POTENTIAL HEALTH BENEFITS OF BIOLOGICALLY ACTIVE PEPTIDES DERIVED FROM UNDERUTILIZED GRAINS:

RECENT ADVANCES IN THEIR ISOLATION, IDENTIFICATION, BIOACTIVITY AND MOLECULAR ANALYSIS

Editors:
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(Volume 4)

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CONTENTS

FOREWORD

In the last decade, bioactive compounds, particularly peptides from underutilized grains, have been the subject of extensive scientific and technological research worldwide. The results achieved try to respond to the interest of human beings in consuming nutritious and healthy foods that contribute to their well-being, and to biodiversity, and community development.

The advancement of knowledge in this field has given rise to the publication of numerous articles, reviews, chapters, and books, mainly in recent years. However, challenges and questions constantly arise new and sophisticated methodologies, and innovative visions based on interdisciplinary collaboration that stimulate the writing of new books.

One such example is "Potential Health Benefits of Biologically Active Peptides Derived from Underutilized Grains: Recent Advances in their Isolation, Identification, Bioactivity and Molecular Analysis."

Its contents are divided into three main axes:

- Progress made in aspects related to the identification, production, bioactivity and molecular characterization of bioactive peptides.
- Identification and description of the main biological activities exhibited by peptides encrypted in the protein sequences of the main grains, whose use is scarce in human nutrition, despite the fact that in several cases the nutritional characteristics of their proteins are better than those of cereals or other grains of massive consumption.
- The possibilities that these peptides have of being applied in different fields such as food, cosmetics, pharmaceuticals and medicine, whether in the design and formulation of functional foods, nutraceuticals, cosmeceuticals, antimicrobials and antivirals.

Mexico in particular, as well as other countries in the American continent, have strong historical ties with most of the underutilized crops included in this publication, which have a high nutritional value and great potential in the world of functional foods, a fact that is reflected in the text.

This book has been rigorously written and combines the knowledge and insights developed to date by researchers around the world. Reading it will provide up-to-date knowledge and will surely raise questions and pose new challenges at a scientific level, as well as stimulate the development of novel products that have an impact on the benefit of society.

The authors of the different chapters have solid knowledge about the different topics addressed, which is evident in the clarity and depth of the treatment of the topics.

It is a compliment that they have chosen me to write this prologue.

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PREFACE

"Potential Health Benefits of Biologically Active Peptides Derived from Underutilized Grains" is a complete review based on the cutting-edge scientific pieces of evidence about the isolation, identification, bioactivity and molecular analysis of the biologically active peptides (BAPs), specifically obtained from underutilized grains. The aspects related to the bioprospecting of BAPs in grains of several varieties of amaranth, quinoa, millet, buckwheat, sorghum, lupin, mung bean, chickpea, broad bean, cocoa bean and chia, are extensively described along the different chapters. Additionally, the chapters provide information about the characteristics of the crop, its main varieties, traditional uses, economic importance, structure and chemical composition of the grains, as well as the classification and distribution of the grain protein fractions. Moreover, the advances in the analytical techniques used for the concentration, purification and molecular characterization of BAPs are described. Furthermore, the impact of BAPs in the promotion of health is highlighted, as well as their potential incorporation as promising ingredients in the development of functional foods, nutraceuticals and cosmeceuticals. Finally, the main findings related to the potential antiviral and anti-COVID-19 activities of BAPs derived from underutilized grains are discussed. The present book will be of great interest to academics, professionals and researchers focused on food science, biotechnology, pharmacology and agriculture, and to professionals involved in the research and development of natural products, pharmaceuticals and cosmetics.

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CHAPTER 1

Biologically Active Peptides: Identification, Production and Biofunctionality

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Abstract: According to reports from the World Health Organization (WHO), non-transmissible chronic diseases, like diabetes, cardiovascular disorders, hypertension, and cancer, among others, are the main causes of death worldwide, comprising 70% of the total deaths. Therefore, there is a great interest in the search for alternative biofunctional agents that can contribute to the prevention and treatment of these types of diseases. Particularly, biologically active peptides (BAPs) represent an attractive and promising alternative due to their therapeutic potential, since they can act in similar ways to synthetic drugs. In this respect, BAPs extracted from food proteins of vegetable origin have shown antioxidant, antihypertensive, antidiabetic, anticancer, antithrombotic, anticholesterolemic, immunomodulatory, antiobesity, antiaging, and antimicrobial properties, thus showing great potential as bioactive ingredients in functional foods and pharmaceutical formulas. This chapter describes the main procedures performed for the identification and production of BAPs, as well as the health benefits of their biofunctionalities found in bioassays *in vitro* and *in vivo*, the elucidation of their mechanisms of action and the therapeutic applications of BAPs originated from underutilized vegetable sources.

Keywords: Bioassays, Bio-functionality, Bioinformatics, Biotechnological processes, Endopeptidases, Enzymatic activity, Enzymes, Exopeptidases, Fermentation, Health benefits, Hydrophobicity, IC_{50}, Multifunctionality, Peptides, Protein hydrolysate, Proteolytic enzymes, Therapeutic potential, Vegetal sources.

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INTRODUCTION

Food ingredients from vegetable sources have an important role in the diet of the less favored people, in economic terms, and of those who for several reasons (philosophy, religion and health) have chosen animal-free nutritional regimens [1]. Additionally, from an ecological and agricultural approach, a great advantage of ingredients from vegetable sources is their higher sustainability [2]. Despite great diversity in available vegetable sources, only a few varieties are widely exploited. Therefore, there is a great interest in increasing the added value of underutilized vegetable sources through the obtention of compounds with techno- and bio-functionalities, so they can be considered by the food and pharmaceutical industries [2, 3].

Proteins are essential macronutrients for human nutrition since they give energy, nitrogen and essential amino acids [3]. Moreover, proteins are one of the main components in several food products due to their techno-functional attributes (production and stability of emulsions, foams and gels; capacity to form films; and viscosity promoters) [2, 4]. In this sense, vegetable proteins are mainly obtained from grains of cereals and pseudocereals, legumes, oleaginous seeds (Table. **1**), and in less extent, from green leaves [1]; this group of proteins has been classified (based on their solubility) according to the Osborne fractionation technique [5] in: water-soluble albumins, globulins soluble in aqueous saline solutions, glutelins soluble in acid and alkaline diluted solutions, and in prolamins soluble in aqueous alcohol solutions. On the other hand, vegetable proteins can be classified in different groups, according to their biological functions: structural or metabolic, defense, stress resistance and storage [1].

The potential uses of vegetable proteins depend on their resistance to processing and bioavailability, digestibility, amino acid profile and antinutritional compounds present in the vegetable source of interest [1 - 3]. Modifications of vegetable proteins by altering their physicochemical/structural characteristics (molecular weight, charge, hydrophobicity and hydrophilicity, among others) have opened the possibility to diversify and improve their nutritional properties (digestibility and allergenicity), as well as their technofunctional attributes and biological functionalities (biofunctionality) [2, 6]. In this respect, one of the most outstanding and promising research lines is the extraction or production of specific fragments of proteins with amino acid sequences with biofunctional effects in humans; they have been named biologically active biopeptides (BAPs). The presence of these peptides with diverse biological functions (antioxidant, anticancer, antihypertensive, hypoglycemic, hypocholesterolemic, and immunomodulatory, among others) has been described in vegetable proteins [6]. Likewise, over the last few years, there has been an increasing interest in bioprospecting and production of new BAPs from less explored sources, with potential therapeutic applications beneficial to health [4, 6]. Hence, this chapter aims to describe the state-of-the-art identification and production of BAPs; besides describing the potential biofunctionalities (beneficial to health), mechanisms of action and therapeutic applications of BAPs. Moreover, this chapter exclusively focuses on BAPs extracted from vegetable sources (underutilized grain proteins); the information regarding the BAPs of each vegetable source will be discussed in the following chapters.

Table 1. Protein content of grain crops [1].

Crop	Total Protein (%)
Cereals	
Maize	7.2 – 9.4
Barley	8.2 – 11.6
Oat	12.1 – 14.2
Rice	7.5 – 9.0
Wheat	11.0 – 14.0
Sorghum	6.0 – 11.3
Pseudocereals	
Amaranth	13.2 – 18.2
Quinoa	12.0 – 20.0
Buckwheat	11.0 – 16.0
Legumes	
Soy bean	32.0 – 42.0
Chickpea	20.0 – 28.0
Broad bean	20.0 – 30.0
Lentil	23.0 – 29.0
Bean	19.0 – 21.0
Pea	21.0 – 28.0
Peanut	25.0 – 28.0
Oleaginous seeds	
Sesame seed	17.7 – 25.0
Sunflower	20.7 – 27.0
Rapeseed	20.0 – 25.0

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CHAPTER 2

Molecular Characterization of Biologically Active Peptides

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Abstract: Biologically active peptides (BAPs) have gained significant research attention in the last few years due to their potential human health benefits and diverse applications. Moreover, the use of food by-products or food wastes as a protein source is highlighted as a viable technological alternative to produce BAPs, reducing food losses. However, the functionality of BAPs depends on the protein source, synthesis method, degree of hydrolysis, molecular weight, purity, and amino acid sequence and composition. Therefore, multiple efforts in the purification and molecular characterization of these compounds have been reported in recent years to know their molecular structure complexity and related bioactivity. BAPs can be obtained from different protein sources and synthesized by chemical, biological, and physical routes, alone or combined, which significantly influence their degree of hydrolysis and molecular weights. Additionally, advanced analytical techniques have been used to separate and identify BAPs. In this context, ultrafiltration membrane systems and electrodialysis systems are the most common methods used to separate BAPs with desirable molecular weights.

Likewise, multiple chromatographic techniques (HPLC, HPLC-MS/MS, UPLC/MS, RT-HPLC, and ion exchange and exclusion) have been widely used for quantitative, qualitative, and sequencing analysis of BAPs. Furthermore, emerging computational and statistical tools (*in silico* analysis) have been used to predict, sequence, and characterize BAPs using chemometric and chemoinformatic data. These tools facilitated the discovery and analysis of new peptides with desirable applications and functionality. Therefore, this chapter aims to discuss the current information about the molecular characterization of BAPs.

Keywords: Amino acid composition, Bioinformatics, Chromatographic separation, Electrodialysis, Electrophoresis, Hydrolysates, Hydrolysis degree, Mass spectrometry, Molecular docking, Peptide characterization, Peptide purification, Peptide sequence, Peptidomics, Proteomics, Ultrafiltration.

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INTRODUCTION

There is currently a particular interest in obtaining bioactive peptides (BAPs), mainly by their human health benefits beyond nutritional capability. Since 2017, more than 3100 publications have been reported in the SCOPUS database, focusing on the obtention, purification, identification, and evaluation of their potential applications (www.scopus.com). BAPs are short protein fractions obtained after protein hydrolysis, containing a sequence of 2 to 20 amino acids per molecule with a molecular weight < 10 kDa [1]. They can be produced from different animal and plant protein sources such as bovine blood plasma protein, gelatin, meat, egg, fish, corn, rice, soybean, pumpkin, sorghum, amaranth, and fungi and bacteria [2]. The hydrolysis can be chemical (acid or alkaline hydrolysis), biological (microbial fermentation, gastrointestinal digestion, or enzymatic hydrolysis), and physical (ultrasonic-assisted extraction and high hydrostatic pressure) routes (alone or combined) [2 - 4]. The reported biological properties of BAPs include antimicrobial, antioxidant, antihypertensive, antidiabetic, and immunomodulatory properties, among others [5, 6], in the concentration- and structure-dependent response [1]. In this context, BAPs have been recognized as functional compounds with potential food and pharmaceutical applications, developing novel functional foods and healthcare products [7].

The obtention of purified BAPs with desired biological activities comprises a series of steps (Fig. **1**). It could be performed by conventional and *in silico* approaches [8]. The traditional route for the isolation of BAPs starts with protein hydrolysis [9]. Then, hydrolysates are separated, fractionated, or purified (*i.e.*, ultrafiltration, electrodialysis, and chromatographic techniques) according to their molecular weights, charges, or sizes; later, the identification of the amino acid composition and sequencing by mass spectrometry and the evaluation of the potential *in vitro*and *in vivo* biological properties are performed [7, 10]. However, due to the diversity of BAPs associated with the protein source, the synthesis method, and the experimental conditions, the purification of BAPs may be a complex process [11].

On the other hand, computational or *in silico* approaches are an active research area used to produce specific peptide sequences using chemometric information of proteins from databases [12]. These tools permit the prediction of the structure-function of lab-synthetic peptides and their potential biological properties [13]. Thus, the combined *in vitro*and *in silico* evaluation of the molecular characteristics of BAPs (hydrolysis degree, molecular weight, and amino acid composition and sequence) is necessary for the acquisition of peptides with desired functionality in less chemical-reagent use and time, resulting in cost-effectiveness for industrial-scale applications [7, 14]. Therefore, this chapter aims to discuss the current information about the molecular characterization of BAPs.

