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METHODS FOR DETERMINING PROTEINS AND AMINO ACIDS AND THE IMPORTANCE OF THEIR USE FOR IDENTIFYING SUSTAINABLE SOURCES OF DIETARY PROTEINS. A REVIEW

Gheorghe MATEI[1], Alina Mădălina PLEȘOIANU[1], Lorena Diana POPA[2], Simona Florina ISTICIOAIA[2], Valentin Nicolae VLĂDUȚ[3]

[1]University of Craiova, Faculty of Agriculture, 19 Libertatii Street, Craiova, Dolj, Romania
[2]Secuieni Agriculture Research Statione, 377 Principala Street, Secuieni, Neamț, Romania
[3]National Institute of Research - Development for Machines and Installations Designed for Agriculture and Food Industry - INMA Bucharest, Romania

Corresponding author email: alina.plesoianu20@gmail.com

Abstract

The aimed of this review is to highlight the importance of sustainable vegetarian protein sources in a world with a growing population in which dietary principles have also changed. This involves using sustainable non-animal protein sources. Nevertheless, the quantification of the nutritional quality of food sources also involves the normalization of the essential amino acid content with the protein content. For this, new methods for the determination and analysis of proteins individually or simultaneously with amino acids are needed, with possible advantages such as being simple, inexpensive, fast and accurate, etc. In this review, we try to summarize the benefits of a sustainable vegetarian diet (rich source of proteins), but also the importance of the continued development of simpler and faster methods to determine protein quality and content. The use of spectrophotometric methods for the measurement and analysis of proteins is of major interest, as they are generally simple, fast and with high sensitivity, as well as the adaptation of traditional methods such as the traditional biuret reagent.

Key words: *protein, amino acids, sustainable sources, vegetarian diet.*

INTRODUCTION

Our food systems play a major role in global greenhouse gas (GHG) emissions, making them a key target for addressing the climate crisis (Poore & Nemecek, 2018). Given the many elements of a sustainable food system (e.g., water, biodiversity, energy use, and food security), a holistic approach is clearly needed to achieve sustainability (Béné et al., 2019). Knowing that food system represents 20 to 30% of the global GHG emissions (Chai et al., 2019), we may understand that promoting sustainable foods that have minimal environmental impact, we can enhance health and contribute to the well-being of local communities. According to the official United Nations website, these foods are grown, produced, and distributed in ways that protect natural resources, prevent pollution, and reduce greenhouse gas emissions.
Although the technological innovations in agricultural sector, food availability and security have increased significantly (Kalmpourtzidou et al., 2022), diets with low vegetable, fruit, wholegrain and high in red and processed meat intake are dominant worldwide (Springmann et al., 2018; Kalmpourtzidou et al., 2020).
Sustainable food features include local sourcing, minimized food waste, organic farming methods, and low water and energy usage. Shifting towards plant-rich diets is expected to deliver benefits for human health, the environment, and animal welfare (Allenden et al., 2022).
Proteins are the building blocks of life. The basic structure of protein is a chain of amino acids - protein foods (plant and animal sources) are broken down into parts called amino acids during digestion. A balanced diet, rich in carbohydrates, proteins, and lipids, is crucial for supporting physiological functions and maintaining good health. Among these, proteins are particularly important as they are essential macronutrients for cell growth and repair in the body. The demand for protein sources has been rising, especially among

athletes, who seek to increase muscle mass and prevent muscle breakdown. While animal proteins are highly digestible, they may not always be accessible or widely consumed due to factors like high cost or lifestyle choices (such as vegetarianism and veganism). As a result, scientists and the food industry are actively exploring alternative protein sources, including plant-based and fungal proteins.

It is known that vegan diets might reduce the risk of premature mortality, NCDs, and excessive weight gain (Moreno et al., 2021). However, it is important to keep in mind that the health benefits of any diet partly depend on the variety of foods consumed and the way in which food is stored, processed, and prepared (Comerford et al., 2021). Complete proteins, typically found in animal-based sources, are highly bioavailable, but also contribute to environmental harm and an increased risk of diet-related chronic diseases. In recent years there has been growing awareness about the health benefits of replacing animal-based proteins with plant-based alternatives, particularly in developed countries. Higher protein quality per meal in the vegan scenario is possible when smart combinations are made by also replacing other meal components and real-life practice shows that people usually replace an animal-based product with a similar plant-based alternative, without making other changes within the same meal (Gehring et al., 2021). Key challenges to improve the consumer acceptability and market value of plant-based proteins would be in achieving nutrient equivalency and enhance bioavailability and digestibility while maintaining the same physicochemical properties, taste, texture, as animal proteins (Ulhas et al., 2023).

Standardized analytical methods are essential for accurately determining the protein and amino acids content in food. Various methods, such as Kjeldahl, Lowry, Bradford, and total amino acid content analysis, are employed across different food industries to quantify protein levels. Accurate measurement of protein content is crucial, as it often influences the economic value of food products and can affect the financial viability of emerging industries focused on alternative protein production.

MATERIALS AND METHODS

In order to achieve our goals, we used the method of documentation on site and on line, both letric and electronic sources, and start to identifying, accessing and selecting of several significant scientific results from the area of our proposed topic, published in different journals indexed in WOS, Clarivate Analytics, Scopus and Springer databases.

The most valuable were highlighted in the present review and were also cited and included in the references chapter.

RESULTS AND DISCUSSIONS

Methods for protein and amino acids determination

The methods used for quantitative protein determination can vary significantly in terms of relative response factors, matrix dependency, sensitivity, sample preparation effort, instrumentation, cost, duration, and sample size (ranging from picogram to kilogram) (Reinmuth-Selzle et al., 2022).

Spectrophotometric methods for protein determination and analysis are highly valued due to their simplicity, speed, and high sensitivity. These protein assays have many uses in the food, plant breeding, pharmaceutical and biomedical industries, as well as in forensic science and a variety of other research fields (Mihalcea et al., 2024). However, the measurement of the nutritive quality of food samples also requires the nominalization of essential amino acid content to that of protein (Mihalcea et al., 2024).

