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THE USE OF VEGETABLE-DERIVED PROTEINS FOR NEW FOOD PRODUCTS

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Abstract. Nowadays there is a high concern with newly-identified protein sources to substitute all kinds of proteins derived from animals. The food industry faces a challenge to produce quality food products that can feed more than nine billion people by 2050, upholding the principles of a sustainable and environmentally affordable way. This idea can be supported by the use of legumes that stand out with appreciable protein content, rich in essential amino acids that increase the foaming and emulsifying properties as well as the dietary fiber content. In recent years, in order to solve environmental and social problems and to diversify food products to cover different nutritional types, proteins of plant origin are used to replace those of animal origin. The present work described a review about emerging alternative proteins for nutrition which focuses on its properties and characteristics.The work analyzes the influence of alternative proteins sources on the food products'sustainability.

Keywords: *plant-based proteins, animal proteins, legumes, aquafaba, meat analogues.*

Rezumat. În zilele noastre există o mare preocupare în ceea ce privește substituirea tipurile de proteine derivate de la animale cu surse de proteine nou-identificate. Industria alimentară se confruntă cu o provocare de a produce produse alimentare de calitate, care să poată hrăni mai mult de nouă miliarde de oameni până în 2050, susținând principiile unui mod durabil și accesibil din punct de vedere ecologic. Această idee poate fi susținută prin utilizarea de leguminoase care se remarcă printr-un conţinut apreciabil de proteine bogate în aminoacizi esențiali cu proprietățile de spumare și emulsionare importante precum și conținutul de fibre alimentare. În ultimii ani, pentru a rezolva problemele legate de mediu, probleme sociale și pentru a diversifica sortimentul de produse alimentare în vederea satisfacerii cerințelor diferitor grupuri de consumatori, proteinele de origine vegetală sunt folosite pentru a le înlocui pe cele de origine animală. Lucrarea de față prezintă o analiză de sinteză despre proteinele alternative emergente specifice industriei alimentare şi influenţa acestora asupra sustenabilității produselor alimentare.

Cuvinte cheie: *proteine vegetale, proteine animale, leguminoase, aquafaba, analogi de carne.*

1. Introduction

The wonder of leguminous crops lies in their capacity to accumulate substantial quantities of proteins and other valuable biologically active organic and inorganic substances in fruits and seeds [1].

On the international stage there is a rich experience in utilizing compounds derived from plant raw materials as additives in food technology to enhance structural and mechanical properties of the end product and improve organoleptic characteristics.The use of natural additives from plant materials not only improves the quality and expands the range of food products but also allows for the rational use of local resources.Therefore, besides their specified technological functions, natural additives from vegetable materials, due to the content of native biologically active substances, augment the biological value of the end product [2].

The use of plant materials with surface-active properties as foaming and emulsifying agents from the composition of natural plant tissues is, primary being natural compounds with a high degree of absorption in the consumer's body [3]. Most of these compounds contain surfactants such as proteins and pectins.

Additionally, a group of compounds containing saponins alongside proteins and pectins is notable. Similar to pectins, saponins reduce blood cholesterol levels, thereby reducing the risk of atherosclerotic vascular lesions, and exhibit anticancer properties [4].