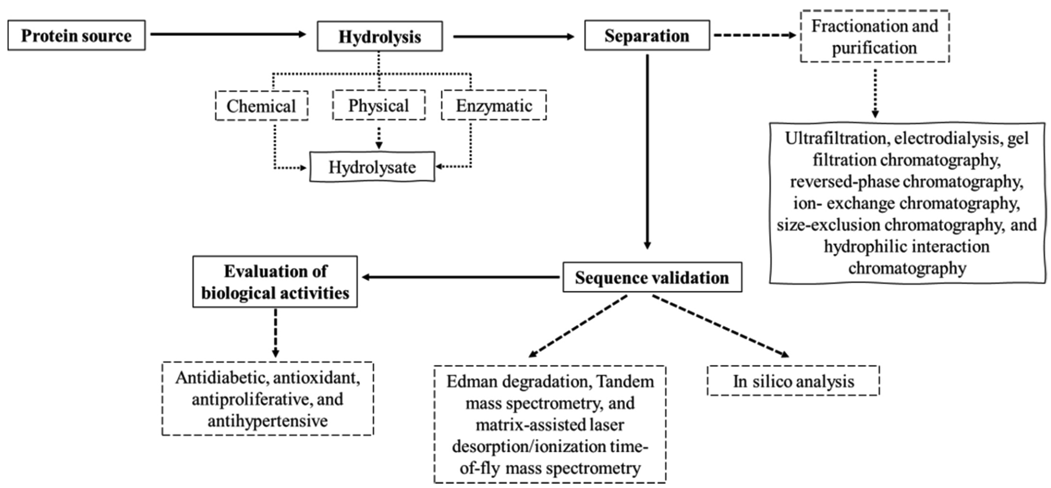

Fig. (1). Flow chart for the protein hydrolysis and separation, purification, and identification of the amino acid sequence of biologically active peptides.

PROTEIN HYDROLYSIS: PROCESS TO OBTAIN BAPS

BAPs are peptides encrypted within the primary protein source. They exert their beneficial properties once released after proteolysis (independently of the hydrolysis route) in a structural-dependent manner [15]. In this context, after the protein hydrolysis, it is vital to evaluate the degree of hydrolysis (DH) and molecular weights of the resulting peptide molecules; these analytical methods provide essential information about the physicochemical properties of BAPs [16].

In general, DH describes the efficiency of the protein hydrolysis process, which is dependent on the protein source, synthesis method, and experimental conditions such as temperature, hydrolysis time, substrate concentration, and pH, among others [16]. According to the literature, enzymatic hydrolysis (mainly alcalase or fermentation process) is one of the most common methods to produce hydrolysates with high DH [11]. It is an analytical method based on quantifying peptide bonds after proteolysis regarding the total number of peptide bonds of the whole protein (expressed as a percentage) [17]. DH plays a crucial role in

CHAPTER 3

Biologically Active Peptides from Amaranth (*Amaranthus* spp.) Grain

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Abstract: Amaranth (*Amaranthus* spp.) is one of the few plant species where both leaves and grains can be consumed. Among the main species used as producers of edible grains are *Amaranthus hypochondriacus*, *A. cruentus*, *A. caudatus*, and *A. mantegazzianus*. The grains of these species are characterized by their protein content (13-18%), which has high nutritional value. Additionally, the different protein fractions of the amaranth grain have been shown to contain sequences of biologically active peptides (BAPs) with multiple beneficial bio-functionalities for health. Among these, we can highlight antihypertensive, antioxidant, antitumor, antidiabetic, antithrombotic, anticholesterolemic and immunomodulatory activities. The aim of this chapter is to describe the different biological functionalities of BAPs obtained from the proteins of the amaranth grain, which can be considered a promising natural source of therapeutic agents with potential use in the prevention and treatment of various chronic diseases.

Keywords: *Amaranthus*, Anticholesterolemic antihypertensive, Antioxidant, Antithrombotic, Antitumor, Bioactive compounds, Biomolecules, Biotechnological processes, Pseudocereal, Functional food, Grain, Health benefits, Hypoglycemic, Immunomodulatory, Nutrition, Peptides, Proteins.

INTRODUCTION

Amaranth has been classified as a pseudocereal since it produces grain-type cereal [1]. Currently, amaranth is cultivated mainly in North America (Canada and Mexico), Central and South America, and in the areas of Asia, Africa, and Australia. China is the main producer worldwide [2].

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Amaranth is a fast-growing dicotyledonous plant because it uses the C4 pathway for carbon fixation (which allows for more efficient use of carbon and water consumption) [3]. That is why it can be grown under diverse and adverse agroclimatic conditions (tropical, subtropical, and temperate climates) and different types of soil (including poor soils), in addition to being resistant to different pests [3, 4].

Amaranth belongs to the Amaranthaceae family, which includes more than 60 genera, including the *Amaranthus* genus [3]. About 70 species of *Amaranthus* have been identified, of which only some (approximately 20 species) have been domesticated for integral use (root, stem, leaves and grain). The most important species are *Amaranthus cruentus* (native to Mexico and Guatemala), *A. caudatus* (native to Peru), *A. hypochondriacus* (native to Mexico), and *A. mantegazzianus* (native to Argentina and Bolivia) [4]. It should be mentioned that these species are characterized by having large inflorescences full of edible grains [3, 4].

The potential of the amaranth grain as food was recognized by ancestral cultures in America, as it formed a basic part of their diet until was replaced by other grains, such as corn and beans. However, in the last two decades, interest in this pseudocereal has been revived [2]. In this sense, several studies have been carried out regarding the nutritional and functional value of the different components of the amaranth grain [5]. Among the most outstanding property is the high protein content (17-19% of the dry weight of the grain) compared to conventional cereal grains, which have an average of 10% protein. Likewise, the protein of the amaranth grain is rich in essential amino acids such as lysine, tryptophan, cysteine, and methionine [6, 7].

That is why amaranth is consumed mainly as a supplement (either as popped grain or as flour) in breakfast cereals, breads, crackers whole grains, energy bars, pasta, snacks, and granola, among others [6]. Furthermore, because the amaranth grain does not contain gluten, it can be used as an ingredient in bakery products (partially replacing wheat) [2, 6].

Additionally, there is an application that due to its interest, novelty and potential requires a special mention. This constitutes the extraction or production of biologically active peptides (BAPs) from hydrolyzed proteins. The presence of these types of compounds with biological functionalities has been described (antihypertensive, anticancer, antidiabetic, antithrombotic, among others) in the proteins of the amaranth grain. Thus, the published scientific evidence so far, allows the amaranth grain to be considered a promising source of BAPs [4].

This chapter aims to describe the current knowledge regarding the biological functions of peptides derived from protein fractions of the amaranth grain, which can potentially be used as bio-functional ingredients or therapeutic agents in the food and/or pharmaceutical industry.

PROXIMAL COMPOSITION OF AMARANTH GRAIN

The amaranth grain is obtained from a mature plant that reaches more than two meters tall. This grain is smooth, bright, and light-colored (between yellow and gold), it has a lenticular shape with a diameter of 1-1.5 mm and a weight of 0.6-1.2 mg. The main parts of the amaranth grain are the seed coat (episperm), the endosperm, the embryo that is made up of two cotyledons, and the perisperm (Fig. **1**) [4].

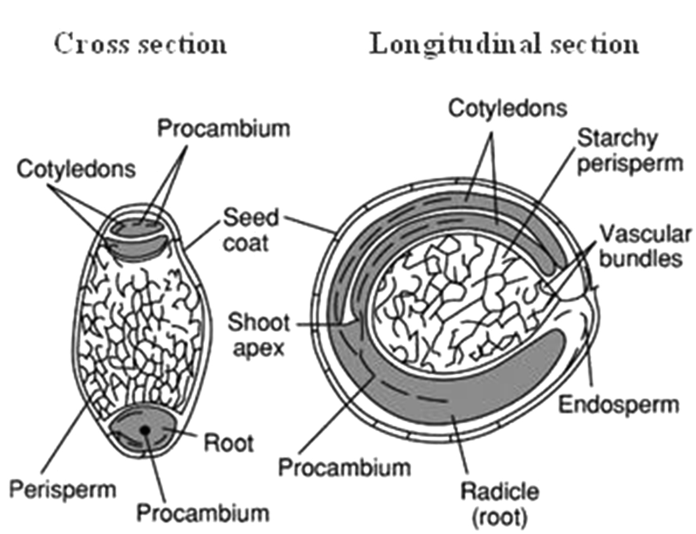

Fig. (1). Scheme of cross and longitudinal sections of amaranth grain. Taken from Montoya-Rodríguez *et al.* [8].

As mentioned above, there are mainly four species of amaranth (*A. cruentus. A. caudatus, A. hypochondriacus and A. mantegazzianus*) that produce grains with a high nutritional value. However, the chemical composition and proximal (average) grain size are inconsistent between species (Table **1**).

Table 1. Average proximal composition of the principal grain producing amaranth species. (Taken and modified from Tovar-Pérez *et al.* [4]).

Component	*A. hypochondriacus*	*A. cruentus*	*A. caudatus*	*A. mantegazzianus*
	(g/100 g dry basis)			
Crude protein	15.6	14.1	13.0	17.5
Fat	6.1	6.0	6.0	7.8

CHAPTER 4

Biologically Active Peptides from Quinoa (*Chenopodium quinoa* Willd) Grain

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Abstract: Dietary proteins and peptides can exert a wide variety of well-studied bioactivities, some of which are related to human well-being, health maintenance and disease prevention. These peptides can be generated by enzymatic hydrolysis, gastrointestinal simulation, or by fermentation with microorganisms. Quinoa is a pseudo cereal consumed by ancient populations for hundreds of years. It does not contain gluten, but it does contain proteins with encrypted sequences that can be released by enzymatic hydrolysis. These sequences contained in quinoa hydrolysates and peptides can exert beneficial effects on health, as they present antidiabetic, antihypertensive, antioxidant, anticancer and anti-inflammatory activities, among others. In addition, quinoa has other nutritional and bioactive compounds such as flavonoids, phenolic acids, fatty acids, vitamins and minerals. There are many studies that demonstrate the activities mentioned above, however, the exploration of *in vivo* models explaining the associated mechanisms are still needed. This chapter aims to understand quinoa from a functional point of view, along with presenting the biotechnological potential of grain proteins, which is currently very poorly exploited. We aim to promote quinoa cultivation, since its beneficial properties are adequate for its use in the prevention of chronic-degenerative diseases.

Keywords: Albumins, Antidiabetic, Antihypertensive, Antiinflammatory, Antioxidant, Bioactive peptides, Enzymatic hydrolysates, Globulins, Glutelins, Hypolipidemic, Prolamins, Pseudocereals, Quinoa, Seed storage proteins, Solubility.

INTRODUCTION

Plant-based food compounds have been increasingly applied in different research areas, such as food packaging, encapsulation of nutraceuticals, functional foods or food fortification. Plant-based materials contain a wide range of different compo-

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

unds, including fibers, proteins, carbohydrates, lipids, polyphenols and many bioactive compounds [1 - 3].

We define "underutilized foods" as those foods that were part of the diets of different ancestral populations for centuries and which were then replaced at the beginning of the 20th century by other foods that currently prevail in the diet of the world population. Pseudocereals could be considered as "underutilized foods". They are a food group including non-herbaceous plant species that do not belong to the cereal family, but that have similar properties and uses. According to their botanical characteristics, pseudocereals are dicotyledons and, therefore, differ from cereals (monocotyledons) [4, 5]. Despite this difference, pseudocereals have a similar composition of starch content; likewise, their texture, palatability and cooking methods are very similar to those of cereals. The most cultivated and studied pseudocereals are quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus* spp.), chia (*Salvia hispanica*) and buckwheat (*Fagopyrum* spp.).

Quinoa (*Chenopodium quinoa* Willd) is a flowering plant from the *Amaranthaceae* family. It was first cultivated 7,000 years ago by the Incas around Lake Titicaca and later it spread to Ecuador and Chile. Today it is mainly cultivated in Bolivia, Chile, Peru, Ecuador and Colombia, as well as in some other countries such as England, Sweden, Denmark, the Netherlands, Italy, Kenya and France [6 - 8]. Bolivia and Peru are the two largest producers of quinoa with a total yield of 67,135 and 89,775 tons, respectively, in 2019 [9].

Quinoa is a dicotyledonous, gynomonoecious annual plant, with an erect stem, bearing alternating leaves of various colors that range from white, yellow, or light brown to red, due to the presence of β-cyanins. Quinoa varieties mainly differ in morphology and phenology, but also present differences in the chemical composition of their tissues [10]. Quinoa, depending on planting density, can grow from 1m up to 3m in height. Their roots can reach a depth of up to 1.5 m below the surface, which protects the plant against drought. The stem is cylindrical, averaging 3.5 cm in diameter, and it can be straight or branched. Leaves are polymorphic, with the upper ones lanceolate, while the lower ones are rhomboid [11]. Flowers are incomplete and do not have petals. As a gynomonoecious plant, quinoa has both hermaphroditic flowers, which are located at the distal end of a group, and female flowers, which are located at the proximal end [12, 13]. The arrangement of flowers in a cluster is considered the panicle; panicle length varies between 15 and 70 cm. Specifically, the quinoa inflorescence is a panicle with a main axis, from which secondary and tertiary axes depart [14]. Two types of inflorescences have been described for quinoa: amarantiform and glomerulate. In the amarantiform type, the glomeruli (short branches bearing a cluster of flowers or grains) are inserted directly into second-order axes, whereas in the glomerulate type, the glomeruli are inserted into third-order axes. The quinoa fruit is an achene. Quinoa produces small, flattened, circular seeds that can measure from 1.5 mm up to 4 mm in diameter (about 350 seeds weigh 1g) [15]. The color of the seeds varies from white to gray or black, and can also include shades of yellow, pink, red, purple and violet, often with very colorful mixtures within the same panicle. Black is dominant over red and yellow, which in turn are dominant over white. This crop has great tolerance to extreme climate and soil conditions, such as frost, drought and high salinity; and it can also be grown in a wide variety of environments in latitudes ranging from 1°39′N in Colombia to 42°S in Chile (from sea level to the plateau). These characteristics make it a promising crop when facing the challenges of global climate change [16, 17].

