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Designing and developing a protein-enriched cracker using food ingredients with adaptogenic properties for physically active individuals

By

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Dedication

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-It always seems impossible until it's done-

By Nelson Mandela

Abstract

Despite health and fitness market growth, a gap persists in convenient foods addressing nutritional and psychological needs of active individuals. Protein enrichment and motivation critically influence activity performance and engagement, respectively. Thus, innovative high-protein foods with mood-enhancing ingredients are needed to sustain participation. The objective of the present research was to develop a high-protein cereal-based food product containing powdered roots of *Rhodiola rosea* and *Eleutherococcus senticosus*, with tailored functional and technological properties to potentially enhance the mood of physically active individuals during the pre-workout phase of exercise.

Social media Twitter was used in 2022, to analyze physically active individuals' demands related to food and nutrition, while bibliometric analysis and market research were done to study food products containing adaptogens. Physicochemical properties of roots from *E. senticosus* and *R. rosea* as adaptogens sources were assessed. Crackers made with protein isolates (55 g/ 100 g) from pulses (faba bean, lupin, pea and lentil) and both roots, were also characterized.

Results revealed that motivation for physical activity engagement is a recurrent theme in Twitter. Moreover, while research has focused on the physiological effects of adaptogens such as *Rhodiola rosea* and *Eleutherococcus senticosus*, their incorporation into marketed foods such as snacks, is limited. *R. rosea* and *E. senticosus* roots were rich in fiber and bioactive compounds, with high stability under acidic and thermal conditions. This potential was explored through their incorporation into high-protein crackers based on faba bean isolate. Their inclusion modified dough rheology, reduced cracker hardness, and enhanced the stability of bioactives such as salidroside, rosavin, and eleutheroside E. Investigation of protein–bioactive interactions revealed that the choice of protein isolates from different sources, influenced texture, color, and the *in vitro* release of adaptogenic compounds. Structural changes in protein matrices, particularly β -sheet formation and aggregation, were key factors in

regulating bioaccessibility, while random coil structures promoted bioactive release.

Overall, the research provides an integrative understanding of consumer behavior, adaptogen functionality, and protein-based delivery systems, establishing the basis for innovative protein-rich snacks that combine health benefits with consumer demand.

Resumen

A pesar del crecimiento del mercado de la salud y el deporte, persiste una brecha en alimentos que atiendan convenientemente tanto las necesidades nutricionales como psicológicas de los individuos activos. El enriquecimiento proteico y la motivación influyen de manera crítica en el rendimiento y la continuidad de la actividad física. Por ello, se requieren alimentos innovadores, ricos en proteínas e ingredientes que mejoren el estado de ánimo, para sostener la participación.

El objetivo de esta investigación fue desarrollar un producto alimenticio a base de cereales que incorporara raíces en polvo de *Rhodiola rosea* y *Eleutherococcus senticosus*, con propiedades funcionales y tecnológicas específicas para potenciar el ánimo de individuos físicamente activos en la fase previa al ejercicio.

Se empleó Twitter para analizar las demandas nutricionales de los usuarios físicamente activos, mientras que se realizaron análisis bibliométricos y de mercado sobre productos que contienen adaptógenos. Asimismo, se evaluaron las propiedades fisicoquímicas de ambas raíces y se elaboraron crackers con aislados proteicos de legumbres (haba, altramuza, guisante y lenteja; 55 g/100 g) incorporando las raíces.

Los resultados mostraron que la motivación es un tema recurrente en Twitter. Aunque la investigación científica ha abordado principalmente los efectos fisiológicos de los adaptógenos, su presencia en alimentos de consumo, como los snacks, sigue siendo limitada. Las raíces presentaron un alto contenido en fibra y compuestos bioactivos con buena estabilidad frente a condiciones ácidas y térmicas. Su incorporación en galletas de aislado de habas modificó la reología de la masa, redujo la dureza y aumentó la estabilidad de bioactivos como salidroside, rosavina y eleuterósido E. El estudio de las interacciones proteína-bioactivos reveló que la fuente proteica influye en la textura, el color y la liberación *in vitro* de los compuestos activos. Los cambios estructurales en las matrices proteicas, especialmente la formación de láminas β y la agregación, regularon la bioaccesibilidad, mientras que las estructuras de espiral aleatoria favorecieron la liberación.

