UNIVERSITÀ DEGLI STUDI DI PADOVA

Comparative Analysis of Protein Profiles in Commercial Soy Milk and Rice Milk: Insights into Nutritional Composition, Allergenic Potential, and Market Trends.

Master's Final Thesis

MSc. Food Science and Health

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Preface

Throughout this significant journey, I want to extend my sincere gratitude to the academic community for their invaluable guidance and steadfast support. I am deeply appreciative of my family, especially my grandparents Marco and Rosa and my mom Paula, who have been by my side unconditionally. I also want to express my heartfelt thanks to my dear friends, both those I met during this international experience and those from my home country, Ecuador, who consistently encouraged and supported me. Furthermore, I hold a special place in my heart for my boyfriend Daniel, who has been my constant companion, inspiring me to be a better person. I deeply appreciate your warmth and love, which have been a source of great strength and motivation.

ABSTRACT

In the past decade, the production of plant-based milk substitutes has grown significantly worldwide. This growth is driven by increased demand for sustainable, high-quality, and nutritionally valuable plant-based products. Lactose intolerance and other diseases have also contributed to this trend. Approximately, around 65% of the global population is lactose intolerant. Studies have highlighted the health benefits of plant-based milk, with comparisons showing that soy milk is rich in protein, healthy fats, vitamins, and minerals, while rice milk is lower in saturated fat but higher in natural sugars compared to cow's milk. It's important to note that non-dairy beverages do not contain the allergenic proteins found in cow's milk, but they may still contain other proteins that could cause allergies in some individuals. Therefore, consumers should be cautious when choosing such products. Given this context, understanding the proteins present in plant-based milk is crucial for assessing their allergenic potential, nutritional value, and suitability as cow's milk substitutes. Characterizing these proteins supports research and innovation in developing safer options for individuals with health issues. This thesis aims to provide detailed information on protein content and other key nutrients of soy and rice milk to contribute to scientific and market knowledge. The report goes on to discuss: the market trend, health and nutritional insights, and the analysis of various techniques used to characterize protein profiles. The report also provides the similarities and differences between rice and soy milk in terms of their protein content and nutritional benefits.

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1 Introduction

1.1 Background

In the last decade, the food industry responsible for the production of plant-based milk substitutes has been growing quickly and acquired more popularity in the world due to the increasing demand for plant-based sustainable products with high quality and nutritional value. Another factor to consider regarding the increase in the production of these drinks is lactose intolerance and its related allergies (Aydar et al., 2020).

Lactose and casein are the predominating proteins in animal milk, representing 80% of the total proteins, followed by whey proteins, such as beta-lactoglobulin and alpha-lactalbumin. These proteins, present in cow's milk, have been identified as highly potential allergens, and allergic reactions to cow's milk are one of the most common food allergies in children. A study published in the Journal of Allergy and Clinical Immunology in 2017 (Schatz et al., 2017) concluded that 2.5% of the population of children between 0 and 5 years old presents complications due to allergies. On the other hand, according to the National Institute of Diabetes and Digestive and Kidney Diseases of the United States, it is estimated that approximately 65% of the world population is lactose-intolerant as shown in Figure 1.1 (Chavarria, 2010).

Figure 1.1 Worldwide prevalence of lactose intolerance in recent populations. (Food Intolerance Network, 2022)

The consumption of animal milk can lead to several complications and cause symptoms that harm not only children' but also adults' health, making vegetable milk the preferred alternative for consumers (Del Castillo Bilbao et al., 2014). Various studies support the growing popularity of the consumption of vegetable drinks and their benefits for health and well-being. A study published by the Journal of the American College of Nutrition in 2020 (Jakše et al., 2020) made a comparison between the nutritional profiles of soy milk and rice milk against cow's milk, and it was determined that plant-based beverages offer significant benefits. The study showed that soy milk is a rich source of protein, healthy fats, essential vitamins, and minerals, whereas rice milk has a high natural sugar content and a low amount of saturated fat. Table 1.1 shows the nutritional content of rice and soy milk in contrast to whole milk, and provides information regarding nutritional values established by the Food and Drug Administration; these values are approximate per 100 ml (Deswal, 2018).

Table 1.1 Nutritional content of rice, soy, and cow’s milk.

	Energy	**Calcium**	**Fats**	**Saturated Fats**	**Sugars**	**Proteins**
	[kcal]	[mg]	[g]	[g]	[g]	[g]
Whole cow’s milk	68	122	4	2.6	4.7	3.4
Soy milk	37	120	1.7	0.26	0.8	3.1
Rice milk	47	120	1	0.1	4	0.1

It is important to emphasize that, although non-dairy beverages do not contain the allergenic proteins found in cow's milk, they may contain other proteins that could cause allergic reactions in certain people. Therefore, as a customer, it is key to identify which products could represent a potential risk to health despite their origin (Durán & Valenzuela, 2010).

It is due to this context that studying in detail the proteins present in plant-based milk has become an important factor in understanding its allergenic potential, nutritional value, and its suitability as substitutes of cow's milk. It is essential to say that the characterization of the proteins in these products promotes the development, research, and innovation in the design and manufacturing process to elaborate safer options for all those people with health problems (Faccin et al., 2009). Hence, there is a need to analyze or make comparisons of different protein profiles of the foods of interest. For this reason, this thesis aims to understand more in detail the protein profiles of soy and rice milk to provide relevant information to scientific and market knowledge.

1.2 Justification

In a world of constant changes, food decisions have evolved remarkably, mainly due to environmental, human well-being awareness, and the rising interest in the analysis of protein profiles and their importance in the food industry (Food and Agriculture Organization, 2004). This study emphasizes the importance of proteins as fundamental components of the human diet since protein consumption plays an indispensable role in human health. Their study and characterization are important because the world population depends on proteins from dairy or substitute sources as an integral part of their daily intake (Fuentes, 2019).

Vegetable drinks, specifically soy and rice drinks, are some of the leading consumption options on the market. However, as these alternatives are gaining acceptance by consumers, it is still crucial to understand their nutritional profile, benefits and detriments, and how the characterization of proteins of interest is carried out. The proteomic characterization of commercial beverages of plant origin opens a path toward a deeper understanding of the protein composition of foods since these differ substantially compared to those of animal origin (Garcia & Perez, 2017).

With the proteomic characterization, it will be possible to help both consumers and producers. On the one hand, consumers are interested in knowing where their food comes from and what nutritional composition they can provide, especially those used for special diets. On the other hand, producers need to acquire all the relevant information on their products in order to offer food that ensures quality, authenticity, and food safety (Gil, 2010).

1.3 Objectives

This study attempts to make a detailed analysis of the protein profiles of soy milk and rice milk. This study will seek a deeper understanding of these substitute beverages on the market, addressing their nutritional value, allergenic implications, and their importance in terms of human health.

To complete this bibliographic thesis, the following objectives are taken into consideration:

1. To analyze and discuss from literature about the proteomic profiles of rice and soy milk, considering specifically the abundance and types of proteins present in both vegetable drinks, and determining their nutritional benefits and their possible limitations.

2. To analyze relevant information from the literature about the characterization of protein profiles in rice and soy milk by using specialized techniques of proteomic analysis such as liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the determination and quantification of proteins.

3. To investigate relevant information collected regarding the market, and its relation to the consumption of plant-based beverages, focusing on understanding how the market for these beverages has been in constant development, and the relation between its growing demand and the well-being and consumption awareness culture.

4. To evaluate the health insights and allergenic potential of proteins from soy and rice beverages due to their relevance in food safety according to the information available in the scientific databases.

1.4 Research methodology

The aim of this qualitative bibliographic study was to make an in-depth and exhaustive review of the scientific literature available about the comparative analysis of the protein profiles of soy and rice milk. This information search was carried out using the academic and scientific databases available on the web, including Google Scholar, PubMed, Scielo, Redalyc, Dialnet, Scopus, Medline, and organizations such as the Food and Drug Administration (FDA), World Health Organization (WHO), United Nations Agricultural Organization (FAO), United States Department of Agriculture (USDA), Science Direct, etc.

For the search, specific keywords were used, including soy milk, rice milk, protein profiles, nutritional composition, and market trends. Inclusion criteria were applied to select relevant studies, including those published in the last fifteen years, including articles written in English or Spanish. Studies that did not directly address aspects of protein profiles, nutritional composition, allergenic potential, or market trends of soy milk and rice milk were excluded.

The collected information was subjected to a critical analysis to highlight the relevance of existing methodologies used for determining protein profiles, and additionally much attention was paid to the differences and similarities that could exist between soy and rice beverages in order to make a more precise analysis when discussing the topic addressed in this project.

To cover all the objectives abovementioned, the following strategy was applied. Primarily, an investigation into the functioning of the vegetable drinks market and an exploration of the underlying factors that sustain its persistent expansion were undertaken. Second, research about the benefits that these drinks have on the daily diet was carried out to understand the nutritional quality that these products can offer to consumers. Furthermore, an analysis of the literature regarding the health problems was taken into consideration, such as allergies and lactose intolerance from milk of animal origin. This analysis was followed by a study focused on the proteins typical of rice and soy, which are used to make healthy and functional drinks that can replace cow's milk, and that provide vitamins, minerals, and components necessary for human nutrition. Additionally, a description of the methods or techniques commonly used to characterize proteins was made, with a special focus on liquid chromatography techniques and mass spectrometry. Finally, a comparative evaluation of all the information gathered was made to determine the importance of soy and rice milk in contrast to cow's milk.

