MSc THESIS

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Evaluation of protein nutritional value of commercially available protein bars

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1 Introduction

Protein bars are a popular food choice for individuals looking to increase their protein consumption, gain muscle mass, or as a convenient on-the-go snack. These bars are easily found in grocery stores, fitness facilities, and internet, with a variety of flavors, ingredients and nutritional profiles. Protein sources like whey protein, soy protein, casein protein, or pea protein are typically used to make protein bars. Personal tastes, nutritional demands, and fitness objectives can all influence protein source selection. The type of protein utilized can have a significant impact on the nutritional value of protein bars. Also, the unique composition of different proteins may have diverse impacts on physiological function inside the human body. The nutritional advantages of a protein are contingent upon its quality. The quality of a protein is evaluated by analyzing its essential amino acid content, as well as examining its digestibility and the bioavailability of its amino acids.

Different protein sources have variable amino acid compositions and digestibility, which might impact the body's efficiency to utilize the protein effectively. Besides the type and quality of protein, the amount of protein per serving is a big factor to consider. Animal and plant protein sources exhibit variations in their molecular structure, amino acid composition, digestibility, and functional attributes such as gelling and emulsifying capacities. These differences have an influence on the bioavailability and sensory qualities of a food.

Protein quality evaluation techniques include *in vivo,* And *in vitro*. *In vivo* methods include expensive and time-intensive examinations, while *in vitro* approaches provide quicker assessments. Infogest, a multi-enzyme assay, provides a more precise simulation of human digestive processes compared to single-enzyme testing, which helps in predicting digestibility. These diverse methods, including those based on amino acid concentrations and digestibility, play a crucial role in evaluating protein quality for designing healthy and sustainable diets.

Understanding the protein quality of protein bars made from diverse protein sources is critical because it has a substantial influence on the physicochemical characteristics, nutritional value, and structural aspects of the finished product. Various protein types, such as wheat, sunflower, and pea, may cause changes in the texture, water activity, viscosity, color, amino acid content, and microstructure of high-protein bars. Furthermore, protein quality is important for developing healthy and sustainable diets

because it effects protein source complementarity, meal-level protein consumption, and total dietary protein quality. As a result, understanding the quality of protein from various sources is essential for creating healthy and attractive protein bars.

The objective of my research is to properly assess the nutritional value of protein bars on the market today. To do this, I conducted an extensive assessment of a wide range of protein bars, using data carefully obtained from OpenFoodFacts, four protein bars were chosen based on their different protein sources (plan, animal, combinations of plan and animal). Furthermore, to obtain insight into how the protein composition of these bars behaves during digestion, I used the Infogest digestion simulation approach. This cutting-edge technology enabled a thorough knowledge of the breakdown process, revealing insight on the bioavailability of protein components. By taking these intricate components into account, my study seeks to enable consumers to make educated decisions when choosing protein bars, ensuring they not only enjoy a tasty snack but also benefit from a nutritious selection.

2 Literature

2.1 Protein bars

Protein bars are food products that offer a convenient supply of protein while being low in carbohydrates and fats. These bars usually have more protein content (>20 g of protein per serving) with some variations in other macronutrients such as carbohydrates and lipids (Keyser & Zielinski, 1976). Protein bars may include a variety of ingredients such as protein powder, collagen protein, peptides, amino acids, gelling agents, flavoring agents, sweetening agents, syrups, honey, edible and medicinal components, dried food materials, chocolate, grease, glycerin, and prebiotic additives. They show varying textures and consistencies, with some ones prioritizing the preservation of a consistent texture over a period of time. These bars are designed to provide advantages such as enhanced athletic performance, decreased tiredness, and a wide range of capabilities (Banach et al., 2014).

Protein bars were originally developed with particular intentions. The formulas were designed to meet the demands of people with different requirements, including the reduction of blood sugar levels in persons with diabetes and hyperglycemia who participate in physical activities (Jovanov et al., 2021). Subsequently, they have been developed to provide a practical and effective protein and energy source with a combination of high in nutrients ingredients such soy protein, whey protein, and dietary fiber, strategically aimed at aiding in post-workout recovery and alleviating fatigue (Keyser & Zielinski, 1976). The variety of protein bar compositions demonstrate the flexibility and capacity to adjust of this practical and nutritious snack choice. Furthermore, this evolution of protein bars is shifting to accommodate various dietary demands, reflecting a growing trend towards individualized nutrition. In addition, the marketing of protein bars has evolved to highlight sustainability, nutritional advantages, and product traceability in order to meet customer expectations for environmentally-friendly and health-conscious goods (Kirkpatrick & Marshall, 2022). These adjustments emphasize how the manufacturer is adapting to changing customer tastes and the significance of meeting various dietary requirements via inventive product development and marketing techniques.

2.2 Protein sources

Protein bars derive their protein content from various sources, catering to different dietary preferences and nutritional needs.

2.2.1 Animal Sources

Animal protein is found in beef, dairy products, eggs, fish, lamb, pork, poultry, seafood and products that are made from these foods. Animal proteins are often regarded as a source of complete protein due to the presence of all the essential amino acids required by the human body (Marcus, 2013a). Essential amino acids (EAAs) are amino acids that are not able to be produced by the human body and so must be acquired via dietary sources. The amino acids that make up the list consist of phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine (Yiğit et al., 2023). Leucine, isoleucine, and valine are branched-chain amino acids (BCAAs) that are crucial for muscle recovery and post-exercise nutrition in sports performance (Martínez Sanz et al., 2018). These amino acids have unique properties and are considered important.

2.2.1.1 Whey protein

Whey protein, an important component of a number of nutritional supplements and food products, is mainly obtained from milk, which is a secondary product of cheese manufacturing (Soltani et al., 2017). This substance is high in protein and includes a range of nutrients such as proteins, lactose, minerals, and vitamins, which makes it a useful source of nourishment (Bleoussi et al., 2020). Whey contains important proteins such as β-lactoglobulin and α-lactalbumin, which play a significant role in its nutritional composition.

The quality of whey protein is assessed based on many variables, such as its amino acid composition, digestibility, and the presence of essential amino acids (BLAZIC et al., 2018). These variables impact the body's ability to efficiently use whey protein for different physiological activities. Whey protein is offered in several forms, such as concentrates, isolates, and hydrolysates, each with specific functions in commercial applications.

An important use of whey protein is in baby formula, where it has a vital function in replicating the protein makeup of human breast milk (Yiğit et al., 2023). Infant formula may be enhanced by adding whey protein, which offers a well-rounded and readily digested nutritional supply for babies, promoting optimal growth and development. This emphasizes the significance of whey protein in nutritional formulations and its role in fulfilling the dietary requirements of various populations.

2.2.1.2 Milk protein

The main sources of milk protein consist of casein, which makes up 80% of the total protein content, and whey proteins, which make up the remaining 20%. Casein plays a vital role in the production of cheese, while whey proteins are used in a variety of processed foods because of their nutritious value (Dimitrovska et al., 2016). Milk proteins include a high amount of essential amino acids, which makes them beneficial for fulfilling the body's nitrogen and vital amino acids needs.

Several variables affect the quality of milk protein, which in turn has an effect on consumer well-being. Processing techniques like as heating may result in denaturation, aggregation, and chemical alterations of amino acids in milk proteins, which can impact their capacity to be digested and the availability of amino acids (Pellegrino & D'Incecco, 2022). Therefore, controlling processing conditions is crucial to preserve the quality and nutritional benefits of milk proteins.

2.2.1.3 Egg white protein

The nutritional advantages and functional features of egg white protein make it a great addition in protein bars. Studies suggest that egg white protein has been shown to improve the thickness, durability, and protein concentration in food, making it an appropriate choice for protein bars (Karami et al., 2019). In addition, egg-white protein peptides are used in enteral nutrition preparations, providing readily digested and complete nutrients, which is particularly helpful for people with intestinal dysfunction.

The physicochemical composition, hydrolysis, denaturation, and functional characteristics are among the elements that affect the quality of egg white protein (EWP). Various studies have emphasized crucial elements of EWP quality. Meziani

et al. conducted an assessment of the physicochemical composition and protein content of egg white protein (EWP) from different bird species. Their findings revealed differences in the kinds and amounts of proteins present (Samira et al., 2021). Yan et al. showed that electron beam irradiation may increase the hydrolysis of EWP without causing any harm to its secondary structure, hence enhancing its functional characteristics (Jin et al., 2017). According to Asaithambi et al., the use of hydrodynamic cavitation treatment resulted in improvements in the functional, rheological, and structural characteristics of EWP. This treatment also enhanced the digestibility of EWP and made it more suitable for different culinary applications (Asaithambi et al., 2022). These studies indicate that the quality of EWP may be improved by different treatments and analytical methods.

2.2.2 Plant Sources

Plant proteins are found in legumes, nuts, vegetables, seeds, some fruits and the products that are made from these foods. Plant proteins are classified as incomplete or of poor quality due to their lack of some amino acids required by humans. Certain vegetable proteins possess a unique characteristic of that includes all of the necessary amino acids. They involve soybeans as well as two types of grains, namely amaranth and quinoa (Marcus, 2013b).

2.2.2.1 Soy protein

Soy protein, obtained from soybeans, is a versatile biomaterial that has many uses in numerous sectors. Due to its high-quality protein content, it serves as a vital source of plant-based protein (Shankar et al., 2023). Moreover, research has shown that include

Soy protein in protein bar recipes might improve insulin resistance, especially when replacing whey protein in different ratios (Jovanov et al., 2021b). In general, soy protein has a substantial impact on improving the nutritional composition and functional characteristics of protein bars.

The nutritional composition of soy protein varies considerably based on the method of the synthesis process. Various processing techniques have an effect on the protein quality, digestibility, and functional characteristics of soy products. Research has

shown that various processing techniques, such as ultrafiltration, acid precipitation, and heat treatments, roasting, boiling, de-hulling, and drying, may modify the protein, carbohydrate, and fat levels in soybeans, hence impacting their nutritional composition (van den Berg et al., 2022). Hence, it is important to give thorough thought to processing methods in order to maintain the quality and bioavailability of soy protein in protein bars.

2.2.2.2 Peanut protein

The nutritional content of peanut protein makes it a great addition in protein bars (Ağagündüz et al., 2023). Peanuts have become known for their substantial protein content and rich amino acid profile, making them an appropriate source of plant-based protein.

Various variables may alter the protein content and quality of peanuts. The protein content and structure may be modified by storage conditions, including temperature and time (Liu et al., 2022). Furthermore, the biochemical quality of peanuts is influenced by environmental conditions and genotypic characteristics. Certain genotypes exhibit differences in oil, protein, carbohydrate, and sugar levels, which in turn alter the ultrastructural qualities associated with these components (Sun et al., 2022). To improve the protein content and quality of peanuts, it is essential to have a thorough understanding of several elements such as storage conditions, genetic features, and environmental impacts. This knowledge is necessary for implementing specific breeding and cultivation techniques.

2.2.2.3 Almond protein

The nutritional advantages and cost-efficiency of almond protein make it a great component for protein bars. Multiple studies have emphasized the efficacy of almond protein powder in formulations (Wanxin Cao, 2005), highlighting its appropriateness for individuals of all age groups, absence of negative effects, and capacity to improve physical fitness and immunity. However, almonds also contain allergenic proteins that have the potential to cause a range of allergic responses, from moderate to severe and even life-threatening. In addition, almond protein bars have been formulated to include vital nutrients such as vitamin E and unsaturated fatty acids, which provide

advantages in preventing cancer and cardiovascular diseases (Du Zengpeng, 2013). By using almond protein in protein bars, a healthful and well-liked snack choice can be offered.

Several variables may impact the protein quality of almond protein bars. Factors such as the specific extraction process used, the digestibility of proteins, the potential to cause allergies, the composition of amino acids, and the solubility of proteins all have significant impacts (Furlan Goncalves Dias et al., 2023). Understanding these factors is essential for formulating almond protein bars with optimal nutritional quality and allergenic safety.

2.2.2.4 Pea protein

Pea protein (PP) is a widely used component in protein bars because of its elevated protein concentration and advantageous effects on health. PP is obtained from yellow split peas and is recognized for its anabolic characteristics, strong satiety impact, and ability to reduce blood pressure (Kaden Stilling, 2020). Pea proteins are offered in many forms including flour, concentrate, and isolate. The isolate form contains more than 80% protein. The PPI snack bars, which were developed using INMUCAL-Nutrients V.4.0, included a protein content of 20-25g per 100g sample. These bars offered a well-balanced mix of carbs, proteins, and fats, ensuring a harmonious distribution of energy (Kushal Narayan Chandak, 2019).