En conjunto, esta investigación aporta una comprensión integral del comportamiento del consumidor, la funcionalidad de los adaptógenos y los sistemas proteicos de liberación, estableciendo bases para snacks proteicos innovadores que integren beneficios para la salud y demanda del consumidor.

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List of abbreviations

ES: *Eleutherococcus senticosus*

RR: *Rhodiola rosea*

FBPI: faba protein isolate

PPI: pea protein isolate

LPI: lentil protein isolate

LuPI: lupin protein isolate



INTRODUCTION

In recent years, there has been a remarkable rise in the popularity of participation in sports activities among individuals from all walks of life. Whether it is the thrill of competition, the desire for physical fitness, or the sense of community that sports offer, people around the world are increasingly engaging in various physical activities (Harvard Health Publishing, 2024). From amateur enthusiasts to dedicated athletes, the rise of sports has created a global phenomenon that transcends age, gender, and cultural boundaries. According to data obtained from the Statista database, the sports and fitness industry generated an estimated revenue of US \$112 billion worldwide in 2023 and is expected to reach USD 202.8 billion in 2030 (Statista, 2025a).

The rapid evolution of digital media has provided new opportunities for individuals to be connected and socially engaged. In fact, physically active individuals tend to use social media as a source to find information and acquire knowledge related to exercise techniques, nutrition and food choices (Bourke et al., 2019). In this context, social media platforms such as X, Facebook and Instagram have been widely employed to retrieve information across different topics. For instance, Vidal et al. (2015) investigated food-related consumer behavior on Twitter, focusing on different eating situations. Similarly, another study analyzed the content from nutrition-related posts on Instagram (Denniss et al., 2023). Within the field of sport, Lauricella & Koster

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(2016) examined the influence of the social media platform X (or Twitter as it was before July 2023) on the consumption of a post-workout beverage made with chocolate and its perceived health benefits. Social media has also been used to gather in-depth insights into the ongoing practices of sport and exercise practitioners (Goodyear et al., 2018). More recently, Stanford & Salles (2021) conducted research exploring the impact of X and Instagram on promoting physical fitness during the COVID-19 pandemic and its consequences on mental health. Overall, these studies highlighted the diverse ways in which social media can influence, particularly in terms of motivation and inspiration.

When it comes to physical performance, there are three different phases: pre-workout, intra-workout (or during exercise) and post-workout (Thomas et al., 2016). In this regard, mood plays a crucial role in the pre-workout phase, since exercise involves not only muscular qualities but also behavioral ones (Roumanille et al., 2020). Mood is known to be a group of persistent feelings or emotions that can be associated with athletic success and that persist for longer periods of time (Renger, 1993). This concept has been described by different studies as a factor that can have an important role in physical activity, as a good mood can influence performance to some extent (Heikura et al., 2023; Knittle et al., 2018; Renger, 1993). In fact, many people experience a lack of motivation to engage in exercise due to two main factors: lack of time and the feeling of not being sufficiently competent (Teixeira et al., 2012). Therefore, mood enhancement is a priority as it not only determines whether people will attempt to modify their physical activity behavior initially, but also whether they will engage or persist with physical activity in the long term (Knittle et al., 2018).

There are different strategies that help increase both physical and psychological well-being, thereby playing a supportive role in sustaining mood. In this regard, medicinal herbs, defined as plants with widespread health properties, represent a promising avenue in terms of mood and physical performance enhancement. Their history goes back as far as to 60,000 years ago, when their use was mainly based on instinct and the experience of using plants, as there was little information regarding their

benefits or health effects (Masoud et al., 2019). Consequently, more than 50,000 species have been used worldwide (Chen et al., 2016). The distribution of medicinal plants around the world is not equal. Specifically, China and India have the highest number of medicinal herbs, with 11,000 and 7,500 species, respectively (Chen et al., 2016).