2

Plant Based Milk Substitutes: Popularity and Market Trends

2.1 Growth within the vegetable milk market:

In recent years, there has been a notable increase in the demand and consumption of alternative milk products. This trend has garnered significant attention, primarily driven by a societal shift toward healthier lifestyles (Giugliano et al., 2023). Furthermore, the growing interest in these milk substitute options has stimulated considerable expansion in both economic and research domains within the food industry. As public awareness continues to rise, companies are actively pursuing innovative strategies to elevate their products to a higher standard. These endeavors encompass the adoption of novel technologies, enhancements in research and development capabilities, and refinement of processing techniques, among other initiatives (Gil, 2010).

It is noteworthy to highlight that the consumption of plant-based milk represents one of the most universally favored categories within the beverage industry. A projected growth rate of 15% anticipated from 2022 until 2029 (Gomez, 2014). This upward trajectory is attributed to several key demographic segments, including the millennial generation, individuals adhering to vegan or vegetarian dietary preferences, environmentally conscious consumers, and those with specific dietary restrictions motivated by health considerations. For instance, soy milk presently stands as a preeminent choice, closely followed by rice milk. This preference is attributable to their nutritional benefits, and mild flavor, characterized by a subtleness that renders them particularly amenable to blending with other products or incorporation into culinary preparations (Hernandez & Rodriguez, 2021; Guevara-Villalobos et al., 2019).

2.2 Causes of the plant-based beverage boom

A substantial percentage of the world population is estimated to exhibit lactose intolerance, constituting a prominent contributing factor to the increased consumption of plant-based beverages. It is imperative to highlight that intolerance is significantly higher in Latin America compared to Europe, primarily attributed to genetic predispositions and gastrointestinal pathologies affecting the small intestine (Gomez, 2014). The reported data reveals varying degrees of lactose intolerance across different regions, ranging from 15% for people in Europe, followed by, Latin America (60%), Asia (62%), and Africa (90%). Investigations conducted in Mexico (Guevara-Villalobos et al., 2019) found that almost half of the adult population experiences gastrointestinal discomfort related to dairy consumption, with 70% of these individuals testing positively for meagre lactose digestion. A similar case occurred in Chile, with more than 50% of the population experiencing gastrointestinal distress, triggered by a discernible genetic predisposition to lactose intolerance (Guevara-Villalobos et al., 2019).

Given the substantial prevalence of lactose intolerance within Latin America, individuals have increasingly chosen to embrace alternative options provided by the food industry. Recent data indicates a decrease in the consumption of cow's milk in Latin America, with a reduction of approximately 12% over the past five years, translating from 77 million metric tons to 69 million metric tons. For instance, in Chile, cow's milk consumption witnessed a decrease from 33,400 tons to 29,800 tons in 2022, with the expectation that this declining trend will persist in the coming years (Hernandez & Rodriguez, 2021).

While lactose intolerance is the norm in Asia, Africa, and Latin America, the opposite occurs in Europe, North America, and Australia. These variations are primarily attributed to ethnic origins rather than geographical location. In Europe, individuals of Caucasian descent, particularly those hailing from northern Europe, exhibit a lower likelihood of lactose intolerance, followed by individuals of Mediterranean origin (Issara & Rawdkuen, 2016). As previously noted, lactose intolerance does not manifest uniformly across all geographical regions, and that is the case of North America. Within the North American context, the native American descendants' lactose intolerance is considerably more prevalent compared to individuals of European roots (Chavarria, 2010).

Individuals who exhibit lactose tolerance possess specific genetic mutations enabling the sustained production of the lactase enzyme, responsible for the hydrolysis of lactose; this genetic adaptation facilitates the digestion of lactose without further complications. These mutations have arisen at various junctures and locales in human evolutionary history (Faccin et al., 2009). Notably, within human populations closely associated with milk-producing domesticated animals, such mutations conferred a distinct adaptive advantage and were preserved via natural selection. This phenomenon is exemplified by the populations inhabiting northern and central Europe, as well as select nomadic and pastoral societies in North Africa and the Middle East. In contrast, in cultures that had little or no association with milk-producing animals, as it has traditionally been the case in the

Japanese, Chinese, Indian, most African, and Native American cultures, the presence of this mutation was irrelevant in evolutionary terms (Jorcin et al., 2012).

A 2017 investigation conducted by EAE Business School (Vila, 2017) highlighted that the highest expenditures on animal-derived dairy products, such as cow's milk, are primarily concentrated within European nations. Specifically, France, Germany, the Netherlands, Belgium, Denmark, and Sweden, exhibited substantial annual spending ranging from 15,698 to 39,406 million euros. In contrast, Latin American countries such as Venezuela, Chile, and Peru demonstrated significantly lower expenditure levels, amounting to 688, 1,107, 1,136, 1,168, and 1,492 million euros annually, respectively (Kandylis et al., 2016).

According to the United Nations Organization for Food and Agriculture (FAO, 2004), significant expansion is anticipated within the food industries specializing in non-dairy beverages. Projections indicate that within the upcoming 2-3 years, approximately 30% of the global population will incorporate plant-based beverages into their diets. Notably, among these alternatives, the most favored substitute milks are ranked in the following order: oat milk, soy milk, rice milk, and almond milk (Lamothe et al., 2021).

2.3 Market trends

Trends in the milk market change rapidly around the world. In Europe, milk, both fresh and pasteurized, is gradually giving way to so-called plant-based drinks. The European Union annually produces about 170 million tons of milk and dairy products, with a cow's milk production of 144 million tons. In the case of Spain, production reaches 8.70 million tons, of which 5.9 million correspond to cow's milk (Lopes et al., 2020).

Spain ranks as the seventh-largest producer of cow's milk within the European Union (Lopez, 2020), following behind Germany, France, the United Kingdom, the Netherlands, Italy, and Poland. Spain's market encompasses a commitment to both plant-based beverages and various lactose-inclusive and lactose-free milk products. Nevertheless, cow's milk consumption in this country has exhibited a decline since 2016, and this downward trajectory is expected to persist until 2025 (Lopez, 2020). This trend can be attributed to intense competition for alternative beverages perceived as healthier options by consumers, such as rice, almond, and soy derivatives. Consequently, the market valuation of non-animal-origin milk alternatives has experienced a notable surge where the soy market is emerging as one of the most lucrative, boasting a value of 7.4 million USD. Figure 2.1 illustrates the market values of diverse plant-based beverages in the year 2022 (Mañes & Molto, 2022).

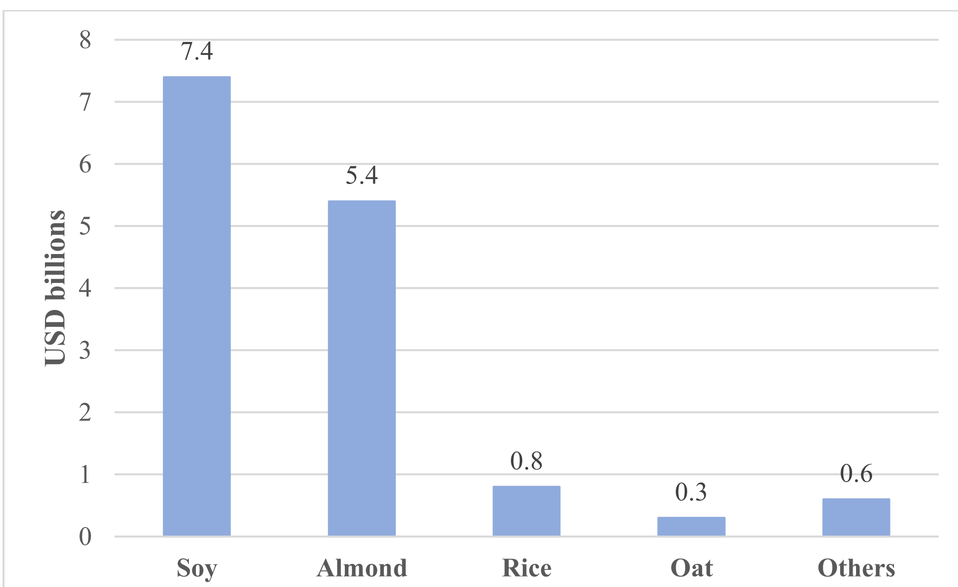

Figure 2.1 Global market value of alternatives to milk of animal origin (vegetable drinks), by category (2022).

Currently, Spain assumes a leadership position in Europe concerning per capita sales volume of plant-based beverages. Non-dairy milk sales in Spain have reached 246 million liters, indicating a growth rate of 14% since 2018 (Murcia, 2015). Furthermore, the global rice milk market is projected to exhibit a Compound Annual Growth Rate (CAGR) of 14% during the forecast period spanning from 2020 to 2025, as reported in Figure 2.2 In Spain, the market value of plant-based milk alternatives has ascended by 15% during this same period, currently amounting to 318 million euros (Lopes, 2020).