In recent years, due to concerns for health and the environment, there is a rising trend to replace eggs with plant-based ingredients, based on plant proteins [5, 6] or starch [7], which have the same foaming and emulsifying properties as chicken eggs, containing not only proteins and pectins but also saponins. The increasing interest in egg-free products is supported by three main aspects: health, dietary preferences, and economic factors. One of the reasons for excluding eggs from the diet is the presence of phenylketonuria. Phenylketonuria is a disorder of amino acid metabolism that, if untreated, can cause irreversible mental health disability [8]. The diet for treating phenylketonuria is based on avoiding foods with high protein content, such as meat, seafood, eggs, milk, nuts, and related products [9]. Another health-related issue is egg allergy, which is one of the most common food allergies [10], second only to cow's milk protein allergy [11]. Major egg allergens are mainly present in egg whites and can trigger life-threatening symptoms [12]. Concerns also persist regarding the cholesterol content in eggs due to associations between cholesterol and cardiovascular diseases [13]. Another health concern for consumers is so called bird flu, an asymptomatic viral infection, leading to international declines in egg sales [14]. Consequently, bird flu outbreaks have affected not only consumption but also egg production, causing serious financial problems in the global poultry industry [15]. Different food preferences and needs, based on consumer attitudes and beliefs, influence consumption and purchasing power and can lead to the development of new food products [16, 17]. Consumer food choices can also be dictated by religion and certain beliefs that establish dietary rules regarding permitted and prohibited foods [18]. Economic factors associated with egg replacement take into account the high cost, transportation difficulties, and storage of fresh eggs based on the limited distribution of the refrigeration chain [19], and their short shelf life, prompting producers to seek alternatives to eggs.

Egg substitutes can be classified into three main groups:

- Based on concentrated and isolated protein forms.

- Sources of plant-based proteins.
- Additives, namely hydrocolloids and/or emulsifiers.

In recent years, numerous studies have been published on the partial or total replacement of eggs in food products using egg substitutes from various sources. The potential use of bovine plasma [20] as an egg substitute in cake production has been analyzed. Additionally, egg replacement in cake production with whey [21]; legumes, especially soy [22], lentils [23], peas [24], and lupin [25], as well as combinations of hydrocolloids such as xanthan gum, guar gum, carboxymethyl cellulose, hydroxypropyl methylcellulose, carrageenan, and/or arabic gum. Some researchers have evaluated the possibility of using gel from chia or banana seeds [26] as an egg substitute. Furthermore, a growing trend is the use of aquafaba, the cooking water of legumes, especially chickpeas [27], an ingredient with high foaming, emulsifying, and thickening capabilities due to its protein, carbohydrate, and saponin content [28], at an appropriate pH and NaCl concentration [29]. Thus, aquafaba has earned a reputation for transitioning from food waste to a nutritionally and biologically valuable ingredient [30]. In this regard, aquafaba could represent a cost-effective and accessible egg substitute for the production of food products such as ice cream, mayonnaise, and confectionery items. Although aquafaba has been classified as a functional ingredient for confectionery products [31], it has been used by few researchers for cake production [32].

Legumes, due to their elevated protein content, can be used in food as meat substitutes. The method of preparing meat-type products such as sausages, salami, burgers, etc., involves the total or partial replacement of meat with legume flour [33]. However, the acceptance of reduced meat consumption and meat replacement with alternative proteins is still generally low in western countries [34-37]. Consumers need to recognize the importance of adopting plant-based food products to support campaigns advocating reduced meat consumption by animal rights/welfare organizations and address increased greenhouse gas emissions detrimental to the environment caused by livestock farming [38].Perspectives on consumer practices related to the use of plant-based substitutes in obtaining meat analogs are crucial/important, first of all, because nutritional analogs with improved nutritional status and high biological value can be produced. This aligns with the promotion of a healthy lifestyle, the preservation of animal life, and the enhancement of environmental sustainability in the context of a sustainable circular bioeconomy [39].

2. The importance of legumes in human nutrition

Legumes (*Fabaceae*) are angiosperm, dicotyledonous plants, numerous (approximately 10,000 species), and diverse. They originate from tropical and subtropical regions around the globe. Their seeds contain 19.5-40.3% protein, which is 1.5-3 times more than cereals. Plant proteins are accessible nutrients with a favorable impact on consumer health, despite the high demand for animal-origin protein [1]. Food Agricultural Organization (FAO) statistics show that the demand for animal-origin protein-rich food products exceed the supply by four times. The deficit of animal protein can be alleviated only by increasing legume crop production [1] and utilizing them in food production.