The most commonly used methods for measuring protein content in foods include the Kjeldahl method, Dumas method, and direct measurement techniques, like UV-spectroscopy and refractive index measurement, each have their own set of advantages and disadvantages.

The Kjeldahl method is a reference technique for protein quantification in food, but it is time-consuming and requires large protein amounts (typically 5 g), making it less suitable for small sample sizes (Wang et al., 2016). The Kjeldahl method involves the digestion of food with a strong acid so that nitrogen is released, which is then quantified using a titration technique (Hayes, 2020). Protein quantity is calculated from the nitrogen concentration of the food using a conversion factor (usually 6.25, equivalent to 0.16 g nitrogen per gram of protein), which is the standard method for protein measurement but has its disadvantages (Hayes, 2020). As discussed previously by Maehre et al. (2018) and Harrysson et al. (2018), it does not measure true protein and the conversion/correction factor of 6.25 is not suitable for all protein types and should be corrected based on the amino acid composition of the protein in question. Several studies have determined species-specific nitrogen correction/conversion factors. For instance, a conversion factor of 5.6 is recommended for shrimp and fish, 5.4 for cereal products, and 4.59 for red seaweed. However, other researchers have suggested conversion factors of 4.9 for fish and 4.7 for flour (Maehre et al., 2018).

The Dumas method is fast and chemical-free but costly to set up and lacks accuracy as it does not measure true protein, while UV spectrophotometric methods such as the Biuret, Bradford, and Lowry methods are easy to use, inexpensive, and capable of quantifying small amounts of protein (Hayes, 2020). However, they can give false positive protein readings depending on the sample preparation method used and solubility of the test sample; while direct amino acid analysis involves hydrolysis of the protein with HCl and subsequent quantification of the amino acids using HPLC, but unfortunately is required initial investment in HPLC equipment, hydrolysis step and is time consuming but is the most accurate method (Hayes, 2020).

Protein content is a critical factor in the technological performance of products. Currently, the ISO standard method for determining protein content is the Kjeldahl method, which is time-consuming and requires specialized instrumentation. For this reason, the use of rapid methods to quantify protein would greatly facilitate the monitoring (Hueso et al., 2022). Methods as Bradford assay, Dumas method and the bicinchoninic acid assay (BCA) have been taken into consideration. For a milk sample, the protein content obtained from Bradford assay and Dumas method was consistent with Kjeldahl values (Hueso et al., 2022). In contrast, BCA protein levels were significantly different when compared to Kjeldahl and no method was proved to be suitable for protein determination in permeate samples (Hueso et al., 2022). In comparison with the official method, the Bradford assay provided the best quantitative results and is recommended for a quick, economical, and easy determination of total protein content in milk and retentate samples, as its simple protocol requires neither expensive equipment nor experienced analysts for data acquisition (Hueso et al., 2022).

Another example of an improved method is the modification of the traditional biuret reagent by replacing copper sulfate and Seignette salt with an alkaline alcohol solution and insoluble copper phosphate, which requires only about 50 mg of biological material to quantify three different components in each sample (Mihalcea et al., 2024). The method offers several advantages, including simplicity, low cost, speed, and accuracy. It involves weighing a single sample to determine both protein content and essential amino acids, lysine and tryptophan in the same biuret supernatant. It is important that new methods for the simultaneous determination of protein and two of the most important amino acids, lysine and tryptophan, are emerging.

Amino acids (AAs) are essential nutrients for life, playing a crucial role in maintaining vital body functions, including protein synthesis, healthy growth, tissue repair, and balanced metabolism. The most of AAs, except tryptophan, phenylalanine and tyrosine, lack a strong chromophore (Yuliarti et al., 2021), such as no ultraviolet absorption and no fluorescence. For example, the amino acids composition in the samples of some herbal raw materials was studied by ***gas chromatography-mass spectrometry (GC-MS) method*** (Savych et al., 2021). Good results were obtained analysing the amino acids composition of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. by HPLC method with a pre-column derivatization 9-fluorenylmethyl chloroformate (FMOC) and o-phthalaldehyde (OPA) (Budniak et al., 2021). ***A new hydrophilic interaction chromatography (HILIC) method*** for free amino acid analysis in honey samples was developed, involving pre-column derivatization with dansyl chloride

(Dns-Cl), sample pretreatment with modified QuEChERS extraction, and liquid chromatographic analysis with UV detection (Kırkan & Aydoğan, 2021). Therefore, AAs are always determined by derivatization to increase detection sensitivity, but there are some drawbacks, such as complex derivatization procedure, incomplete derivatization, derivative instability, reagent interference, expensive reagents and long analysis time (Wada et al., 2021; Figueroa et al., 2023). Therefore, a quick, simple and accurate analytical method for amino acid is needed (Chen et al., 2021). ***A direct method for determining amino acids in plants using HILIC and HPLC-MS/MS*** was established, with sample extraction completed in 10 minutes, making it simpler, faster, and more environmentally friendly, while the determination of 21 underivatized amino acids was completed in 15 minutes with satisfactory recovery, precision, and stability (Chen et al., 2021).

Thin-layer chromatography (TLC) is classically used for routine separations, identification of the individual amino acids or vitamins, and their quantitative determinations. With the advent of the automated multiple development (AMD) technique all steps from application to mixing solvents, development, and drying can be automated. Although such automation is obviously expensive, it is a prerequisite for the wider use of HPTLC in the pharmaceutical industry (Mohammad et al., 2012). High performance thin layer chromatography (HPTLC) involving the use of smaller average particle size of adsorbent in the preparation of TLC plates provides faster separations with reduced zone diffusion and enhanced sensitivity (Mohammad et al., 2012).

An analytical method for the direct determination of amino acids (methionine, proline, glycine, histidine, arginine, and lysine) in biologics was developed using hydrophilic interaction HPLC with UV detection, offering the advantages of simplified sample preparation by eliminating the derivatization step and enabling simultaneous selective quantitative determination of multiple amino acids (Runova et al., 2021).

This ultrasound-assisted acid hydrolysis procedure using HPLC-UV offers a reliable and efficient alternative for AA analysis in plant-based proteins, offering significant cost and time savings (Custodio-Mendoza et al., 2024).