Quinoa can be eaten as a hot breakfast cereal, as a substitute for rice, or it can be boiled in water to make baby food. Quinoa seeds can even be popped, like popcorn, ground and used as flour, or sprouted. Quinoa flour can be mixed with corn or wheat flour to produce bread, noodles and pasta, and sweet cookies. In addition, quinoa flour can also be drum dried and extruded, yielding products with favorable sensory and nutritional qualities. Similarly, solid state fermentation of quinoa with *Rhizopus oligosporus* Saito provides good quality tempeh [13].

From an economic and sustainability perspective, sweet quinoa varieties are the most promising since they provide more high-quality protein than bitter crops. The whole grain can be used for fermenting, thus for making beer, bread, soups, cookies and beverages. Furthermore, the whole plant is often used as green fodder for cattle, pigs and poultry [10, 18 - 21]. The aim of this chapter is to present the structural characteristics, chemical composition, nutritional content and main protein fractions of quinoa, as well as to point out functional properties of its protein and peptide hydrolysates, along with their various activities and effects on human health.

PROXIMAL COMPOSITION OF QUINOA GRAIN

Quinoa seeds contain several layers: pericarp, seed coat and perisperm, from outside to inside [14]. They can be conical, cylindrical or ellipsoidal and contain saponins concentrated in the pericarp. In the mature seed, endosperm is present only in the micropylar region of the seed and consists of one or two tissues with cell layers surrounding the hypocotyl-radicle axis of the embryo (Fig. **1**).

CHAPTER 5

Biologically Active Peptides from Pearl Millet [*Pennisetum glaucum* (L.) R.Br.], Foxtail Millet [*Setaria italica* (L.) P.Beauv.] and Finger Millet [*Eleusine coracana* (L.) Gaertn] Grains

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Abstract: Millet is a small grain. Africa and Asia are the largest producers and consumers. Millet is hardier than large grain cereals and could be added to multi-season, multi-crop agriculture. It has nutritional properties such as proteins and carbohydrates, high content of fiber and unsaturated fats, essential amino acids, minerals, and vitamins, and it is considered a highly energetic cereal. The health benefits of whole millet grain consumption are risk reduction of various chronic diseases. In addition, it is characterized by having short growing seasons; it grows in poor soils and with efficient use of water. The consumption of this cereal is indicated for high-performance athletes, pregnant women, people suffering from stress or weakness, vegetarians and those seeking nutritional balance. Its largest protein fractions are albumin, prolamins and glutelins. Cereal peptides from their protein fractions have been found to possess biological activities of high interest to maintain and improve human health. Peptides from millet grains have been shown to have antioxidant, anti-inflammatory and hypoglycemic activities.

Keywords: Agronomic practices, Albumins, Amino acids, Bioactive peptides, Distribution, Environmental agents, Globulins, Glutelins, IC_{50}, Millet grain, Minerals, Niacin, Prolamins, Protein, Protein fractions, Riboflavin, Thiamin, Varieties, Vitamins, Yield.

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INTRODUCTION

Millets include proso millet (*Panicum miliaceum* L.), broom millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), little millet (*P. sumatrense*), finger millet or ragi (*Eleusine coracana* L.), fonio (*Digitaria* spp.), Teff (*Eragrostis tef*), free-range or Japanese millet (*Echinochloa crusgalli*), Guinea millet (*Bracharia deflexa*), jungle rice millet (*E. colonum*), Kodo millet (*Paspalum scrobiculatum*) and Job's tears (*Coix lachryma-jobi*), among others [1]. Moreover, it is also known by several different common names including bajra, cumbu and cattail millet [2]. The most commercial varieties are pearl millet, heart, prose and foxtail [3]. Millet is smaller than rice, corn and wheat. Millets arrived in Europe from Asia at the same time that wheat and barley were introduced to Asia in the opposite way. Their cultivation allowed the development of cities and civilizations. Even though indigenous African millets are also being used as a grain food and forage, primarily [4]. The world population has been confronting the challenge to feed a forecasted 9,000 million people. Cereals like rice, wheat, and maize constitute an extensive portion of the total population's intake. An annual global production of 34 million tons has been calculated, of which 90% is assigned to developing countries, with 75% of this being used for human consumption, while the rest is diversified between forage for small livestock and for the elaboration of beverages (The Quality Aspect and Safety of Some Traditional Fermented Product from Sorghum and millet). Millet has a nutty sweet taste and is gluten-free [5].

Millet grain is a food source providing high levels of energy. It fights tiredness, fatigue, asthenia, stress, muscle cramps, and depression and it helps to improve memory. It also has antioxidant effects; it benefits the immune system and tones the nervous system. Additionally, it has a high content of purines, which prevents the formation of atheroma plaques. Furthermore, it increases digestibility once fermented, strengthens bones, reduces blood glucose levels and weight, and protects gut health [6].

Agronomically, millet is a crop of direct sowing; it requires full sun and intermediate irrigation, labor and fertilization. It grows in a temperature range of 15-40 °C, in low fertility soils, and it can be developed in high salinity soils or in high pH values 5.5 - 7.5; it has high photosynthetic efficiency; it grows at shallow depth, and it withstand adverse climatic conditions such as floods or droughts; kernels can be harvested only 40 days after flowering when kernels emerge from the head. In addition, due to the fact that it is a C4 plant, it has dry matter production ability, fewer disease and pest issues and a high tolerance to abiotic stresses like salinity and drought [7, 8]. Its main use is for human and animal consumption [5]. Grain cereals are very dry, hard and small [9] and they are conserved dry, making them durable for long-term storage. Moreover, they are the major international commodity market [9, 10]. The health benefits of whole millet grain consumption are a risk reduction of various chronic diseases, and the production of satiety, with less calories than rice and wheat grains because it is rich in fiber; moreover, it reduces constipation [11], heart and coronary heart disease risk and it can be helpful on weight management [10, 12].

Three to eight ounces of millet grains are recommended per day, depending on the required calories [8]. Additionally, this grain helps to maintain optimal health as a result of the phytochemicals it contains [13], and it provides a good proportion of the daily energy and nutrient requirements [14]. Consuming millet grain products fortified with folates before and during pregnancy also helps to prevent neural tube defects during fetal development [10]. Furthermore, Millet grains are essential sources of B vitamins (riboflavin, niacin, folate and thiamine) [15] and minerals (selenium, iron and magnesium) [16, 17].

Pearl Millet [*Pennisetum glaucum* (L.) R.Br.]

Pearl millet [*Pennisetum glaucum* (L.)] is the principal cereal grown in the semi-arid tropic in the Sahara [18]. The highest annual production is of 9.7 million tons in India [14]. As shown in Fig. (**1A**), the grain structure of the pearl millet (Fig. **1B**) variety is schematic. This variety has a texture similar to brown rice [19].

Pearl millet flour is used to make various baking products such as cakes, ciabattas, cookies, muffins and flatbreads [1]. This variety contains high levels of protein, fiber, minerals and phytochemicals [24]. Besides, it is used as forage in summer seasons [25]. Pearl millet is resistant to salinity (2.0 dS/m), drought stress (350 mm of water per cycle) and elevated temperatures; while for other millet varieties, the yield would be compromised [26]. High salt concentrations in soil provoked a chain of changes in properties, water, and nutrients' availability, number of leaves reduced, accumulation of ions causing leaf senescence and cellular plasmolysis.

Nitrogen doses such as 60 and 90 kg with bio-fertilizer increased fresh and dry matter, yield and plant height [27]. In the grain, high contents of Fe, Zn and protein (100-120 mg/kg, 47-87 mg/kg and 6-18%, respectively) can be found in 281 advanced lines bred of millet grains [19]. The usefulness of this high availability of Zn and Fe in lines of pearl millet grains is the reduction of anemia problems through micronutrients. Furthermore, in the SOSAT-C88 × ICTP 8203 cross of millet grain, significant amounts of Fe (50.55 ppm) and Zn (39.81 ppm) were found [18]. Meanwhile, the ICMV 167006 × ICMV 221 cross grains have higher concentrations of Fe (55.67 ppm) and Zn (38.59 ppm) [19].

CHAPTER 6

Biologically Active Peptides from Buckwheat (*Fagopyrum esculentum* Moench) Grain

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Abstract: Buckwheat (*Fagopyrum esculentum* Moench) is a pseudocereal with a high content of amino acids, such as lysine; its lack of gluten makes it an attractive product with high nutritional potential that could be included in the human diet. It contains several compounds such as phenolics, carotenoids, organic acids, vitamin E and B-complex vitamins, and macro and microelements. Its high protein quality and peptide content are relevant in *F. esculentum*. Additionally, biologically active peptides (BAPs) have been associated with the prevention of some chronic and neurodegenerative diseases. Consequently, the agro-industrial, cosmetic, food and pharmaceutical sectors have shown a strong interest in this pseudocereal due to its wide range of compounds. This chapter is a compilation of scientific contributions related to buckwheat properties, including generalities, nutritional value, bioactive compounds, BAPs, and finally, perspectives and conclusions.

Keywords: Agroindustry, Amino acids, Bioactive properties, Biopeptides, Buckwheat, Chronic diseases, *Fagopyrum esculentum*, Macroelements, Microelements, Neurodegenerative diseases, Nutraceutical, Phenolics, Proteins, Pseudocereal, Organic acids, Secondary metabolites, Vitamins.

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

INTRODUCTION

Common buckwheat is a pseudocereal belonging to the Polygonaceae family, *Fagopyrum* genus and *Fagopyrum esculentum* Moench species. This plant is native to the Asian continent and was domesticated for cultivation since 6000 BC, spreading rapidly throughout the European continent. It is currently grown on several continents, with China as the main producer with 55% of the total world production, followed by Russia with 20%, Ukraine with 15%, and Poland with 3%. *F. esculentum* and *F. tataricum* of the Fagopyrumen genus are the only two cultivable species for human consumption with 90% of the world's production [1, 2].

Buckwheat food products are flour, sprouts and seeds. The latter have no gluten content and a higher amount of high-quality protein than other cereals such as wheat, corn and rice, which determine their potential use in gluten-free diets, a market with a tremendous growth in recent years. Moreover, benefits to human health have been attributed to these plants due to their broad biochemical diversity and biological activity. For example, leaves and roots of F. esculentum have a high content of phenolic compounds, mainly rutoside or rutin, between 2% and 3% in leaves. Due to the presence of quercetin and rutoside, their use in the prevention of oxidative brain damage has been studied in neurological disorders such as Alzheimer's disease. Additionally, the antiinflammatory activity of extracts obtained from germinated seeds showed an inhibition in the production of inflammatory mediators (IL-6 and TNFα) in macrophages. Hypocholesterolemic, hepatoprotective, antioxidant, antiinflammatory, vasorelaxant, hypoglycemic, antidiabetic, neuroprotective and mineralizing properties have been associated with buckwheat food products for their high protein and flavonoid content [3 - 5].

In addition to the functional food application that reduces the incidence and prevents diseases [6], biopeptides in buckwheat have aroused great scientific interest as potential components in applications in various sectors such as agriculture. This chapter focuses on the description and applications of buckwheat biopeptides. Scientific information was compiled to describe the general aspects, nutritional value and bioactive compounds of this pseudocereal. Finally, a conclusive analysis and future perspectives of the biological contributions of buckwheat are presented.

PROXIMAL COMPOSITION OF THE BUCKWHEAT GRAIN

Fagopyrum esculentum Moench is an annual plant with an erect, articulated, and hollow stem, reaching 40-90 cm in height. Its leaves are heart-shaped with a sharp pointed end, alternated and sagittated. The flowers are small, white or pink, and grouped in clusters. The fruit is a trigonous achene of 4 to 6 mm long by 2 to 3 mm wide, containing a single seed, smaller than the fruit that can be dark gray, silver, or black, depending on the species. Its flowering period extends from 1 to 3 months, which means that there exist flowers and seeds simultaneously, making difficult the establishment of harvesting times [7].

F. esculentum seeds are composed of 60-63% carbohydrates, 13-16% proteins, 10.5% maximum fiber, 2-3% fats, flavonoids (*e.g.*, routine), phytoestrogens, organic acids (*e.g.*, maleic, menolenic, oxalic, malic, citric), carotenoids (precursors of vitamin A), group B vitamins (*i.e.*, B1, B2, B3, B6, B8 (inositol) and B9), vitamin E and macro and microelements (*i.e.*, sodium, potassium, magnesium, calcium, phosphorus, iron, manganese, silicon, sulfur, selenium, copper, zinc, chromium, iodine, nickel, cobalt, aluminum, boron, vanadium, fluorine) (Table **1**). Buckwheat has higher amounts of iron than other cereals, thereby conferring it anemia preventive properties [8, 9]. The contribution of vitamin B1 or thiamine and B2 or riboflavin is considerable by doubling the amount found in brown rice, which prevents atherosclerosis and blood cholesterol increments. Vitamin E and phospholipids such as choline are also present; however, buckwheat does not contain vitamin A [10, 11].

Table 1. Grain and flour proximate composition of *Fagopyrum esculentum* Moench.