Among these medicinal herbs, there is a small group named as adaptogenic plants, which contain active compounds that can increase resistance against physical, chemical, biological and psychological stressors (Amir et al., 2023). Plant adaptogens can produce a normalizing physiological response and are non-toxic when used in normal doses (Rusu, 2018). Their consumption has been related to the improvement of cognitive function and mood, as well as focus and performance enhancement of physically active individuals during exercise (Cropley et al., 2015; Holford, 2003). In this regard, adaptogenic plants have been traditionally consumed as herbal teas containing root powder (Winston, 2019). In fact, the use of adaptogenic plants as root powders has been limited to teas and some beverages (Boolani et al., 2020, 2022). No studies have incorporated the root powders in a solid food matrix. In the past last years, they have been consumed in the form of supplements made from extracts from different parts of the plants (Kuo et al., 2010; Williams, 2021). These extracts are standardized to contain certain amounts of active compounds to produce a specific effect such as enhancement of physical performance and mood (Wal et al., 2019).

In the pre-workout phase, where a good mood is pivotal, physically active individuals should also ensure that their nutritional requirements are met. These nutritional requirements and energy consumption vary within the different phases of workout, depending on type, intensity and duration of training, as well as age and body composition of the physically active individual, among others (EFSA, 2015). Thus, for the pre-workout phase, physically active individuals should maintain sufficient glycogen stores for energy, and this requires a recommended intake of around 6 to 10 g of carbohydrates/kg/day. In addition, it is also necessary to consume around 1.2

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to 2 g of protein/kg/day and to have proper hydration (Thomas et al., 2016). In the case of protein intake, although there is a controversy on whether it should be taken before or after workout, a pre-exercise intake can provide amino acids during and after exercise and therefore it stands to reason there is less need for immediate post-exercise protein ingestion (Jäger et al., 2017; Peeters et al., 2023). Overall, a progressive increase in protein consumption is recommended, to avoid liver overload and renal issues (Pérez-Guisado, 2008). Furthermore, it is very important to ensure that all the essential amino acids are provided by eating complementary proteins (Schoenfeld et al., 2017). Within the recommended foods that are rich in protein, meat, poultry, eggs, dairy and plant-based sources, such as legumes and grains, are the most consumed (Grozenski & Kiel, 2020).

However, despite the nutritional recommendations, many physically active individuals do not meet their nutritional goals due to busy lifestyles, the shift towards a plant-based diet, and the indiscriminate use of supplements for athletes that have not been evaluated and proven under scientific trials, among others (Cui et al., 2022). In addition, a diet based solely on conventional foods makes it difficult to meet the specific nutritional needs for engaging in exercise. Thus, supplements are widely used because they can provide targeted nutrients, such as creatine, glutamine or beta-alanine (Thomas et al., 2016). Among these, creatine and protein-based supplements, with isolated or concentrated proteins from whey, casein, soy or egg, are especially popular. In the last few years, the food industry's continuous innovation has led to a wide range of specific food products that align with the dietary needs of physically active individuals (Roumanille et al., 2020). This includes protein or energy bars, gels, puddings made with rice or maize, cheese and protein shakes, among others (Cui et al., 2022).

The growing demand for plant-based protein sources has emerged in the past decade driven by both environmental and health considerations. Although animal protein sources have a higher digestibility compared to plant-based ones, animal-based proteins have been associated with higher greenhouse gas emissions due to animal agriculture (Bianchi & Simonato, 2025). For this reason, pulse proteins have garnered attention as promising sources of

sustainable protein. Indeed, the global pulse protein market was valued at USD 18.93 billion in 2023 and is expected to reach USD 33.26 billion by 2031 (Data Bridge Market Research, 2024). With the proliferation of protein supplementation, pulse protein isolates have become an ideal ingredient in many products aimed at athletic performance. Protein isolates typically contain 90% or more protein by weight and a very low content of sugar and fat.

In the context of fitness and exercise, pulse protein isolates have been recently used as core ingredients for protein shakes, ready-to-drink shakes and beverages, providing a quick source of amino acids (Cui et al., 2022). Though most plant proteins are limited in certain amino acids (methionine, cysteine and tryptophan), this can be addressed by blending protein sources like cereals and legumes (Higa et al., 2024). Due to this, another major application is in high-protein bars and baked goods, where pulse protein isolates are incorporated to increase their protein content. Even though pulse protein isolates can mimic most of the functional properties of those from animal sources, their incorporation in food products is still challenging as they also have some downsides like off-flavors and antinutrients (Bianchi & Simonato, 2025).