Legumes are the second most important group of crops after cereals in human nutrition [40]. This is attributed to their low cultivation cost, nutritional properties, and beneficial physiological effects [2]. The most common edible legumes include beans, peas, chickpeas, lentils, soybeans, peanuts, and lupins. In the Republic of Moldova, the average production of legumes is around 56 thousand tons annually. The most prevalent crops are beans, constituting about 34% of the total production, peas 19%, chickpeas 13%, and lentils 5% [41].

Legumes are cultivated on a large scale for their nutritional properties, high protein content in seeds, and varied mineral substance content. The mineral content of legumes varies depending on the type and variety of legumes [42]. The regular consumption of legumes reduces individuals' susceptibility to chronic diseases such as cardiovascular diseases, diabetes, cancer, and excess body weight [3]. This may be due to the high content of protein, dietary fiber, essential fatty acids, and isoflavones [2].

3. Chemical composition of legumes

Due to their high nutritional value, legumes are widely used as a primary source of protein, especially in vegan diets. In addition to proteins, legumes are rich in essential components for the human body, such as minerals and vitamins B, and other vital health-protective compounds (phenolics, inositol phosphates, and oligosaccharides). They boast a low glycemic index, making them suitable for individuals with diabetes. Leguminous plants comprise numerous species, with seeds differing significantly in chemical composition and nutritional value, Table 1 [43].

Table 1

Chemical composition of legumes

Legume	Protein content, %	Fat content, %	Carbohydrate content, %	Mineral content, %	Source
Chickpeas	25.1	4.5	63.0	4.7	[1]
Beans	22.3	1.2	72.5	4.0	[44]
Green Peas	22.9	1.5	55.8	3.3	[45]
Lentils	30.4	2.1	54.0	4.2	[46]
Soybeans	32.7	15.7	30.1	3.6	[47]
Lupins	40.8	4.4	41.6	4.3	[48]

Legumes are characterized by a high dry matter content, with significant variation in minerals content among different legume varieties, ranging from 2.5% to 4.4% [44, 49]. They also feature a high protein content, with some varieties such as soy and lupins containing up to 48.2%, while lentils and beans have recorded the lowest protein content at 21.9% [43, 50]. The nutritional role of legumes in the human body is determined not only by their protein content but also by their structure and functions. Two protein fractions, albumins and globulins, are distinguished. Albumins, comprising approximately 10-25% of total proteins, serve structural and enzymatic functions in the human body. Legumes with higher albumin content exhibit increased nutritional value. Globulins, mainly considered immune proteins, constitute about 60-75% of total proteins in peas, soy, and lupins and 80−90% in beans and chickpeas [52]. The fat content of legumes varies from about 1% to 19.4% [43, 49, 50, 53]. Nutritionists emphasize the necessity of legume consumption due to their dietary fiber content, essential for the digestion process [54]. Scientific data indicate significant variation in legume fiber content, ranging from 11.7% to 22.8% [55]. The amino acid profile of legume proteins demonstrates the diversity of amino acids in their composition and the difference in endo- and exogenous amino acid content between species. Peas, chickpeas, and beans exhibit the highest amounts of essential amino acids [56, 57]. Antinutritional substances include compounds that significantly diminish the nutritional value of legumes, such as chymotrypsin and trypsin inhibitors. These negatively impact protein digestion processes by complexing with proteins and blocking proteolytic enzyme activity [58]. The highest content of these compounds is identified in peas, while lupins and beans exhibit the lowest values [59]. Tannins in legume seeds are classified as antinutritional compounds. Lupins show the highest tannin content, while other legume species analyzed present relatively low tannin levels [60]. Tannins, as polyphenolic compounds, can form stable complexes with proteins, minerals, and vitamins A and B_{12}, resulting in digestibility inhibition. This negative effect of tannins can be mitigated through heat treatment of legumes [61].