Kowalska et al. (2022) studied and optimized the simple extraction of flours samples followed by free amino acids determination procedures; the conditions of amino acids derivatisation reaction with ninhydrin for chromatographic determination of free amino acids sum.

The drawbacks of HPLC methods are the usage of high volumes of toxic solvents and sample derivatization (Pukleš et al., 2024). Listed drawbacks led to a trend where such products are often analyzed by analytical methods developed by the industries themselves, even though they have not been standardized or validated (Gilani et al., 2008). ***Capillary electrophoresis (CE)*** is often preferred over HPLC as a faster and more environmentally friendly analytical technique, commonly used for analyzing pharmaceuticals and biopharmaceuticals. (Costa et al., 2019; Piestansky et al., 2021).

Microchip electrophoresis (ME) devices have proven to be powerful tools for analyzing amino acids (AAs), offering advantages such as low cost, rapid analysis, minimal sample and reagent consumption, and effective separation, establishing their importance in analytical science (Pukleš et al., 2024). Compared to CE, the method optimization is more demanding due to the short separation path (Tüma et al., 2007).

Sustainable sources of dietary protein

In the European Union, food proteins are predominantly animal-based, primarily from meat and dairy products; almost all soy but also a larger part of pulses and cereals consumed in the European Union are used for animal nutrition (Detzela et al., 2021). The global population is expected to surpass 9 billion by 2050, driving a substantial rise in the demand for food, particularly protein-rich foods and animal feed (Godfray et al., 2010). The rising awareness about the relevance of high-quality proteins in the diet has encouraged the researchers and nutritionists to search for eco-friendly and sustainable protein sources (Kumar et al., 2021).

Innovation and interest in both novel protein sources such as plant, algae, and mycoprotein, and their respective processing technologies have been rising steeply (Rolland et al., 2020).
Some common alternative protein sources for vegan diets include legumes (lentils, chickpeas, peas, and beans), grains (quinoa, barley, and bulgur wheat), nuts and seeds (almonds, chia seeds, and pumpkin seeds), mycoprotein (single cell protein from fungus) and algal proteins (Kaur et al., 2021; Zeng et al., 2022; Qin et al.,2022).
Animal based proteins such as dairy, meat, poultry, and eggs are considered complete proteins because they contain all of the essential amino acids in sufficient amounts (Ulhas et al., 2023). Alternative proteins, on the other hand, are often considered incomplete proteins because they may lack one or more of the essential amino acids (Gorissen et al., 2018). For example, legumes such as beans, lentils, and chickpeas are high in lysine, while grains such as rice and quinoa are high in methionine and cysteine (Hertzler et al., 2020). Most of cereal species have low protein quality due to their small amounts of lysine and tryptophan (Babu & Prasanna, 2014). Therefore, by combining these different plant-based proteins, an individual can obtain a complete protein source that provides all of the essential amino acids needed for optimal health (Gorissen et al., 2018). For consumers not interested in vegan or vegetarian alternatives, meat hybrids, which replace a fraction of meat (e.g., 20% to 50%) with plant-based proteins, could offer a low-threshold option for more sustainable food consumption (Profeta et al., 2021).
Plant-based protein sources including legumes, seeds and nuts, and by-products/waste from food production have been studied and utilized to simulate meat and other analogues (Tachie et al., 2023). Some seeds such as pumpkin, sunflower and hemp have also been used to produce alternative eggs, cheese and flour products respectively (Watson, 2021). Meat, egg, dairy products, bakery products (snacks), beverages, and dietary supplements have been developed from plant and alternative protein sources (Bunge, 2022).
Due to the fact that demand for sustainable sources of dietary protein is steadily growing, providing dietary protein to a growing world population with rising living standards requires the development of sustainable protein sources other than animal meat (Yuliarti et al., 2021). Sesame meal holds significant potential as a source of plant-based protein. Because of its unique amino acid composition, high in methionine and cysteine, and low in lysine, means that consuming it together with soybean protein provides a more complete profile of essential amino acids (S´a et al., 2022). Sesame meal is a by-product rich in protein, produced during the industrial extraction of sesame oil which is currently treated as waste. That it could help meet this need if high quality protein could be extracted from the meal in an environmentally friendly way; the usually method of traditional alkali-soluble acid precipitation extraction process produces proteins with poor functionality and harmful to the environment (Cao et al., 2023).
Hempseed protein is another nutritious and sustainable alternative, offering a rich source of amino acids. It has gained increasing attention for its sustainability and nourishment; therefore, hempseed protein is an excellent potential protein ingredient to be applied to food systems to add nutritional value and promote environmental sustainability (Dong et al., 2024). From a nutritional standpoint, hempseed protein isolate encompasses a complete profile of all the essential amino acids required by adults according to FAO/WHO standards and presents potential bioactive peptides after digestion (Mamone et al., 2019).
Microalgae and macroalgae (Seaweed) are traditional food sources in many cultures, some algal species can have comparable or even higher protein content compared to traditional meat, milk, egg, or plant protein sources (Boukid et al., 2022; Fleurence et al., 2018). Laver (*Porphyra* spp.) is one of the most commonly cultivated seaweeds, typically consumed in its dried form. Seaweed is not only nutritionally excellent, as it is rich in protein, dietary fibre, and vitamin B, but is also used as a raw material for various foods such as sushi and gimbap (Wada et al., 2021). Red seaweeds, including laver, are known for their wide-ranging protein content, varying from 2.7 % to 47 % (Figueroa et al., 2023). The protein content is the most important quality

indicator of laver. Laver's high protein content not only provides nutritional value, but the amino acids such as taurine, alanine, and glutamic acid derived from this protein are also the basis for its distinctive flavour (Jeong et al., 2023). Additionally, dried laver is produced by grinding raw laver and then drying it, which results in minimal visual differences based on its composition and gives it a characteristic black colour (Kim et al., 2024). Consequently, it is challenging to determine the protein content of dried laver without specific quantitative methods such as Kjeldahl Method (Kim et al., 2024).