HYPOTHESIS

Social media analytics and market research can help identify the nutritional needs and food product demands of physically active individuals. In addition, incorporating *Rhodiola rosea* and *Eleutherococcus senticosus* adaptogenic root powders into high-protein crackers formulated with different pulse protein isolates will induce protein interactions with the bioactive compounds and fiber of the root powders, which (i) will alter dough rheology and cracker texture, (ii) enhance thermal retention of key bioactives during processing, and (iii) modulate in vitro bioaccessibility in a protein-source-dependent manner.

OBJECTIVES

The objective of the present research is to develop a cereal-based food product containing powdered roots of *Rhodiola rosea* and *Eleutherococcus senticosus*, with tailored functional and technological properties to potentially enhance the mood of physically active individuals during the pre-workout phase of exercise.

Furthermore, the specific objectives of this research are the following:

1. Exploration of consumer perceptions of food and nutrient requirements for physical activity by using Twitter data.
2. Assessment of adaptogenic plants in food products on the market, with a critical comparison of market trends and peer-reviewed scientific evidence.
3. Characterization of the adaptogens *Rhodiola rosea* and *Eleutherococcus senticosus* in terms of chemical composition and bioactive compounds of interest.
4. Development of high-protein crackers incorporating adaptogenic root powders to evaluate their impact on physicochemical characteristics, and to examine the effect of processing on the stability of the bioactive compounds.
5. Investigation of how *Rhodiola rosea* and *Eleutherococcus senticosus* root powders interact with different pulse protein isolates and

influence the physicochemical properties and *in vitro* bioaccessibility of high-protein crackers.