Nutrients (g/100 g DW of Sample)	**Grain**	**Flour**
Total carbohydrates	72.9	69.6
Total fiber	10.7	8.4
Crude protein	11.2	12.0
Ashes	2.0	1.8
Fat	7.4	3.16
Total Minerals	2.0-2.5	0.8-9
Minerals (mg/100 g DW of sample)	**Grain**	**Flour**
Potassium	450	410
Magnesium	390	190
Phosphorus	330	400
Calcium	110	17
Iron	4	2.8
Manganese	3.4	1.09
Zinc	0.8	2.4

Modified from [11].

The lipid contribution (1.5 to 4.0%) of buckwheat consists of oleic oil (30-45%); linoleic acid or omega-six fatty acids (31-41%), which represent a third of the

CHAPTER 7

Biologically Active Peptides from Sorghum [*Sorghum bicolor* (L.) Moench] Grain

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Abstract: Sorghum (*Sorghum bicolor* L. Moench) is an important staple food crop grown worldwide, easy to grow and drought tolerant. In recent years, sorghum grain has been increasingly regarded as a promising feedstock for the production of bioactive compounds. In particular, its main protein (kafirins fraction) has been used for the generation of biologically active peptides due to its high hydrophobic amino acid content and its hypoallergenicity. Several studies have shown that sorghum-derived peptides have antioxidant, anticancer, antimicrobial and anti-inflammatory activities, among others. These beneficial health effects could be attributed to the fact that in general, peptides derived from kafirins present glutamic acid and non-polar amino acids: proline, leucine and alanine. It has been reported that the presence of these hydrophobic amino acids in peptides facilitates their interaction with free radicals by neutralizing them, in addition to their low molecular weight and the position of the amino acids in the peptide sequence. Therefore, sorghum is a cereal with high potential for the production of biologically active peptides with benefits for human health.

Keywords: Alcalase, Bioactivity, Cereal, Health properties, Hydrophobicity, Kafirins, Peptide extract, Peptide fraction, Peptide sequence, Prolamins, Protein hydrolysate, Purified peptides, Sorghum flour, Sorghum grain, Sweet sorghum, White sorghum.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an annual C4 plant belonging to the *Sorghum* genus of the grass family (Poaceae). This plant likely originated in Africa where it is a major food crop and has numerous varieties, including grain sorghums [1]. Sorghum is considered one of the main cereal crops worldwide after wheat, maize, rice and barley [2]. This cereal is unique due to its high bio-

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

mass yield, short production cycle, tolerance to droughts and floods, high tolerance to salinity, and by its adaptation to dry tropical and subtropical climates [3, 4]. Therefore, it can be cultivated in tropical arid and semi-arid regions of Africa, Asia and the Americas [5].

The survival of sorghum is higher than that of other cereal food crops, and hence more economical to produce. Sorghum is a strong grass and usually grows to a height of 0.6 to 2.4 meters (2 to 8 feet), sometimes reaching as high as 4.6 meters (15 feet) tall. Stalks and leaves are coated with a white wax, and the pith, or central portion, of the stalks of certain varieties, is juicy and sweet. Leaves are about 5 cm (2 inches) broad and 76 cm (2.5 feet) long. The tiny flowers are produced in panicles that range from loose to dense; each flower cluster bears 800-3000 kernels [3, 4].

Sorghum is mainly used as livestock feed and as a starch source for biofuels. Recently, there has been an increase in incorporating sorghum into human foods because its proteins are considered safe for people with celiac disease [3, 6]. Therefore, sorghum flour is incorporated into several manufactured products, such as bread, cookies, snacks, tortillas, pasta and beers [3]. Demand for sorghum is expected to increase in the midst of global climate changes, since this grain is drought tolerant, viable for non-irrigated and semiarid farming areas, and could also be a potential alternative to wheat and maize [6, 7]. However, in western countries (where 40% of the sorghum produced worldwide is harvested) sorghum is mainly used as animal feed [8].

Recent scientific evidence has shown that peptides with different biological activities, including antihypertensive, antiviral, antioxidant, anticancer, anti-inflammatory, antiaging, antidiabetic and antimicrobial, have been isolated from sorghum protein hydrolysates produced by enzymatic digestion. Thus, the published scientific studies allow to consider sorghum grain as a potential source of biologically active peptides [9]. This chapter aims to describe the state of the art about the use of sorghum grain as a promising raw material for the production of peptides with beneficial health properties.

PROXIMAL COMPOSITION OF SORGHUM GRAIN

Sorghum grain can take many shapes and sizes, from a tight-headed, round panicle, to an open, droopy panicle that can be short or tall; nevertheless, they are smaller than those of wheat [3, 4]. Fig. (**1A**) shows the different types of inflorescences of the sorghum varieties. Sorghum grain is obtained from the mature plant and morphologically it is spherical and oblong (3 mm in diameter), with a weight ranging from 1.8 to 2.4 mg. The grain-producing varieties generally bear grains with several colors, including red, orange, bronze, white and black [3, 4]. Sorghum grain is composed of five main parts: pericarp, testa, germ, endosperm and floury endosperm [2]. Fig. (**1B**) shows a diagram and a scanning electron microscopy of the sorghum grain.

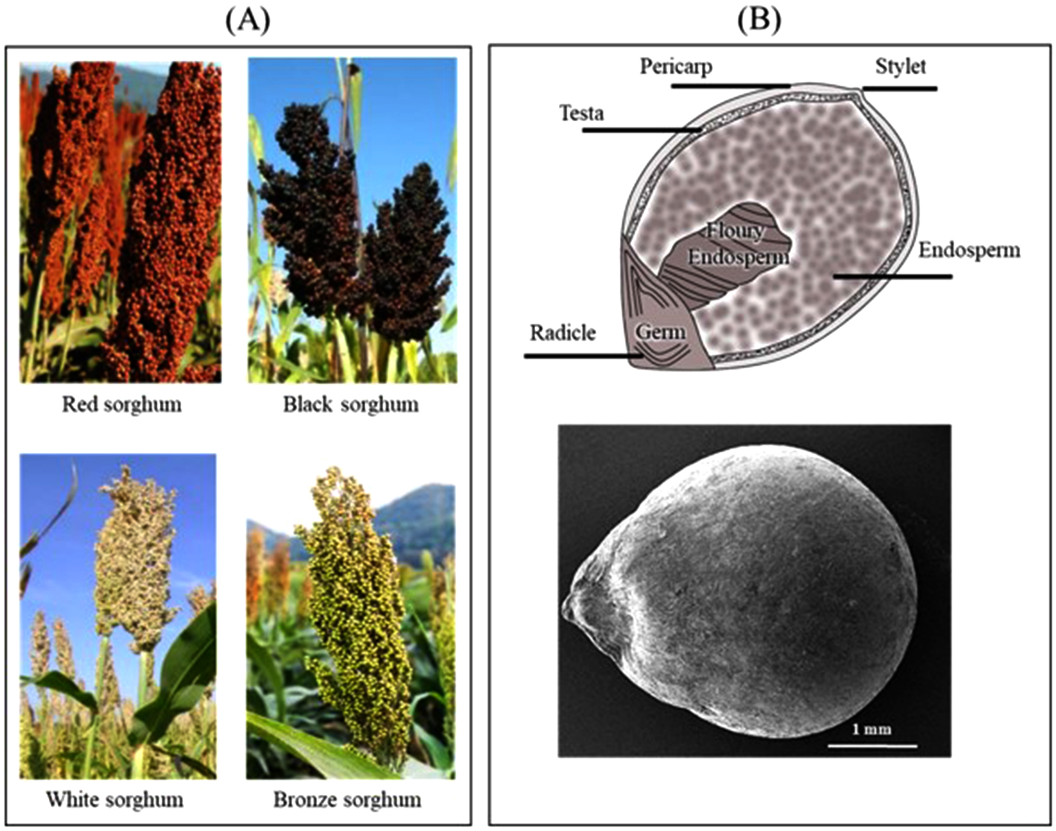

Fig. (1). (**A**) Inflorescences of the principal sorghum varieties that produce grain [10 - 13]. (**B**) Diagram and scanning electron micrography of sorghum grain [14, 15].

Whole grain sorghum is a great source of fiber and protein that adds a hearty, nutty flavor to recipes. The grain contains 8 to 12% of protein and 65 to 76% of starch, with approximately 2% of fiber. Milling sorghum using a roller mill, by following a similar procedure used for wheat and corn, results in a gritty and speckled flour with an astringent taste [16]. The germ contains a rich source of oil (28%), high levels of protein (19%) and 10% of ash [1, 16]. The bran is mainly composed of cellulose and hemicellulose; appreciable amounts of starch are deposited in the mesocarp tissue of this fraction [16]. The lipids in the bran mostly consist of wax rather than oil. The composition of sorghum grain from different origins may vary due to several factors, such as climatic conditions, the nature of the hybrid, and the way the crop is managed [2, 17].

CHAPTER 8

Biologically Active Peptides from Lupin (*Lupinus angustifolius* L.) Grain

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Abstract: There is a great diversity of lupin grains; however, their varieties share a high protein content, thus making them an essential ingredient for substituting or complementing other foods, as well as a source of interest for obtaining hydrolysates and biologically active peptides. At present, it should be noted that numerous studies have shown positive effects of these hydrolysates and peptides in the prevention and/or treatment of diseases, due to their anti-inflammatory, hypocholesterolemic, antidiabetic and hypoglycemic effects; however, it is still necessary to carry out bioavailability and biodigestibility studies to develop forms of inclusion in foods that allow their greater absorption, distribution and metabolism.

Keywords: Blue lupin, Bioactive peptides, Bioavailability, Biodigestibility, Biological properties, Composition, Grain characteristics, Hydrolysates, Lupin, Peptides, Protein, Protein fraction, Yellow lupin.

INTRODUCTION

The lupin seed is a non-genetically modified grain of low cost when compared to soybean, thus representing an interesting alternative, and it is an important source of protein and oil [1]; generally, lupin can be classified into yellow and blue [2]. Despite Lupinus is a very diverse genus, only four species (L. *albus*, L. *angustifolius*, L. *luteus* and L. *mutabilis*) are cultivated. Within each of them, there are subspecies of interest, for example, in the northwestern region of Ethiopia, the growth and yield of seven varieties of narrow-leaved sweet lupin

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

(*Lupinus angustifolius* L.) were studied: *Bora*, *Probor, Sanabor, Vitabor, Haags blaue, Borlu* and *Boregine* [3]; studies of Australian lupin cultivars have also been reported to find the most valuable one, as shown by Mazumder and co-workers [4], who studied six cultivars of *Lupinus angustifolius* L. named *Barlock, Gunyadi, Jenabillup, Jindalee, Jurien, Mandelup* and three of *Lupinus albus* L. named *Luxor, Rosetta* and *WK388*.

Lupin, in general, is tolerant to a wide variety of abiotic stresses, it can fix nitrogen, it efficiently absorbs phosphates from the soil through its specialized cluster root structures, and it has a proven potential for the recovery of poor soils, making it a very sustainable crop [1]. The nutritional value of lupin seeds depends on the quantity and quality of amino acid composition of protein, fat, carbohydrates and antinutritional substances. There are variations in protein content between species and cultivars as a result of growing conditions and soil types, accounting for 28% to 48% of the dry weight of the whole seed [4]. The reported studies agree on its high protein and oil content [5]; moreover, the utilization of lupin as a source of dietary protein is an excellent ecological alternative to animal products for human nutrition [6], in addition, to its advantage of having several potential health effects, such as the protection of microglial cells, neuroprotection of the brain, protection against oxidative stress, prevention of non-alcoholic fatty liver disease [7], and an anti-inflammatory effect in retinal pigment epithelial cells [8], among others. Therefore, considering the importance of this little exploited and underutilized grain, this chapter aims to present the results of studies carried out on lupin grain as well as the biological effect of the peptides obtained from it.

PROXIMAL COMPOSITION OF LUPIN GRAIN

Legumes are the most consumed seed due to their high protein, minerals and fiber content [9]. At present, the grain of *Lupinus angustifolius* (Fig. **1**) has been widely investigated because it is a source of peptides with biological activity [7, 10]. This chapter describes important information about the lupin grain chemical composition.

Mera [11] describes the differences between blue lupin (L. *angustifolius*) and yellow lupin (L. *luteus*) kernels. First, the average weight of the blue lupin grain varies from 150 to 180 mg, with its shape being relatively oval; the color of the grain is mottled, varying from light to intense, although there are also white ones; additionally, the pods usually contain 4 to 6 grains. Furthermore, the yellow lupin grain has an oval and slightly flattened shape with light to strong intensity pigmented spots [2].

Fig. (1). *Lupinus angustifolius*, plant and grains. Sources: http://herbarivirtual.uib.es/ and https://agric.wa.gov.au/n/169.

In a comparative study between blue and yellow lupin seeds, it was found that yellow lupin seeds had a higher protein content than blue lupin seeds; the highest amounts found in blue lupin were in Graf and Tango cultivars, while for yellow lupin, they were found in Dukat, Talar, Lord and Baryt cultivars [2].

In general, lupin has a higher protein content than other legumes [12], presenting a biological value of 91% [10], which makes it an important source of protein for human consumption; in addition, it has been shown to have essential amino acids (g/16 g N) such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and Cys + Met [10].

Naumann and co-workers [13] characterized the molecular interactions between primary bile acids and fractionated lupin (*Lupinus angustifolius* L.) cotyledons, finding that when lupin proteins were isolated by ultrafiltration and spray drying, protein content increased in value up to 63% in the dry matter [13]. Moreover, they found that ash content was higher (15 g/100g dry matter) than carbohydrate content 4.8 g/100g dry matter) and that lupin has high levels of lysine, an amino acid underestimated in cereals.