Legumes have been the world's second most important source of staple food after cereals as "poor man's meat". To date, several legumes have been studied and proposed as protein alternatives for human consumption and among them: soybean is the most prominent in terms of economics (Cheng et al., 2019). Knowing that soy protein is rich in all the nine amino acids that are necessary for human nutrition (Kudełka et al., 2021), plant-based meat alternatives have commonly been based on products derived from soy due to its high protein content (Cober et al., 2022). Heaving a higher protein level than both cereals and potatoes, they are filled with all the important amino acids that the human body needs (Guo et al., 2023). It can be utilized as a replacement for animal proteins in the food sector since it has nutritional and biological benefits similar to those of animal proteins (Huang et al., 2019). However, natural soy protein isolate contains a large number of hydrophobic groups, which seriously affects the solubility and emulsification of soy protein, resulting in its functional properties not being able to be well developed, limiting the application of soy protein in industry (Arfat & Benjakul, 2013). Phosphorylation of sodium tripolyphosphate reduces protein molecule size and increases intermolecular interactions, making it a dispersing additive in food processing and offering a reference for developing functional properties of industrially modified soy isolate proteins in the food industry (Guo et al., 2023).

Wheat gluten, also known as 'seitan,' is used as a binding agent combined with soy to create plant-based meat. The unique viscoelastic properties of wheat gluten allow it to form a resilient gel making it useful in the preparation of thickeners, fortifiers, and texturing agents (Pietsch et al., 2019). Chickpea is also a rich source of dietary protein (17-22%), has low allergenicity, high solubility and bio-functionality making it an interesting candidate as an alternative ingredient for plant-based meat (Boukid et al., 2021). In addition to chickpea, pea protein has also drawn interest from researchers as a potential additive in alternative meat products (Lu et al., 2020).

Extensive research has been conducted on several underutilized legumes with similar nutritional properties to soybean; one of these legumes is velvet beans (Baby et al., 2023). Though it is rich in protein (27%), complementary amino acid pattern to that of cereal grains exerts health beneficial properties, including antiparkinsonian, antidiabetic, and anticancer properties, it is limited for human consumption due to the presence of antinutrients (Baby et al., 2023).

Pulses (beans, peas, lentils, cowpeas, chickpeas, pigeon peas, etc.) being the second most important food source for humans, following cereals, play a key role in human nutrition, being rich in proteins, complex carbohydrates, essential vitamins, minerals, and phytochemicals, while being low in fats. Pulses are also considered the most suitable for preparing protein ingredients (Concentrates and isolates) because of their high protein content, wide acceptability and low cost.

Protein content of different pulses varies with genotypes, germination, environmental conditions and application of fertilizers during growth and development (Singh, 2017; Isticioaia et al., 2020). The pulse proteins have essential amino acids composition complementary to cereals and are naturally gluten-free, thus, safe for the patients suffering from gluten intolerance/allergies (Shevkani et al., 2019).

Applications in which pulses are used includes bakery and pasta (because of the essential amino acid content complimentary to cereals makes them particularly suitable for improving the protein quality of cereal-based foods (Shevkani et al., 2019), imitation milk and bean curds (preparation of non-dairy/imitation milks, beverages and bean curds), meet products (the effect of incorporation of lower levels of (1.5, 2.5 and 5%) chickpea protein concentrates on the properties of cooked sausage and reported that chickpea proteins not only reduced cooking yield and lipid oxidation but also increased color stability, total antioxidant capacities, protein content and acceptability by consumers (Ghribi et al., 2018), encapsulation material and edible/biodegradable films.

The seeds of flowering plants typically store significant amounts of seed protein, including storage proteins and lectins. The amount of protein found in seed varies from 10% (in cereals) to 40% (in certain legumes and oilseeds) of the seed dry weight, which forms a major source of dietary protein (Liu et al., 2017). The 16 proteins identified in faba bean were mainly composed of glutamic, arginine, lysine, glycine, leucine, cysteine, and methionine, which are essential to human life (Liu et al., 2017). The flours are essential and the most commonly used raw material for bakery products, however, due to the properties of proteins in gluten-free flours, their usage is limited (Kowalska et al., 2022). On the other hand, the nutritional properties of gluten-free flours are their main advantage due to the well-balanced composition of the amino acids with high biological value (Ciesarová et al., 2021).

Another rich source is found in fresh and preserved mushrooms which have been appreciated by humans since ancient times for their organoleptic, nutritional and medicinal properties (Wani et al., 2010). Although they are low in calories, mushrooms are a good source of high-quality protein, amino acids, dietary fibre, vitamins (vitamin B2, folate and niacin), as well as many healthy minerals (iron, calcium, phosphorus, potassium, selenium, zinc and copper) and phenolic compounds (Mattila et al., 2001).

Although not a source of plant protein, fish by-products, such as frames, trimmings, and viscera from commercial fish species, are rich in proteins and could serve as an economical source of bioactive peptides and functional protein hydrolysates for the food and nutraceutical industries (Nikoo et al., 2023). Despite this necessity, within the seafood sector, the increased use by-products is happening relatively slowly because the seafood industry focuses on its primary raw materials and products that require minimal processing (Wassef et al., 2023).

Sustainable diets and environmental impact

Sustainable diets focus on enhancing public health, food security, and reducing the environmental impact of the food system by promoting a plant-based diet and reducing the consumption of animal products (Lonnie & Johnstone, 2020). Meat and dairy, the main protein sources in an omnivorous diet, contribute the most to the food-related ecological footprint (Borkent et al., 2024). The food systems underlying these diets are responsible for a third of global greenhouse gas emissions (Crippa et al., 2021) and influence several other environmental indicators (Willett et al., 2019). A transition to a more sustainable diet-one that contains more plant-based products and less meat, could increase food availability while releasing the burden on the environment (Chen et al., 2019).

Studies suggest that the environmental impact of food production is higher for omnivorous diets and lower for vegetarian/vegan diets (Moreno et al., 2021). Fortunately, the alternative protein market is rapidly growing as consumers are becoming more conscious of the impact of their food choices (Kaur et al., 2021). The effects of food production on climate change have led to decreased agricultural yields and increased food insecurity in some regions of the world (Rockstrom et al., 2009). This, combined with the rapidly growing global population, makes current food systems unsustainable. According to the WRI, a combination of changes will be required to reduce the impact of our diets on the environment, including a shift in our food choices, changes in food production (sourcing and transformation), and reductions in food losses and waste (World Resources Institute, 2018).