The quality of pea protein in protein bars is greatly influenced by the production method. The solubility and heat stability of pea protein isolate (PPI) are affected by several parameters, including protein content, pH, ionic strength, and heat treatment settings. Promising options for enhancing these qualities use optimization techniques (Hall & Moraru, 2021). Incorporating advanced methods such as Lorentz force-assisted charge carrier separation may improve the quality and quantity of pea protein concentrates, so making them more acceptable for inclusion in protein bars (Bogahawaththa et al., 2019). Hence, it is essential to comprehend and enhance the production procedures to maintain the quality of pea protein in protein bars.

2.3 Protein

2.3.1 What is protein?

Proteins are crucial nutrients for the functioning of the human body. They are the main structural component of muscle and other tissues in the body like hair, muscle, enzymes, and more. They play a role during the synthesis of hormones, enzymes, and hemoglobin. Proteins can serve as an energy source but are not the most common choice (Hoffman & Falvo, 2004).

Proteins are intricate compounds composed of lengthy sequences of simpler entities known as amino acids. Amino acids are connected by peptide bonds to create polymer chains. The polymer chains form the fundamental structure of the protein (Khavinson et al., 2015). The arrangement and order of amino acids along the chain, controlled by the peptide bonds, play a role in defining the protein molecule's folding into its three-dimensional structure. Protein folding is crucial since it ultimately governs the function and stability of the protein. Peptide bonds serve the dual purpose of linking amino acids and enhancing the overall structural integrity of proteins. The proteins rely on the presence of these components to provide the required stability and adaptability, enabling them to assume their distinct structure and perform their biological activities with high efficiency (Schiene-Fischer et al., 2011).

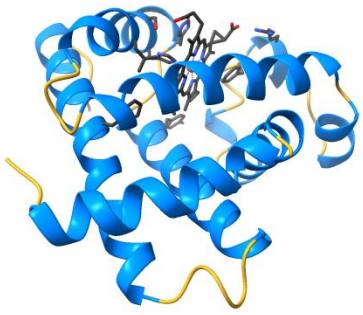

Figure 1. A 3D structure of the protein myoglobin (Xie et al., 2021)

There are nine amino acids referred to as essential amino acids because they are essential for human health, but the body cannot synthesize them independently. Therefore, it is crucial to consume foods that contain these essential amino acids to

prevent deficiencies and maintain optimal health. They are histidine, lysine, leucine, isoleucine, valine, threonine, tryptophan, and methionine.

Humans are capable of producing five different amino acids in their bodies. Alanine, aspartic acid, asparagine, glutamic acid, and serine are the five. Six amino acids are conditionally essential, meaning that their synthesis may be restricted under certain pathophysiological circumstances, such as newborn immaturity or acute catabolic distress. Arginine, cysteine, glycine, glutamine, proline, and tyrosine are the six (Trumbo et al., 2002).

2.3.2 Amino acids

Proteins are composed of monomers known as amino acids. The essential structure of each amino acid is same, including a core carbon atom, referred to as the alpha (α) carbon, that is connected to an amino group (NH2), a carboxyl group (COOH), and a hydrogen atom. Each amino acid is accompanied by an additional atom or set of atoms that are chemically connected to the core atom, often referred to as the R group.

The term "amino acid" is derived from its fundamental structure, which consists of both an amino group and a carboxyl-acid group. As previously stated, proteins consist of a total of 20 amino acids. Nine amino acids are classified as essential in humans due to their inability to be synthesized by the human body, necessitating their acquisition from dietary sources. The R group (or side chain) varies for each amino acid (Flissi et al., 2020).

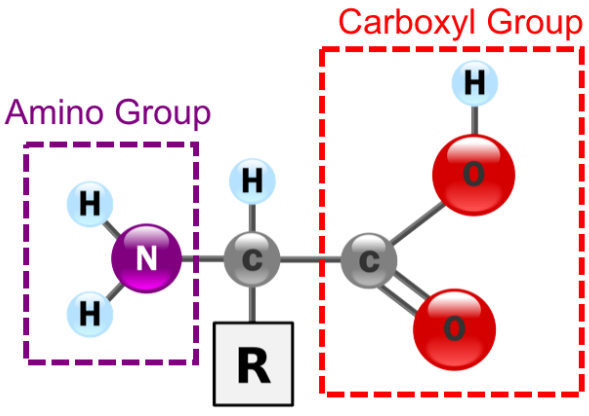

Figure 2. Amino acids possess a core carbon atom that is asymmetric, connecting an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) (Dewangan et al., 2023)

The amino acid's nature, such as its acidity, basicity, polarity, or non-polarity, is determined by the chemical composition of its side chain. The amino acid glycine has a hydrogen atom. Valine, methionine, and alanine are classified as nonpolar or hydrophobic amino acids, while serine, threonine, and cysteine are characterized as polar amino acids with hydrophilic side chains. The amino acids lysine and arginine are classified as basic amino acids due to the positive charge of their side chains. The amino group of proline is connected to the R group, resulting in the formation of a ring-like structure. Proline deviates from the conventional structure of an amino acid due to the absence of a distinct amino group from the side chain (Rye et al., 2013).

The protein's form, size, and function are ultimately determined by the sequence and quantity of amino acids. The covalent link between amino acids, referred to as a peptide bond, is established by a dehydration process. The coupling of the carboxyl group of a specific amino acid with the amino group of the entering amino acid results in the liberation of a water molecule. The bond formed is referred to as the peptide bond (Flissi et al., 2020).

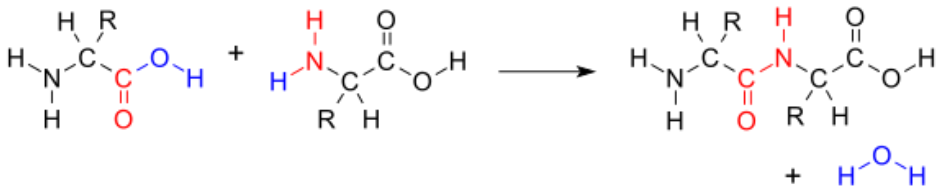

Figure 3. The production of peptide bonds is a process involving dehydration synthesis. The amino group of the entering amino acid is connected to the carboxyl group of one amino acid. During the procedure, a water molecule is liberated (Trapp et al., 2021)

2.3.3 Structure

As previously mentioned, the shape of a protein plays a crucial role in determining its functionality. As an example, an enzyme has the ability to attach to a particular substrate at a location referred to as the active site. In the event of modifications to the active site due to localized alterations or alterations in the general structure of the protein, the enzyme's ability to bind to the substrate may be compromised. In order to

gain insight into the process by which proteins acquire their ultimate shape or conformation, it is essential to comprehend the significance of the four distinct stages of protein structure, namely primary, secondary, tertiary, and quaternary (Rye et al., 2013).

Primary structure: The primary structure is the specific sequence of amino acids in a polypeptide chain.

Secondary structure: The secondary structure of polypeptide chains is generated by local folding into α-helices or β-pleated sheets, supported by hydrogen bonds.

Tertiary structure: A polypeptide's tertiary structure is shaped by chemical interactions between amino acid side chains (R groups).

Quaternary structure: The quaternary structure involves the assembly of multiple polypeptide subunits, stabilized by weak interactions, to form a functional protein complex.

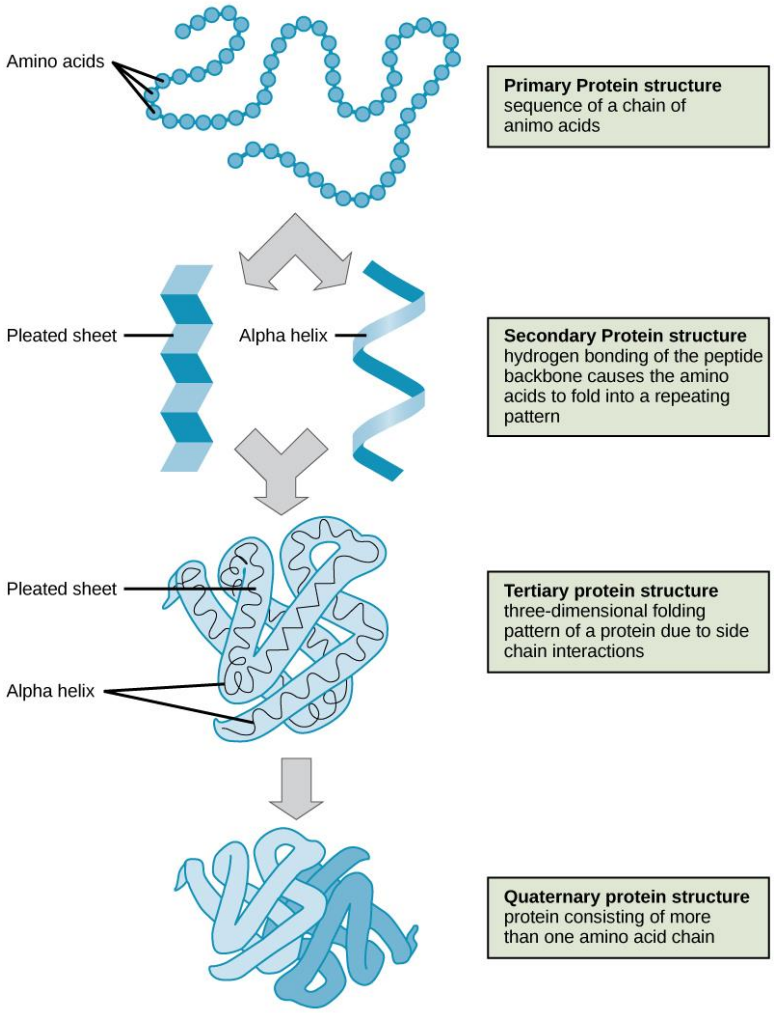

Figure 4. The pictures provided depict the four distinct levels of protein structure (Rye et al., 2013)

2.3.4 Dietary recommendations

The US and Canadian Dietary Reference Intake recommendations state that in order to reduce the risk of deficiency, men and women aged 19 to 70 should eat 56 grams and 46 grams of protein daily, respectively. The 0.8 grams of protein per kilogram of body weight and the average body weights of 70 kg (154 pounds) and 57 kg (126 pounds), respectively, were used to compute these Recommended Dietary Allowances (RDAs) (Trumbo et al., 2002). The European Union (EU) recommends a daily protein intake of approximately 0.8 grams per kilogram of body weight for the average adult. This recommendation is based on the body's daily requirements for amino acids, the building blocks of protein, and is intended to meet the body's needs for growth, maintenance, and repair (Russell et al., 2023).

A number of studies have shown that due to increased muscle mass and sweat losses, as well as the requirement for body repair and energy, athletes and active individuals may need to consume higher amounts of protein (higher than 0.8 g/kg). For endurance exercisers, recommended intake ranges from 1.2 to 1.4 g/kg to 1.6-1.8 g/kg for strength athletes and up to 2.0 g/kg/day for older adults. A maximum daily protein intake of roughly 25% of energy requirements, or 2 to 2.5 g/kg, is suggested (Bilsborough & Mann, 2006).

A diet high in protein may cause an increase in the excretion of calcium in the urine as a means of regulating the pH imbalance caused by the oxidation of sulfur amino acids. This might increase the likelihood that calcium in the renal circulatory system will result in high risk of kidney stones (By Janice R. Hermann, 2021).

2.3.5 Digestion

2.3.5.1 Oral phase

Mastication, the act of chewing in the mouth, is vital for protein digestion since it initiates the breakdown of food into smaller particles, facilitating subsequent digestion. Mastication is the process of using rhythmic jaw motions and coordinated muscular actions to break down food into a bolus that may be easily swallowed (“Neural Mechanisms of Mastication,” 2015). The brainstem provides the central

pattern generator responsible for mastication. This generator produces motor orders for chewing by integrating sensory information from mechanoreceptors and muscle spindles. Its purpose is to optimize the process of food digestion. In addition, mastication includes mechanical actions like as occlusion, mixing, and sensory evaluation, all of which aid in the creation of a bolus that may be swallowed (Gray-Stuart et al., 2012). The salivary glands in the mouth release saliva during the process of chewing. Saliva includes salivary amylase, an enzyme that starts breaking down carbohydrates. It also contains trace levels of lingual lipase, which begins the digestion of lipids. Nevertheless, the process of breaking down proteins does not start in the oral cavity (Shang et al., 2023).

