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AMINO ACIDS PROFILE IN HYBRID BURGERS WITH PARTIAL MEAT REPLACEMENT BY PROTEIN EXTRUDATES FROM SUNFLOWER SEED CAKE AND FABA BEAN FLOUR

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I. INTRODUCTION

The growing interest in sustainable, plant-based protein sources, along with the growing consumer choice for healthy and sustainable food products, has prompted the exploration of agro-industrial by-products, particularly those derived from legumes and seeds, as raw materials for protein recovery [1]. This study aims to evaluate the use of high-moisture extrudates (HME) made from sunflower seed cake and faba bean protein sources without impairing the aminoacidic profile of hybrid burgers, thereby valorizing this agro-food waste.

II. MATERIALS AND METHODS

Sunflower protein isolate preparation. The sunflower seed cakes were grinded and added with a cysteine solution (8.25 mM at pH=7.0, cake:extracting solution = 1:15 (w/v). The mixture was stirred to achieve the colorless complex between the thiol group of cysteine and the o-quinone of chlorogenic acid of sunflower seed. The mixture was brought to pH= 9 with 4M NaOH, to solubilize protein and 6N HCl was added until pH=5 to precipitate proteins (pH shift). After cooling at 0-4 °C, the solution was acidified at pH=4.5 (isoelectric point). The proteins were separated by centrifugation (6000 rpm) and freeze-dried.*HME production*. HME were elaborated using a laboratory-scale twin-screw extruder (Process 11 extruder, Thermo Fisher Scientific, USA) operating at IRTA [2]. The protein mix was faba bean concentrate (44.8% protein): sunflower (64.2% protein) in proportion 58.7:41.3. The obtained HME moisture was higher than 50% and protein 29%. *Hybrid burger production*. The texturized HME was used to elaborate a hybrid meat/plant protein burger formulation with pork loin and chicken breast. Meat protein replacement with the plant-based extrudate was tested at 28%, according to the protein content of each component. To ensure color homogeneity between meat and plant components, two natural colorants derived from non-selective water extraction of edible fruits and vegetables (Exberry®, Netherlands) were used: Veggie Red (from carrots and radishes) was tested at 0.3%, and Brown (from fresh and caramelized carrots), at 0.03%. *Amino acid analysis*. Amino acids were determined as in [3]. In brief, 0.5 g of the samples (the plant-based protein isolates, the HME and the hybrid burger), underwent hydrolysis in 6 mL of 6N HCl at 110 °C for 23 h. After cooling, 1.5 mL of 50 mM norvaline in 0.1 N HCl was added as internal standard. The mixture was filtered over paper and brought to 250 mL with deionized water. Ten µL of the extract were derivatized with the AccQ Fluor reagent kit (Waters, Milford, MA, USA) according to the manufacturer's instructions. Sulphured amino acids were determined (UNI 22619) in a sample containing 15 mg of protein, oxidated with 2 mL of performic acid and left 15 h in an ice bath. Then, 30 µL of 48% hydrobromic acid were added, and the samples were dried under nitrogen and hydrolysed with 0.6 mL of 6N HCl as described above. After cooling, 25 µL of 50 mM norvaline in 0.1 N HCl was added as internal standard and the mixtures were filtered through paper and brought to 250 mL with deionized water. The extract (10 µL) was derivatized with the AccQ Fluor reagent kit. Tryptophan was determined as follows (UNI 22618): a sample containing 50 mg of protein underwent alkaline hydrolysis adding 4 mL of 4.2 M NaOH and 150 µL of 5-methyltryptophan as internal standard. Amino acids were determined with RP-UPLC/ESI-MS by an UHPLC system coupled with ESI-MS (UHPLC Acquity H Class with a single quadrupole detector, Waters, Milford, MA, USA), in Single Ion Recording (SIR) mode. Results were expressed as mean ± std. dev. over two sample replicates and two injections. Quantification was performed with a calibration curve for each amino acid.

III. RESULTS AND DISCUSSION

The nutritional profiles of the single plant-based protein isolates, the plant-mixed extrudate, and the hybrid burger were evaluated by comparing essential, sulfur-containing, and aromatic amino acids contents to the World Health Organization (WHO) reference pattern (1–2 years) [4], with identification of the limiting amino acids. Sunflower protein isolate was limited in lysine (score: 0.59), while faba bean concentrate was limited in sulfur amino acids (score: 0.67) (Table 1). The mixed HME showed scores above 1.0 for all essential amino acids, giving a complete and balanced nutritional profile (Table 1). The sunflower isolate contributed with sulfur amino acids, resulting in a nutritionally enhanced blend with no limiting amino acids. A complete amino acid profile was confirmed in hybrid burgers (Figure 1).