Although the environmental impact of animal-sourced foods can be reduced by reducing our consumption of red meat, further benefits can be gained from changes in farming practices, such as recycling manure as fertilizer for crops, converting manure into biogas, and/or feeding cattle on grasslands instead of (or alongside) animal feeds (Moreno et al., 2021).

In terms of plant-sourced foods, agricultural practices increasingly rely on the use of chemical fertilizers to restore nutrients to the soil, thereby enabling farmers to plant just 1 crop, without the need for rotational cropping (Lacour et al., 2018; Partal et al., 2020, 2023).

The urgent need to shift to plant-rich diets is now considered a vital strategy in achieving a global sustainable food system and in combating an important driver of climate change (Allenden et al., 2022). Both collective and individual level, food behaviours and food choices represent major levers of action against the ongoing environmental disaster (Rabès et al., 2020).

CONCLUSIONS

The development of new methods for the determination of protein and amino acids is essential for the advancement of research and the food industry, with multiple positive implications, such as: increased accuracy and reliability (more accurate determination of protein and amino acid content, essential for assessing the nutritional quality of food products), fast and efficient (modern methods, such as spectrophotometric and machine learning techniques, provide fast and efficient results, saving time and resources in laboratories and industries), wide applicability (these new methods are versatile and can be applied to a wide range of food products), promoting healthy diets (the correct determination of proteins and amino acids is essential for creating food products with adequate nutrient content) and innovation and sustainability (the development of these methods can support research into alternative protein sources, such as plant protein or protein from sustainable sources, contributing to innovative solutions to global food challenges).

The use of protein from sustainable sources is not only a necessity to protect the environment and ensure a balanced diet, but also an opportunity to encourage more responsible and affordable agriculture.

In conclusion, progress in the development of protein and amino acid determination methods is essential to improve food quality, production process efficiency and nutritional sustainability on a global scale.

REFERENCES

Aleksandrowicz, L., Green, R., Joy, E. J. M., Smith, P., & Haines, A. (2016). The Impacts of Dietary Change on Greenhouse Gas Emissions, Land Use, Water Use, and Health: A Systematic Review. *PLoS ONE, 11*(11): e0165797.

Allenden, N., Hine, D. W., Craig, B. M., Cowie, A. L., McGreevy, P. D., & Lykins, A. D. (2022). What should we eat? Realistic solutions for reducing our food footprint. *Sustainable Production and Consumption*, 32, 541–549.

Arfat, Y. A., & Benjakul, S. (2013). Effect of zinc sulphate on gelling properties of phosphorylated protein isolate from yellow stripe trevally. *Food Chem.*, 141, 2848–2857.

Babu, R., & Prasanna, B. M. (2014). Molecular breeding for quality protein maize (QPM). In Tuberosa R et al. (eds.), Genomics of Plant Genetic Resources. *Springer Netherlands*, pp 489–505.

Baby, C., Kaur, S., Singh, J., & Prasad, R. (2023). Velvet bean (Mucuna pruriens): A sustainable protein source for tomorrow. *Legume Science*, 5(3), e178.

Béné, C., Prager, S. D., Achicanoy, H. A. E., Toro, P. A., Lamotte, L., Bonilla, C., & Mapes, B. R. (2019). Global map and indicators of food system sustainability. *Sci. Data*, 6, 279.

Bengtsson, J., Bullock, J. M., Egoh, B., Everson, C., Everson, T., O'Connor, T., et al. (2019). Grasslands - more important for ecosystem services than you might think. *Ecosphere*, 10(2): e02582.

Borkent, J. W., Grootswagers, P., Linschooten, J., Roodenburg, A. J. C., Ocké, M., & de van der Schueren, M. A. E. (2024). A vegan dietary pattern is associated with high prevalence of inadequate protein intake in older adults; a simulation study. *The Journal of Nutrition, Health and Aging*, 28, 100361.

Boukid, F., Rosell, C. M., & Castellari, M. (2021). Pea protein ingredients: A mainstream ingredient to (re) formulate innovative foods and beverages. *Trends in Food Science & Technology*, 110, 729–742.

Boukid, F., Rosell, C. M., Rosene, S., Bover-Cid, S., & Castellari, M. (2022). Non-animal proteins as cutting-edge ingredients to reformulate animal-free foodstuffs: Present status and future perspectives. *Critical Reviews in Food Science and Nutrition*, 62(23), 6390–6420.

Budniak, L., Slobodianiuk, L., Marchyshyn, S., Demydiak, O., & Dakhym, I. (2021). Determination of amino acids of some plants from Gentianaceae family. *Pharmacia* 68(2): 441–448.

Bunge. (2022). Plant Protein Portfolio for Meat Alternatives. *In Protein Vision, Food navigator*.

Cao, P. H., Zhang, C. X., Ma, Y. X., Yu, Y. M., Liu, H. M., Wang, X., & Zheng, Y. Z. (2023). Extraction of protein from sesame meal: Impact of deep eutectic solvents on protein structure and functionality. *LWT - Food Science and Technology*, 187, 115366.

Chai, B. C., van der Voort, J. R., Grofelnik, K., Eliasdottir, H. G., Klöss, I., & Perez-Cueto, F. J. A. (2019). Which Diet Has the Least Environmental

Impact on Our Planet? A Systematic Review of Vegan, Vegetarian and Omnivorous Diets. *Sustainability*, 11(15):4110.

Chen, C., Chaudhary, A., & Mathys, A. (2019). Dietary change scenarios and implications for environmental, nutrition, human health and economic dimensions of food sustainability. *Nutrients, 11*(4):856.

Chen, X., Feng, D., Zou, Y., Li, H., & Song, H. (2021). Quick extraction and direct determination of amino acids from plants by hydrophilic interaction liquid chromatography (HILIC) and high-performance liquid chromatography-mass spectrometry without derivatization. *E3S Web of Conferences* 252, 02055.