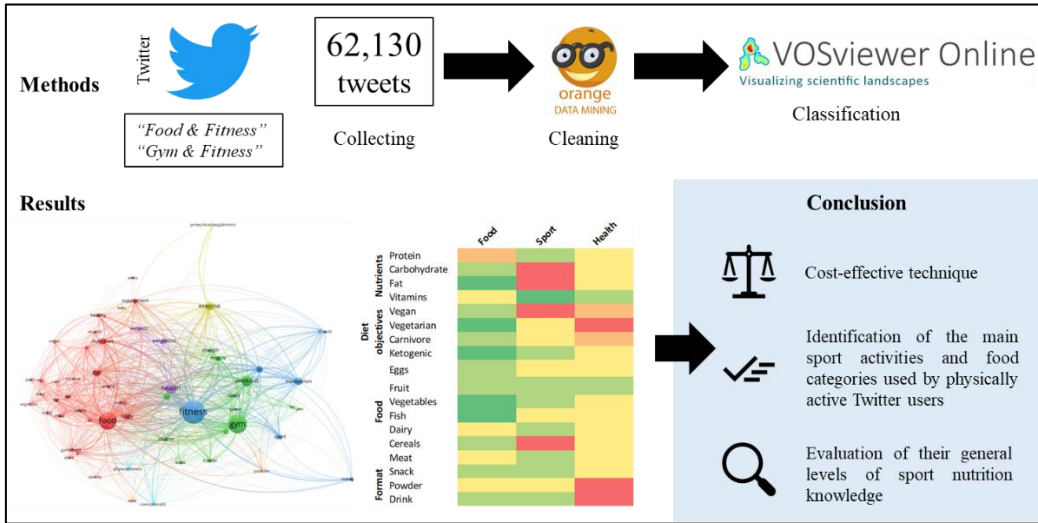


WORKING PLAN

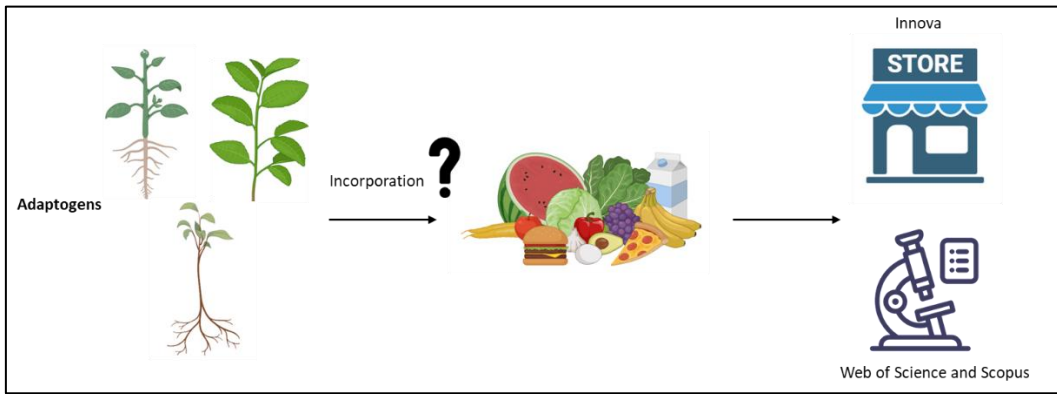
The study is organized into different chapters that cover the following subject matter:

- The introduction provides background information and context relevant to understanding the importance of mood on the pre-workout phase and the use of adaptogenic plants as a strategy to improve motivation, leading to an enhancement in physical performance. In addition, this chapter also includes the importance of protein for the pre-workout phase and the need to develop a new vehicle to deliver functional compounds in a protein enriched matrix.
- Chapter 1. Exploring consumers' perceptions and biases on eating behaviors and sport nutrition: A twitter perspective.
- Chapter 2. Mapping the growth and trends of adaptogens in food: a comprehensive analysis of literature and market insights.
- Chapter 3. Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*.
- Chapter 4. Development of high-protein functional crackers enriched with adaptogenic compounds.
- Chapter 5. Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*.
- The general discussion covers the summary of the key findings and identifies potential avenues for future research based on the outcomes of the study.