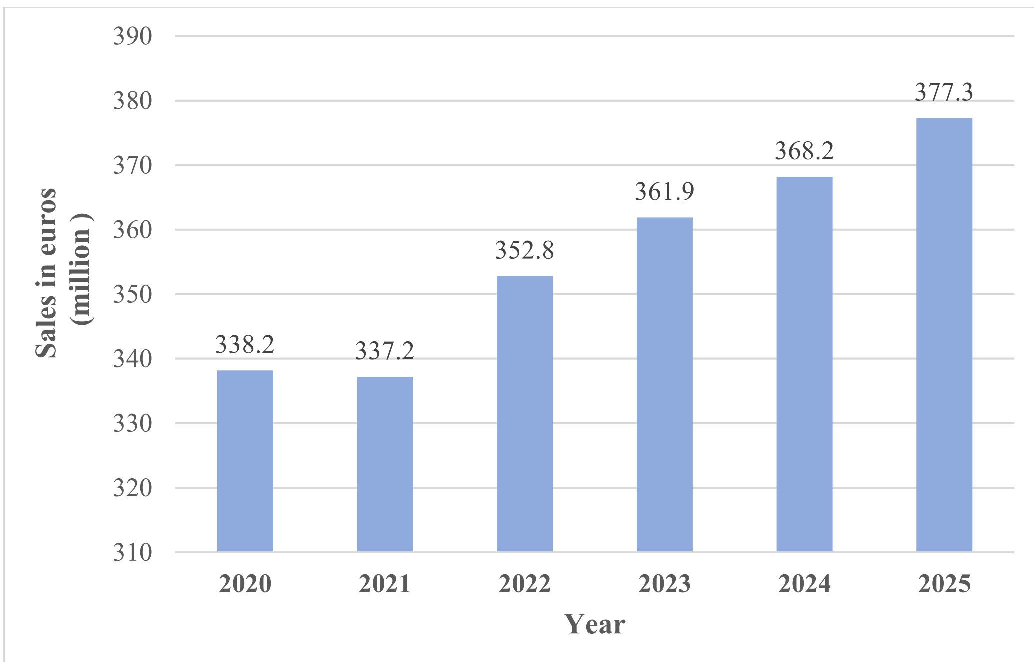

Figure 2.2 Sales of plant-based milk from 2020 to 2022 and their projected trends through 2025 popularity (Lopes, 2020).

It is noteworthy that, despite the comparatively higher dairy tolerance within the European populace, the predominant impetus for transitioning from animal-derived milk to plant-based alternatives primarily stems from heightened health concerns. This shift in dietary habits has led to substantial variations in dairy consumption patterns. Liquid milk consumption has waned, while products like Greek yogurt, characterized by lower fat and reduced sugar content, have witnessed an increase in popularity (Pérez-Rodríguez et al., 2023).

3

Soy Milk and Rice Milk: Widely Consumed Plant-Based Milk Substitutes

With the increasing popularity of veganism, the market has witnessed a substantial demand for alternatives to animal-derived products. Although traditional cow's milk has long been recognized as a valuable source of high-quality protein and essential fats, it is important to note that plant-based milk options, such as rice milk and soy milk, have now become essential items on the shopping lists of individuals seeking both nutritional value and sustainability in their dietary choices (Pontonio & Rizzello, 2021).

While cow's milk undeniably provides a commendable source of protein, it is worth emphasizing that plant-based alternatives have become increasingly attractive for a variety of reasons. For instance, rice milk is not only lactose-free but also fortified with carbohydrates, B vitamins, and antioxidants, offering a unique nutritional profile that caters to specific dietary needs (Rivera & Muñoz, 2011). Conversely, soy milk has an exceptional protein content of high quality, providing an appealing option for those seeking a plant-based protein source that rivals traditional dairy. This expanding array of plant-based options underscores the evolving landscape of dietary choices and the appealing nutritional benefits they bring to light, drawing the interest of a growing number of consumers (Rivera & Muñoz, 2011).

3.1 Soy Milk

Soy milk is a whitish beverage that is obtained from the aqueous emulsion resulting from the hydration of whole soybeans (*Glycine max*), selected and cleaned, followed by adequate technological processing. Its formula may contain sugar, dyes, flavorings, and preservatives (Rodriguez, 2007).

It is obtained from soybeans, which are ground with water and subsequently heated with steam water for 15-20 minutes, obtaining a milky-looking product that receives this name. It is then subjected to different processes: technological pasteurization or sterilization, and packaging to obtain commercial products. Soy milk is enriched with minerals and vitamins, and it is the main food for infants and children who cannot tolerate cow's milk. In addition, the nutritional interest in soy is due to its fatty acid and phytosterol content. Its beneficial effects on health have determined the development, in the food industry, of new products based on soy milk (Torres & Tovar, 2009).

Pasteurized soy milk refers to a liquid soy-based product that has undergone a pasteurization procedure. This process entails subjecting the product to a temperature not less than 65°C, followed by rapid cooling. This process is used to mitigate public health risks by eradicating pathogenic microorganisms while minimizing alterations to the product's organoleptic and nutritional attributes. It is imperative and mandatory that soy milk complies with established quality standards aligning with regulatory requirements in the food industry (Vallath et al., 2022).

3.2 Rice Milk

Rice milk is a plant-based non-dairy beverage made primarily from ground rice and water. It is marketed as a vegan substitute for cow's milk, easy to digest, and suitable for allergy sufferers. Like other plant-based drinks, rice milk has generally an opaque white or beige color and a creamy texture that resembles dairy milk (Vitoria Miñana, 2017).

The industrial process of making rice milk involves a structured sequence of steps. First, the rice grains are ground, either fully or partially. The term "fully milled" entails the complete removal of the hull, germ, and bran, resulting in white rice exclusively. "Partially milled" denotes the removal of the hull only, obtaining as a result brown rice. While the "fully milled" process may create the optimal texture, it currently results in the depletion of essential vitamins, minerals, and dietary fiber (Vallath et al., 2022). For both processes, the ground rice is then pulverized, made into a slurry by combining it with water, and filtered to remove any particles of excessive size or coarseness. The resultant slurry is then subjected to enzymatic treatment to partially hydrolyze starch, facilitating a more homogenous mixture (Verduci et al., 2019).

Once the desired viscosity (density) is reached, other ingredients are added, such as oil, salt, stabilizers, vitamins, minerals, flavors, and sweeteners. After the addition of oil, an emulsion is produced through homogenization, creating creaminess and stability in the rice milk (Torres & Tovar, 2009). Although commercially produced rice milk can typically be found in major supermarket chains and health food stores, there are many simple recipes online for making rice milk with basic ingredients in a home kitchen. In most commercial brands of rice milk, the first four ingredients are water, ground rice, vegetable oil (canola, sunflower, or safflower), and salt. In some varieties of rice milk, there may be additional ingredients, which may include natural or artificial flavors, such as vanilla and chocolate; thickeners, such as xanthan gum, tapioca starch, or carrageenan; and vitamins and minerals for nutrient fortification, such as calcium phosphate and vitamins A, D and B12 (Rivera & Muñoz, 2011).

Exogenous proteins are proteins that are not naturally present in a particular food or beverage but are added intentionally or unintentionally during processing or manufacturing. In the context of the study, exogenous proteins refer to α-amylases from Aspergillus niger, Bacillus licheniformis, and Bacillus amyloliquefaciens that were identified in commercial rice milks. These exogenous proteins can serve various purposes, such as improving the texture, flavor, and nutritional value of the food or beverage. In the case of rice milk, the addition of exogenous α-amylases can help to hydrolyze starch and achieve a desired level of sweetness and viscosity. However, the presence of exogenous proteins in food or beverage products can also pose a risk to consumers with food allergies or intolerances, which highlights the importance of accurate labeling and allergen management in the food industry.

4

Health Benefits and Allergenic Potential

4.1 Soy

4.1.1 Health insights

Soybeans have been consumed in Asian countries for centuries. Epidemiological investigations have established a noteworthy correlation between soy intake and various health advantages (Chavarria, 2010). These encompass a reduced occurrence of coronary heart disease, atherosclerosis, and type 2 diabetes, a diminished susceptibility to specific cancer types such as breast and prostate cancer, enhanced bone health, and alleviation of menopausal-related symptoms (Chavarria, 2010). As a result of these associations, numerous scientific studies have attempted to elucidate the biological effects of the different compounds in soy and their possible mechanism of action in the body (Palleiro, 2019).

One of the pioneering investigations in this area pertains to the hypocholesterolemic effect, initially proposed by Meeker and Kesten in 1940 (Lichtenstein, 2001). Their proposal stemmed from the observation that rabbits fed with soy flour did not manifest hypercholesterolemia, in contrast to those fed with casein. Subsequent to this observation, an extensive body of research has explored this effect in human subjects. The existing evidence suggests that the amino acid composition of soy protein, as well as certain nonprotein constituents such as saponins, isoflavones, and phytic acid, may exert influence over serum cholesterol levels. However, it is important to note that at the present time, there is no universally agreed-upon consensus regarding the precise components responsible for these effects and the mechanisms through which they elicit them (Lopez, 2020).