Cheng, A., Raai, M. N., Zain, N. A. M., Massawe, F., Singh, A., & Wan-Mohtar, W. A. A. Q. I. (2019). In search of alternative proteins: Unlocking the potential of underutilized tropical legumes. *Food Security*, *11*(6), 1205–1215.

Ciesarová, Z., Kukurová, K., Torbica, A., Belović, M., Horváthová, J., Daško, L., & Jelemenská, V. (2021) Acrylamide and 5-hydroxymethylfurfural in thermally treated non-wheat flours and respective breads. *Food Chem*., 15:365:130491.

Cober, E. R., Daba, K. A., Warkentin, T. D., Tomasiewicz, D. J., Mooleki, P., Karppinen, E. M., … Hadinezhad, M. (2022). Soybean seed protein content is lower but protein quality is higher in Western Canada compared to Eastern Canada. *Canadian Journal of Plant Science*.

Comerford, K. B., Miller, G. D., Reinhardt Kapsak, W., & Brown, K. R. (2021). The complementary roles for plant-source and animal-source foods in sustainable healthy diets. *Nutrients*, 13(10):3469–84.

Costa, B. M. C., Prado, A. A. Oliveira, T. C., Bressan, L. P., Munoz, R. A. A., Batista, A. D., da Silva, J. A. F., & Richter, E. M. (2019). Fast methods for simultaneous determination of arginine, ascorbic acid and aspartic acid by capillary electrophoresis. *Talanta*, 204, 353–358.

Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nat. Food*, 2, 198–209.

Custodio-Mendoza, J. A., Pokorski, P., Aktaș, H., & Kurek, M. A. (2024). Rapid and efficient high-performance liquid chromatography-ultraviolet determination of total amino acids in protein isolates by ultrasound-assisted acid hydrolysis. *Ultrasonics Sonochemistry*, 111, 107082.

Detzela, A., Krügera, M., Buscha, M., Blancobc, I., Varelabc, C., Mannersd, R., Beze, J., & Zanninif, E. (2021). Life cycle assessment of animal-based foods and plant-based protein-rich alternatives: an environmental perspective. *Journal of the Science of Food and Agriculture*.

Dong, X., Woo, M. W., & Quek, S. Y. (2024). The physicochemical properties, functionality, and digestibility of hempseed protein isolate as impacted by spray drying and freeze drying. *Food Chemistry*, 433.

Figueroa, V., Farf́an, M., & Aguilera, J. (2023). Seaweeds as novel foods and source of culinary flavors. *Food Reviews International*, 39(1), 1–26.

Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In Proteins in food processing (pp. 245–262). *Woodhead Publishing*.

Gehring, J., Touvier, M., Baudry, J., Julia, C., Buscail, C., Srour, B., et al. (2021). Consumption of ultraprocessed foods by pesco-vegetarians, vegetarians, and vegans: associations with duration and age at diet initiation. *J Nutr*., 2021151(1).

Ghribi, A.M., Amira, A.B., Gafsi, I.M., Lahiani, M., Bejar, M., Triki, M., Zouaria, A., Attia, H., & Besbes, S. (2018). Toward the enhancement of sensory profile of sausage ''Merguez'' with chickpea protein concentrate. *Meat Sci*, 143:74–80.

Gilani, G. S., Xiao, C., & Lee, N. (2008). Need for accurate and standardized determination of amino acids and bioactive peptides for evaluating protein quality and potential health effects of foods and dietary supplements. *J. AOAC Int*., 91, 894–900.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, 327:812–818.

Gorissen, S. H., Crombag, J. J., Senden, J. M., Waterval, W. H., Bierau, J., Verdijk, L. B., & van Loon, L. J. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids*, 50, 1685–1695.

Guo, Y., Liu, C., Ma, Y., Shen, L., Gong, Q., Hu, Z., Liu, Z. W. X., Guo, Z., & Zhou, L. (2023). Study on the Structure, Function, and Interface Characteristics of Soybean Protein Isolate by Industrial Phosphorylation. *Foods*, 5;12(5):1108.

Harrysson, H., Hayes, M., Eimer, F., Carlsson, N.-G., Toth, G. B., & Undeland, I. (2018). Production of protein extracts from Swedish red, green and brown seaweeds, Porphyra umbilicalis Kützing, Ulva lactuca Linnaeus, and *Saccharina latissima* (Linnaeus) J. V Lamouroux using three different methods. *J. Appl. Phycol*., 30, 3565–3580.

Hayes, M. (2020). Measuring Protein Content in Food: An Overview of Methods. *Foods* 9.10: 1340.

Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M., & Allgeier, C. (2020). Plant proteins: Assessing their nutritional quality and effects on health and physical function. *Nutrients*, 12(12), 3704.

Huang, T., Tu, Z., Shangguan, X., Sha, X., Wang, H., Zhang, L., & Bansal, N. (2019) Fish gelatin modifications: A comprehensive review. *Trends Food Sci. Technol*., 86, 260–269.

Hueso, D., Fontecha, J., & Gómez-Cortés, P. (2022). Comparative study of the most commonly used methods for total protein determination in milk of different species and their ultrafiltration products. *Front. Nutr*., 9:925565.

Isticioaia S.F., Leonte A., Popa L.D., Trotuș E., Apostol L., Voicea I., MATEI G., - *Sorghum bicolor* (L.) Moench. - Plant for the future in Moldavia agriculture (Romania) and for the human nutrition.