In a comparative study of blue and yellow lupin conducted by Księżak and co-workers [2], it was shown that the average fat content in blue lupin seeds was slightly higher than that in yellow lupin. These authors also mentioned that among

CHAPTER 9

Biologically Active Peptides from Mung Bean [*Vigna radiata* (L.) R. Wilczek]

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Abstract: Mung bean (*Vigna radiata* L.) is a protein-rich pulse mainly cultivated in Asia, where its consumption has been associated with positive health outcomes. Mung bean protein is especially rich in leucine, lysine, phenylalanine, and tyrosine amino acids and it contains the 8S α-globulin as the major seed storage protein. Protein-derived products from pulses, such as protein concentrates, hydrolysates, and purified peptide fractions are becoming popular functional foods. Mung bean peptides are enzymatically generated using gastrointestinal and non-gastrointestinal proteases. Protein hydrolysates generated by one or a combination of enzymes have been demonstrated to exert different biological potentials, including antioxidant, antihypertensive, anticancer, and hypocholesterolemic effects. These properties are attributed to the amino acid sequences, the type of enzyme used for hydrolysis, and the purification method. More robust experimental designs must be performed to understand the role and mechanisms of these bioactive peptides with *in vivo* studies and clinical trials. Furthermore, there is a lack of information related to the incorporation of bioactive peptides into a food matrix while preserving their bioactivity. This chapter provides an overview of the central aspects of mung bean physical structure and chemical composition, protein characteristics, enzymatic production, and the biological potential of mung bean protein hydrolysates and peptides.

Keywords: 11S legumin, 7S globulin, 8S α-globulin, Anticancer, Antihypertensive, Antiinflammatory, Antioxidant, Bioactive peptides, Biological potential, Enzyme hydrolysis, Globulins, Green gram, Hypocholesterolemic, Legumes, Protein concentrates, Protein hydrolysates, Protein ingredients, Pulses, Storage proteins, Vicilin.

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INTRODUCTION

Mung beans, also known as green gram (*Vigna radiata* L.), are believed to be a native crop from India. Its cultivation extends throughout Asia, some regions of China, Central Africa, South and North America, and Australia [1]. To date, there are more than 5,000 mung bean accessions identified, hence the nutritional content of each variety is diverse [2]. It is consumed in Asia as a snack, bean noodles, or bean sprouts. Furthermore, mung beans are an important food in China due to their biological effects, such as detoxifying, hypocholesterolemic, antitumor, and anti-inflammatory effects [3]. This pulse is a source of vitamins, minerals, and has a high nutrient value compared to soybean and kidney bean. Its protein content ranges from 20.9 to 32.0%, making it a good protein source, except for the lack of essential amino acids such as cysteine and methionine [4]. Also, there is a great interest in its protein hydrolysates due to their potential applications as functional ingredients. The peptides obtained from the hydrolysis of proteins, in addition to being a source of amino acids, present different health benefits. These properties are attributed to the amino acid sequences and length of the chain, the type of enzyme used for the hydrolysis, and the purification methods used to obtain the peptides [5]. Recent scientific evidence supports the relevance of peptides as bioactive compounds in disease prevention and treatment due to their beneficial effects. The peptides obtained from mung beans could be used as ingredients to create healthier foods and reduce the risk of cardiovascular diseases, cancer, reduce oxidative stress, enhance the immune response, among others. This chapter aimed to provide an overview of the central aspects of mung bean physical structure and chemical composition, protein characteristics, enzymatic production, and the biological potential of mung bean protein hydrolysates and peptides.

PROXIMAL COMPOSITION OF THE MUNG BEAN

The *Vigna radiata* L. species comprises more than 5,000 cultivars, each with differences in color, size, and shape. The most relevant cultivars related to production and distribution in Asia are baliv, henan green gram, habei green gram, and different jilv varieties [6]. However, on average, the typical shape and size are described as round green to gold small seeds with a mean size of 4.7 mm in length, 3.9 mm in width, and 3.8 mm in thickness, and an average weight of 5.1-7.85 g per 100 seeds [7, 8]. The mung bean is a dicotyledon seed that presents similarities with other legume seeds such as common beans (*Phaseolus vulgaris* L.). Its main components are a split cotyledon, the seed coat, the radicle, and the embryo [9]. Mung bean seeds are considered a staple food abundant in starch, essential amino acids, soluble and insoluble fibers, minerals, and phytochemicals [4, 7, 10].

The cotyledon and the embryo of mung beans represent more than 90% of the composition of the seed followed by the seed coat, which comprises less than 10% of the total weight. The moisture reported for whole seeds is around 6.8 to 11.7% and is distributed by equals among cotyledon and seed coat [7, 11]. Table **1** shows a compilation of ranges reported of the main components of the mung beans. The most abundant nutrients are starch and proteins, which are in the cotyledon. The fiber and most of its phytochemicals are predominant in seed coats, with traces in the cotyledon. Total minerals are present in both parts, seed coat and cotyledons.

The nutritional composition reported for mung bean seed-may have variations depending on the cultivar, environmental conditions during harvesting, and differences in techniques applied during nutrient quantification. Carbohydrates are the predominant nutrients in seed composition with a total of 54.2 to 64.7 of the total dry weight of the seed [8, 11, 12]. The contribution of fiber carbohydrates in whole seed ranges from 4.8 to 12.8%. Unfortunately, there is no abundant information about dietary fiber and carbohydrates characterization allowing a deep understanding of the different digestible and non-digestible polysaccharides present in the seed. Cooked seeds contain resistant starch and no resistant starch in a range of 5.62-6.27% of 36.3 to 42.1%, respectively (reported on a dry weight basis) and a predictable glycemic index of 41.2 to 42.1 [13]. The oligosaccharides raffinose and stachyose are also reported as components of the seeds [14].

As it is reported for other pulse seeds such as common beans, lentils, and chickpeas, mung beans are known to be a good source of proteins, with tryptophan and sulfur-containing amino acids (cysteine and methionine) as limiting amino acids [4, 15]. Mung bean contains about 20.9 to 32.0% of total protein. However, it contains antinutritional factors such as proteinase inhibitors (trypsin and chymotrypsin inhibitors) and lectin/ hemagglutinin proteins. Therefore, soaking and heat denaturation treatments should be applied to the seeds for safe consumption. The seed oil content in whole grains is low and ranges from 1.2 to 1.56%; with linoleic, palmitic, oleic, and stearic fatty acids being its main components. Also, the content of α, δ, and γ carotenes is high compared to other seeds, meaning that the seed is a good source of these antioxidants and vitamin A precursors [10, 14].

For total phenolics, seed coats are the main reservoir with 29.58 mg of gallic acid equivalents (GAE)/g, contrarily in cotyledons, only 0.59 mg GAE/g were detected. More than 83% of flavonoids and 60% of saponins are present in the seed coat. However, condensed tannins are reported to be in higher proportion in mung beans cotyledons. The most relevant phenolics in mung beans are phenolic acids (1.87-5.6 mg of rutin equivalents/g) and flavonoids (1.49-1.78 mg of

CHAPTER 10

Biologically Active Peptides from Chickpea (*Cicer arietinum* L.) Grain

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Abstract: At present, it is relevant to investigate new sources of nutrients with beneficial activity for humans, so the scientific community has proposed to investigate different legumes such as beans, soybeans, and chickpeas. Chickpea is a grain with high nutrient (lipids, minerals, protein, and carbohydrates) content and is commonly used in Mediterranean cuisine. However, chickpea is a grain with high protein content, which has attracted the attention of researchers, as it is a new source for the obtainment of peptides with biological activity; in that sense, peptides with antioxidant, antihypertensive, hypocholesterolemic, anticancer, and antidiabetic activity have already been reported. This chapter summarizes the most recent information about the biological activity of peptides from chickpea (*Cicer arietinum* L.) grain.

Keywords: Anticancer, Antidiabetic, Antihypertensive, Anti-inflammatory, Antioxidant, Amino acid, Bioactive peptides, Chickpea, *Cicer arietinum*, Desi, Functional food, Health-promoting, Hypocholesterolemic, Kabuli, Legumes, Nutrient-rich food, Proximal composition, Protein fractions, Protein hydrolysate.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume in the world after beans and peas; belonging to the *Fabaceae* family, it is an annual harvest plant and is considered a dry bean. Its grain is nutrient-rich food because besides having high values of carbohydrates, fiber, and protein, the latter is the one with the highest quality among legumes. Moreover, this grain also contains minerals,

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

vitamins, dietary fiber, unsaturated fatty acids, and isoflavones, and it is also a good source of vitamins B9, riboflavin, niacin, thiamine and β-carotene [1 - 4].

Chickpea grains are prepared in various forms according to ethnic and religious factors [1]. In the Indian subcontinent, chickpea is split in half (dhal) and ground to make flour (besan) used to prepare different snacks. In other parts of the world, especially in Asia and Africa, it is used in stews, soups, salads and consumed in many ways such as raw, fried, cooked, salted, or fermented, in most cases providing the consumer with the nutrients that characterize chickpea [1]. Moreover, water that remains from cooking chickpea ("aquafaba") is used as a substitute for egg white or milk in vegan recipes [5].

Chickpea is a food that has accompanied the human species since the fertile crescent of the Near East. Its domestication dates back to 7000 BCE, with *C. arietinum* being identified as the wild species in southeastern Turkey. The plant began to be distributed in the Mediterranean area in 6000 BCE and in India around 3000 BCE; then, it was exported to the "new world" by the Spanish and Portuguese in the 16th century [2].

Currently, there are two main varieties of chickpea, the Kabuli variety with a large grain of relatively smooth, rounded, light-colored surface and pale pink flowers, which is abundant in the Mediterranean countries and near the East, and the Desi variety with tiny wrinkled grains, covered by a dark color and generally purple flowers that are abundant in India, Afghanistan and Ethiopia [2].

Chickpea crop is characterized as an herbaceous plant, growing above 100 cm in height, being hairy and glandular, with pale pink or purple flowers from which a pod develops, containing between 2 or 3 grains. It also has deep roots up to 2 meters deep, with a rounded hairy stem, with numerous excretory glands that allow it to adapt to arid environments; the branches are usually imparipinnate with 3 to 8 leaves on the stem and one at the top [6].

Chickpea regularly grows in temperate or arid zones. It is cultivated mainly in Asia, Europe, Australia, and North America, generally as a winter crop. In the year 2020, 15.1 million tons of chickpea were produced, with India as the primary producer (73.38% of the global production), followed by Turkey (4.17%), Pakistan (3.29%), Myanmar (3.19%), Ethiopia (3.03%) and Russia (1.93%); however, despite its extensive production, only 2 million tons of chickpea are traded internationally, with Australia being the first exporter (423 thousand tons) of this legume and India the first importer (305 thousand tons) [7]. The most common problems affecting production are environmental stress (abiotic stress) like drought, salt, temperature, UV and nutrient deficiency of the soil, and biotic stress like fungi, bacteria and nematodes that decrease production yields [8].

Recent studies have shown that chickpea grains have components with antineoplastic, antimicrobial, anti-inflammatory, antioxidant, antihypertensive and hypocholesterolemic properties [9 - 13]. However, its use is limited only as an ingredient at cooking. This chapter aims to describe the characteristics of chickpea and its biologically active peptides.

PROXIMAL COMPOSITION OF CHICKPEA GRAIN

Legumes are a vital crop due to their high protein, essential amino acid, and carbohydrate content, which is why it plays a key role in the diet of many developing countries, consumed in various form, supplementing the elementary nutrients for most people; this is why it is referred as the “poor man's meat.” However, this role is limited due to low protein and starch digestibility, low mineral bioavailability, and high antinutritional factors [14].

This legume usually contains two cotyledons covered by a coat and it is generally non-endospermic. The grain coat has an outer integument (cuticle, palisade layer, hourglass cells, parenchymatous layer, inner epidermis) with or without the remnants of an inner integument (aleurone layer and endosperm) at the abaxial interface (between the grain coat and cotyledons) [15].

Chickpea is a type of legume that contains a variety of nutrients and compounds beneficial to health, such as carbohydrates, proteins, unsaturated fatty acids, minerals such as Ca, P, Mg, K, vitamins such as riboflavin, niacin, thiamin, folate, vitamin A, dietary fiber and isoflavones [1, 16].

The chickpea carbohydrates constitute about 60-65% of the cotyledons, with starch being the most present polysaccharide in chickpeas (30.8-37.9% of dry matter), and the remaining portion of carbohydrates composed of soluble sugars, crude fiber, and dietary fibers (18-22%) [17, 18]. Chickpea carbohydrates are mainly composed of oligosaccharides (α-galactosidase) divided into two groups: the first is composed of raffinose, stachyose, and verbascose, while the second consists of galactosyl-cyclitol, such as ciceritol, with ciceritol and stachyose being the most abundant in the grain [12].

Furthermore, chickpea grain has resistant starch, which represents 35% of the chickpea’s total starch; it has a physiological function similar to dietary fiber, only being hydrolyzed in the large intestine, where it produces methane, carbon dioxide, and short chains of amino acids by fermentation, acting also as a probiotic and generating flatulence to the consumer [18, 19].

Protein represents an essential chickpea component due to its functional properties and balanced amino acid content. According to the Osborne fractions’

CHAPTER 11

Biologically Active Peptides from Broad Bean (*Vicia faba* L.)