In general, research into the potential cholesterol-lowering effect of soy has followed two paths: some researchers have studied the effect of soy protein, mainly in isolated form, while many others have examined the role of isolated soy supplements. A meta-analysis of several studies investigating the effects of soy protein on serum lipids concluded that daily consumption of 31 to 47 g of soy protein can lead to reductions in total cholesterol and low-density lipoprotein-cholesterol (LDL-C) of 9.3% and 12.9%, respectively. However, it is important to note that changes in cholesterol concentrations also depend on initial cholesterol levels. Probably, alterations in other dietary components, such as the type of fat and dietary cholesterol itself, could explain these effects (Hernandez & Rodriguez, 2021).

In addition, an explanation for the hypocholesterolemic impact of soy protein rely on its capacity to bind with bile acids, consequently impeding the intestinal reabsorption of cholesterol and thereby reducing its serum concentration. Other works seem to indicate a possible decrease in hepatic cholesterol production mediated by the action of soy peptides (Liu et al., 2022). An activation of LDL receptors, depressed in states of hypercholesterolemia, after the administration of soy protein, has also been proposed. But these premises are actually raised in isolated works that have not been confirmed yet, so more studies are required to try to clarify this issue.

Scientific investigations (Giugliano et al., 2023) have elucidated that the effectiveness of soy protein in reducing blood lipids in humans is notably enhanced when it is consumed in conjunction with natural isoflavones. Studies indicate (Faccin et al., 2009) that both soy protein without isoflavones and isoflavone-containing tablets exhibit less pronounced hypocholesterolemic effects. Additionally, various soy products hold the potential for cardiovascular disease (CVD) mitigation and overall health improvement. This is attributable to their rich composition of polyunsaturated fats, dietary fiber, vitamins, and minerals, combined with a low concentration of saturated fats (Faccin et al., 2009).

Another effect linked to CVD pertains to the potential antihypertensive influence. Similar to peptides derived from milk, soy contains peptides that exhibit the capacity to inhibit the angiotensin-converting enzyme, thereby inducing an antihypertensive response (Marcone et al., 2017). Numerous animal studies (Bhat et al., 2015), primarily involving spontaneously hypertensive rats, have demonstrated a reduction in blood pressure following the administration of soy peptide fractions obtained through enzymatic digestion. Importantly, this reduction in blood pressure is shown to be dose-dependent. Conversely, these peptides have minimal impact on blood pressure in normotensive animals (Del Castillo Bilbao et al., 2014).

Finally, one of the bases for the development of atherosclerosis is the oxidation of LDL. The antioxidant effect of soy peptides can be framed within the cardioprotective properties of soy. It has been shown that tripeptides with tryptophan and tyrosine residues at the C-terminus response present in soy protein have a great capacity to scavenge oxygen-free radicals (Marcone et al., 2017). This capacity is increased in peptides released by soy protein digestion, compared to the whole protein. In particular, the tripeptide Pro-His-His has been identified as the active antioxidant center of such peptides. It is believed (Aydar et al., 2020) that the presence of histidine in the peptides allows the chelation of metals and the sequestration of hydroxyl radicals and reactive oxygen species, thus providing antioxidant capacity to said peptides. Furthermore, an antimutagenic and antioxidant capacity has been associated with the consumption of soy milk (Aydar et al., 2020), which is the reason why soy milk is a promising functional food.

The consumption of soy milk has been shown to have beneficial effects (Kandylis et al., 2016) on metabolic syndromes in both animal models and humans. The beneficial role of soy consumption in diabetics is another reason that justifies its inclusion in the daily diet. Recent studies using pigs as an animal model suggest that high consumption of soy protein can reduce the appetite, thus reducing food consumption and fat deposits, and consequently improving insulin sensitivity (Kandylis et al., 2016).

4.1.2 Components

The composition of soybean differs depending on the variety and the environmental conditions in which the crop is cultivated. Typically, soybeans are made up of 35-40% protein, 15-20% fat, 30% carbohydrates, 10-13% moisture, and about 5% minerals and ash. Soy does not contain cholesterol and lactose, and it comprises various minor constituents such as: minerals, vitamins, protease inhibitors, phenolic compounds including isoflavones, saponins, phytates, among others, which possess biologically active properties that contribute to its recognized health benefits. (Kandylis et al., 2016).

Proteins are the most abundant component of soy, of which between 80-90% are storage proteins. Soy proteins can also be classified based on their sucrose gradient sedimentation coefficients. According to this arrangement, the soy protein fraction has four fundamental components 15S, 11S, 7S and 2S. Fractions 11S (glycinin-rich), 7S (composed of conglycinin and β-conglycinin), and 2S (trypsin-inhibiting proteins) constitute more than 85% of the storage proteins (Del Castillo Bilbao et al., 2014).

Various commercially available formats of soy protein, serving both as supplements and protein sources, exhibit significant promise. Soy proteins, classified among non-dairy protein options, have consistently demonstrated robust biological activity across a spectrum of experimental settings, encompassing in vitro, in vivo, and clinical trials. These versatile soy protein formulations play a pivotal role in nutritional and therapeutic contexts (Del Castillo Bilbao et al., 2014).

4.1.3 Allergenic potential

In recent years, soy consumption has experienced a significant increase, not only due to its nutritional value but also because of its numerous health benefits. This rise in consumption has consequently led to an increased occurrence of individuals allergic to this legume (Giugliano et al., 2023).

Legume allergies typically manifest in childhood and, in the case of soy, tend to persist with age. The average age at which this type of allergy usually appears is around three months of age. Approximately 30-40% of infants are allergic to cow's milk protein and exhibit allergies to the protein in soy-based infant formulas. In adults, soy allergies are rare but can still occur (Giugliano et al., 2023).

The World Health Organization (WHO) identifies 8 allergenic proteins in soy, 7 of which can cause food allergies. These proteins are often resistant to high temperatures and action of acidic gastric juices and digestive enzymes. Cooking can enhance their allergenic potential. These proteins are Gly-m (*Glycine max*): 5, 7, 8, 20, 21, 24, 25 (Kandylis et al., 2016).

Soy milk allergies are more common in kids; nevertheless, soy-based formulas can serve as an alternative source of nutrition for infants with cow's milk protein allergy (CMA). However, it is important to note that soy allergy is prevalent since soy is among the top eight food allergens. Several soy allergens have the ability to bind to IgE antibodies in allergic patients, but only a few of them are considered immunodominant allergens (Chavarria, 2010).

These allergens include proteins Gly-m 5 (beta-conglycinin) and Gly-m 6 (glycinin), which have been associated with severe allergic reactions to soy. Moreover, a study conducted in Northern European children revealed that 86% of children with soy anaphylaxis had IgE binding to Gly-m 5 or Gly-m 6, whereas in children with moderate symptoms, this occurred in 55%, and only in 33% of subjects with mild symptoms (Verduci et al., 2020).

However, it is important to emphasize that sensitization to soy allergens does not always result in clinical symptoms. The clinical and immunological basis of soy allergy appears to be complex. For instance, in a double-blind, placebo-controlled food challenge study (Ballmer et al., 2007), it was found that only 1% of soy-allergic patients reacted subjectively (symptoms reported by the patient) and objectively (symptoms observed by physicians) to 0.21 and 37.2 mg of soy protein, respectively. Moreover, approximately 50% of children allergic to soy protein become tolerant by the age of 7.

As a result of soy allergy, a range of symptoms may manifest, including skin conditions like acne or eczema, nasal congestion, swelling, asthma, shortness of breath, hay fever, pollen allergy, itching and hives, conjunctivitis, fever, fatigue, weakness, and nausea, mouth ulcers or fever blisters, gastrointestinal issues such as diarrhea and colitis, and, in severe cases, it can potentially lead to anaphylaxis (Verduci et al., 2020).

4.2 Rice

4.2.1 Health insights

Rice (*Oryza sativa* L.) is a globally prevalent crop and serves as a primary staple for over half of the world's population. Uruguay stands as an exemplary country, notable for its robust exportation of rice and related products. Extensive research endeavors in Uruguay have been dedicated to enhancing rice quality, with a primary focus on crop improvement, varietal enhancements, and heightened production yields (Chavarria, 2010).

In white rice, the predominant protein fraction is glutelin, distinguished by its elevated lysine content, a feature unique among cereals (except oats), which predominantly contain prolamin. Notably, white rice exhibits low values of crude fiber and lipids, with phosphorus (P) and potassium (K) being the primary mineral components. Furthermore, the predominant vitamins present in white rice are niacin, inositol, and choline. White rice primarily consists of the endosperm, characterized by a high starch content, approximately 78% on a wet basis or 90% on a dry basis (Garcia & Perez, 2017).