Legumes are increasingly recognized as a potential source of antioxidants, containing phenolic compounds with significant antioxidant activity. These compounds are primarily present in the legume seed coat, with colored flower varieties containing higher antioxidant amounts [62]. Chickpeas and beans exhibit the highest values for total phenols, while peas contain approximately 50% fewer phenolic compounds [63-65]. Lentils and lupins show the highest antioxidant activity, beans present average values, and chickpeas and peas have the lowest antioxidant activity [66, 67].

4. Chemical composition and properties of legume boiling water

Legumes are suitable for use in the food industry due to their high nutritional value, relatively low allergy risk, high yield, and low cost [68]. Thus, legumes are considered an adequate protein source as an alternative to animal proteins.

Legumes primarily consist of starch and water-soluble fibres and are rich in polyphenols, carbohydrates, and proteins, with nutritional and structural characteristics varying by variety, Table 2 [69].

Cooking water from legumes, known as aquafaba, has been widely used as a substitute for egg whites due to its excellent functional properties in forming stable foam and emulsion, similar to those of egg whites [74].

Table 2

Chemical composition of aquafaba

Aquafaba	Dry substance content, g/100g	Mineral content, g/100g	Protein content, g/100g	Carbohydrate content, g/100g	Saponine content, mg/g	Source
Chickpeas	5.1	0.6	1.0	3.6	4.5	[70]
Soybeans	5.5	0.8	0.7	4.1	6.4	[71]
Beans	3.3	0.8	0.7	1.8	5.9	[72]
Lentils	4.7	0.5	1.5	2.7	12.0	[72]
Peas	1.8	0.26	0.6	0.7	3.5	[73]

Therefore, it was explored the possibility of using legume boiling water in food manufacturing. It has been demonstrated that the carbohydrates and proteins transferred from legumes to the cooking water during boiling are responsible for the formation of stable foam and emulsion [69]. However, the quality of aquafaba varies depending on the legume variety and the production technology [74, 75]. To ensure the consistency of aquafaba and the quality of products obtained using this ingredient, standardizing aquafaba production is necessary.

This involves selecting specific legume varieties for aquafaba production and determining manufacturing conditions, including the water-to-bean ratio, the use of additives, temperature, pressure, and time [76, 77].

5. Possibilities of using aquafaba in the production of food products

Mayonnaise is a popular semisolid food product that enhances the texture and flavor of dishes such as salads and sandwiches. In recent years, due to health concerns, there has been a growing trend to replace eggs with plant-based ingredients in mayonnaise production [5]. One notable attempt involves creating plant-based mayonnaise using aquafaba from chickpeas [78]. Aquafaba was obtained from Kabuli chickpeas, known for its high emulsifying properties [75]. The process to obtain liquid aquafaba involved soaking chickpeas in water at 4 ºC for 16 hours, followed by boiling for 30 minutes. Ingredients like rapeseed oil, chicken eggs, table salt, vinegar, and crystalline sugar were used. In the production of vegan mayonnaise was utilized dried aquafaba. The most effective drying method for aquafaba, which preserved its high emulsification properties, was identified. Five different drying methods were employed: freeze-drying, spray drying, convection oven drying, rotary evaporator drying, and vacuum drying. The powdered form of aquafaba, dried in a convection oven at a temperature of 80 °C until a constant mass was achieved, was used in the preparation of vegan mayonnaise [79, 77].

The stability of the mayonnaise was assessed by studying the microstructure and particle size distribution. The vegan mayonnaise remained stable during storage, maintaining a consistency identical to mayonnaise prepared with eggs. The sample of vegan mayonnaise exhibited good stability upon heating, on the first day, the 14th day, the 21st day, with peak values on the 28th day compared to egg mayonnaise. It is noted that chickpea aquafaba obtained by boiling contains heat-stable proteins [76], which could contribute to the stability of vegan mayonnaise during heating. The sample of vegan mayonnaise had a less acidic environment (pH ranging from 3.74 to 4.66) compared to egg yolk mayonnaise (pH=3.24 to 3.96). Color parameter values showed a distinct difference between egg mayonnaise and vegan mayonnaise, with the latter having a darker appearance and lower color intensity [77]. Previous research has indicated that the emulsion color can shift from gray to increasingly bright white as droplet size decreases, likely due to increased light scattering [80, 81].