2.3.5.2 Stomach

In the stomach, the process of protein digestion is initiated by the action of gastric juice, a secretion rich in hydrochloric acid (HCl) produced by gastric parietal cells. This acidic environment is crucial for activating pepsinogen, secreted by chief cells, which then converts into its active form, pepsin. Pepsin is a proteolytic enzyme that hydrolyzes peptide bonds within proteins, breaking them down into smaller peptides and amino acids. Concurrently, gastric lipase is also present, facilitating the hydrolysis of fatty acids (Blanco & Blanco, 2022). The gastric phase of digestion is activated by gastrin, histamine, and acetylcholine, which are triggered by the presence of dietary amino acids and the stretching of the stomach walls (Ganapathy, 2012). In summary, the stomach's digestive processes, which include the activities of pepsin and lipase in the presence of hydrochloric acid (HCl), are crucial for the breakdown of proteins into peptides and their subsequent preparation for further digestion in the intestines.

2.3.5.3 Intestinal phase

In the intestinal phase, the pancreatic exocrine glands release crucial digestive enzymes required for further digestion. Trypsinogen, which is secreted as an inactive form, is activated by enteropeptidase in the mucous membrane of the intestine. Trypsin specifically cleaves peptide bonds at the carboxyl end of lysine and arginine residues by hydrolysis. Chymotrypsin, a pancreatic enzyme, breaks down peptide

bonds that are next to aromatic amino acids. Carboxypeptidase catalyzes the hydrolysis of peptides by cleaving amino acids off the carboxyl terminus of the peptide chain, resulting in the release of individual amino acids. Moreover, the lining of the intestines generates enteropeptidase, an enzyme that triggers the conversion of trypsinogen into trypsin. In addition, aminopeptidases catalyze the hydrolysis of peptide bonds located at the N-terminus of proteins. Disaccharidases break down disaccharides, whereas nucleotidases, phosphatases, and nucleosidases break down nucleic acids into nucleotides. Furthermore, the liver synthesizes bile, which consists of cholic and chenodeoxycholic acids. These acids possess the ability to emulsify lipids. Bile facilitates the process of breaking down dietary lipids into smaller droplets, so increasing the surface area available for lipase to work upon, which in turn assists in the digestion and absorption of these fats. Together, these digestive enzymes and bile aid in the decomposition and assimilation of nutrients in the small intestine (Blanco & Blanco, 2022; Ganapathy, 2012; Jahan-Mihan et al., 2011)

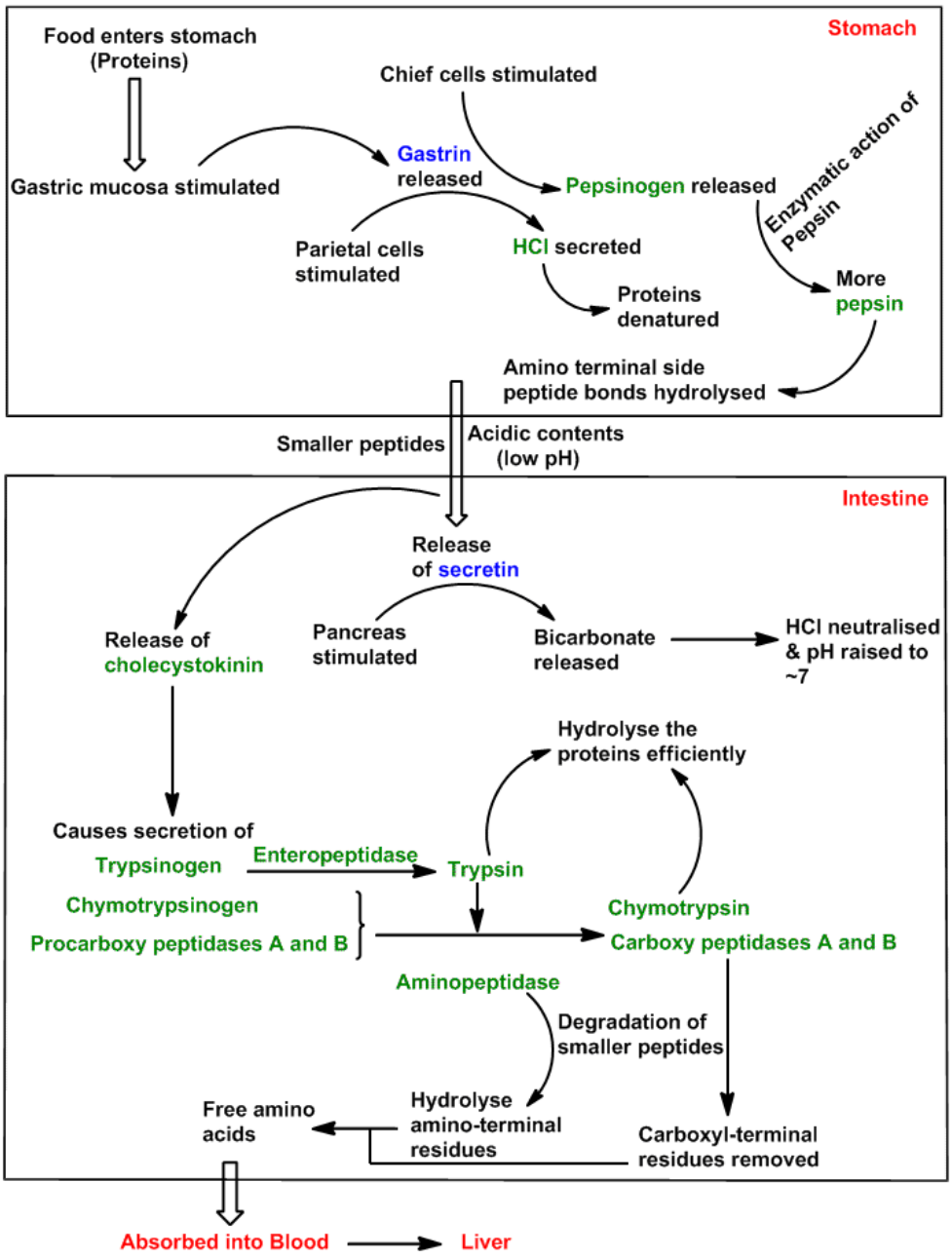

Figure 5. Overview of protein digestion. The organs are shown in the color red. Enzymes are shown in the color green. Hormones are shown in the color blue (Blanco & Blanco, 2022)

When the body takes more amino acids than it needs, the extra amino acids are taken up by the liver, which then deaminates them, turning the nitrogen into ammonia, which the liver then uses to produce urea via the urea cycle. Urea is eliminated by the kidneys. It is possible to turn other amino acid molecule components into glucose and utilize as energy(Bilsborough & Mann, 2006).

2.4 Protein quality

The amino acid content of protein is considered the most crucial element and distinguishing feature from a nutritional perspective. Various methods exist that assess the utility of proteins to an organism by considering their proportion of amino acids and, in some systems, the digestibility of the protein source.

2.4.1 Biological value

Biological Value (BV) is needed to evaluate dietary proteins in protein synthesis efficiency. BV shows how much protein is used for tissue growth and repair rather than waste. Assessing nitrogen (N) intake and excretion.

$$BV = (\text{Retained N} / \text{Absorbed N}) \times 100.$$

It represents the body's retained amino acid ratio, omitting stool protein. BV values over 100 indicate sufficient amino acids for biological requirements, while below 100 indicate insufficient essential amino acids. This formula calculates a protein's % body need satisfaction (McAuliffe et al., 2023).

2.4.2 Net Protein Utilization

Net Protein Utilization (NPU) is a critical measure utilized to assess the efficiency of dietary protein utilization by the body for various physiological functions. Similar to Biological Value (BV), NPU hinges upon measurements of urinary and fecal nitrogen (N) losses, as well as those observed with the consumption of the test protein. The formula for NPU involves the calculation of retained nitrogen relative to consumed nitrogen, thus providing insights into the proportion of ingested protein retained and utilized by the body.

The equation for NPU scrutinizes the extent to which consumed protein is retained by the body, contrasting with BV, which evaluates the retention of absorbed protein. This practical metric holds significance in agriculture, particularly for poultry farming, where NPU values are instrumental in evaluating new feeds and optimizing strategies

for cost-effective poultry meat production. The carcass analysis technique is often employed to determine NPU, facilitating informed decision-making in agricultural practices (Jahan-Mihan et al., 2011)

2.4.3 Protein Digestibility Corrected Amino Acid Score

The evaluation of dietary protein quality involves the use of the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which encompasses the assessment of both the amino acid content and digestibility of the protein. It offers a thorough assessment of protein quality by considering the protein's essential amino acid composition and its efficiency in digestion and absorption by the body.

The (PDCAAS) is determined by comparing the amino acid composition of the protein under investigation to a predetermined set of necessary amino acids defined by the Food and Agriculture Organization of the United Nations (FAO). Subsequently, the score is modified in accordance with the digestibility of the protein, confirmed by examinations including human feeding experiments or chemical analysis. The PDCAAS scale ranges from 0 to 1, where a score of 1 signifies that the protein in question satisfies the necessary amino acid requirements in the appropriate ratios and exhibits a high level of digestibility. A protein source is deemed to possess good quality when it achieves a score of 0.5 or above (Craddock et al., 2021a).

2.4.4 Digestible Indispensable Amino Acid Score

The Food and Agriculture Organization (FAO) launched the Digestible Indispensable Amino Acid Score (DIAAS) in March 2013 as an enhanced approach to evaluate protein quality. The proposition was put out as a substitute for the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which is now regarded as the established mechanism for assessing protein quality.

DIAAS is a notable innovation by including the assessment of amino acid digestibility, particularly at the terminal segment of the small intestine. This methodology offers a more precise representation of the quantity of amino acids assimilated by the organism and the protein's role in satisfying human amino acid and nitrogen needs. On the other hand, the PDCAAS method is dependent on an

assessment of digestibility across the whole of the digestive system, which may result in an overestimation of the absorption of amino acids.

The primary objective of DIAAS is to enhance the accuracy of protein quality assessment by directing attention on the digestibility of amino acids at the terminal stage of the small intestine. Enhanced precision may optimize dietary recommendations and facilitate the use of protein sources that better align with people nutritional requirements (Craddock et al., 2021b)

3 Materials and Methods

The objective of this study was to comprehensively evaluate the protein nutritional quality of protein bars available in the market. This assessment employed a multi-step approach, with a primary focus on utilizing the Infogest digestive simulation technique to mimic human gastrointestinal digestion.

3.1 Materials

The experiments took place at the Buda Campus of The Hungarian University of Agriculture and Life Sciences (MATE) in Budapest, Hungary. Four different commercial protein bars were utilized for the experiments based on the market research results, including: Abso Crispy (28% protein) containing pea protein isolate and rice protein concentrate. BiotechUSA Zero Bar (40% protein) comprising whey protein isolate and soy protein isolate. BiotechUSA Protein Bar (30% protein) containing milk protein concentrate and whey protein isolate. DM Sportness (34% protein) incorporating peanuts and milk protein.

Analytical balances were used for sample measurement, along with pipettes and cylinders for solution transfer, beakers for solution mixing, volumetric flasks for dilution, spoons for solid material transfer, and Eppendorf tubes for solution preparation and storage.

The enzymes and solvent required for the digestibility experiment were obtained from Sigma-Aldrich Company. These included simulated salivary solution (SSF), simulated gastric solution (SGF), simulated intestinal solution (SIF), bile solution (prepared by dissolving 11.4 mg of porcine bile extract in 7.1 ml of SIF), pancreatin stock solution, $CaCl_2(H_2O)_2$, distilled water, HCl, and NaOH.

3.2 Methods

3.2.1 Market research

Open source, and user-added data was pulled from the site OpenFoodFacts.com based on the search for “protein bars”. A total of 4469 protein bars were found, however after excluding protein bars with no ingredient information and those with ingredients

listed in languages other than English, the final dataset had 1661 protein bars which were included into the evaluation. The protein bars were analyzed for their nutritional profile, including protein, lipid and carbohydrate content and for their protein sources used. Analysis was done in Microsoft excel (version 2021). Based on the results four protein bars were selected with different protein sources. These protein bars were used to assess the effect of protein source on protein digestibility of protein bars.