E3S Web of Conferences 180, 03004 (2020) TE-RE-RD 2020.
Jeong, G.-T., Lee, C., Cha, E., Moon, S., Cha, Y.-J., & Yu, D. (2023). Determination of optimum processing condition of high protein laver Chip using air-frying and reaction flavour technologies. *Foods*, 12(24), 4450.
Kalmpourtzidou, A., & Scazzina, F. (2022). Changes in terms of risks/benefits of shifting diets towards healthier and more sustainable dietary models. *EFSA Journal*, 20(S2): e200904.
Kalmpourtzidou, A., Eilander, A, & Talsma, E. F. (2020). Global vegetable intake and supply compared to recommendations: a systematic review. *Nutrients*, 12, 1558.
Kang, Z., Zhao, Y., Chen, L., Guo, Y., Mu, Q., & Wang, S. (2022). Advances in machine learning and hyperspectral imaging in the food supply chain. *Food Engineering Reviews*, *14*(4), 596–616.
Kaur, L., Lamsar, H., L´opez, I. F., Filippi, M., Ong Shu Min, D., Ah-Sing, K., & Singh, J. (2021). Physico-chemical characteristics and in vitro gastro-small intestinal digestion of New Zealand ryegrass proteins. *Foods*, 10(2), 331.
Kim, E., Park, J. J., Lee, G., Cho, J. S., Park, S. K., Yun, D. Y., Park, K. Y., & Lim, J. H. (2024). Innovative strategies for protein content determination in dried laver (Porphyra spp.): Evaluation of preprocessing methods and machine learning algorithms through short-wave infrared imaging. *Food Chemistry*: X 23, 101763.
Kırkan, E., & Aydoğan, C. (2021). Free Amino Acid Analysis in Honey Samples by Hydrophilic Interaction Liquid Chromatography with UV Detection Using Precolumn Derivatization with Dansyl Chloride. *Chromatographia*, 84:127–133.
Kowalska, S., Szłyk, E., & Jastrzębska, A. (2022). Simple extraction procedure for free amino acids determination in selected gluten-free flour samples. *European Food Research and Technology*, 248:507–517.
Kudełka, W., Kowalska, M., & Popis, M. (2021). Quality of soybean products in terms of essential amino acids composition. *Molecules*, 26(16), 5071.
Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., Belwal, T., Dahuja, A., Joshi, S., Berwal, M. K., Satankar, V., Bhoite, A. G., Amarowicz, R., Kaur, C., & Kennedy, J. F. (2021). Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids*, 115, 106595.
Lacour, C., Seconda, L., Alles, B., Hercberg, S., Langevin, B., Pointereau, P., Lairon, D., Baudry, J., & Kesse-Guyot, E. (2018). Environmental impacts of plant-based diets: how does organic food consumption contribute to environmental sustainability?. *Front Nutr*., 5:8.
Liu, Y., Wu, X., Hou, W., Li, P., Sha, W., & Tian, Y. (2017). Structure and function of seed storage proteins in faba bean (*Vicia faba* L.). *3 Biotech*, 7:74.
Lonnie, M., & Johnstone, A. M. (2020). The public health rationale for promoting plant protein as an important part of a sustainable and healthy diet. *Nutrition Bulletin*, 45(3), 281–293.
Lu, Z. X., He, J. F., Zhang, Y. C., & Bing, D. J. (2020). Composition, physicochemical properties of pea protein and its application in functional foods. *Critical Reviews in Food Science and Nutrition*, 60(15), 2593–2605.
Maehre, H. K., Dalheim, L., Edvinsen, S. K., Elvevoll, E. O., & Jensen, I.-J. (2018). Protein determination—Methods Matter. *Foods*, 1, 5.
Mamone, G., Picariello, G., Ramondo, A., Nicolai, M. A., & Ferranti, P. (2019). Production, digestibility and allergenicity of hemp (*Cannabis sativa* L.) protein isolates. *Food Research International*, 115, 562–571.
Mattila, P., Könkö, K., Eurola, M., Pihlava, J. M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., & Piironen, V. (2001). Contents of vitamins, mineral elements, and phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem*., 49, 2343–2348.
Mihalcea, E., Enache, A. C., Gradinaru, V. R., & Drochioiu, G. (2024). Simultaneous determination of protein and essential amino acids in biological samples. *Acta Chemica Iasi*, 32_1, 1–12.
Mohammad, A., Moheman, A., & El-Desoky, G. E. (2012). Amino acid and vitamin determinations by TLC/HPTLC: review of the current state. *Central European Journal of Chemistry*, 10:3, 731–750.
Moreno, L. A., Meyer, R., Donovan, S. M., Goulet, O., Haines, J., Kok, F. J., & Pieter van't Veer (2021). Perspective: Striking a Balance between Planetary and Human Health. Is There a Path Forward?. *Adv Nutr*., 13(2):355–375.
Nagy, M. M., Wang, S., & Farag, M. A. (2022). Quality analysis and authentication of nutraceuticals using near IR (NIR) spectroscopy: A comprehensive review of novel trends and applications. *Trends in Food Science & Technology*, 123, 290–309.
Nikoo, M., Regenstein, J. M., & Yasemi, M. (2023). Protein Hydrolysates from Fishery Processing By-Products: Production, Characteristics, Food Applications, and Challenges. *Foods*., 12(24):4470.
Özdoğan, G., Lin, X., & Sun, D.-W. (2021). Rapid and noninvasive sensory analyses of food products by hyperspectral imaging: Recent application developments. *Trends in Food Science & Technology*, 111, 151–165.
Ozturk, S., Bowler, A., Rady, A., & Watson, N. J. (2023). Near-infrared spectroscopy and machine learning for classification of food powders during a continuous process. *Journal of Food Engineering*, 341, Article 111339.
Partal E, Paraschivu M. (2020). Results regarding the effect of crop rotation and fertilization on the yield and qualities at wheat and maize in South of Romania. *Scientific Papers. Series A. Agronomy*, vol LXIII, no.2, 184–189.
Partal E., Oltenacu C.V., Paraschivu M., Cotuna O., Contescu L.E. (2023). Wheat yield and quality under the influence of sowing date, plant density and

variety in South of Romania, *Scientific Papers. Series A. Agronomy*, Vol. LXVI, No.2, 339–345.

Partal E., Oltenacu C.V., Paraschivu M., Cotuna O., Dima M., Contescu L.E. – 2023b. *Effects of different soil tillage on soil moisture, weed control, yield and quality of maize (Zea mays L.).* Romanian Agricultural Research, no. 40, 475–482.

Piestansky, J., Matuskova, M., Cizmarova, I., Olesova, D., & Mikus, P. (2021). Determination of branched-chain amino acids in food supplements and human plasma by a CE-MS/MS method with enhanced resolution. *Int. J. Mol. Sci*., 22, 8261.

Pietsch, V. L., Werner, R., Karbstein, H. P., & Emin, M. A. (2019). High moisture extrusion of wheat gluten: Relationship between process parameters, protein polymerization, and final product characteristics. *Journal of Food Engineering*, 259, 3–11.