In the production of Macaron-type biscuits, chickpea aquafaba and flaxseed gel were used as plant-based foaming agents [82]. The quality of the formed foam and the volume of the dough obtained after beating were analyzed. The dough volume of aquafaba biscuits was similar to the control sample, while the dough obtained from flaxseed gel resulted in a 40% reduction in volume compared to the control sample. Additionally, the height of the semifinished product for aquafaba-based biscuits was similar to the control sample, while the flaxseed gel biscuits had significantly lower height. Texture parameters and organoleptic characteristics for aquafaba biscuits showed high values close to the control results [83].

In the category of products that include brioche, spray-dried legume aquafaba was used as a foaming agent [22, 84-87]. The specific volume of these brioche was lower compared to egg-based muffins [88], but the results regarding the mass loss during baking showed much lower values for aquafaba muffins compared to the control sample. The parameter, mass loss, is closely related to the hydration capacity of legume aquafaba powder [89]. Therefore, mass loss during baking is low when the hydration capacity is high.

For the color parameters CIELab (L*, a*, b*) of the muffins, the best results were obtained for those with eggs. Significant differences were observed between the values of the crumb and crust; the carbohydrate components present on the product's surface lightly brown through heating [90]. This could be attributed to the Maillard reaction that occurs during baking [91]. The textural characteristics of the muffin samples evaluated (hardness, adhesiveness, cohesiveness, elasticity, gumminess, and chewiness) did not show significantly different results. According to a previous study [88], firmness, gumminess, and chewiness depend on the hygroscopicity of the product, by affecting the state of starch when subjected to the processes of gelatinization or retrogradation [23, 92]. The results of the sensory analysis did not show significant differences between characteristics for all samples. It was found that the evaluated attributes were closely related to each other. Overall acceptability was primarily influenced by aroma, followed by appearance, uniformity, and the volume of the muffin [93].

6. The use of legumes as meat analogues

Approximately one third of all global greenhouse gas emissions (GHG) are attributed to the food production system. Cattle breeding generates 7% of it [94]. This goes against the objectives of a circular bioeconomy, especially since beef production can have GHG emissions of almost 20 times that of tofu, or more than 100 times that of nuts [95]. On the other hand, based on the data presented by FAO (2020) meat is a good source of proteins but frequently is considered not efficient regarding use of resources, mainly passing the harvest calories to meat calories, utilization of 80% of cultivable areas and 70% of fresh water [96].

Another aspect is the health problems associated with eating meat while meat substitutes are sources of plant-based protein that are cholesterol-free and low in saturated fat and contain essential amino acids beneficial for health [4].

Legumes can also be used in the formulation and production of meat analogues, which are rich in protein, starch, fiber and essential amino acids (arginine, lysine, glutamic acid, leucine and aspartic acid) [97, 98]. Legume proteins are recommended in the production of these products due to their foaming and gelling properties, but they have a lower digestibility compared to proteins of animal origin, Figure 1 [99].

Firstly, because of structural differences between these two kind of proteins. Plant based proteins are rich in β-sheet structures and poor in α-helixes in comparation with animal proteins. Such structure negatively influenced the digestion process. Secondly, legumes have a large amount of fibres, a feature that slows down the digestion process. The quantity of antinutritive factors of plant-based proteins, is an other element that affect the digestion [101].

However, there are methods for improving the properties that slow down the digestion of these proteins. On the one hand to adjust the protein digestibility-corrected amino acid score to the value of 1, thus approaching the value of this index for animal proteins. On the other hand, to balance the anabolic response of plant-based proteins as the consumed amount increases. An other way to increase the quality indices of plant based proteins is the fortification with amino acids. Good results were obtained after blending diferent sources of plant proteins, producing a complex source of amino acids [102] as in case of rise and pea that could increase the PDCASS indices to 1.00 [103].