3.2.2 *In vitro* digestion simulation

The evaluation of protein quality in the selected protein bars involved in vitro digestion simulation using the Infogest protocol. The simulation was designed to mimic the three main stages of digestion: oral, gastric, and intestinal.

First, 1 g of each protein bar were transferred to three separate centrifuge tubes, The triplicate samples were prepared to ensure the accuracy and reliability of the evaluation process. The pH of the protein samples was adjusted to mimic the acidic environment of the stomach (pH=3) and the alkaline environment of the small intestine (pH=7).

During the oral phase, the protein samples were mixed with 0.80 ml simulated salivary solution (SSF), 5 µl of $CaCl_2(H_2O)_2$, and 0.195 ml distilled water, mixed and incubated at 37 °C for 2 minutes.

For the gastric phase, 1.28 ml of simulated gastric solution (SGF), 1µl $CaCl_2(H_2O)_2$, pre-measured volume of 6 M HCl, 0.32 ml pepsin stock solution, and distilled water were added to the protein samples (table 1). The mixture was then incubated for 2 hours at 37 °C.

Table 1. The amount of HCl and water that was added (from the experiments).

	HCl Added (ul)	distilled water (ul)
A. Abso Crispy	15	0.384
B. BiotechUSA Protein Bar	25	0.374
C. BiotechUSA Zero Bar	40	0.359
D. DM Sportness	15	0.384

For the intestinal phase, 1.7 ml of simulated intestinal solution (SIF), along with 8 µl (0.001 ml) of $CaCl_2(H_2O)_2$, 0.50 ml of bile solution (prepared by dissolving 11.4 mg of porcine bile extract in 7.1 ml of SIF), and 1 ml of pancreatin stock solution were added to the protein samples. Additionally, pre-measured volumes of 1 M NaOH and water were included (Table 2). The mixture was then incubated for 2 hours at 37 °C to simulate the intestinal phase of digestion.

Table 2. The amount of NaOH and water that was added (from the experiments).

	NaOH Added (ul)	distilled water (ul)
A. Abso Crispy	0	0.792
B. BiotechUSA Protein Bar	0	0.792
C. BiotechUSA Zero Bar	0	0.792
D. DM Sportness	0	0.792

3.2.3 Procedure assessment of protein nutritional quality

3.2.3.1 Methanolic Precipitation

Following the process of digestion, the portion that remained undigested was isolated by the method of methanol (CH_3OH) precipitation. Methanol was introduced into the digests to achieve a concentration of 80 v/v %. The mixture was then vigorously mixed for 30 seconds using a vortex and incubated at a temperature of -20°C for a duration of 1 hour. The samples were thereafter subjected to centrifugation at a speed of 6000 RPM for a duration of 20 minutes at a temperature of 4 degrees Celsius. The supernatants produced following centrifugation were used for the measurement of amino acids and free amino group content.

3.2.3.2 Hydrolysis Procedure for amino acid determination

To prepare, 1000 µL of the supernatant was placed into a 1.5 mL microtube and evaporated using a rotary vacuum centrifuge. The residual material was then dissolved in 6 M HCl (with 1% phenol) for hydrolysis using a Milestone Ethos One microwave oven.

The hydrolysis of all samples was carried out using a Milestone Ethos microwave oven to guarantee a thorough study of amino acids. Two different techniques, customized to meet particular analytical needs, were used. The standard procedure included exposing the samples to a progressive temperature rise of 10 °C per minute until reaching 180 °C, then allowing them to incubate for 20 minutes before cooling. Similarly, the procedure for determining tryptophan followed the same temperature profile. During hydrolysis, the samples were prepared by dissolving them in a borate buffer solution with a pH of 8.51. The amounts used were either 5 mL for powders or 2.5 mL for digests. Afterwards, any solid particles were eliminated via filtering using a 22 µm HPLC filter prior to derivatization. The process of derivatization consisted of combining 10 µL of the sample with 70 µL of borate buffer and 20 µL of Waters AccQTag reagent (AQC). This mixture was then incubated at a temperature of 55 °C for a duration of 10 minutes to facilitate the derivatization process. After the incubation period, the samples that had been treated with a derivative underwent an additional filtering step to purify them. Subsequently, a Waters Acquit UPLC H-Class apparatus, fitted with an AccQ UPLC BEH C18 column (2.1x100 mm, 1.7 µm particle size), was used to conduct chromatographic analysis. The column was kept at a temperature of 43 °C. Each sample was injected with a volume of 10 µL and a flow rate of 0.7 mL/min. A PDA detector, set at a wavelength of 260 nm, was used to detect individual amino acids that were separated. An assessment of both the quality and quantity was performed by using well-established amino acid standards as points of comparison.

3.2.3.3 Free amino group content determination

After digestion, undigested intact proteins and larger peptides are precipitated from the digestive mixture, leaving behind smaller peptides and amino acids. In order to get comparable data hydrolysis of the supernatants were done before determination. To prepare, 220 µL of the supernatant was placed into a 1.5 mL microtube and evaporated using a rotary vacuum centrifuge. The residual material was then dissolved in a mixture of 260 uL of H_2O, 120 uL of 1 M NaOH/ 1% DDP, 120 uL of 2 M HCl and 500 uL of 6 M HCl and placed in a 110 °C drying oven for 15 h.

In this experiment, we prepare a hydrolysis buffer by combining three components. First, we add 260 uL of distilled water to each sample. Then, for the 1% DPP

component, we measure 0.12 g of DPP powder into a centrifuge tube, add 2.4 ml of 1M NaOH, and 9.6 ml of distilled water, and dissolve the mixture using an ultrasonic bath. From this solution, we measure 120 µL and add it to all samples. Next, for the 2M HCl component, we prepare it from 6M HCl by adding 0.4 ml of 6M HCl to 11.6 ml of distilled water in a centrifuge tube, and then we add 120 uL to each sample. Finally, we add 500 uL of pure 37% HCl to each sample. The samples are then placed in a 110°C drying oven for 15 hours. Through the use of the hydrolysis technique, our theory suggests that peptides undergo decomposition into individual amino acids, leading to a solution mostly composed of amino acids.

Using the ortho-phthalaldehyde (OPA) approach, which involves reacting amino acids with OPA to produce molecules that absorb UV-visible light, we were able to measure the amount of free amino acids in the hydrolyzed supernatant. These molecules may be quantitatively analyzed by spectrophotometry since their absorbance is directly proportional to the content of amino acids. Insightful information on protein sample composition may be gained by using this method, which enables the quantitative analysis of amino acids by means of the synthesis of UV-visible molecules.

OPA reagent was made by dissolving 1.905g of borax in a beaker containing 30 ml of distilled water. Ultrasonicate the mixture for 25 minutes. Weigh 50 mg of SDS and 44 mg of DTT separately. Dissolve 1 ml of OPA in ethanol in an Eppendorf tube. Once the borax is dissolved, add the measured SDS and ultrasonicate for 5 minutes. Then I add the DTT and continue ultrasonication for another 2 minutes. Then, add the OPA solution, wash the mixture several times with water, and ultrasonicate for an additional 2 minutes. The mixture was transferred to a volumetric flask and dilute it with water to the 50 ml mark. Warp in aluminum foil. The OPA reagent must be fresh before the measurement.

From each sample, we transferred 50 µl into an Eppendorf tube and diluted it with 250 µl of Trichloroacetic Acid (TCA). Then, from the diluted sample, we took 25 µl and added 750 µl of OPA reagent, allowing it to react for 10 minutes. It's important to note that we conducted two parallel samples for this measurement. Subsequently, we placed 750 µl of each sample in a cuvette for spectrophotometric analysis at 340 nm.

For calibration L-Serine standard was used for that preparing a stock solution by dissolving approximately 25 mg of serine in 5 ml of water in a 15 ml centrifuge tube.

Diluting the stock solution to obtain a second stock solution by combining 400 µl of 5000mg/l stock solution with 1600 µl of water in an Eppendorf tube. The calibration curve acts as an indicator for converting instrument results into meaningful concentration values, guaranteeing the precision and dependability of the analytical technique.

Table 3. Presents calibration data, detailing the absorbance measured at 10 minutes for the mixture of the 2nd diluted stock solution and the 5% stock solution.

2nd stock solution, ul	5% TCA, ul	app. Absorbance (10 min R)
0	1000	0,168
25	975	0,228
50	950	0,254
75	925	0,301
100	900	~~0,125~~
125	875	0,298
180	820	0,395
250	750	0,5

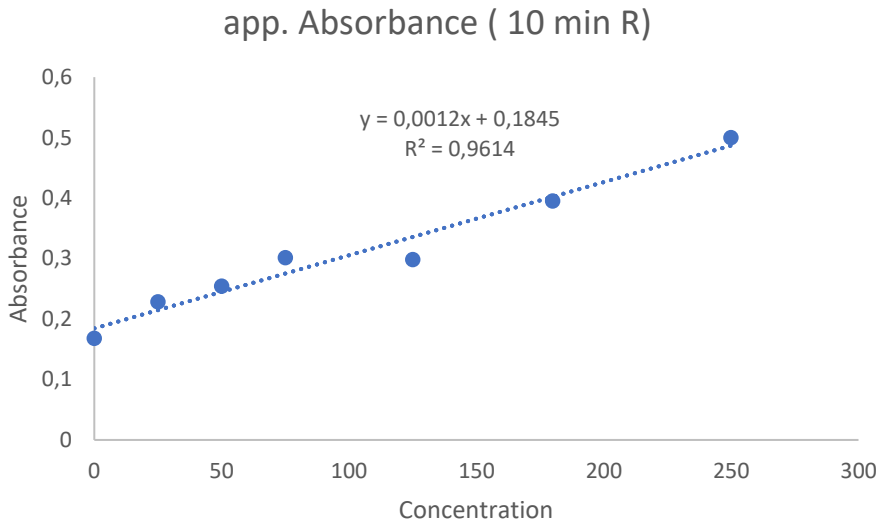

Figure 6. Calibration curve results with the results of y=0,0012x+0,1845, R^2 = 0,9614

3.2.3.4 Digestible Indispensable Amino Score (DIAAS) calculations

Indispensable amino acid (IAA) composition and standardized ileal digestibility (SID) were used to calculate the respective Digestible Indispensable Amino Score (DIAAS) according to the three age categories defined by the Food and Agriculture Organization (FAO) for infants (0-6 months), children (6-36 months), and older children, adolescents, and adults. Protein sources are categorized as excellent quality proteins when their average Digestible Indispensable Amino Acid Score (DIAAS) is over 100. They are considered high-quality proteins when their average DIAAS is equal to or more than 75. Protein sources that do not meet these criteria fall into the category of no quality claim, with a DIAAS score below 75 (Herreman et al., 2020). The DIAAS value of a protein is calculated based on the quantity of the most limiting digestible indispensable amino acid (DIAA).

4 Results and Discussion

4.1 Market research

I started my investigation into the protein bar market by doing thorough market research, with the objective of understanding formulation patterns and dietary preferences. By using open-source data from OpenFoodFacts.com and specifically focused on protein bars, a total of 4469 products were originally found. Nevertheless, in order to guarantee the dependability of the dataset, a specific filtration process was implemented, which included eliminating bars that lacked enough component information or were listed in languages other than English.

The final dataset consisted of 1661 protein bars. Afterwards, I conducted a dimensional experiment to evaluate the effectiveness of different proteins used in these bars. In this section, I will thoroughly examine the precise nutritional compositions of the chosen protein bars and show the results obtained from both the market research and the experimental study.