Rice bran offers a valuable energy source with its fat content ranging from 12 to 15%. It boasts a high fiber content of approximately 20% and contains about 13% protein of excellent biological quality, primarily comprising hypoallergenic albumin and globulin. Within its lipid fraction, rice bran exhibits a well-balanced profile of fatty acids and it is rich in antioxidants, including various B complex vitamins and alpha-tocopherol (Kandylis et al., 2016).

Furthermore, the serous portion of rice bran contains policosanol, a compound known for its efficacy in reducing LDL-cholesterol levels (low-density lipoproteins responsible for cholesterol transport) while simultaneously increasing HDL-cholesterol levels (high-density lipoproteins), facilitating cholesterol transport (Gil, 2010).

There is a percentage of broken rice that is sold at a low price or destined for animal feed. On the other hand, defatted rice bran is undervalued when it is used for animal feed or as fuel and a source of silica. Due to their low cost and composition, it is pertinent to consider these fractions to be ingredients of rice milk formulations and functional rice drinks. Consequently, rice-based beverages are composed of rice starch, grain proteins, and added lipids (high-oleic sunflower oil). Compared to bovine milk, they contain lower protein and higher carbohydrate amounts and can be considered a hypoallergenic alternative. They also present deficiencies in their mineral content such as calcium and vitamins, which should be considered for inclusion for a better nutritional contribution (Faccin et al., 2009).

4.2.2 Components

Rice primarily consists of starch, with smaller quantities of proteins, lipids, fiber, and ash. However, the grain's composition and its fractions can vary due to several factors such as varietal differences, environmental conditions, management practices, processing, and storage, resulting in grains with distinct nutritional characteristics. Additionally, nutrients are not evenly distributed among different grain fractions. The outer layers contain higher concentrations of proteins, lipids, fiber, minerals, and vitamins, whereas the center is rich in starch. Therefore, the polishing process results in a reduction in nutrient content, except for starch, leading to differences in composition between whole and polished rice (Faccin et al., 2009).

Rice is a valuable source of energy, as its primary component is carbohydrates (81.2%). It has low-fat content, it is easily digestible and hypoallergenic, making it the first cereal recommended by pediatricians when introducing complementary foods. Polishing rice removes the bran, where most vitamins, minerals, and fiber are concentrated. Additionally, the bran of whole rice contains lipids, pentosans, and antioxidants present in the rice grain. These lipids are rich in nutrients such as vitamin E and have been shown to reduce consumers' blood cholesterol levels (Gil, 2010).

Whole rice was historically less consumed due to its longer cooking time. Nowadays, it is known to offer significant nutritional benefits and natural antioxidants, sparking increased interest in its consumption as a healthy food, especially in the production of plant-based beverages (Gil, 2010).

4.2.3 Allergenic potential

Rice allergy is common in East Asian countries, but its prevalence is much lower in Latin America and Europe; therefore, its consumption does not represent a potential major risk for the world population. There is not much research regarding this specific cereal, but it has been reported that allergic hives can occur due to contact with rice and ingestion of both cooked and uncooked forms (Faccin et al., 2009).

Currently, there are no exact values for the number of people allergic to rice, but it is estimated that only 0.2% of the world population is affected by this case. A study about functional food published in Chile (Durán & Valenzuela, 2010), indicates that the Japanese are those who suffer the most from this type of allergy. It was indicated that both the young and adult populations were allergic to the globulin protein (Durán & Valenzuela, 2010). Rice allergy can cause reddish lesions on the skin in large areas of the body, affect the gastrointestinal tract, the cardiovascular system and the respiratory tract, and may include abdominal pain, diarrhea, nausea, vomiting or stomach cramps, tight throat or snoring, difficulty swallowing and anaphylaxis (Giugliano et al., 2023).

It is worth mentioning that despite this problem, the Asian countries that suffer the most from this type of allergy produce a genetically modified rice known as “Shiseido rice” free of globulins to be consumed by allergic people. This modified and physiologically functional rice is also used in this region for the preparation of drinks such as rice milk, including others such as sake or rice liquor (Durán & Valenzuela, 2010).

5 Methodology

5.1 Relevant literary methodologies

This bibliographic review comprehensively discusses the principal analytical methodologies employed in the protein characterization of plant-based beverages, with a particular focus on rice and soy. The investigation encompasses the treatment protocols applied to these beverages to facilitate protein extraction, along with the instrumental techniques utilized for subsequent protein analysis and quantification.

In general, the use of a combination of techniques such as liquid chromatography or capillary electrophoresis, and mass spectrometry is proven to obtain reliable and complete information on the identification, characterization, and quantification of amino acids for cereal samples (Palleiro, 2019).

Proteomics is essentially the study and characterization of the entire set of proteins (proteome) expressed by a genome, with the aim of obtaining an integrated global view of cellular processes (Mañes & Molto, 2022). Proteins can be identified and categorized based on their function and interactions with each other, and for their analysis mass spectrometry techniques are available. Mass spectrometry is based on the differential behavior exhibited by ionized molecules when traversing electric and magnetic fields. This technique allows these ions to be separated based on their mass-to-charge ratio and subsequently detected (Lopez, 2020).

Through mass spectrometry, it is possible to obtain structural information about proteins, such as their mass and amino acid sequence. This information can be used to identify proteins by searching in databases. Mass spectrometry also proves useful in identifying and locating post-translational modifications in proteins (Mañes & Molto, 2022).

Protein separation processes are primarily dominated by liquid chromatography (LC). The resolution of protein mixtures through LC is based on the principle that, under a given set of conditions, solutes dissolved in a mobile phase interact to varying degrees with the stationary phase contained within the column (Mañes & Molto, 2022). The type of interaction depends on the physical and chemical properties of the stationary phase. Thus, LC exploits inherent differences among proteins (e.g., size, hydrophobicity, binding specificity, and charge) in order to achieve their separation (Palleiro, 2019). Depending on the separating principle, liquid chromatography techniques can be classified into size exclusion chromatography (SEC) or gel filtration, ion exchange chromatography (IEC), hydrophobic interaction chromatography (HIC), and reverse phase chromatography (RPC).

Chromatographic techniques have been employed in the analysis and purification of proteins, however, various studies have also utilized them to detect conformational changes in proteins, either due to the chromatographic process itself or the nature of the sample (Pontonio & Rizzello, 2021). Consequently, it is possible to assess the stability of a protein. Moreover, due to the expression of proteins as inclusion bodies in recombinant microorganisms, the process of protein refolding using chromatographic techniques has seen a notable increase in importance over the past decade. Currently, innovative methods are under development to further enhance this process (Pontonio & Rizzello, 2021).

In a study conducted by Battisti et al. (2021), the protein composition of commercial soy milk products was analyzed using a label-free quantitative proteomics approach. The methods used include protein precipitation, protein digestion, high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS), and proteomic data analysis using MaxQuant software (Cox & Mann, 2008). Furthermore, statistical analyses such as principal component analysis and unsupervised clustering were performed to evaluate the differences in protein composition among soy milk samples (Battisti et al., 2021).

In the same way, there are protein profile studies carried out on rice milk where the same techniques and similar and effective procedures are applied when carrying out this type of study. For example, a current ongoing study conducted by Battisti, et al., 2023, analyzed different commercially available rice milk samples have been analyzed using a label-free quantitative proteomics approach where computational analyzes were used to obtain detailed information on the proteomics of rice milk. Mass spectrometry analyses were performed and bioinformatics tools were used to identify the amino acid sequences of the identified proteins.

These techniques are highly sensitive and accurate, allowing for the identification and quantification of proteins in complex samples such as soy and rice milk. Furthermore, these techniques enable the identification of proteins that may not be detectable by other protein analysis methods, providing a more comprehensive view of the protein composition of the sample. Overall, label-free quantitative proteomic techniques are a valuable tool for the analysis of the protein composition of food and other biological products (Mañes & Molto, 2022).

6

Analysis and Discussion

Based on the foregoing discussion and the pertinent data gathered, it is established that milk represents one of the most widely consumed staple food products on a global scale (Chavarria, 2010). Nonetheless, this consumption trend has experienced a decline in recent years, gradually supplanted by the rising popularity and demand for plant-based beverages. This shift is evident in the increasing variety of plant-based drinks now accessible in the market (Carta, 2021).

6.1 Health and Nutritional Composition

It is noteworthy that vegetable emulsions or beverages exhibit a visual resemblance to bovine milk rather than a nutritional equivalence. Nevertheless, the substantial consumer demand for rice and soy-based beverages has propelled their popularity within the market. Consequently, the industry has directed efforts toward enhancing the nutritional compositions of these products, thereby elevating their nutritional profiles. Considering this, during the overall manufacturing process, the careful selection and incorporation of ingredients is principal to achieve a vegetable beverage of elevated nutritional value (Faccin et al., 2009).

Furthermore, although plant-based beverages can serve as a suitable choice for individuals with allergies or dietary intolerances, it is essential to recognize that not all plant-based beverages are equal in terms of their nutritional composition. For instance, rice milk may contain fewer proteins and less calcium compared to cow's milk, whereas soy milk can be a substantial source of proteins and other nutrients.