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Abstract: The broad bean progenitor was a local wild vegetation, which was discovered in a prehistoric Natufian culture site. It is in symbiosis with *Rhizobium leguminosarum* for nitrogen fixation. It has a large genetic diversity and belongs to the third largest family of angiosperms, with over 16,000-19,000 species. Commonly, the genotypes are *V. faba var. major*, *minor*, *equina* and *paucijuga.* Some of its applications, for example, are as pills for Parkinson´s disease or hypertensive patients due to its L-Dopa, and high potassium and low sodium contents, respectively. Likewise, bread, biscuits, pasta, emulsions and beverages can be fortified with broad-bean flour, improving the protein content. The majority of proteins contained in the broad bean are globulins (80%), followed by albumins (20%), and, in a lesser amount, glutelins (15%) and prolamins (6%). Globulins are composed of legumin and vicilin/convicilin. Broad bean is a cheap and healthy source of protein. Therefore, it can produce biologically active peptides; for example, NPN-1 can decrease muscle wasting; protein hydrolysates are hypocholesterolemic; VFTI-G1 is anticarcinogenic (IC_{50}=30µM); moreover, a protease inhibitor isolated from a broad bean, is useful in the treatment of fungal disease in HIV-infected patients (51.2% inhibition at 32 µM). In addition, fraction F1 has antityrosinase activity IC_{50}=0.140; and fabatins have moderate activity against *E. coli*, *E. Hirae* and *P. aeruginosa.* The future of product developments in food and pharmacology lies in a combination of breakthroughs in genetics, physiology of the gut, hydrolysis, extrusion and purification of BAPs.

Keywords: Anticancer, Antioxidant activity, Antityrosinase activity, Bioactive peptides, Broad bean, Enhance protein, Genes, Genotypes, Globulins, Grain legumes, Hydrolysates, Parkinson's disease, Peptides, Sequence amino acid, Storage proteins, *Vicia faba*.

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INTRODUCTION

Broad bean (*Vicia faba* L.), also known as field bean, faba bean, horse bean, or tick bean, is an annual grain legume growing in different climatic zones. Before it was domesticated three millennials ago, the broad bean progenitor was a local wild plant. It was discovered in the prehistoric Natufian culture site of el-WAD Terrace, Mount Carmel, Israel [1]. Later, when the broad bean was domesticated, it was assigned to Central Asian, the Mediterranean, and South America [2].

Broad bean is herbaceous, annual, and perennial. It has a pivoting primary root with a lot of secondary and tertiary roots [3]. Broad bean cultivars grow in clay-lime, chalky, well-drained, and textured soils, with neutral pH, although they acclimate to a wide pH range (6 to 9); likewise, they have grown poorly in sandy-loam soils, especially in humid regions [4]. This crop can withstand winter frost, but its optimal temperature is 18-27°C. Furthermore, the broad bean is linked through symbiosis with the bacteria *Rhizobium leguminosarum* for nitrogen fixation, as a result, they consume less energy than mineral N fertilizers [5 - 7]. The plant can grow 90-130 cm tall, depending on the genotype. At the 8-10 node growth stage, when the plant is around 30 cm tall, it produces its first flowers [8].

The genus Vicia, tribe Fabeae, family Fabaceae (syn. *Leguminosae, Papilionaceous*), order Fabales, has a large genetic diversity, and it is the third largest family of angiosperms (Fabaceae family) with over 16,000-19,000 species [9]. Broad bean is generally considered as a large-seed crop, within each cultivar the seed size also varies greatly depending on the cultivar location. Commonly, their genotypes are categorized into four main botanical varieties (Fig. **1**) according to seed size: (a) *V. faba* var. *majo*r is ovoidal and flattened in shape, formed by 3-4 large seeds, with indehiscent pod (> 1.0 g seed-1); it has been traditionally used for human consumption because of their edible pods and softer and sweeter seeds; (b) *V. faba* var. *minor* is ellipsoidal in shape, formed by 3-4 small seeds, with 6-13 mm diameter (< 0.5 g seed-1); (c) *V. faba* var. *equina* is flattened in shape, formed by medium seeds, with dehiscent pod (0.5-1.0 g seed-1); and (d) *Paucijuga* is round and has small seeds (0.2 g seed-1) [10]. Likewise, they are classified based on their ability to acclimatize to oceanic or continental weather. Also, they are categorized into spring or winter types based on their frost resistance [8, 15].

Fig. (1). *Vicia Faba* seeds varieties and plants **a)***major*, **b)***minor*, **c)***equina*, **d)***paucijuga* [11 - 14].

Broad bean is one of the most important pulse crops in the world due to the superior nutritional values of protein, carbohydrates, B group vitamins, and minerals [16]. L-Dopa, which is stored in various parts of the broad bean, is a precursor of dopamine; L-Dopa is used as an ingredient in some of the pills for Parkinson´s disease. However, L-Dopa causes side effects such as nausea, vomiting, low blood pressure, drowsiness and restlessness; besides, synthesized L- Dopa is expensive. Consequently, broad bean consumption is recommended, which is ranked second after velvet bean in terms of L-Dopa content. Nevertheless, the L-Dopa quantity is conditional on genotype, environmental conditions, and growth stage [8]. Moreover, mature broad bean has a high content of potassium (1,062 mg/100 g) and low sodium (13 mg/100 g); therefore, hypertensive people can eat it. Moreover, the synthesis of pyrimidines, purines, and amino acids needs folate as a cofactor, thus, broad bean is a good source of it [17].

Additionally, the human diets in the Middle East, the Mediterranean region, China and Ethiopia contain broad beans as an important food. They use it as a green or dried, fresh or canned vegetable because its nutritional value is higher than those of peas or other grain legumes [5]. Likewise, *equina* and *minor* types are employed for animal feeding, but all types can be used for it.

CHAPTER 12

Biologically Active Peptides from Cocoa Bean (*Theobroma cacao* L.)

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Abstract: Recently, proteins and peptides have become important ingredients in functional foods due to the emergence of knowledge about their properties and biological activities. Many biologically active peptides (BAPs) have been studied from different animal and vegetal origins, principally legumes, such as soybeans and beans. BAPs have been shown to have anticancer, antitumoral, antioxidant and antimutagenic activities. Cacao, an ancient crop that originated in the Mesoamerican region, contains several bioactive compounds, *e.g.,* polyphenols, alkaloids, flavanols, procyanidins and peptides. Cocoa beans contain 50% lipids and 14% protein, with albumins and globulins prevailing. The principal amino acids from cocoa protein are lysine, arginine, serine, proline, alanine, leucine, valine and methionine, and they allow the elucidation of the peptide's bioactivity. Cocoa BAPs show antitumoral, antioxidant, antihypertensive and antidiabetic activities and obesity prevention. This work demonstrates the potential of all its BAPs.

Keywords: ACE, Albumins, Antihypertensive, Antioxidant, Antitumoral, Bioactive peptides, Cocoa bean, Cocoa protein, Criollo, Forastero, Free radicals, Globulins, Obesity, Proteins, Trinitario.

INTRODUCTION

Cocoa (*Theobroma cacao* L) is a crop known worldwide due to its fruit, which is one of the most economically important and popular agricultural products in the world of gastronomy and in the food industry for being the raw material for the manufacture of cocoa, chocolate, and other byproducts of industrial interest, such as cocoa liquor, cocoa powder and cocoa butter [1]. In addition, in recent years, the significant growth of scientific studies that show and support the beneficial

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

effects of the components of cocoa beans on human health has increased the interest in its consumption and demand due to its great potential as functional food, which opens up new perspectives in its market [2, 3].

Cocoa is a small, evergreen tree, 8-15 m tall, belonging to the family *Malvaceae* (alternatively, S*terculiaceae*) and to the genus *Theobroma* [4]. Its flowering produces fruits in the form of capsular-oval pods that hang directly from the trunk of the tree. Inside, fruits contain a cluster of seeds called “cocoa beans”. Each pod contains approximately 30-40 cocoa beans, and it is estimated that a tree is capable of producing up to 2 kg of beans per year. Cocoa production extends within 20 degrees of latitude to the north and south of the equatorial plane of the earth, where hot conditions, with temperatures ranging between 20 and 30 °C, and high environmental humidity favor its development and spread [5]. In this way, cocoa cultivation covers more than 70,000 km^2 of land in multiple countries around the world, including Ivory Coast, Ghana, Nigeria and Cameroon in West and Central Africa; Mexico, Brazil, Ecuador, Venezuela, Colombia, the Dominican Republic and Peru in the Americas; and Malaysia, Indonesia and Papua New Guinea in Asia and Oceania. This contributes to the annual world production of cocoa of more than 4 million tons per season (4,794 million tons in the 2018/2019 growing season), and with a growth trend of an estimated production of 5,175 million tons in the 2020/2021 growing season, it represents the economic livelihood of approximately 40 to 50 million people worldwide [4, 6, 7].

Within the species *Theobroma cacao* L., at least four botanically different subspecies are cultivated in the different producing countries: Criollo, Forastero, Trinitario and Nacional cultivars [8]. However, on the American continent, a classification of at least 10 main genetic groups of *Theobroma cacao* has been defined, including Marañón, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Nacional and Guayana, which reflects the great genetic diversity of this crop worldwide [3]. These are distinguished by the morphological characteristics of the fruit, the geographical origin and the particular textural and organoleptic properties associated with their different chemical compositions.

Cocoa of the Forastero genotype (*Theobroma cacao* L. ssp. *shaerocarpum* Cuat) constitutes a group of cocoa subvarieties, which includes Amelonado, the best known and most cultivated subvariety in West Africa (except Cameroon) and the most used for improvement in the main cocoa-producing countries. The ears of these subspecies are ribbed and slightly rough with smooth, rounded ends that turn yellow when ripe. Its grains are characterized by being small and flat, with violet cotyledons, and by having a strong robust sour-bitter flavor with moderate acidity. Chemically, they are rich in cocoa butter and have a higher content of phenolic compounds -as well as nutrients such as carbohydrates, fats, proteins, peptides and amino acids- than the rest of the cocoa subspecies. It is classified as bulk-grade cocoa, as it represents more than 90% of world cocoa production. Therefore, it is mainly used for the manufacture of cocoa mass, cocoa powder, cocoa butter and milk or bitter chocolate [2, 9, 10].

The Criollo-type cacao (*Theobroma cacao* L. ssp. *Cacao* Cuat.) is a subspecies cultivated since prehistoric times in Central America, Venezuela, Madagascar, Sri Lanka and Samoa. It is characterized by green and red presenting immature cobs, and as they mature, they turn yellow and orange-red. The grains of this subspecies are large, rounded, and have ivory-white cotyledons. They have a mild flavor and low bitterness, but they are very aromatic, standing out for their nutty and fruity notes, and so they are known commercially as "fine cocoa". It is currently a relatively rare cocoa species, as it exhibits low resistance to damage from pests and climatic changes and produces low yields [2, 5, 10].

Trinitario cocoa is a polymorphic hybrid of the Criollo and Forastero subspecies grown in the West Indies, South America and Central America, which has higher yields. It is less susceptible to diseases, and it has a basic and strong chocolate flavor with characteristics of particular organoleptic properties, such as wine.

The Nacional subspecies, cultivated only in Ecuador, are characterized by the larger size of pale purple beans that produce fine cocoa ("arriba" flavor) emphasized by aromatic notes of spices and flowers [11]. Together, these last three subspecies, unlike Forastero, are used mainly for the production of dark chocolates and represent between 5-10% of the world cocoa market [2].

PROXIMAL COMPOSITION OF THE COCOA BEAN

Structurally, cocoa beans are made up of four well-identified parts. The first is constituted by the shell and comprises between 10-14% of the dry weight of the grain. The second is represented by the embryo or germ, followed by two cotyledons that together represent between 86-90% of the dry weight of the grain. Finally, the cocoa pulp, which is similar to a mucilaginous membrane with sweet-acid characteristics, is very aromatic, pleasant to the palate, and surrounds the grain, representing approximately 40% of its fresh weight [1, 11].

The chemical composition of cocoa beans and their byproducts is complex. It varies according to genotype, geographical area of origin, cultivation conditions, degree of maturity and postharvest processing to which the grains are subjected, such as fermentation and drying [8, 10], as shown in Table **1**. In general, nutritionally, cocoa beans are mainly composed of fat (cocoa butter) -representing between 48-59% of the dry weight of the bean, and consisting mainly of

CHAPTER 13

Biologically Active Peptides from Chia (*Salvia hispanica* L.) Seed

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Abstract: Chia seed (*Salvia hispanica* L.) is rich in nutraceutical compounds with multiple benefits for human health and with great potential for its use in food. Interest in the study of this seed has been increasing year by year. Currently, studies have been based on the beneficial potential of chia proteins as a low-cost source of vegetable protein. Meanwhile, other studies have been based on the residual use of chia cake, a residue from the extraction of the chia oil industry. Bioactive peptides from chia have been shown to possess the inhibitory potential of molecular targets of hypertension, diabetes, adipogenesis, microbial, cancer, and aging. This chapter aims to provide an overview of chia bioactive peptides and their importance as a seed with high value for its protein content.

Keywords: α-amylase, α-glucosidase, ACE, Adipogenesis, Anticancer, Antidiabetic, Anti-inflammatory, Antihypertensive, Antiaging, Antioxidant, Bioactivity, Chia seed, DPPIV, Hydrolysis, *In vitro*, *In vivo*, *In silico*, Protein, Peptides, Superfood.