4.1.1 protein sources

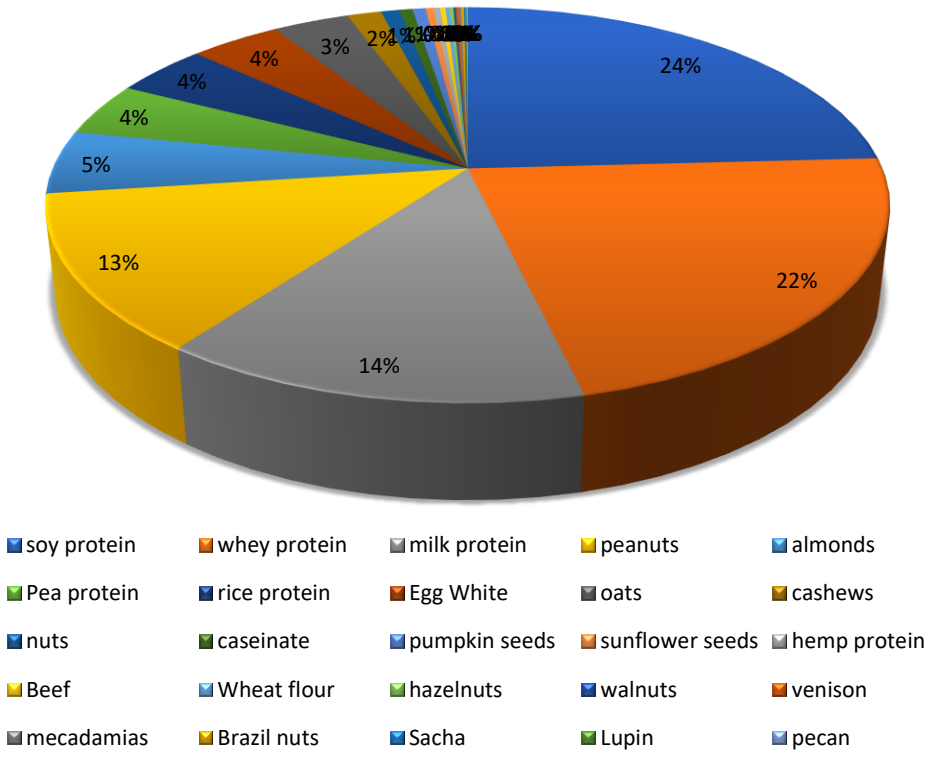

Figure 7. Visualizing protein sources diversity and their percentage

Out of the protein bars analyzed, 58.82% included protein derived from plants, whereas 41.18% contained protein derived from animals. In our analysis, we considered ingredients with a protein content of 20-30% or higher as significant protein sources within the food bars. You can find a detailed breakdown of the protein sources along with their quantities bellow (Table 4).

Table 4. Based on the search results provided, showing the protein sources and their quantities, as well as their classification as either animal-based or plant-based protein sources.

Protein Source	Quantity	Animal-Based	Plant-Based	Percentage of Total
Soy protein	397	No	Yes	23.89%
Whey protein	368	Yes	No	22.15%
Milk protein	230	Yes	No	13.85%
Peanuts	213	No	Yes	12.83%
Almonds	86	No	Yes	5.18%
Pea protein	73	No	Yes	4.40%
Rice protein	71	No	Yes	4.27%
Egg White	69	Yes	No	4.15%
Oats	58	No	Yes	3.49%
Cashews	26	No	Yes	1.57%
Nuts	14	No	Yes	0.84%
Caseinate	10	Yes	No	0.60%
Pumpkin seeds	10	No	Yes	0.60%
Sunflower seeds	6	No	Yes	0.36%
Hemp protein	5	No	Yes	0.30%
Wheat flour	3	No	Yes	0.18%
Hazelnuts	3	No	Yes	0.18%
Walnuts	2	No	Yes	0.12%
Venison	2	Yes	No	0.12%
Macadamias	2	No	Yes	0.12%
Brazil nuts	2	No	Yes	0.12%
Sacha	1	No	Yes	0.06%

Lupin Protein Isolate	1	No	Yes	0.06%
Pecan	1	No	Yes	0.06%
Totale	1661	683 (41.18%)	978 (58.82%)	100%

4.1.1.1 Animal-based protein sources

In this subsection, we delve into the various animal-based protein sources prevalent in the market landscape. By analyzing the distribution and prevalence of these sources, we gain valuable insights into the formulation trends within the protein bar industry. Through examining the utilization rates of key animal-derived proteins such as whey, milk, and egg, we aim to elucidate their respective contributions to the nutritional composition of protein bars.

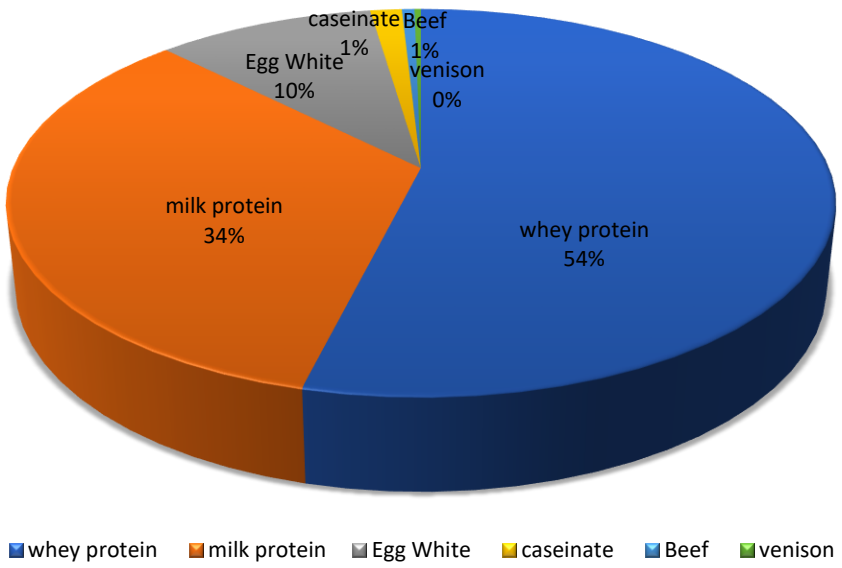

Figure 8. Animal-based protein sources and their percentages

Whey protein emerged as the predominant animal-based protein source, featuring in 22% of the products surveyed. Following closely behind was milk protein, present in 14% of the items, while egg protein accounted for 4%. Additionally, lesser-utilized sources such as caseinate, beef, and venison each constituted less than 1% of the protein bars examined. Beef proteins boost meat product yield, versatility, and

profitability. Venison proteins may boost protein bar rheology and fiber content (Dvoryaninova et al., 2018). The neuroprotective antioxidant peptide (APVPH I) in venison protein was found in by (Kim et al., 2010). This boosts protein bar antioxidants, particularly compared to beef.

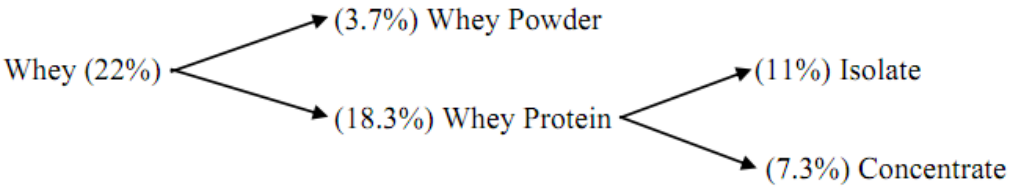

Figure 9. Analysis of whey protein variants in protein bars: powder vs. isolate vs. concentrate

In our analysis, we found that whey protein accounted for 22% of the protein sources in protein bars. This comprises 3.7% whey powder and 18.3% whey protein. Whey powder refers to a processed form of whey protein. Within whey protein, 11% was identified as isolate, while 7.3% was categorized as concentrate. Whey protein isolate is a more refined form containing a higher percentage of protein with minimal fats and carbohydrates. On the other hand, whey protein concentrate retains more of the natural components found in whey, including some fats and carbohydrates. The key difference lies in their processing methods and protein content, with isolate being purer and faster-absorbing, making it favored for post-workout recovery, while concentrate offers a more balanced nutritional profile (Kobelkova et al., 2022).

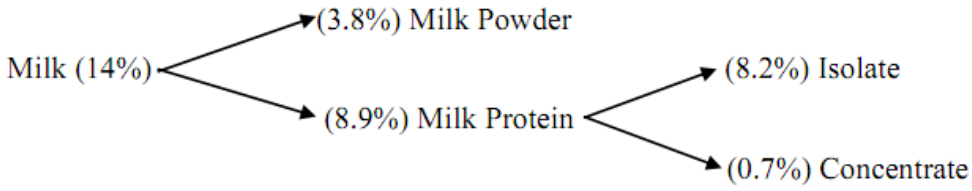

Figure 10. Analysis of milk protein variants in protein bars: powder vs. isolate vs. concentrate.

During our research, we found that milk protein accounted for 14% of the protein sources in protein bars. Particularly, 3.8% of the protein derived from milk powder

and 8.9% derived from milk protein. Milk powder is a dehydrated version of milk, often used as a useful source of dairy protein. Milk protein, obtained from milk, consists of a blend of whey and casein proteins, which results in a gradual and continuous release of amino acids.

Out of the total milk protein, 8.2% was determined to be isolate, while 0.7% was classified as concentrate. Milk protein isolate is a refined version of milk protein, characterized by a greater proportion of protein and low amounts of lipids and carbs. In contrast, milk protein concentrate preserves a greater amount of the inherent constituents of milk, such as certain lipids and carbs (Patel et al., 2022).

4.1.1.2 Plant-based protein sources

In this section, we turn our focus towards plant-based protein sources prevalent in the protein bar market. With growing consumer interest in plant-derived alternatives, understanding the distribution and utilization of these sources provides valuable insights into industry trends.

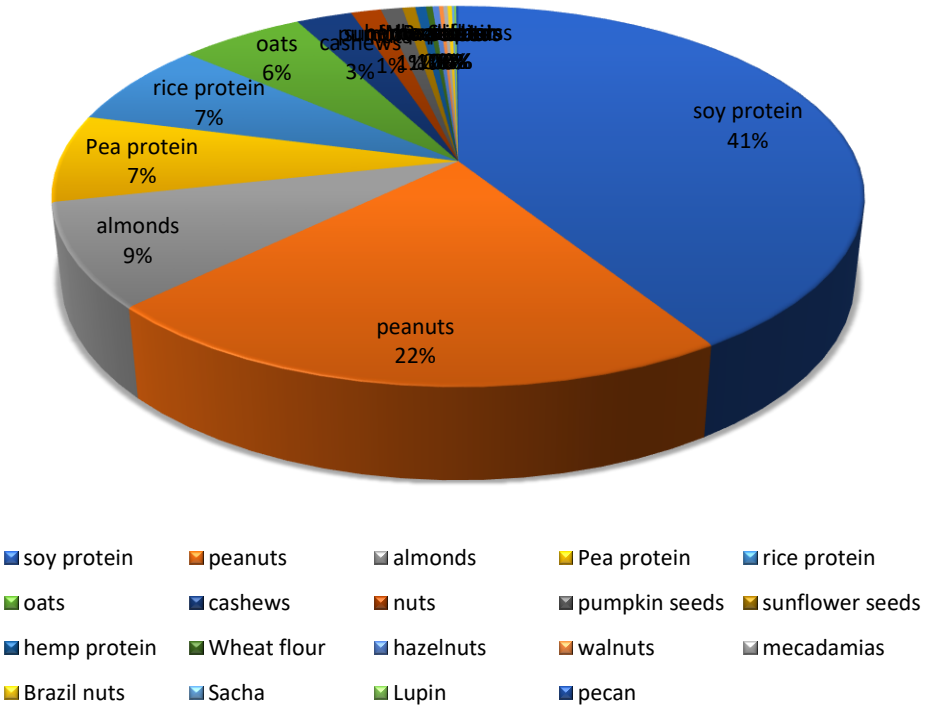

Figure 11. Plant-based protein sources and their percentages

Among plant-based protein sources, soy protein stood out, appearing in 24% of the products. Second most common plant-based protein source was peanuts (13% of items), followed by almonds (5% of items), pea protein (4% of items), and rice (4% of items).