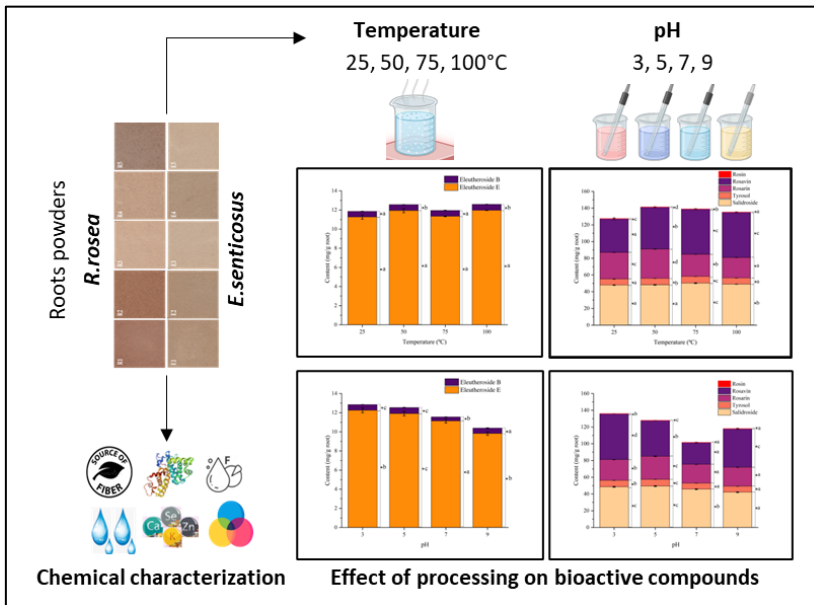
CHAPTER 1



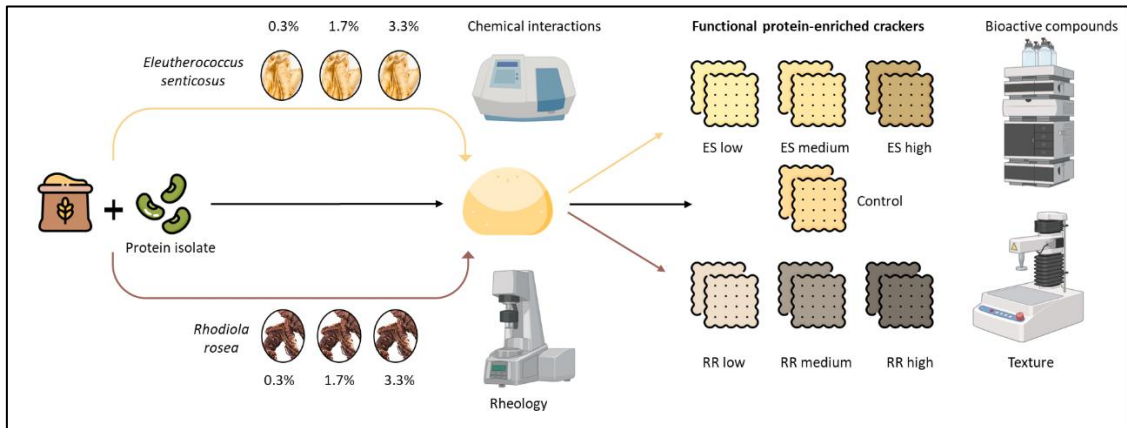
CHAPTER 2



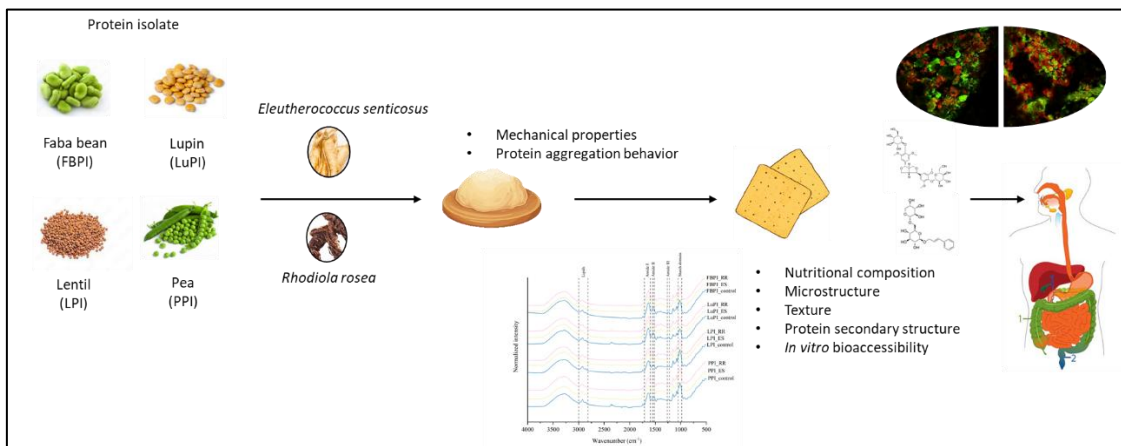
CHAPTER 3



CHAPTER 4



CHAPTER 5



Publications:

This thesis has resulted in the publication of five research papers:

1. Molina, C. N., Gasparre, N., Garzon, R., Vinuesa, J. M., & Scanlon, M. G. (2024). Exploring consumers' perceptions and biases on eating behaviors and sport nutrition: A twitter perspective. *Food and Humanity*, 2, 100286.
2. Molina, C. N., Gasparre, N., Mañes Vinuesa, J., & Scanlon, M. G. (2025). Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*. *International Journal of Food Science and Technology*, 60(1), waf049.
3. Molina, C. N., Gasparre, N., Mañes, J., & Scanlon, M. G. (2025). Development of high-protein functional crackers enriched with adaptogenic compounds. *LWT*, 130, 118257.
4. Molina, C. N., Gasparre, N., Bandara N., Vinuesa, J. M., & Scanlon, M. G. (2025). Protein matrix interactions and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*. *Journal of Functional Foods*, 135, 107086.
5. Molina, C. N., Boukid F, Vinuesa, J. M., & Scanlon, M. G. Mapping the growth and trends of adaptogens in food: A comprehensive analysis of literature and market insights. (Submitted to *Journal of Agriculture and Food Research* on January 12, 2026).