INTRODUCTION

Chia seed (*Salvia hispanica* L.) is native to western Mexico and Guatemala [1, 2]. It is an herbaceous annual plant that belongs to the Lamiales order, Lamiaceae family, Nepetoidae subfamily, and *Salvia* genus [3, 4]. It was used as a food mixed with beans, amaranth, and corn by the Mayan and Aztec civilizations in the pre-Columbian period [1]. For the Mayans and Aztecs, chia represented an essential grain for their food, preparation of medicines, preparation of paintings, and was used for ceremonies [5]. It has been reported that there are around 900 species that occur in Mexico, Guatemala, Bolivia, Australia, Argentina, Peru, Eur-

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

ope, and America [3]. The word “chia” is derived from “chian”, which means oily and oilseed [6].

Chia is considered a superfood; chia seeds are a good source of nutrients, such as omega-3 fatty acids, gluten-free proteins, and antioxidants that protect the seeds against chemical and microbial degradation [6]. It has been reported that it contains 15 to 24% protein, 40% lipids with 60% omega-3 fatty acids, dietary fiber (18-30%), carbohydrates (24-41%), and vitamins and minerals [7]. It is considered functional food [2] since it is a source of healthy bioactive compounds and primary antioxidants with potential applications [1]. The industrial importance of the chia seed has focused on obtaining oil [8], leaving aside the residual cake. Some of the current uses of the seed are texturizing agents adding chia in ice cream and milk [9, 10] and finally in oil microencapsulation [11].

Currently, scientific evidence reflects that chia's omega-3 and omega-6 fatty acids have anti-inflammatory potential [2] and phytochemicals such as phenolic compounds with antioxidant and anti-inflammatory potential [12]. In addition, the consumption of the seed has been shown to possess a preventive effect against type 2 diabetes mellitus [13]. Recent research has evaluated the beneficial properties of the bioactive peptides present in chia seeds, such as the antioxidant, antidiabetic, anti-inflammatory, antiadipogenic, anticancer, antiaging, and antimicrobial potentials [8, 14 - 20].

This chapter aims to present the state-of-the-art of the potential use of chia seed in the food industry, focusing on its proteins and beneficial health properties of the peptides contained in this seed.

PROXIMAL COMPOSITION OF CHIA SEED

The composition of chia has been studied in many studies. Chia seeds are small, oval in shape, and flat, measuring between 2.0 to 2.5 mm long, 1.5 mm wide, and 0.8 to 1.0 mm thick [21]. There are black and white chia seeds, which are slightly different from each other; the white ones are larger, thicker, and broader than the black ones. Both seeds' protein and fatty acid content vary significantly [22]. Table **1** shows the proximal composition of chia seeds in different regions currently being reported, the regions where these seeds were cultivated cause variations in their proximal content.

Chia seeds have a high nutritional value, mainly thanks to their high lipids, fiber, and protein content. Interest in the chemical composition of chia seeds grown in different parts of the world has increased over the years. Current data show that chia seed contains approximately 30 to 34% dietary fiber (insoluble and soluble fractions). In terms of dietary fiber content, chia seeds overcome dried fruits, nuts, or cereals [23]. The lipid content of chia seed is 30 to 35% and is characterized by a high polyunsaturated fatty acid content. The primary fatty acid is linolenic acid, representing 60% of all fatty acids [24]. Chia seeds are also a good source of vegetable protein, accounting for approximately 15 to 25% of their mass. Protein variations are associated with soil type, climate, where these seeds are grown, and the methodology used for protein fractionation [8, 25]. The protein content of chia seed is higher compared to other traditional cereals such as wheat (14%), barley (9.2%), oats (15.3%), rice (8.5%), and corn (14%) [3].

Chia seeds provide minerals, such as phosphorus, calcium, potassium, and magnesium. Furthermore, they contain 26 to 41% carbohydrates and 4 to 5% of ashes [1, 4, 6, 23, 25, 26]. The presence of vitamins such as vitamin B1, B2, and niacin has also been reported [23].

Table 1. Proximate composition of chia seeds in different regions.

Component	Chile [26][b]	Yungay, Ancash, Peru [27][b]	Maringá-Paraná-Brazil [28][b]	Buenos Aires, Argentina [29][a]	Acatic, Jalisco, Mexico [1][b]
Carbohydrates	34.57±0.26	32.90±0.09	NR	37.45	21.8
Fiber	37.50±1.07	10.10±0.21	NR	NR	35.1
Lipids	30.22±0.08	27.90±0.32	27.88 ± 0.89	30.23	18.3
Protein	25.32±0.21	17.80±0.18	23.54 ± 0.08	21.19	18.8
Ash	4.07±0.02	4.50±0.21	4.29 ± 0.02	NR	4.7
Moisture	5.82±0.04	6.80±0.18	6.27±0.19	NR	5.8
Energy (kcal)	576.50±9.60	NR	NR	NR	388.3

Expressed in g/100g to chia seed, *NR: Not reported, [a]: Chia seed white variety, and [b]: chia seed black variety.

NUTRITIONAL ASPECTS OF CHIA SEEDS

Mexico is currently the world's leading producer of chia seeds [30]. The state of Jalisco is the leading producer, producing 4,771.04 tons annually [31]. Today, chia is part of the diet of Mexicans and several Central American countries. It is becoming increasingly popular and common in supermarkets and health food stores worldwide. The interest is mainly due to its bioactive composition and nutritional value when incorporated into a balanced diet [32]. The approval in 2019 of chia seeds as a new food by the European Parliament allows their consumption and incorporation into food [32]. As mentioned above, chia seeds are an excellent source of fat (18 to 30%) and proteins (18 to 25%), particularly polyunsaturated fatty acids such as α-linolenic (60%) and linoleic (20%) acids. It

CHAPTER 14

Challenges and Opportunities of Biologically Active Peptides in the Design and Formulation of Nutraceuticals and Functional Foods

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Abstract: Consumer demand for products with health benefits beyond simple nutrition is the market driver for nutraceuticals and functional foods. The development of these products has been on the rise since the last decade as consumers became more aware of the consequences of lifestyle diseases. This scenario has also benefited from the growing economy, changing lifestyles and consumption patterns. Researchers and the food industry are exploring novel sources of bioactive components and attention has been given to underutilized grain proteins. This chapter aims to review the incorporation of underutilized grains into nutraceuticals and functional foods. The recent advances and challenges in food processing techniques to develop these products are presented. Moreover, comprehensive details on the improvement of product properties with the incorporation of underutilized grains, by means of nutritional, techno-functional and bioactivity, as well as sensorial analysis are given. Finally, the health promoting effects of peptides encrypted in the protein fraction of these grains will be discussed.

Keywords: Amaranth, Boactive, Bioactivity, Buckwheat, Chia, Cocoa, Food, Formulation, Functional foods, Health benefits, Ingredients, Lupin, Mung bean, Nutraceuticals, Peptides, Proteins, Quinoa, Techno-functional, Underutilized grains, White sorghum.

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

INTRODUCTION

The suggestion that certain food constituents might provide some unique health benefits and improve the quality of life has led to an increased interest in functional foods and nutraceuticals. Food constituents that might be responsible for those unique health benefits are diverse and include vitamins, polyunsaturated fatty acids, polyphenols, pre and probiotics, or peptides, among others.

The concept of nutraceutical was initially coined by DeFelice in 1995 [1]. This concept considers that nutraceuticals work to prevent disease and provide benefits to sustain the healthy state of the body. This concept has evolved over the years and at present, nutraceuticals are also being viewed as therapeutic agents against non-communicable diseases (NCDs). However, since they can be consumed in the form of pills, tablets, or powders, just like supplements, both concepts are used interchangeably [2]. However, nutraceuticals can be composed of a combination of bioactive compounds which have been isolated or extracted from whole foods.

Although there is not a universally accepted definition for functional foods, they are recognized as foods that, being taken as part of a daily diet, provide health benefits beyond their nutritional effects [3]. Thus, functional foods are beneficial for enhancing the nutritional status, promoting health and well-being, and reducing risk factors for several NCDs. Functional foods include both traditional products such as yogurt to newer and tailored foods. The physiological benefits of functional foods include the support of development and growth, modulation of gastrointestinal functions, stimulation of metabolism, reinforcement of the cardiovascular system, and the enhancement of psychological functions [4]. Assessing the legal framework of functional foods in Europe, health claims are authorized under Regulation No. 1924/2006. The different types of claims in the category of health claims are "Function health claims" (Article 13 claims), "Risk reduction claims" (Article 14(1)(a)), and "Claims referring to children's development" (Article 14(1)(b)) [5].

The global market of nutraceuticals and functional foods is diversifying rapidly. Indeed, the global market of dietary supplements, functional foods, and functional beverages was valued at more than $454 billion in 2021 and is predicted to be at a 9.0% compound annual growth rate from 2021 to 2030 [6]. Consumer attitudes toward functional foods have been reviewed [7]. In general, consumers have a positive attitude to purchase and consume functional foods not only in western areas, such as North America and European countries but also in developing countries. Thus, functional foods should retain organoleptic and nutritional benefits apart from their beneficial added value [8]. Moreover, an integration between credible science and effective communication with consumers seems essential for the future development of functional foods [7].

RECENT ADVANCES IN THE DEVELOPMENT OF NUTRACEUTICALS AND FUNCTIONAL FOODS

Over the last decades, the development of nutraceuticals and functional foods has shown enormous potential and a rapid increase to meet consumers' demand for healthier products. A search for related patents on Espacenet (keywords: functional food AND nutraceutical) provides 8975 results with 6611 of them published over the last 10 years (https://worldwide.espacenet.com/, accessed February 2022). Some of the most recent innovations patented on the development of nutraceuticals and functional foods are shown in Table **1**. This table includes a summary of techniques that are applied for the extraction or formulation of food ingredients as homogenization, spray drying, titration, enzyme hydrolysis or microbial fermentation.

Food processing techniques should preserve the quality, functionality, and nutritional characteristics of the products, which is of particular interest when it comes to nutraceuticals and functional foods. Therefore, emerging technologies with minimal treatment are finding numerous applications in research and the food industry. High-hydrostatic pressure, ultrasound-assisted and microwave-assisted extraction, pulsed electric field, radio-frequency drying, high voltage electrical discharge, and supercritical fluids are among the most common emerging technologies applied to preserve the functionality of food components [9]. For example, high-pressure processing is used for microbial inactivation or whey protein hydrophobicity modification [9]. This technique can also be used to improve the storage stability of bioactive ingredients in beverages [10].

Ultrasound-assisted extraction has been applied to produce functional ingredients from fruit, vegetables, herbs, spices, seeds, or microorganisms [11]. The extraction process is influenced by physical parameters such as frequency, length, and amplitude of the wave, solvent choice, temperature, or matrix characteristics. Together with conventional ultrasound extraction techniques, hybrid combinations with Soxhlet or supercritical fluid extraction have been applied [11]. One interesting application of ultrasound-assisted extraction is the recovery of biologically active peptides from chia seeds or sesame bran [12, 13].

Supercritical fluid extraction is extensibility used to obtain bioactive components from natural sources such as polyphenols, carotenoids, or polyunsaturated fatty acids [14]. Moreover, it can be applied to pre-treat quinoa protein hydrolysates, removing oil from the flour, and increasing its antioxidant activity [15]. Pre-treatment of canola seeds with supercritical extraction protected the functional

CHAPTER 15

Challenges and Opportunities of Biologically Active Peptides in the Design and Formulation of Cosmeceuticals

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Abstract: In recent years, the use of cosmetics and personal care products has increased worldwide, due to their corrective, decorative and hygienic functions. The development of new cosmetic formulations based on the use of bioactive compounds has expanded rapidly due to consumer demand for products with protective and therapeutic functions derived from natural sources. Skin aging is a complex biological process influenced by a combination of endogenous or intrinsic and exogenous or extrinsic factors. Since skin health and beauty are considered to be one of the main factors representing overall "well-being" and the perception of "health" in humans, several antiaging strategies have been developed in recent years. There are two main groups of agents that can be used as antiaging components, antioxidants and cellular regulators. Antioxidants, such as vitamins, polyphenols and flavonoids, reduce collagen degradation by reducing the concentration of free radicals in tissues. Cell regulators, such as retinols, peptides and growth factors, have direct effects on collagen metabolism and influence collagen synthesis.

Keywords: Antiaging, Antioxidants, Bioceutical, Cell regulators, Collagen, Cosmeceutical, Cosmeceutical, Cosmetics, Enzymes, Health, Nutricosmetic, Peptides, Photoaging, Pigmentation, Skin care.

INTRODUCTION

Skin aging is a biological process characterized by a progressive reduction in the normal functions of the skin and its repair capabilities, manifested by increased susceptibility to disease and harmful environmental stimuli. The skin is a complex tissue with many functions, especially defense, and in which drastic changes in both structure and chemical composition occur with age. Over thousands of years,

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

genes present in our cells have been able to provide, according to a completely physiological evolution, the construction of valid defensive systems adapting to oxidative and environmental stresses. We can therefore say that the conservation of biological systems is the result of a continuous balancing act between molecular damage and cellular repair mechanisms. DNA damage, caused by normal metabolic processes, occurs continuously, at a rate of between one thousand and one million molecular lesions per cell per day. The advent of epigenetic studies has provided insights into how environmental stress can act on the functional activation state of genes [1]. This implies a more careful consideration of how cosmetic and cosmeceutical products should be formulated, delivered and applied to the skin to achieve ideal tissue regeneration to combat and prevent aging. In recent years, dermatological scientific research has made considerable progress in identifying many active ingredients and antioxidant substances, studying their possible direct or indirect effects on the cells of the epidermis and, in general, on our health. There are two main groups of agents that can be used as antiaging components (Fig. **1**): antioxidants and cell regulators. Antioxidants, such as vitamins, polyphenols and flavonoids, reduce collagen degradation by reducing the concentration of free radicals in the tissues. Cell regulators, such as retinols, peptides and growth factors, have direct effects on collagen metabolism and influence collagen production [1 - 5]. For some time now, the concept of cosmeceuticals and nutraceuticals has been on the rise.