Cow's milk is a rich source of protein, as it contains all the essential amino acids in proportions suitable for human needs. Plant-based drinks, on the other hand, tend to have a lower protein content. Rice milk is an example of a low protein content that is between 0.1-0.6 g/ml. The exception is soy milk, which has approximately the same amount of protein as cow's milk, which is around 3-4 g/100 ml. The problem with cow's milk is that it contains lactose and casein, which for allergy sufferers, can be very harmful.

Additionally, soy proteins contain the essential amino acids that the body needs to function properly. Figure 6.1 shows the amino acids profiles of rice milk, soy milk and cow milk in g/100 ml indicating that soy and cow milk are practically on par in comparison to the low values of rice milk. Soy drinks also contain several health-promoting substances, such as isoflavones, which can help reduce the risk of chronic diseases like cardiovascular diseases. Soy proteins are used in human and animal nutrition due to their quality and their ability to improve nutrition and prevent diseases.

Soy milk is characterized by having a higher concentration of several essential amino acids than rice milk. These essential amino acids include lysine about 0.19 g/100 ml, threonine about 0.15 g/100 ml and tryptophan about 0.05 g/100 ml, while for rice milk those values are on average 0.01 g/100 ml (Gil, 2010). Lysine plays a crucial role in protein synthesis and the maintenance of muscle mass. Threonine is important in collagen formation as well as liver function, and tryptophan influences mood, sleep and appetite regulation (Gil, 2010). Soy milk, therefore, can be considered a more complete source of protein compared to rice milk due to its more favorable amino acid profile. Table 6.1 shows the amount of the different amino acids present in vegetable beverages such as soy and rice milk in contrast to cow's milk (g/100 ml).

Table 6.1 Amino acids profiles of rice milk, soy milk, and cow milk (g/100ml) (Palleiro, 2019)

Amino acids	Rice Milk [g/100 ml]	Soy Milk [g/100 ml]	Cow Milk [g/100 ml]
Tryptophan	0.00	0.05	0.03
Threonine	0.01	0.15	0.14
Isoleucine	0.00	0.16	0.20
Leucine	0.02	0.26	0.32
Lysine	0.01	0.19	0.27
Methionine	0.00	0.02	0.04
Cystine	0.00	0.00	0.03
Phenylalanine	0.01	0.17	0.16
Tyrosine	0.01	0.14	0.16
Valine	0.01	0.16	0.22
Arginine	0.02	0.26	0.12
Histidine	0.00	0.09	0.09
Alanine	0.01	0.15	0.11
Aspartic Acid	0.02	0.40	0.25
Glutamic Acid	0.04	0.69	0.69
Glycine	0.01	0.15	0.07
Proline	0.01	0.21	0.40
Serine	0.02	0.22	0.18

Despite of its favorable nutritional profile, soy milk does not fully meet the requirements for nutritional completeness due to its insufficient content of the essential amino acid methionine. Soybeans typically contain methionine within the range of 0.0 to 0.02 g per 100 ml, falling below the recommended threshold of 0.04 - 0.05 g per 100 ml set by the Food and Agriculture Organization.

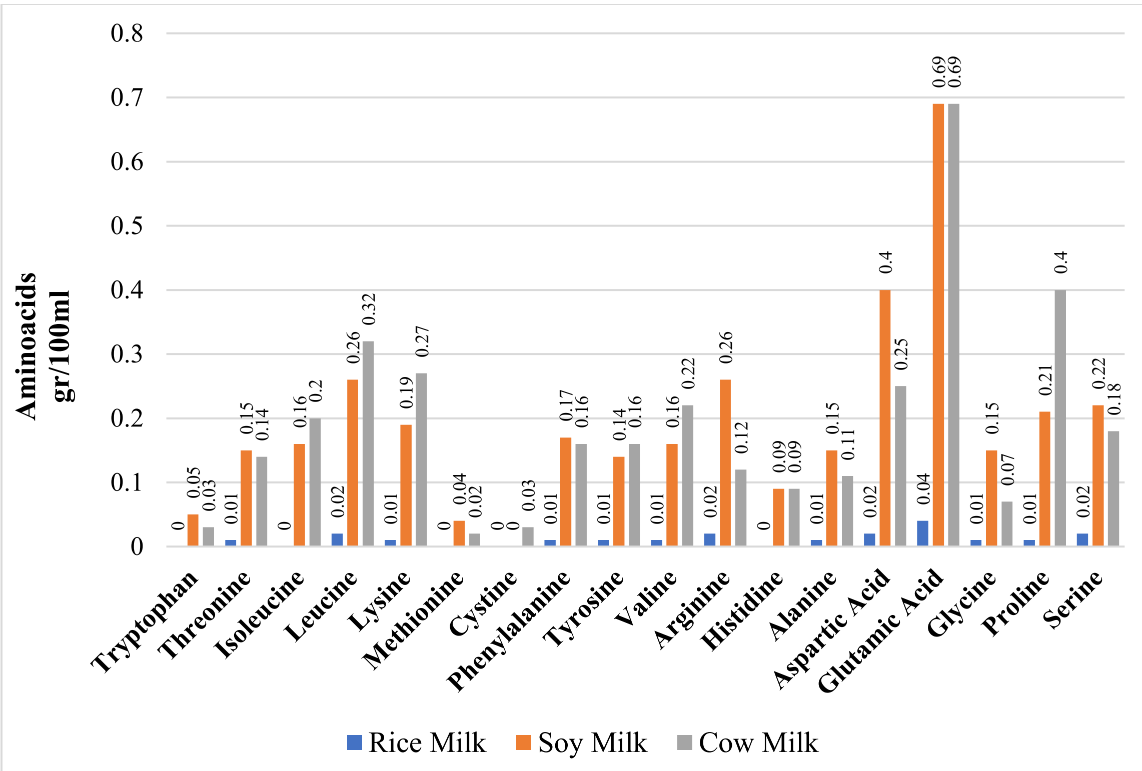

Figure 6.1 Amino acids profiles of rice milk, soy milk and cow milk (g/100 ml) (Palleiro, 2019)

Various global organizations agree that the main factors that promote excess weight and the appearance of chronic diseases are a sedentary lifestyle and poor eating habits. Obesity has emerged as a global epidemic, and this condition is intricately linked with risk factors associated with cardiovascular pathologies, making proper nutrition a focal point for authorities (Giugliano et al., 2023). Hence, soy and rice milk can be utilized as part of diets for obesity control. In the case of soy, it has been observed that the presence of phytosterols can assist in lowering blood cholesterol levels (Cabral & Klein). In the case of rice, it has been found that the presence of policosanol helps in reducing LDL-cholesterol levels in the blood (Reiner et al., 2005).

According to nutritional value, cow's milk provides 68 kcal/100 ml, whereas the average energy yield for the same quantity of soy milk and rice milk is 30 and 48 kcal, respectively (Carta, 2021). In comparison with milk, the caloric contribution of plant-based beverages is significantly lower.

Regarding the total carbohydrate content, cow's milk provides approximately 5 - 6 g in 100 ml. On the contrary, soy beverages contain 1 - 3 g per 100 ml of carbohydrates. On the other hand, rice milk has the highest carbohydrate content with 11-13 g per 100 ml (Lamothe, 2021). In contrast to cow's milk, plant-based beverages generally exhibit lower carbohydrate amounts with the exception of rice milk. Hence, rice milk may not be the best choice for people with diabetes or at risk for diabetes who are trying to limit carbohydrate intake and control their blood glucose.

Another possible health risk of consuming rice milk is its potentially high levels of arsenic. Arsenic is a toxic metal that occurs naturally in soil and water and can subsequently enter the food supply through plants. Rice tends to be a grain that absorbs higher amounts of arsenic and can sometimes reach dangerous levels if not closely monitored. (Rivera & Muñoz, 2011). The Food Safety Authority of Ireland (FSAI) advises against the consumption of rice milk as a substitute for cow's milk, breast milk, or infant formula for babies and young children up to 4.5 years due to the presence of low levels of inorganic arsenic in rice milk (FSAI, 2022). Consequently, the sale of these infant formulas is not in compliance with the legal composition and nutritional standards for infants and is therefore prohibited in the European market (FSAI, 2022).

On the other hand, rice milk has been suggested as a substitute for cow's milk for children older than 5 years with milk protein allergies. If a child follows a vegan diet, fortified rice milk provides a source of some of the essential nutrients commonly found in dairy, like calcium, vitamin A, vitamin D, and vitamin B12. However, rice milk provides very little protein, another much-needed nutrient for child growth. A suitable protein powder, such as soy or pea, may need to be added to rice milk to ensure adequate protein intake and proper growth and development (Rivera & Muñoz, 2011).

Meanwhile, the selection process of soybeans is important to enhance the nutritional value to the final product. Low-quality soybeans affect the chemical composition, physicochemical, and microbiological properties of soy milk. Currently, the producers of this milk carefully select the yellow soybean variety (*Glycine max*) because the recovery of protein content is approximately 70 to 80% (Kandylis et al., 2016); nevertheless, these values vary depending on the stages prior to production as well as processing.