Table 1 - Amino acid scores of sunflower seed protein isolate (64.2% Protein), faba bean protein concentrate (44.8% protein) and HME extrudate (29% protein). Limiting amino acids are in bold letter.

Amino acids	*WHO 2007*	*Sunflower seed protein isolate*		*Faba bean protein concentrate*		*HME mixed extrudate*	
	mg essential AA/g protein	mg essential AA/g protein	WHO 2007 AA score %	mg essential AA/g protein	WHO 2007 AA score %	mg essential AA/g protein	WHO 2007 AA score %
His	18	24.8	1.38	29,9	1.66	23.6	1.31
Thr	27	40.0	1.48	39,4	1.46	42.4	1.57
Lys	52	**30.7**	**0.59***	62,6	1.20	54,3	1.04
Val	42	43.3	1.03	34,2	0.81	44.1	1.05
Ile	31	37.5	1.21	32.5	1.05	40.2	1.30
Leu	63	68.2	1.08	73.6	1.17	74.8	1.19
Trp	7.4	9.68	1.31	7.9	1.07	8.5	1.15
Specific categories of amino acid **							
SAA	26	42.9	1.65	**17.3**	**0.67***	30.8	1.19
AAA	46	90.2	1.96	67.8	1.47	70.4	1.53

*Limiting amino acid; **SAA = Sulfur-containing amino acids (Met+Cys); AAA= Aromatic amino acids (Tyr+Phe)

Table 2 - Amino acid scores of the hybrid pork-poultry/plant extrudate Burger (*31% Chicken + 31% Pork + 28% plant extruded +10% fat).*

Amino acids	*WHO 2007*	*Hybrid burger*	
	mg essential AA/g protein)	mg essential AA/g protein	WHO 2007 AA score %
His	18	31.1	1.73
Thr	27	41.1	1.52
Lys	52	73.0	1.40
Val	42	44.5	1.06
Ile	31	37.5	1.21
Leu	63	68.0	1.08
Trp	7.4	13.7	1.85
Specific categories of amino acid **			
SAA	26	38.7	1.49
AAA	46	65.4	1.42

**SAA = Sulfur-containing amino acids; AAA= Aromatic amino acids



Figure 1. Images of the hybrid burger afetr cooking

IV. CONCLUSION

This study demonstrates the potential for the valorization of sunflower seed cakes as a protein source, preferably blended with other alternative plant-based protein sources, to compensate for limiting amino acids. The aminoacidic profile of the blend is highly suitable to be used to elaborate HMEs intended for partial meat replacement in hybrid burgers. The resulting product, combining meat and suitable plant proteins, preserves a valuable nutritional profile.

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REFERENCES

1. Ribes S., Aubry L., Kristiawan M., Jebalia I., Dupont D., Guillevic M., et al. (2024). Fava Bean protein concentrate added tobeef burgers improves the bioaccessibility of some free essential amino acids after *in vitro* oral and gastrointestinal digestion. Food Research International, 177:113916. https://doi.org/10.1016/j.foodres.2023.113916.
2. Barnés-Calle C., Matas G., Claret A., Guerrero L., Fulladosa E., Gou P. (2024). High moisture extrusion of pea protein isolates to mimic chicken texture: Instrumental and sensory insights. Food Hydrocolloids,154:110129, https://doi.org/10.1016/j.foodhyd.2024.110129.
3. Prandi B, Zurlini C, Maria CI, Cutroneo S, Di Massimo M, Bondi M, Brutti A, Sforza S and Tedeschi T (2021) Targeting the Nutritional Value of Proteins from Legumes By-Products Through Mild Extraction Technologies. Front. Nutr. 8:695793. https://doi.org/10.3389/fnut.2021.695793.
4. Protein and Amino Acid Requirements in Human Nutrition: Report of a Joint WHO/FAO/UNU Expert Consultation, WHO Technical Report Series, n. 935, Geneva 2007, ISBN: 9789241209359.