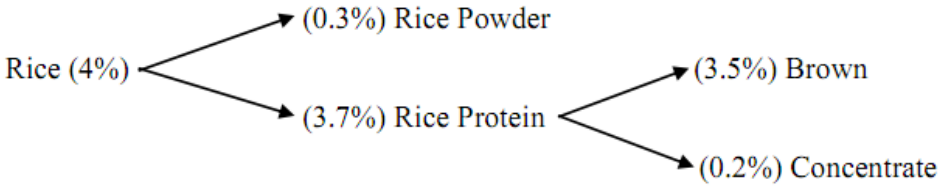

Figure 12. Analysis of rice protein variants in protein bars: powder vs. protein, brown vs. concentrate

In our examination, rice protein accounted for 4% of the plant-based protein sources in protein bars, with 0.3% attributed to rice powder and 3.7% to rice protein. Rice powder is a processed version of rice. Rice protein, obtained from rice, provides a plant-based substitute for proteins produced from animals and is often used in vegan and vegetarian diets. The composition of rice protein was determined to be 3.51% brown rice protein and 0.2% concentrate. Brown rice protein is obtained from the whole grain, preserving a higher proportion of its natural nutrients and fiber in comparison to white rice protein. Rice protein concentrate is a refined version that has a high amount of protein per serving (Zhang & Liu, 2022).

The assessment conducted in the given events examines several protein sources typically used in high-protein bars, including whey, milk, pea, soy, and rice proteins. It's important to note that while the assessment focuses on these specific protein sources, the food sector utilizes a wide range of protein sources with varying qualities. Each protein source brings its own set of attributes to the table, affecting not only the nutritional profile but also the sensory aspects and overall consumer acceptance of high-protein bars (Małecki et al., 2020). Understanding the diverse characteristics of different protein sources is crucial for formulating high-quality protein bars that meet consumer expectations and dietary needs.

4.1.2 Macronutrients

Macronutrients are vital nutrients that provide our body with the necessary energy and building blocks to perform all of its activities. Here, you can see the macronutrient data for various protein bar sources, depending on a 100-gram serving size.

4.1.2.1 Protein Content

In this section, we delve into the protein content of various sources found in protein bars. Through a detailed analysis of protein composition, we aim to discern patterns and variations across different ingredients.

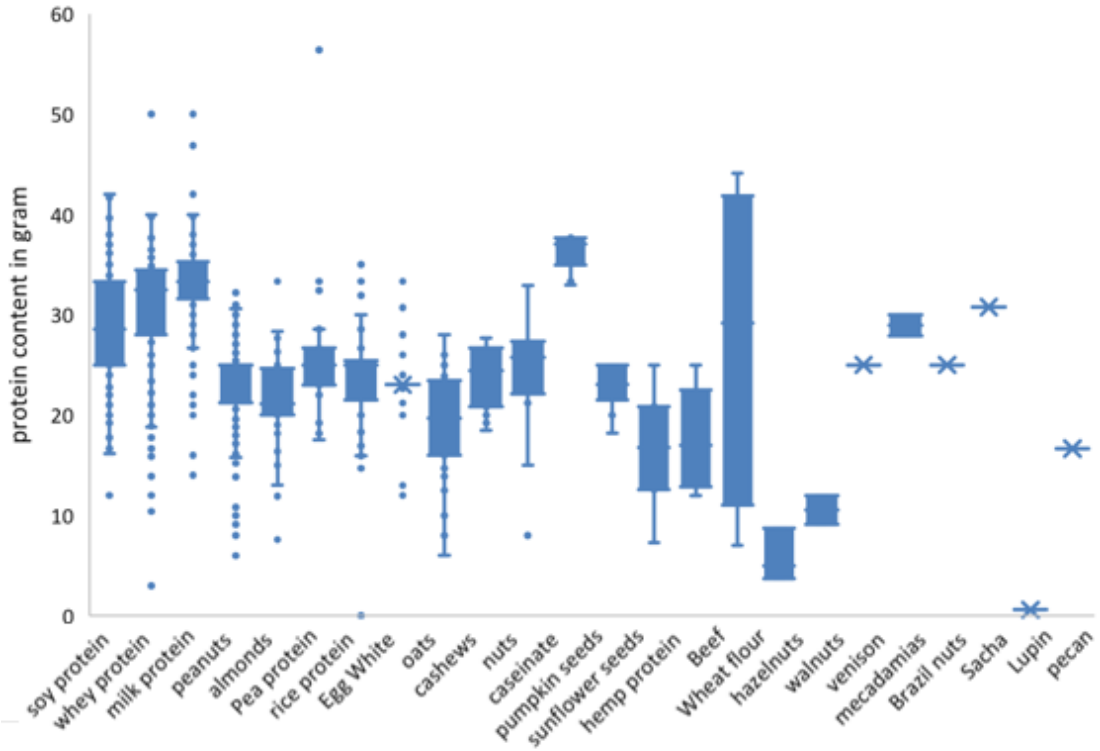

Figure 13. Protein content in protein bars sources

The protein composition of protein bars exhibits significant variability among different sources. Notably, the primary protein sources—soy, whey, and milk protein—demonstrate a consistent range of protein concentrations, typically falling between 15 to 40 grams per 100 grams. Conversely, peanuts, almonds, pea, and rice protein exhibit a narrower range, typically between 20 to 30 grams per 100 grams.

On the contrary, beef protein sources showcase a wide range of protein content, with some variants boasting high protein content while others possess lower levels. Derived from raw materials containing collagen, beef proteins offer notable water-retaining and emulsifying properties, enhancing the texture and structure of food products (Kim et al., 2010).

However, protein bars formulated with wheat flour and lupin protein present notably low protein levels, measuring less than 10 grams per 100 grams. Conversely, caseinate-derived protein bars exhibit exceptionally high protein content, ranging between 30 to 40 grams per 100 grams.

Given that the top 10 protein sources offer the most pertinent insights, our analysis will predominantly focus on these sources, namely soy, whey, milk protein, peanuts, almonds, pea, and rice protein, as well as egg white, oats, and cashews.

4.1.2.2 Carbohydrate Content

In this segment, we explore the carbohydrate content of protein bars sourced from the selected 10 protein origins. Through meticulous analysis, we uncover notable trends and patterns shaping the carbohydrate composition of these bars.

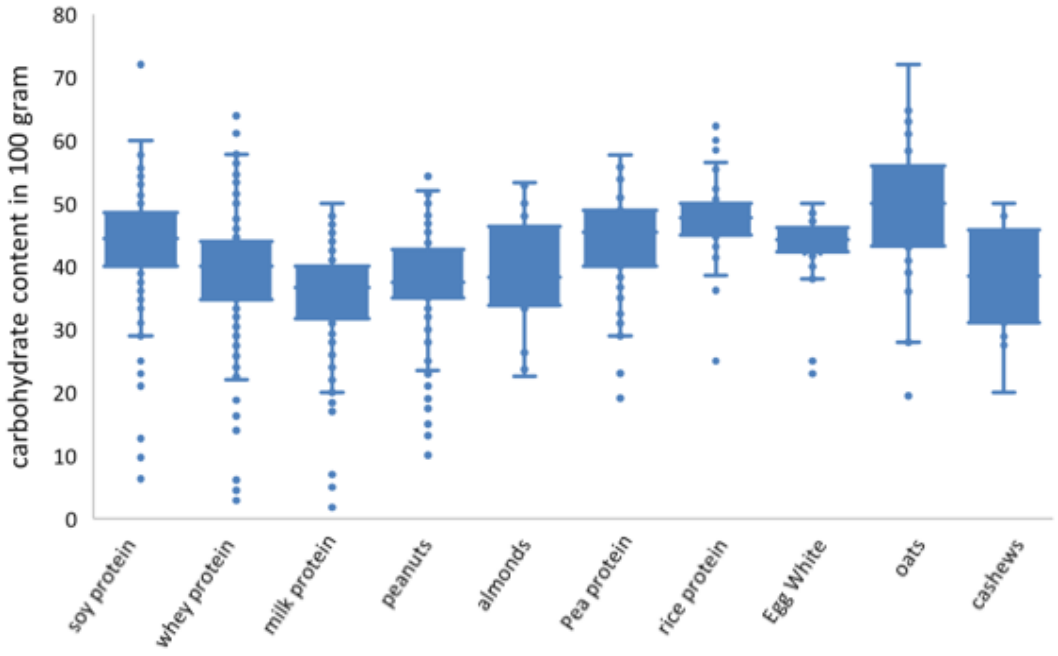

Figure 14. Carbohydrate content in protein bars sources

The analysis of carbohydrate content across various protein sources in protein bars reveals interesting trends. The majority of protein bars, regardless of their protein source, exhibit carbohydrate content within a comparable range of 10g to 60g per 100g. Notably, a significant proportion of protein bars, spanning all sources, demonstrate a narrow range of carbohydrate content, typically falling between 30g and 50g per 100g.

Regarding whey and milk sources, some protein bars exhibit exceptionally low carbohydrate content, measuring less than 10g per 100g. Similarly, protein bars featuring soy and peanut sources also showcase relatively low carbohydrate content, often less than 20g per 100g. Additionally, it's observed that the majority of rice and egg white protein bars sources have similar carbohydrate content, ranging between 40g and 50g per 100g.

This comprehensive assessment highlights the variability in carbohydrate composition across different protein sources and provides valuable insights into the nutritional profile of protein bars.

4.1.2.3 Sugar Content

In this section, we explore the sugar content present in protein bars sourced from different protein origins.

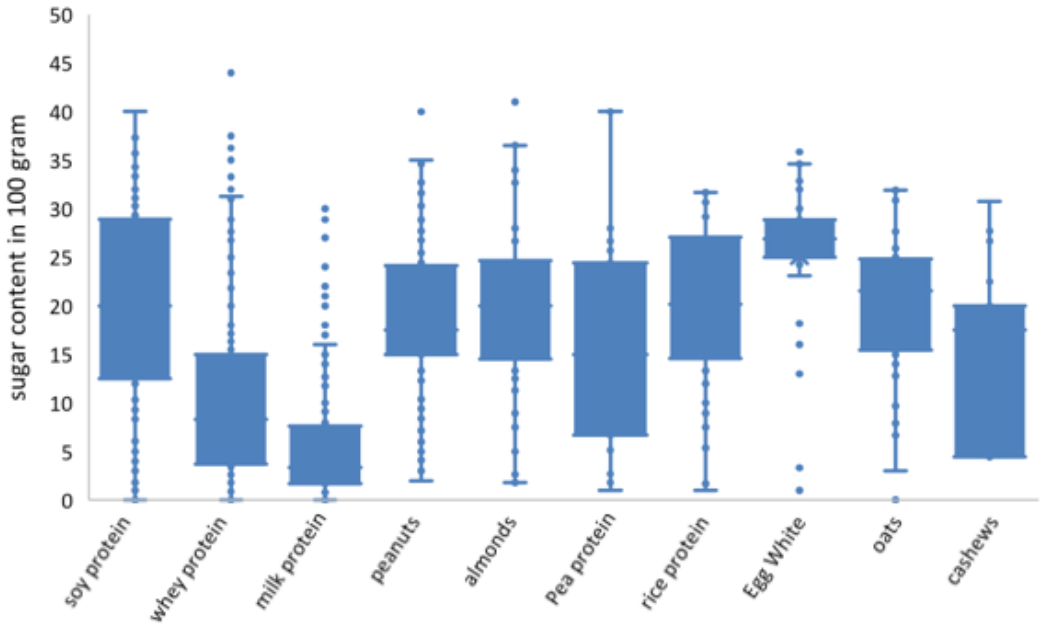

Figure 15. Sugar content in protein bars sources

Examining the sugar level in different protein sources inside protein bars shows significant patterns. Most protein bars have a sugar level that falls between 0g to 30g per 100g, which varies across the majority of products. Nevertheless, certain protein bars derived from soy, whey, peanuts, almonds, and pea protein exhibit elevated levels of sugar, with amounts as high as 40g per 100g. In contrast, it has been noticed that the majority of whey and milk protein bars consist of a low sugar content, usually less than 20g per 100g. This detailed evaluation illuminates the differences in sugar content across various protein sources in protein bars, offering useful insights into their nutritional characteristics.