Soy, for example, is a readily available and widely used ingredient in meat analogues around the world. Soy is famous for its excellent nutritional and functional attributes, it is rich in carbohydrates, fats, fibers, vitamins, micro and macronutrients [104]. Due to the proportional nutrient content of soy, it can be used to replace red meat [105]. Because its proteins have the ability to reduce blood cholesterol, they are indicated in the manufacture of meat analogs intended for consumers suffering from cardiovascular diseases [106]. In the development of this branch of the food industry, obtaining sausages and similar products, nuggets, soy proteins can be used in both untextured and textured form [107, 108].

Defatted soybean meal is used to obtain textured vegetable proteins by stripping soluble carbohydrates and texturing the filtrate by spinning or extrusion [109].

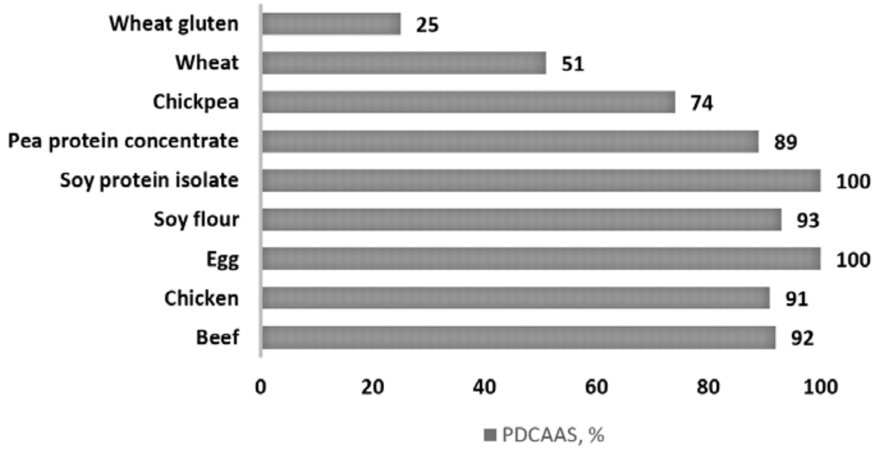

Figure 1. Protein digestibility-corrected amino acid score (PDCAAS), adopted from [100].

Textured soy protein concentrates mimic meat muscle fibers (turkey or chicken breast meat fibers), provide meat analog fibrous characteristics, such as chewiness, firmness [110]. Peas protein meat analogues have a fibrous texture similar to that of fish and chicken meat, which contained 90% protein from pea protein isolates, gluten and high moisture starch to improve the product's moisturizing properties [111].

7. Conclusion

Currently there is a great preoccupation regarding the substitution of proteins of animal origin with vegetable protein sources. The fruits and seeds of legumes contain important amounts of proteins and biologically active substances, as well as other compounds. Plant-based proteins have a favorable impact on the health of consumers. The animal protein deficit can only be alleviated by increasing the production of legumes and their use in food production. In the Republic of Moldova, the most widespread crops are beans, followed by peas, chickpeas, and lentils. Legumes are suitable for use in the food industry due to their high nutritional value, relatively low allergy risk, high yield, and low cost.

Aquafaba is a by-product of the preservation or boiling processes of legumes. It can be widely used as a substitute for egg white due to its excellent functional properties of forming foams and stable emulsions, similar to those of egg whites. Possibilities of using aquafaba in the manufacture of food (mayonnaise, macaron biscuits, etc.) were analysed.

It has been shown that legumes can be used in the formulation and production of meat analogues. Proteins from legumes are recommended in the production of these products due to their foaming and gelling properties, but they have a lower digestibility compared to proteins of animal origin. Thus, to solve the problems related to the environment, social problems and to diversify the assortment of food products in order to satisfy the requirements of different groups of consumers, proteins of plant origin can be used to replace those of animal origin.

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