In the case of rice grains, the chemical composition of rice milk is contingent upon the rice's quality. Among the range of rice byproducts, brown rice emerges as the optimal choice for the production of plant-based beverages due to its nutritional content (Kandylis et al., 2016). This is attributed to the presence of the bran layer and germ, which are abundant in protein content, ranging between 7 – 8 g/100 g (Garcia & Perez, 2015). In contrast, white rice undergoes a process that results in the removal of these nutrient-rich layers, leading to a decline in its nutritional value ranging between 2 - 3 g/100 g (Garcia &

Perez, 2015). Furthermore, brown rice exhibits higher levels of dietary fiber, vitamins, minerals, and antioxidants.

The examination of proteins plays an essential role in addressing various inquiries concerning the composition and chemical properties of food. Hence, protein characterization methods are of major importance, and the choice of the most suitable method is contingent upon the research's objectives and scope.

Based on the research studies within the scope of this report, it was observed that the widely used methods to characterize proteins in soy milk and rice milk use a label-free quantitative proteomic approach in conjunction with other techniques such as mass spectrometry and liquid chromatography.

The proteomic approach stands as the most suitable method for the complete characterization of proteins in soy and rice milks for many reasons: wide detection range, does not require chemical markers, identifies unknown proteins, generates more complete data on the relative abundance of proteins, and their sensitivity and precision in the detection of small amounts of proteins and changes in their abundance (Lopez, 2020), which is crucial for studies of protein composition in foods.

In a study conducted by Battisti et al. (2021), different proteins were identified in soy milk samples, and most of the proteins were found to be multifunctional and could be classified into several categories which are gene ontology (GO) – based molecular function categories, including nutrient storage activity, binding activity, enzyme regulation activity, and antioxidant activity. Moreover, significant differences in protein composition were observed among the different soy milk samples, suggesting that variations in thermal and technological treatments used in soy milk production can affect the protein composition of the final products.

Principal component analysis (PCA) and non-hierarchical clustering analysis using the K-means algorithm were performed to compare the different soy milk samples and highlight the similarities and differences in terms of protein composition (Battisti, et al., 2021). Three different clusters of soy milk samples were found, which were grouped according to their protein composition. These groups could be influenced by variations in the thermal and technological treatments used in the production of soy milk, as well as by the variability due to the genetic background of the soybean varieties used in the production process.

According to the molecular function-based categorization of identified proteins showed in Figure 6.2 A, the majority of the proteins are related to binding (13%), followed by nutrient reservoir activity (12%), and oxidoreductase activity (12%). The highest contribution in terms of percentage mass is given by the proteins related to the category of nutrient reservoir activity (75.9%), followed by endopeptidase inhibitor activity (6.6%), lipid binding (6.4%), and seed maturation (3.0%) (Battisti et al., 2021). These findings suggest that soy milk is a rich source of proteins with nutrient reservoir activity, which is consistent with previous proteomics studies on soy milk and soybean seeds .

Figure 6.2 B shows the results of a GO enrichment analysis, which identifies the most significantly enriched gene sets in the soy milk proteome. The nodes in the graph represent the description of the gene set, and the node size corresponds to the number of associated GO terms within each gene set. The node color corresponds to the significance (p-value) tested by Benjamini-Hochberg FDR threshold at 0.05, and the edge size corresponds to the number of GO terms that overlap between two connected nodes. The GO terms associated with oxidoreductase activity are significantly enriched, which supports the observation made with molecular function-based categorization of identified proteins . Other significantly enriched GO terms include enzyme inhibitor activity, binding, structural constituent of ribosome, protein folding, and translation (Battisti, et al., 2021). These findings suggest that soy milk contains a diverse array of proteins with various molecular functions.

It is worth noting that the molecular function-based categorization of identified proteins in Figure 6.2 A is based on the information available in the UniProt database. Additionally, the categorization is based on the molecular function of individual proteins, whereas the GO enrichment analysis in Figure 6.2 B considers the functional relationships between groups of proteins. Therefore, the two analyses provide complementary information about the soy milk proteome.

Overall, the findings of this study have important implications for individuals with soy allergies or those seeking plant-based protein sources. The high abundance of proteins with nutrient reservoir activity in soy milk suggests that it could be a valuable source of protein. However, the presence of other proteins with potential allergenic or anti-nutritional properties, such as lectin and trypsin inhibitors, should also be considered.

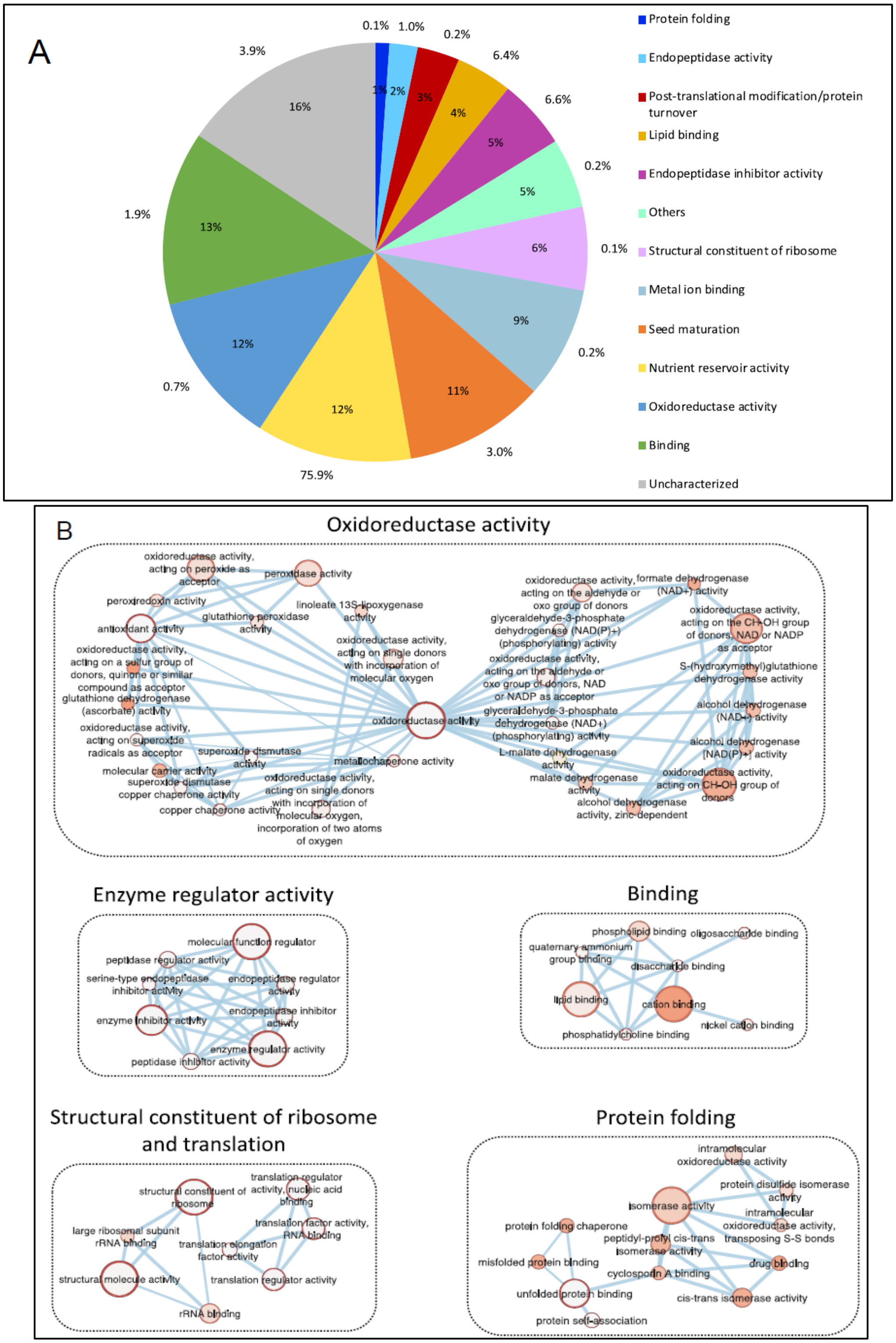

Figure 6.2 A) Molecular function – based categorization of identified proteins in soy milk. The values both within and outside the pie chart represent the proportions of proteins categorized, and the cumulative average mass percentage, respectively. B) GO enrichment analysis (Battisti et al., 2021).

An additional investigation, undertaken by Battisti et al. in 2023, which is still in progress, assessed the protein profile of several commercial rice milks using a combination of proteomic and bioinformatic approaches. The study identified proteins in rice milk, and the proteins were categorized based on their GO-based molecular function.

Furthermore, it was observed that exogenous proteins present in commercial rice milks can serve various purposes, such as improving the texture, flavor, and nutritional value of the food or beverage. This proteins are not naturally present in a particular food or beverage; instead, are added during processing or manufacturing. Exogenous α- and β-amylases can help to hydrolyze starch and achieve a desired level of sweetness and viscosity. However, the presence of exogenous proteins in food and beverage products can potentially pose allergenic risks to individuals with food allergies or intolerances. This underscores the critical importance of precise labeling and vigilant allergen management practices within the food industry.