4.1.2.4 Fat and Energy (kcal) Content

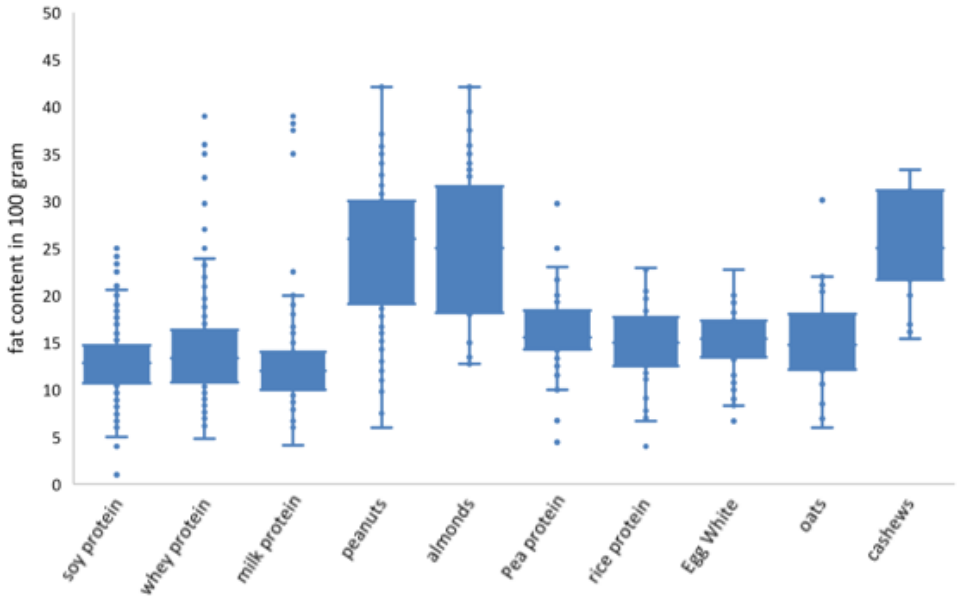

Figure 16. Fat content in protein bars sources

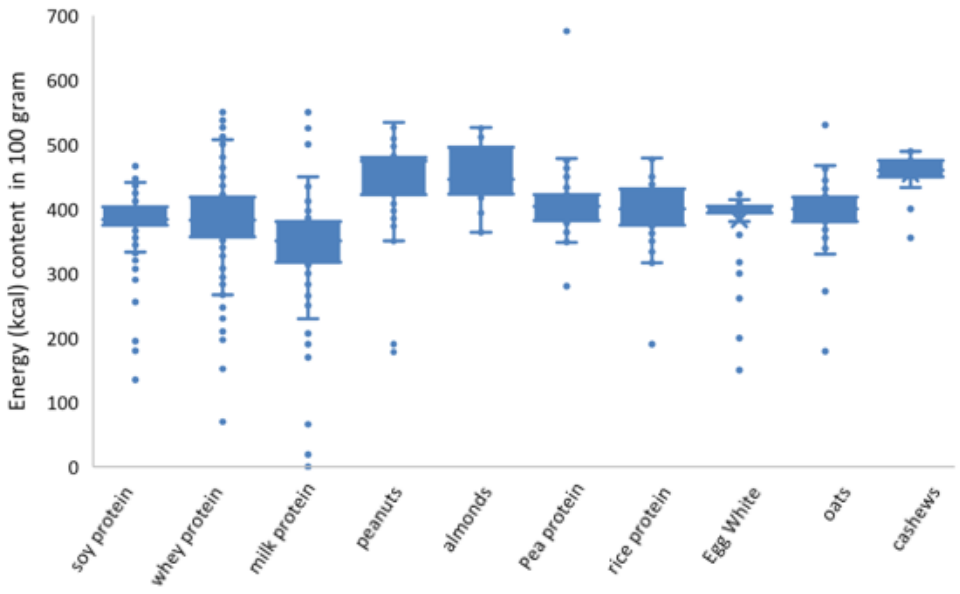

Figure 17. Energy (kcal) content in protein bars sources

The figures revealed notable differences in both fat and energy content across various protein sources found in protein bars. Protein bars containing peanuts, almonds, and certain whey and milk protein varieties were observed to exhibit a high-fat concentration, exceeding 30g per 100g. Conversely, protein bars formulated with soy, pea, and rice protein, as well as those containing egg white and oats, demonstrated a relatively low-fat content of less than 25g per 100g.

In terms of energy content, significant variations were observed among different protein sources. While the majority of protein bars fell within the range of 300 kcal to 500 kcal per 100g, certain bars made from whey and milk displayed very low energy content, measuring less than 100 kcal per 100g. Conversely, protein bars derived from pea protein showcased high energy content, with values reaching almost 700 kcal per 100g.

Upon further examination of the figure, it becomes evident that the first three protein sources soy, whey, and milk protein exhibit low fat content and high energy content, exceeding 300 kcal per 100g. This suggests that energy is derived primarily from protein and carbohydrates, given their high content in these bars. Conversely, peanuts, almonds, and cashews, known for their high-fat content, also displayed high energy content.

Furthermore, protein bars containing pea, rice, egg, and oats protein were found to have low fat content but high energy content, potentially derived from carbohydrates.

This observation underscores the importance of understanding the caloric implications of different protein sources for dietary planning and nutritional considerations.

4.1.2.5 typical pairings

In this section, we delve into the typical pairings of protein sources found in protein bars, as revealed by heatmap analysis (Table 5). Most of the protein bars have at least two protein sources so by examining the frequency and prevalence of these pairings, we gain valuable insights into the formulation trends within the protein bar industry.

Table 5. Heat map of common pairing protein bars sources (my results)

	soy protein	whey protein	milk protein	peanuts	almonds	Pea protein	rice protein	Egg White	oats	cashews
soy protein	0	75	41	78	4	5	0	0	22	0
whey protein	202	0	208	24	12	0	0	0	9	0
milk protein	33	186	0	11	0	0	0	0	0	0
peanuts	48	6	3	0	4	8	6	3	4	0
almonds	25	24	0	34	0	23	15	34	4	0
Pea protein	1	2	5	22	20	0	27	0	8	8
rice protein	12	1	0	12	4	21	0	0	0	5
Egg White	1	0	0	17	12	0	0	0	0	0
oats	2	3	0	1	2	0	13	0	0	0
cashews	5	3	0	0	8	2	2	23	0	0

The heatmap analysis provides insights into the most common pairings of protein sources in protein bars. It reveals that certain combinations are more prevalent than

others, with protein bars paired with soy and whey sources, as well as whey and milk sources, emerging as the most typical pairings. These combinations are found in a large quantity of protein bars, indicating their popularity among manufacturers.

Additionally, soy and milk, as well as soy and peanuts, show an average frequency of occurrence. This suggests that while these pairings are not as dominant as soy and whey or whey and milk, they still represent a significant portion of protein bar formulations.

Furthermore, the analysis highlights specific combinations that are relatively common for certain protein sources. For instance, rice protein bars are often paired with pea and almonds, while egg white protein bars are commonly paired with almond and cashews. Additionally, oats protein bars tend to be paired with soy, whey, or pea sources.

Overall, the heatmap provides valuable insights into the prevalent pairings of protein sources in protein bars, offering guidance for manufacturers and consumers alike in understanding the composition of these products.

4.1.3 Summary of market research

In summary, the comprehensive analysis of protein content, carbohydrate content, sugar content, fat content, energy content, typical pairings, and the formulation of various commercial protein bars provides valuable insights for informed decision-making. Through meticulous examination, it becomes evident that protein bars vary significantly in their nutritional composition, formulation, and pairings of protein sources.

The analysis reveals that protein bars formulated with whey protein isolate, soy protein isolate, and milk protein isolate tend to have higher protein content, often exceeding 30%. These bars also exhibit relatively low-fat content and varying levels of carbohydrate and sugar content, making them suitable choices for individuals focusing on protein intake while moderating other macronutrients. Furthermore, the prevalence of certain pairings, such as soy and whey or whey and milk sources, underscores their popularity among manufacturers. This information offers valuable guidance for understanding formulation trends within the protein bar industry.

Considering the experiment's focus on protein quality, it's essential to highlight the protein sources used in the selected commercial protein bars. Abso Crispy features

pea protein isolate and rice protein concentrate, BiotechUSA Zero Bar incorporates whey protein isolate and soy protein isolate, BiotechUSA Protein Bar contains milk protein concentrate and whey protein isolate, and DM Sportness utilizes peanuts and milk protein.

Based on the analysis and the experiment's objectives, the selection of protein bars would depend on individual dietary preferences, nutritional goals, and ingredient preferences. For instance, individuals seeking a high-protein option with a balanced macronutrient profile may opt for BiotechUSA Zero Bar, while those looking for plant-based options might prefer Abso Crispy.

Table 6. protein bar supplement facts from the protein bar labeling information provided.

Nutrition Information Per Serving (100 g)	**Abso Crispy**	**BiotechUSA Zero Bar**	**BiotechUSA Protein Bar**	**DM Sportness**
Protein source	pea and rice	whey and soy	milk and whey	peanuts and milk
Energy (kcal)	402 kcal	380 kcal	370 kcal	373 kcal
Fat (g)	20.4	15	17	17
of which saturated(g)	8.2	8.50	13	6.8
Carbohydrates (g)	22.3	22	31	26
of which sugars (g)	4.9	1.9	2.6	3
of which polyols (g)	12.8	17		21
Dietary fiber (g)	21.4	12	7.5	10
Protein (g)	28.6	40	30	34
Salt (g)	0.57	0.80	0.57	1.1

The meticulous analysis of various aspects of protein bars provides a comprehensive understanding of their nutritional profiles and formulation trends. By considering these factors alongside individual preferences, informed choices can be made to select protein bars that align with dietary goals and preferences.

4.2 *In vitro* protein digestibility

Evaluating the nutritional quality and efficiency of the protein sources used in commercial protein bars requires a thorough examination of protein digestibility. This

section focuses on the data obtained using the OPA technique to quantify the *in vitro* protein digestibility (IVPD%) of different protein bars. The IVPD% indicates the ratio of proteins that undergo digestion under simulated physiological settings, offering insights into their decomposition into smaller peptides and amino acids. By analyzing the IVPD values of protein bars made with different protein sources, we can enhance our comprehension of their digestibility and the possible effects on food intake and nutritional outcomes.

Table 7. *In vitro* protein digestibility (IVPD%) results in % for the four selected protein bars. Results include protein content of bars in g/100 g product and relative standard deviation (RSD) in %.

protein bars		**Protein content g/100 g**	**IVPD%***	**RSD%**
A	**Abso Crispy**	28,6	45,1±23	51
B	**Biotech USA Protein bar**	30	63±11,2	17,7
C	**Biotech USA Zero bar**	40	62,1±13,4	21,6
D	**Sportness protein bar peanuts**	34	45,2±14,2	31,5

*Results are shown in average±deviation format (n=6).

The average *in vitro* protein digestibility (IVPD) percentage for Abso Crispy is 45.1%±23 (RSD 51%) (table 7). This value suggests a relatively low protein digestibility compared to expectations, there is significant variation in the results obtained from the six parallel samples of Abso Crispy protein bars. In essence, when analyzing multiple samples of the same product, noticeable differences in the measured values of protein digestibility are observed. Factors such as high presence of fats and carbohydrates (Table 6) in the product may contribute to reducing the release of protein from its matrix, fibers also slow down the digestion of carbohydrates, thereby affecting the overall digestibility. Also, soy contains antitrypsin, which has the ability to interfere with the action of trypsin. Trypsin is an enzymatic protein that facilitates the hydrolysis of proteins in the small intestine during the process of digestion. Antitrypsin, a protease inhibitor included in soybeans, affects the function of trypsin, hence decreasing its ability to properly breakdown proteins (Lu et al., 2022). Using numerous parallel samples enables a thorough evaluation of protein digestibility, and possible factors can contribute to variability

may include fluctuations in processing parameters and interactions with other substances included in the protein bars. Furthermore, the protein digestibility may be affected by environmental variables, storage conditions, and the testing procedures used. Abso Crispy is primarily composed of 20%-30% protein blander of pea protein isolate and rice protein concentrate, which are highly regarded plant-based protein sources because to their excellent amino acid profiles and digestible properties (Bailey et al., 2023). Nevertheless, the variations noticed in IVPD% across the parallel samples indicate that the observed deviation is likely caused by different phases of the sample preparation process, such as sampling, digestion simulation, precipitation, supernatant separation, dilutions, and other factors. These stages together contribute the comprehensiveness of the sample preparation process, potentially leading to variability in the assessed protein digestibility (Orlien et al., 2021). It is important to emphasize that the IVPD of the samples appears to be low 45.1% ±23 compared to study by (Qin et al., 2022) indicate that pea protein was 83-90% IVPD, while rice protein percentage of (IVPD) observed was 86.30% by (Khan et al., 2017).

