tests for the effects of partial substitution of FBS for the growth of L929 cells were conducted. The results obtained are presented in Figure 3. Interestingly, applying the hydrolysates with lower concentrations of FBS (1% or 2.5%) resulted in a growth rate equivalent to or higher than that in the standard medium (DMEM with 10% FBS). The results are encouraging, particularly regarding partial FBS replacement. Thus, the hydrolysates have the potential to be utilized alongside other components in formulating alternative mediums for animal cell growth, without relying on animal derivatives. Furthermore, the hydrolysates showed no significant cytotoxicity towards L929 cells at the tested concentrations. Under the same conditions, doxorubicin (Dox; 1 µg/mL), a chemotherapeutic agent with recognized cytotoxic activity, exhibited a significant inhibitory effect ($p<0.05$), reducing cell viability by approximately 53%.

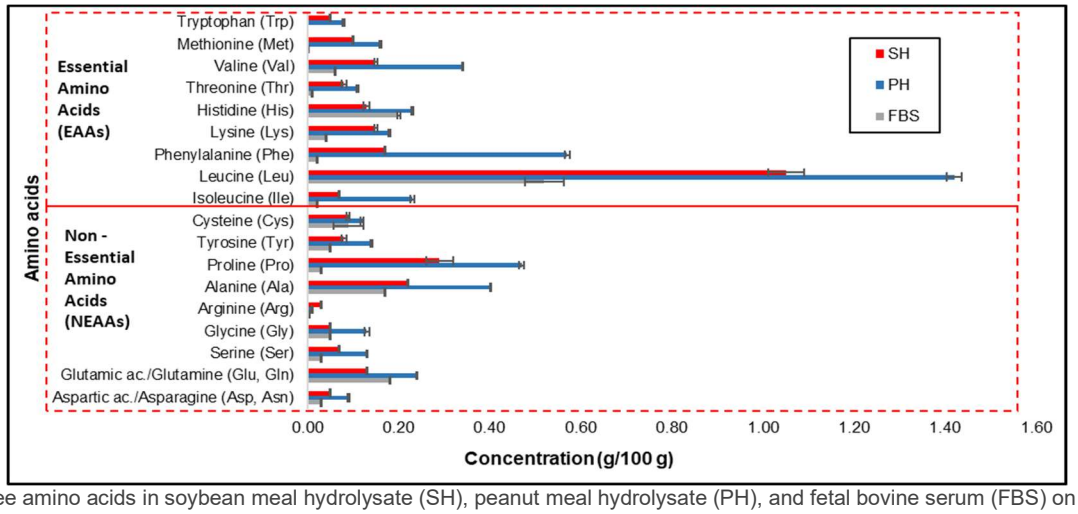

Figure 2 Free amino acids in soybean meal hydrolysate (SH), peanut meal hydrolysate (PH), and fetal bovine serum (FBS) on a dry basis.

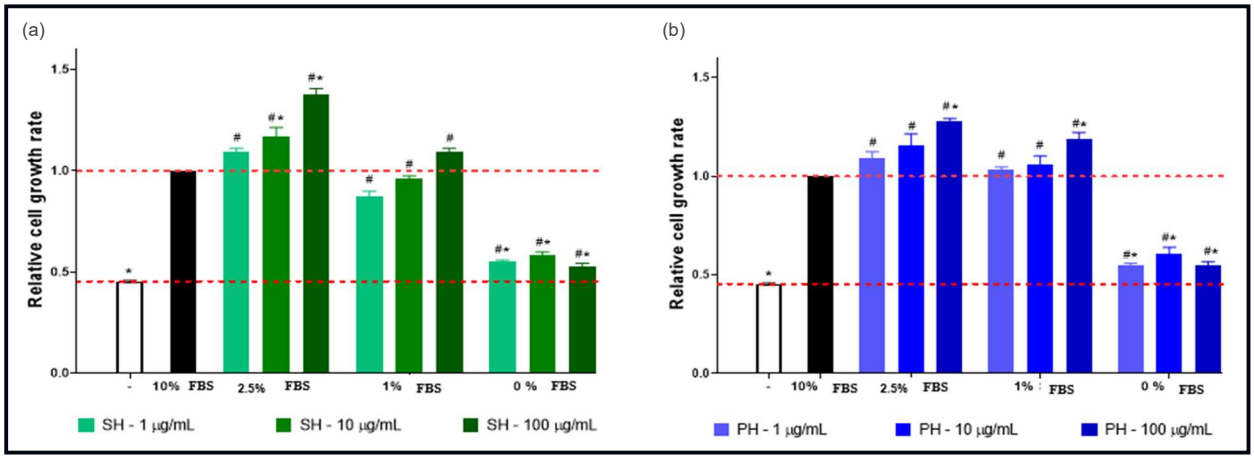

Figure 3 Effect of soybean meal hydrolysate (SH) and peanut meal hydrolysate (PH) on fibroblast growth rate (*p < 0.05 compared to cells cultured in DMEM medium supplemented with 10% FBS (black column), #p < 0.05 compared to cells cultured in DMEM medium without FBS or any other source of protein (-; white column)).

4 CONCLUSION

Protein hydrolysates from plant residues, notably soy and peanut flour, show potential as supplements in animal cell culture media for cultured meat production due to their abundance of free amino acids and low molecular weight peptides. In addition to favorable nutritional properties, these hydrolysates exhibited bioactive peptides, with significant outcomes in terms of cell viability, especially when partially replacing FBS. Therefore, this study holds importance beyond its technological scope, as it may contribute to various UN Sustainable Development Goals. However, further research in cell culture is needed to strengthen the application of these inputs.

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