The molecular function-based categorization of identified proteins without and with exogenous proteins showed in Figure 6.3 provides valuable insights into the molecular function of rice milk proteins. The categorization was carried out manually using the information available in UniProt and revealed that the most represented categories in both datasets were related to binding, followed by nutrient reservoir activity and oxidoreductase activity.

When comparing Figure 6.3 A and B, it is clear that the addition of exogenous proteins significantly altered the molecular function-based categorization of identified proteins. For example, in Figure 6.3 A, about 50% of the total % mass was contributed by the proteins with nutrient reservoir activity, followed by categories such as IgE binding (14%), nucleic acid binding (8%), ion binding (5%), and nucleotide binding (4%). However, in Figure 6.3 B, 46% of the total % mass was contributed by the proteins grouped under nutrient reservoir activity, followed by IgE binding (12%).

Interestingly, 12% of the total % mass in Figure 6.3 B was contributed by proteins with hydrolase activity, which was only 1% in Figure 6.3 A. Of the 12% contributed by proteins with hydrolase activity, 11% was contributed by the exogenous α-amylases.

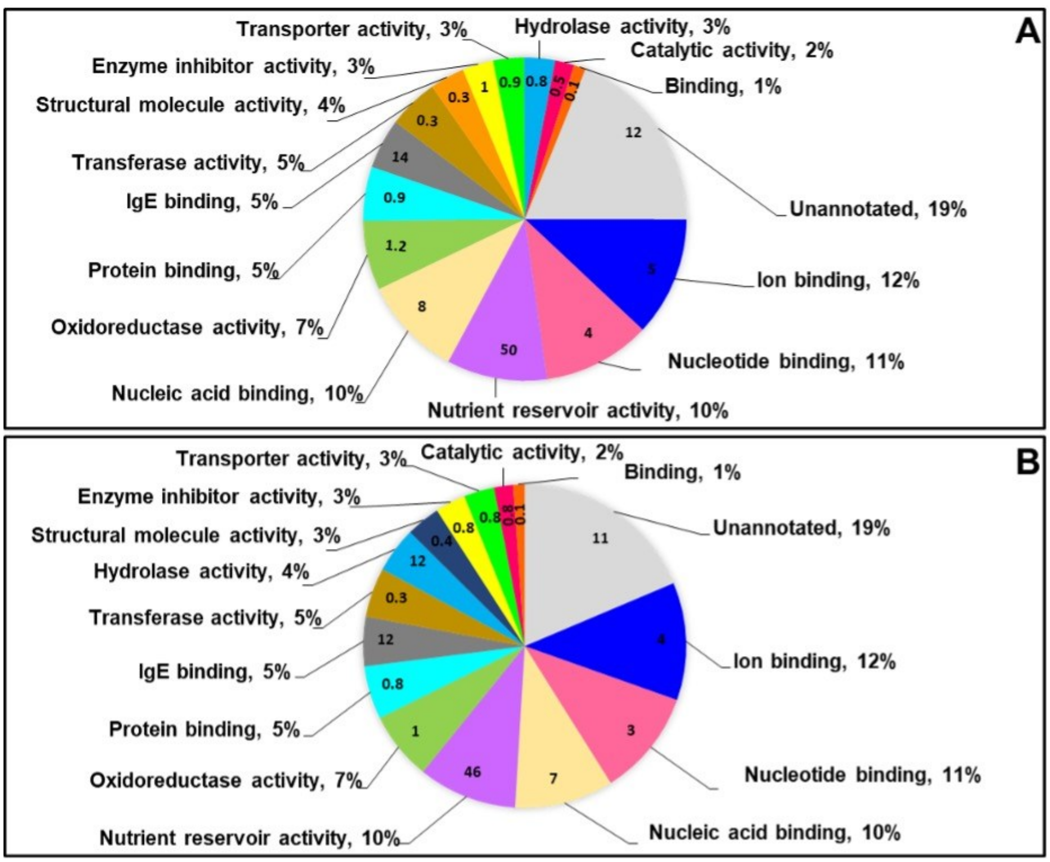

Figure 6.3 Categorization of identified proteins based on molecular function in the absence (A) and presence (B) of exogenous proteins. (Battisti et al., 2023, publication in progress)

In a comprehensive context, the molecular function-based categorization of the identified proteins furnishes valuable insights into the molecular functionality inherent in rice milk proteins. The results of this study can be used to understand more deeply the molecular function of rice milk proteins with those of other plant-based milk substitutes and dairy milk, which can help consumers make informed choices about their milk consumption. The categorization can also be used to identify proteins with specific molecular functions that are of interest to researchers and product developers.

6.2 Market analysis

As previously described, there are several important reasons regarding health that have led people to change their eating habits where vegetable drinks are clearly occupying a large sector in the market. Thus, it can be said that the most commercialized vegetable drink on the market is soy-based milk because it is a familiar product and has been studied for decades, followed by the almond-based beverage, and rice milk. The latter is gaining more acceptance globally in recent times.

Additionally, according to all the information analyzed, it would be useful to have an updated description of the eating habits of each country which would allow redirecting, planning and developing policies, nutritional education programs, and food-nutritional communication strategies based on evidence and directed to combat the main health problems such as allergies, intolerance to certain enzymes, excess weight, among others.

Regarding milk consumption, there is a notable trend of substitution by vegetable-based alternatives. Regions such as Latin America and Asia have witnessed a significant decline in milk consumption levels, notably decreasing by 8% between 1997 and 2019 (Hernandez & Rodriguez, 2021). Specifically, the consumption of dairy desserts and milk, including powdered forms, has experienced a substantial reduction, amounting to nearly 40%. Consequently, there has been a transformative shift in consumption patterns. For instance, in 1997, an average of 179 liters of fluid milk were consumed per capita, whereas by 2019, this had decreased to 115 liters (Garcia & Perez, 2017).

It is plausible that the shifts in consumption patterns share a common underlying origin linked to cultural transformations in the dietary practices of the population, extending beyond mere economic factors. While some instances do exhibit correlations between declining milk consumption and the economic activities of nations, this phenomenon is not universally applicable. For instance, in Chile, despite of its strong tradition of livestock farming, the decline in milk consumption is evidently motivated by health considerations and an inclination towards the consumption of innovative products that promote sound nutrition and well-being customs (Murcia, 2015). The possible motivation for the shift towards plant-based beverages in traditionally livestock-oriented countries may be due to a change in customs (Murcia, 2015).

7
Conclusions

In conclusion, this study delved into the proteomic profiles of rice and soy milk, shedding light on the abundance and variety of proteins, including amino acid composition, within soy- and rice-based beverages. Additionally, this report addressed the limitations associated with these alternative milk sources, and their tendency in the market. In accordance with the previously defined objectives, the following conclusions are drawn:

The analysis of market trends unveiled that the global milk market is in a state of rapid transformation, with Europe at the forefront of the shift towards plant-based alternatives. The industry is prioritizing the improvement of nutritional compositions in these products, resulting in elevated nutritional profiles, and the demand for rice and soy-based beverages has risen significantly. Soy milk, is a frontrunner in the market, reflecting its substantial market value of 7.4 million USD. On the other hand, rice milk market value is 0.8 million USD. Despite the fact that the rice market still needs to grow, it is notable that the global rice milk market is poised for impressive development, with a projected Compound Annual Growth Rate (CAGR) of 14% between 2020 and 2025.

Regarding the health perspective, rice milk tends to be lower in proteins and calcium compared to cow's milk, whereas soy milk emerges as a substantial source of proteins and essential nutrients. Soy and rice milk can play a role in obesity control, with phytosterols in soy milk aiding in cholesterol reduction and policosanol in rice milk contributing to lowering LDL-cholesterol levels. These plant-based beverages offer lower caloric contributions compared to cow's milk, making them suitable options for calorie-conscious individuals. For these reasons, plant-based beverages are a suitable option for individuals with allergies or dietary issues. However, it was observed that with respect to allergenic potential, *Glycine max* present in soy milk can cause problems in children until 7 years old, and rice does not represent a potential risk for consumers.

Moreover, soy milk stands out as a nutritionally robust alternative due to its balanced amino acid profile and the presence of essential amino acids vital bodily functions like lysine, threonine, and tryptophan. Nevertheless, despite this favorable profile, soy milk falls short of meeting the nutritional completeness requirements due to its insufficient content of the essential amino acid methionine.

While soy milk offers a balanced amino acid profile and essential amino acids, rice milk contains fewer proteins than soy and cow milk. However, rice milk has the highest carbohydrate content and can be a less suitable choice for individuals with diabetes or those aiming to control carbohydrate intake. Additionally, rice milk may pose health risks due to potentially high levels of arsenic, particularly in infant formulas. Despite these concerns, rice milk can serve as a substitute for cow milk for children older than 5 years with milk protein allergies and within vegan diets, with the need for protein supplementation.

Finally, it is observed that the choice of an appropriate protein characterization method is crucial for comprehensive analysis in food science research. Label-free quantitative proteomic approaches, complemented by mass spectrometry and liquid chromatography, are widely used to characterize proteins in soy and rice milk, providing comprehensive data on protein composition, abundance, and changes.

8

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