COSMECEUTICAL OR NUTRACEUTICAL?

Cosmeceutical

The term cosmeceutical, coined by Albert M. Kligman in the early 1980s, is used to indicate a category of cosmetic products characterized by the presence in their formulas of biologically active ingredients of synthetic or natural origin (animal or vegetable) with a pharmacological-like action [6 - 10]. These ingredients must: a) be able to pass through the horny state and reach, *in situ*, an adequate concentration to carry out their action; b) have a specific biochemical mechanism of action on the skin; c) be able to demonstrate the claimed efficacy [11 - 15]. However, the term cosmeceutical does not unequivocally define a specific type of product. The Food and Drug Administration (FDA) does not recognize or define the term cosmeceutical and in Europe, there is no legislation to date giving this category of products a legal value (not even the recent introduction of the European Cosmetics Regulation, European Directive 76/768 nor its subsequent amendments, recognize its existence); however, in common usage, this term seems to be the keyword for the new generation of antiaging cosmetics [16 - 20]. In reality, cosmeceuticals were not created exclusively to combat signs of skin aging, but more generally to provide protection for the skin, nails and hair, and to act as a lightening agent or to protect against the sun's rays. The literature contains a wide range of effective active ingredients for the formulation of cosmeceuticals, which are designed to be used as whitening or depigmenting agents, moisturizers, antiaging and antiphotoaging agents, and photo protectors [21 - 23].

The cosmeceutical landscape is quite broad and complex. There are authors who differentiate between pharmacological cosmeceuticals (*e.g.*, topical retinoids not allowed for cosmetic use, topical preparations based on minoxidil), neurocosmeceuticals (topical preparations capable of improving the condition of psychophysical well-being by promoting the release of β-endorphins) and cosmeceuticals (functional cosmetics).

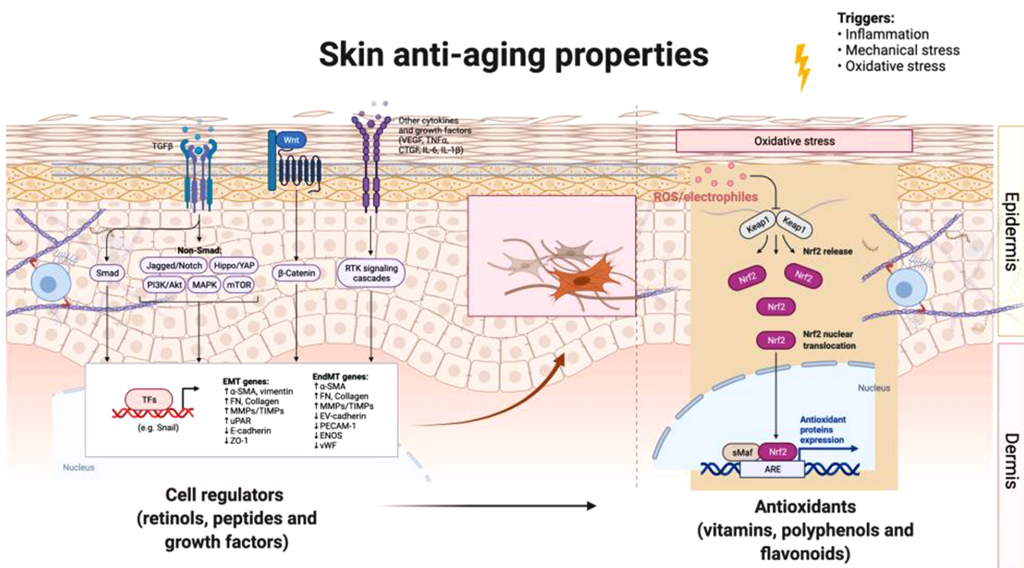

Fig. (1). The skin antiaging strategies attempt to reverse the dermal and epidermal signs of photo- and chronological aging. In recent years, dermatological scientific research has made considerable progress in identifying many active ingredients and antioxidant substances, studying their possible direct or indirect effects on the cells of the epidermis and, in general, on our health. There are two main groups of agents that can be used as antiaging components: antioxidants and cell regulators. Schematic summary of the mechanisms of biologically active peptides (BAPs) related to their potential cosmeceutical application. ROS: Reactive oxygen species; MAPK: mitogen-activated protein kinases; ERK: extracellular-signal-regulated kinases; JNK: c-jun N-terminal kinase; MMPs: matrix metalloproteinases; cAMP: cyclic adenosine monophosphate; MITF: microphthalmia-associated transcription factor; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-6: interleukin-6.

Nutraceuticals

This concept emerged in 1989 by Dr. Stephen de Felice, Director of the Innovative Medicine Foundation. He stated that it would be any substance that can be considered as food or as part of food and that provides medical or health

CHAPTER 16

Biologically Active Peptides with Antiviral and Anti-COVID-19 Potential: Promising Insights for a Healthy Future

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Abstract: Adequate nutrition is essential for good health. The characterization of the molecules present in grains - used in the human diet for thousands of years - and their hydrolysates are currently a growing trend, due to their potential benefits on health, low cost and minimal adverse effects. Grains contain many bioactive compounds, such as carbohydrates, lipids and proteins; the latter can be cleaved by chemical hydrolysis or enzymatic processes and generate smaller fragments named peptides. The continuous attack of pathogenic microorganisms and viruses on humans makes necessary a continuous search for new antimicrobial and antiviral molecules; in this respect, several studies have found antimicrobial and antiviral properties in some peptides, with some of these deriving from underutilized grains. In respect to this, an exhaustive bibliographic search of these findings was performed by the use of the online search engines NCBI, Google Scholar and Google. Therefore, this chapter aims to describe the state-of-the-art scientific findings about the effects of peptides proceeding from underutilized grains against microorganisms, particularly focusing on the antiviral potential effects.

Keywords: Antiviral, Antimicrobial, Biofunctionality, Cereals, COVID-19, Enzyme, Fermentation, Functional, Health, Hydrolysates, Legumes, Nutraceutical, Plant-based food, Peptides, SARS-CoV-2, Virus.

INTRODUCTION

Viruses are a vast and heterogeneous group. Hitherto, the discussion about if they should be considered alive microorganisms continues; at present, the consensus is that they are obligate intracellular parasites that coexist with all living beings.

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Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

They are composed of a genetic material and a capsid – which can be covered by structural proteins-. They are able to alternate between two states: extracellular or inactive and intracellular or active [1]. During the last few years, the SARS-Co--2 – virus that causes COVID-19 – has become highly relevant in the life of the global population. It belongs to the Coronaviridae family, at the Nidovirales order, which includes a big number infectious viruses, which generate respiratory or gastrointestinal disorders in numerous species of birds and mammals. The members of this viral family are characterized by the projection of their surface proteins in a spike-like form [2] and because of their positive-sense single-stranded RNA genomes [3].

Since human beings are in constant contact with microorganisms, such as virus, bacteria and fungi, some of them pathogenic, there has been a constant search for ways to evade, control and cure the diseases they produce. Since good health is of paramount importance to humans, this search is a priority for the well-being of the population worldwide. At present, there is a growing interest in functional foods; which are products that besides providing nutrients, have biologically active components with health benefits, with no adverse effects and which can contribute to reduce the incidence of several diseases [4].

Legumes are a low-cost food source and currently there is great interest in them for their high content of phytochemicals, which are chemical compounds synthesized by the plant itself, with some of them having secondary metabolites with biofunctional effects [5]. Cereals are the traditional base of food worldwide and their consumption is considered important for human well-being [6].

As mentioned before, functional foods possess biological properties associated with health benefits (nutraceuticals); these benefits also comprise protection against viral infections. In this respect, it has been found that bioactive peptides produce beneficial physiological effects that can be helpful in the treatment of immune, antimicrobial, cardiovascular, gastrointestinal and neurological disorders, among others [7]. Small biofunctional peptides (from 3-20 amino acids) are defined as inactive amino acid sequences at the interior of the precursor protein, which exert several biological effects after their liberation by chemical or enzymatic hydrolysis, and which can influence cell metabolism and/or act as vasoregulators, growth factors, hormonal inducers and neurotransmitters, among other effects [8].

ANTI-COVID-19 POTENTIAL REPORTED IN PEPTIDES FROM PSEUDOCEREALS

Pseudocereals are considered underutilized food sources with dicotyledonous seed, such as amaranth, chia, and quinoa, among others. They have high nutritional values; *e.g.* quinoa has a high content of proteins and essential amino acids; in this respect, Rangaswamy and co-workers [9] focused on the bioactivity of quinoa. They identified and characterized its proteins by simulating an *in vitro* gastrointestinal digestion *in vitro*, performing proteomic analyses and using bioinformatic tools. Likewise, they showed that its proteins have *in vitro* antioxidant potential and generated a library to explore more potential bioactivities. Due to the COVID-19 pandemic, and also based on previous studies, the authors evaluated the interaction of the Angiotensin Converting Enzyme-2 (ACE2), which is a functional receptor for the SARS-CoV-2 virus. In this sense, it has been posited that the inhibition of the binding between the SARS-CoV-2 spike protein and ACE2 can be an effective strategy in the treatment of COVID-19 patients [10]. In this study, it was found through molecular docking that the quinoa peptide NWRTVKYG was the most effective one, since it showed the capacity to stabilize the ACE2-Spike composite, showing the potential of these peptides in the treatment of COVID-19 patients.

ANTI-COVID-19 POTENTIAL REPORTED IN PEPTIDES FROM COCOA BEAN

Theobroma cocoa L. produces the cocoa bean, in which the content of polyphenols and their health benefits have already been described to date. Some studies have shown that cocoa supplementation decreases oxidative stress in blood plasma. It also contains proteins such as albumin, globulin, prolamin and glutelin, which can be cleaved at various pH into hydrophilic and hydrophobic peptides, thus suggesting, that it contains bioactive compounds with the potential to be used as dietary supplements and as a source in the production of functional foods and nutraceuticals. Therefore, cocoa (*T. cocoa* L.) grain has been investigated for its inhibitory effect on ACE and for its antioxidant potential. By using two clones of cocoa grown by Malaysian farmers, Sarmadi and co-workers [11] demonstrated this inhibitory effect on ACE activity by first eliminating fats, alkaloids and polyphenols, and subsequently, generating an autolyzed at pH 5.2-3.5, obtaining oligopeptides with hydrophobic amino acid residues. Autolyzates at pH 3.5 excelled in ACE inhibition to those obtained at pH 5.2. They found a significant and moderate correlation between protein content and ACE activity. IC_{50} values ranged from 3 to 9.7 mg/ml. It is also possible that other compounds such as alkaloids and oligosaccharides may contribute to the enzyme inhibitory activity of cocoa autolyzates; however, their results suggest that the ACE inhibitory effect could be the product of cocoa protein hydrolysis, indicating that the high amount of hydrophobic amino acids may be responsible for the antioxidant and ACE inhibitory effects. . In this respect, it is known that the most clinically used ACE inhibitors do not inhibit ACE2; however, both proteins share a high percentage of homology and the affinity of these biopeptides from cocoa to

SUBJECT INDEX

A

Erik G. Tovar-Pérez & Agustin Lugo-Radillo (Eds.)

B

C

D

E

F

G

H

O

P

R

S

T

U

V

Erik G. Tovar-Pérez

Erik G. Tovar-Pérez is the Food Engineer with an MSc. and a PhD in Biotechnology from the Metropolitan Autonomous University [Universidad Autónoma Metropolitana (UAM-I)], at which he was awarded with the Medal of University Merit, the Yearly Prize for Research "Dr. Christopher Augur" and the Prize "Support to Research Projects in Nutrition" by the Institute of Nutrition and Kellogg´s Health. He was a Postdoctoral Fellow at the Food Technology Unit of the Center for Research and Consulting in Technology and Design of the State of Jalisco (CIATEJ, A.C.). At present, he is a Professor in the Researchers for Mexico program by the Science and Technology National Council (CONACYT), commissioned to the Faculty of Engineering of the Autonomous University of Queretaro, Amazcala Campus, where he collaborates with the Biosystems Engineering Group in projects related to the development of metabolites inducing factors to generate bioactive compounds. He is a level 1-member of the National System of Researchers (SNI). His research is focused on the study of the potential health benefits of biologically active peptides from underutilized vegetable grains in Mexico.

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Agustin Lugo-Radillo is the Medical Doctor with an MSc. and a PhD in Molecular and Genetic Medicine from the University of Sheffield. During his doctoral studies he was awarded, by the Ellison Medical Foundation, a full scholarship for the Molecular Biology of Aging course at MBL. He is an expert in aging and anti-aging Medicine. After his PhD, he was a Principal Investigator at the National Institute for Geriatrics (INGER). At present, he is a Professor in the Researchers for Mexico program by the Science and Technology National Council (CONACYT), commissioned to the School of Medicine and Surgery of the Benito Juárez Autonomous University of Oaxaca. He is a level 1-member of the National System of Researchers (SNI). His research is focused on to discover novel molecules and molecular strategies to delay, slow and reverse aging and chronic or degenerative diseases.