The mean *in vitro* protein digestibility percentage for BiotechUSA Zero Bar is 62.1%±13.4 (RSD 21%). The BiotechUSA Zero Bar primarily contains whey protein isolate and soy protein isolate, which are known for their outstanding nutritional profiles and high digestion. The increased percentage of IVPD seen in BiotechUSA Zero Bar, in comparison to Abso Crispy, indicates a potentially enhanced capacity to digest proteins derived from whey and soy isolates. Also, high fat and carbohydrate content in Abso Crispy protein bar the reason that can slow down the digestion process, affecting the absorption of amino acids and ultimately impacting protein digestibility. These protein sources (whey and soy protein) are of excellent quality since they contain a high amount of necessary amino acids and are easily broken down and absorbed by the body's enzymes during digestion (Pozdnyakov et al., 2022). The significant proportion of IVPD highlights the effectiveness of whey protein isolate and soy protein isolate in enhancing the consumption of protein and achieving favorable metabolic results. A study by (Qin et al., 2022) found out that the IVPD percentages of whey protein ranged from 98-100 % and soy protein from 95-98%.

The average IVPD percentage for BiotechUSA Protein Bar has been determined as 63±11.2 (RSD 17.7%). The BiotechUSA Protein Bar, like the BiotechUSA Zero Bar, has a consistent digestibility profile throughout the evaluated samples, as seen by its lowered variation and RSD%. The BiotechUSA Protein Bar contains milk protein

concentrate and whey protein isolate, which provide a combination of rapidly absorbed and slowly released proteins. The increased proportion of IVPD seen in BiotechUSA Protein Bar, similar to BiotechUSA Zero Bar, indicates that these proteins are efficiently digested and absorbed during simulated digestion. Although our findings are somewhat lower than those of (Qin et al., 2022), who assessed that the (IVPD) percentages of milk protein varied from 84-94% and whey protein from 98-100%.

The average In Vitro Protein Digestibility (IVPD) percentage for DM Sportness is recorded as 45.2%±14.2 (RSD 31.5%). The variability identified in Abso Crispy protein bars, which included the use of numerous parallel samples, is also evident in the larger deviation and RSD% in DM Sportness Protein Bar. This suggests that the deviation observed likely arises from the extensive nature of the sample preparation process. The DM Sportness Protein Bar has a unique combination of plant-based and animal-based proteins, including 10% peanuts and 10%-20%milk protein. The observed fluctuations in IVPD% highlight the intricate interaction between different protein sources and their rates of digestion. The IVPD% percentage of milk protein varied from 84-94% (Qin et al., 2022) and peanut protein can reach 93.91% under optimal conditions (Luo et al., 2011), so we can say that our samples showed lower results than what was mentioned.

Upen carefully examining the ANOVA Results, it becomes evident that there is no significant variation in IVPD percentages across the protein bars. This is supported by the non-significant p-value ($0.103 > 0.05$). This indicates insufficient statistical evidence to reject the null hypothesis, suggesting that there are no noticeable differences in protein digestibility – based on the free amino group content data - among the protein bars that were sampled.

4.3 Results of Digestible Indispensable Amino Score (DIAAS) of protein bars

The table 9. below displays the Digestible Indispensable Amino Acid Score (DIAAS) of studied protein bars, highlighting the presence of essential amino acids that are vital for human nutrition. DIAAS is a metric used to assess the quality of protein by measuring the degree to which a protein source supplies essential amino acids that

can be effectively broken down and absorbed by the human body. A protein with a higher DIAAS score indicates a greater capacity to fulfill the body's amino acid needs.

Table 8. Validation measurement of *in vitro* protein digestibility from the amino acids as well as DIAAS values. The results include the relative standard deviation (RSD) in % and t test.

Protein bar	**IVPD%***	**RSD%**	**t test**
Abso Crispy	46,7±4,20	8,98	0.908
BiotechUSA Protein Bar	87,5±3,04	3,48	0,027
BiotechUSA Zero Bar	65,6±2,28	3,48	0,738
DM Sportness	86,6±11,64	13,45	0,003

*Results are shown in average±deviation format (n=3).

Table 8 presents an understanding of the consistency of outcomes acquired from both the OPA and DIAAS techniques. The content-based OPA findings for Abso Crispy (0.908) and BiotechUSA Zero Bar (0.738) protein bars show no significant correlation between the IVPD% calculated from OPA and AA results. This suggests that the results are reliable and cohesive. However, the coherence is absent in the case of BiotechUSA Protein Bar (0.027) and DM Sportness (0.003). Due to the significant levels of carbohydrates and fat found in both BiotechUSA Protein Bar and DM Sportness, it is possible that the interaction of oleic acid during hydrolysis might result in the formation of complexes. These complexes may possibly obscure amino groups and have an impact on OPA readings (Orlien et al., 2021). Other material can affect the efficiency and the yield of OPA reaction so it is crucial to take into account parameters such as macronutrient content when analyzing the outcomes of protein digestibility tests.

Table 9. The table presents the DIAAS values of the chosen protein bars, each formulated with distinct protein sources. These values reflect the amount of digestible indispensable amino acids present in each protein bar (Adults results).

AA	Abso Crispy	Biotech USA Protein bar	Biotech USA Zero bar	DM Sportness protein bar peanuts
His	0.52	1.58	1.18	1.08
Ile	0.25	1.37	1.03	1.86
Leu	0.53	1.11	0.83	1.30
Lys	0.75	1.53	1.15	1.59
SAA	0.19	0.98	0.73	1.23
AAA	0.75	1.12	0.84	0.84
Thr	0.77	1.15	0.87	0.52
Trp	0.84	0.72	0.54	0.49
Val	0.50	1.24	0.93	1.32

SAA: sulfuric amino acids (methionine + cysteine)

AAA : aromatic amino acids (tyrosine + phenylalanine)

The Abso Crispy protein bar, which contains pea and rice protein, displays a very low ratio of essential amino acids. Tryptophan has the greatest ratio (0.84), signifying its comparatively greater percentage in relation to other amino acids. However, the sulfur-containing amino acids (SAA), namely methionine and cysteine, have the lowest ratios (0.19), indicating potential limitations on their abundance compared to other amino acids. This suggests that methionine and cysteine are limiting amino acids in this protein source. A study of (Guillin et al., 2021) show that the DIAAS of pea protein was 0.88, with a limiting AAs of Met + Cys, and for rice protein it was 0.37 according to (Phillips, 2017) with a limiting AAs of Lys. our results do not align with these findings and have different limiting AAs. High fat and carbohydrate content may reduce protein release from the matrix, and fibers decrease carbohydrate breakdown, lowering digestibility.

The Biotech USA Protein Bar, made with a combination of milk and whey protein boasts high ratios across all essential amino acids, owing to its blend of milk and whey protein sources. Histidine exhibits the highest ratio (1.58), indicating its relatively abundant presence in this protein source. Conversely, tryptophan registers the lowest ratio (0.72), suggesting its limited proportion compared to other amino acids and identifying it as the limiting amino acid in this protein source. the study of (Phillips,

2017) show that the (DIAAS) of milk protein was 1.18, with a limiting AAs of Met + Cys, and for whey protein it was 1.09 with a limiting AAs of Val, both sources highlight the bar's favorable amino acid profile. The findings highlight the strong and diverse amino acid composition that is clearly present in the Biotech USA Protein Bar. Although its lower in the amino acids that is limited in the protein bar compared to previous research, it still has a well-balanced composition and significant nutritional value.

The Biotech USA Zero Bar has a blend of whey and soy protein, which results in a high ratio of essential amino acids. Histidine as the greatest ratio (1.18), suggesting that it has a comparatively greater percentage in this protein source. Yet, tryptophan exhibits the lowest ratio (0.54), suggesting its limited abundance relative to other amino acids and identifying it as the limiting amino acid in this protein source. the study of (Phillips, 2017) show that the DIAAS of milk protein was 1.18, with a limiting AAs of Met + Cys, and for soy protein it was 0.898 with a limiting AAs of Met + Cys. Compared to our results we got limiting AAs of tryptophan and the relatively low DIAAS values.

The DM Sportness protein bar contains peanuts and milk protein, displays a approximately the same ratio of essential amino acids. Isoleucine has the greatest ratio (1.86) and methionine and cysteine (SAA) display lower ratios (0.49), indicating possible constraints in their abundance compared to other amino acids and designating them as the limiting amino acids in this protein supply. As we know that the DIAAS of milk protein is 1.18 according to (Phillips, 2017) with limiting AAs of Met + Cys, and its 0.434 for peanuts protein according to (Rutherfurd et al., 2015). Our identification of methionine and cysteine (SAA) as the limiting amino acids and the almost similar ratio in this protein bar is consistent with findings for milk protein and peanuts.

5 Conclusion

In summary, evaluating protein bars is a complex process that takes into account a number of variables, including digestibility, protein type, and quality. Important insights into the nutritional content and bioaccessibility of these products have been gained by a thorough analysis of protein bars derived from various protein origins, including plant-based, animal-based, and combinations thereof, as well as the use of *in vitro* digestion simulation techniques Infogest.

Protein bar market research has shed light on the industry's different protein sources, macronutrient compositions, and nutritional characteristics. Through careful examination of protein bar formulations, including animal- and plant-based protein sources, the assessment of protein bars raises consumer knowledge and encourages informed decision-making, allowing people to reap the advantages of protein supplementation while supporting their health and wellness goals. The heatmap study provides insight into the most popular combinations of protein sources used in protein bars, highlighting current trends and the preferred formulations of those working in the industry. According to the results, many protein bar recipes use a combination of two or more of the following ingredients: soy and whey or whey and milk. Four protein bars were chosen for further experiment: Abso Crispy features pea protein isolate and rice protein concentrate, BiotechUSA Zero Bar incorporates whey protein isolate and soy protein isolate, BiotechUSA Protein Bar contains milk protein concentrate and whey protein isolate, and DM Sportness utilizes peanuts and milk protein.

Findings from the OPA method used to evaluate the IVPD of various protein bars provide important information about how well proteins are digested. Abso Crispy protein bar have an IVPD percentage lower than reported values for individual pea and rice proteins, suggesting challenges in protein digestion potentially influenced by factors like the presence of fats, carbohydrates, and antinutritive compounds in soy. BiotechUSA Zero Bar IVPD percentage is lower compared to individual whey and soy proteins reported in the study. BiotechUSA Protein Bar IVPD percentage show slightly lower value than reported values for individual milk and whey proteins. The IVPD percentage of DM Sportness protein bar shows a lower value than reported values for individual milk and peanut proteins. This suggests that the deviations observed likely arises from the extensive nature of the sample preparation process,

such as sampling, digestion simulation, precipitation, supernatant separation, dilutions, and other factors. In addition, it could be influenced by factors like the presence of fats, carbohydrates, and antinutritives in soy that can slow down the digestion process, affecting the accessibility of amino acids and ultimately impacting protein digestibility.

The DIAAS values of the studied protein bars provide insights into the nutritional quality of protein sources and their ability to meet essential amino acid requirements. Comparing the DIAAS values of the protein bars to literature findings on the protein sources' digestibility, several key observations emerge. Abso Crispy, formulated with pea and rice protein, exhibits a notably low ratio of essential amino acids, with methionine and cysteine identified as limiting amino acids, contrary to previous research findings on individual protein sources. Biotech USA Protein Bar, containing milk and whey protein, its lower in the amino acids that is limited in the protein bar compared to previous research, but it still has a well-balanced composition and significant nutritional value. Biotech USA Zero Bar, with whey and soy protein, similarly demonstrates high essential amino acid ratios compared to previous protein bar, but compared to studies we got different limiting AAs and the relatively low DIAAS values. Lastly, DM Sportness protein bar, composed of peanuts and milk protein, aligns with previous findings regarding the identification of methionine and cysteine as limiting amino acids and almost similar ratio in this protein bar sources, indicating consistency in amino acid ratios with milk protein and peanuts. These comparisons emphasize the significance of comprehending the mix of protein sources and its influence on the nutritional quality when assessing protein bars.

In order to evaluate protein bars, one must take into account digestibility, protein sources, and quality. By employing methodologies such as *in vitro* protein digestion simulation and market research, valuable insights regarding nutritional composition and industry trends are achieved. The OPA method and DIAAS values yield significant insights regarding amino acid profiles and protein content. Although there may be some inconsistencies, these comparisons assist consumers in making well-informed decisions when considering protein bars. In general, this methodology improves comprehension and provides guidance for decision-making regarding protein bars and nutrition.

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