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*, 360, 987–992.

Profeta, A., Baune, M. C., Smetana, S., Bornkessel, S., Broucke, K., Van Royen, G., Enneking, U., Weiss, J., Heinz, V., Hieke, S., & Terjung, N. (2021). Preferences of German Consumers for Meat Products Blended with Plant-Based Proteins. *Sustainability*, 13, 650.

Pukleš, I., Páger, C., Saka˘c, N., Šarkanj, B., Markovi´c, D., Saka˘c, M. K., & Jozanovi´c, M. (2024). Green Microfluidic Method for Sustainable and High-Speed Analysis of Basic Amino Acids in Nutritional Supplements. *Molecules*, 29, 5554.

Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. *Journal of Agriculture and Food Research*, 7, 100265.

Rabès, A., Seconda, L., Langevin, B., Allès, B., Touvier, M., et al. (2020). Greenhouse gas emissions, energy demand and land use associated with omnivorous, pesco-vegetarian, vegetarian, and vegan diets accounting for farming practices. *Sustainable Production and Consumption*, 22, 138–146.

Reinmuth-Selzle, K., Tchipilov, T., Backes, A. T., Tscheuschner, G., Tang, K., Ziegler, K., Lucas, K., Pöschl, U., Fröhlich-Nowoisky, J., & Weller, M. G. (2022). Determination of the protein content of complex samples by aromatic amino acid analysis, liquid chromatography-UV absorbance, and colorimetry. *Analytical and Bioanalytical Chemistry*, 414:4457–4470.

Rockstrom, J., Steffen, W., Noone, K., Persson, A., Chapin, F. S. 3rd, Lambin, E. F., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H.J. et al. (2009). A safe operating space for humanity. *Nature*, 461(7263):472–5.

Rolland, N. C., Markus, C. R., & Post, M. J. (2020). The effect of information content on acceptance of cultured meat in a tasting context. *PLoS One1*, 15(4), e0231176.

Runova, O. B., Korotkov, M. G., & Ustinnikova, O. B. (2021). Development of the conditions for direct quantitative determination of amino acids in biologics by hydrophilic interaction HPLC. *Pharmaceutical Chemistry Journal*, 55, 6.

S´a, A. G. A., Pacheco, M. T. B., Moreno, Y. M. F., & Carciofi, B. A. M. (2022). Cold-pressed sesame seed meal as a protein source: Effect of processing on the protein digestibility, amino acid profile, and functional properties. *Journal of Food Composition and Analysis*, 111.

Savych, A., Marchyshyn, S., Harnyk, M., Kudria, V., & Ocheretniuk, A. (2021) Determination of amino acids content in two samples of the plant mixtures by GC-MS. *Pharmacia* 68(1): 283–289.

Shevkani, K., Singh, N., Chen, Y., Kaur, A., & Yu, L. (2019). Pulse proteins: secondary structure, functionality and applications. *J Food Sci Techno*., 56(6):2787–2798.

Singh, N. (2017) Pulses: an overview. *J Food Sci Technol* 54:853–857.

Springmann, M., Wiebe, K., Mason-D'Croz, D., Sulser, T. B., Rayner, M., & Scarborough, P. (2018). Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail. *Lancet Planet Health*, 2, e451–e461.

Tachie, C., Nwachukwu, I. D., & Aryee, A. N. A. (2023). Trends and innovations in the formulation of plant-based foods. *Food Production, Processing and Nutrition*, 5:16.

Tüma, P., Samcová, E., Opekar, F., Jurka, V., & Stulík, K. (2007). Determination of 1-methylhistidine and 3-methylhistidine by capillary and chip electrophoresis with contactless conductivity detection. *Electrophoresis*, 28, 2174–2180.

Ulhas, R. S., Ravindran, R., Malaviya, A., Priyadarshini, A., Tiwari, B. K., & Rajauria, G. (2023). A review of alternative proteins for vegan diets: Sources, physico-chemical properties, nutritional equivalency, and consumer acceptance. *Food Research International*, 173, 113479.

Wada, K., Tsuji, M., Nakamura, K., Oba, S., Nishizawa, S., Yamamoto, K., & Nagata, C. (2021). Effect of dietary nori (dried laver) on blood pressure in young Japanese children: An intervention study. *Journal of Epidemiology*, *31*(1), 37–42.

Wang, H., Pampati, N., McCormick, W. M., & Bhattacharyya, L. (2016). Protein nitrogen determination by kjeldahl digestion and ion chromatography. *J Pharm Sci*., 105(6):1851–1857.

Wani, B. A., Bodha, R. H., & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *J. Med. Plants Res*., 4, 2598–2604.

Wassef, E., Saleh, N., Abde-Meguid, N., & Abdel-Mohsen, H. (2023). Utilization of fish waste biomass as a fishmeal alternative in European seabass (Dicentrarchus labrax) diets: Effects on immuno-competence and liver and intestinal histomorphology. *Int. Aquat. Res*., 15, 145–161.

Watson, E. (2021). Plant-based should be healthy, says Spero founder: 'We don't want to create a product that's identical [to animal products] and bad for you'. *Food Navigator*.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., Vries, W. D., Sibanda, L. M., Afshin, A., Chaudhary, A., Herrero, M., Agustina, R., Branca, F., Lartey, A., Fan, S., Crona, B., Fox, E., Bignet, V., Troell, M., Lindahl, T., Singh, S., Cornell, S. E., Reddy, K. S., Narain, S., Nishtar, S., & Murray, C. J. L. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet*, 393, 447–492.

World Resources Institute. Creating a sustainable food future. In: A menu of solutions to feed nearly 10 billion people by 2050. Washington (DC): World Resources Institute; 2018.

Yuliarti, O., Kiat Kovis, T. J., & Yi, N. J. (2021). Structuring the meat analogue by using plant-based derived composites. *Journal of Food Engineering*, 288.

Zeng, Y., Chen, E., Zhang, X., Li, D., Wang, Q., & Sun, Y. (2022). Nutritional value and physicochemical characteristics of alternative protein for meat and dairy-A review. *Foods*, 11(21), 3326.