CHAPTER 1

EXPLORING CONSUMERS' PERCEPTIONS AND BIASES ON EATING BEHAVIORS AND SPORT NUTRITION: A TWITTER PERSPECTIVE

1.1. INTRODUCTION

Setting goals for healthier lifestyles is becoming a growing practice worldwide. In fact, sports and endurance activities are already part of the daily lives of many individuals who are pursuing an active lifestyle. Evidence of that is the increase in the health and fitness market in recent years, which generating an estimated revenue of \$96.7 billion worldwide (2019), with a prediction of \$136.6 billion by the end of the 2024 (Statista, 2021a). Activities such as physical activity, exercise, physical fitness and sport are included in definitions for that market, despite their different meanings. While physical activity is defined by the World Health Organization (WHO) as “any bodily movement produced by skeletal muscles that requires energy expenditure”, exercise is used to describe “any physical activity that is planned, structured, repetitive, and purposive in the sense that improvement or maintenance of one or more components of physical fitness is an objective” (World Health Organization, 2020). As far as physical fitness and sport are concerned, the former is defined as a set of attributes/components such as muscular endurance and body composition that are either health- or skill-related, while

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the latter term is related to a subset of exercises undertaken individually or in a team.

The main reason behind practicing a sport is its significant health benefits, mainly associated with blood pressure reduction, maintenance of a healthy body weight, lowering of total- and low-density lipoprotein levels, improvement of cardiorespiratory and muscular fitness, as well as an increase in the amount of high-density lipoprotein (Kapoor et al., 2022).

Some studies have reported the greater understanding of physically active individuals and athletes regarding general nutrition compared to sports nutrition concepts (Devlin et al., 2017; Tam et al., 2021). As a result, they often consume an inadequate diet to support their energy requirements and/or nutritional needs (Tam et al., 2021). Therefore, moved by the need for covering their sport nutrition knowledge, physically active individuals commonly resort to different sources, such as books, Internet, or social media (Rodríguez-Martín & Alberto Castillo, 2017). Nowadays, our digitally connected society has helped social media to become a powerful tool, providing a real-time glimpse on nutrition and sport activities. Moreover, users can have access to a platform to connect, engage in discussions, read articles, watch videos and learn from experts' _opinions. As a result, users can acquire more knowledge about healthy eating patterns, exercise routines or sports techniques, among others. However, this generated knowledge is not always accurate as sometimes it is provided from non-professional sources that could lead to misinformation (Rodríguez-Martín & Alberto Castillo, 2017). Thereby, with its 4.55 billion users worldwide as of 2022, social media like Twitter, Instagram or Facebook, have become the recent principal sources of information for physically active individuals and athletes (Rosen et al., 2022).

In this context, the social media company Twitter stands out, since it allows massive exchange of interpersonal communication about various topics. Because of that, Twitter has attracted the interest of scientific researchers, since its information can be captured, analyzed, and obtained more quickly compared to the use of online surveys or interviews, and because it avoids the

biases or the limitations of asking people questions. The Twitter social media has therefore been used to identify food-related consumer behavior in four eating situations (breakfast, lunch, snack and dinner) (Vidal et al., 2015), the relationship between state-level obesity rates and social media engagement with food and beverage brands (Gu et al., 2021), the impact of environmental programs on society (Zarrabeitia-Bilbao et al., 2022), or consumer perceptions of different foods and beverages, such as eggs (Sass et al., 2020) or beer (Arellano-Covarrubias et al., 2019). Regarding sports and nutrition, there are very few studies that have used social media as a methodology to understand what society thinks and consumes in relation to sports nutrition. Some studies have focused on analyses of different sports brands and their social media activity, the role of social media in the promotion of physical activity during pandemic times, the study of food and beverage brands on social media, or the effect of social media videos on food consumption, appetite and weight loss (Gascoyne et al., 2021; Rosen et al., 2022; Singh & Sharma, 2022; Stanford & Salles, 2021).

Therefore, despite the need to know and understand the demands and opinions of physically active individuals in relation to sports nutrition, there are no previous studies examining the use of social media such as Twitter to address this question. Hence, the purpose of the present study was to provide insight into the most discussed Twitter topics related to fitness, gym, food and nutrient needs, so that we can gain insights into the lifestyle of physically active users.

1.2. MATERIALS AND METHOD

1.2.1. Selection of terms for social media research

An initial search was carried out to identify the most frequent terms that would accurately reflect the habits and knowledge of consumers using Twitter social media. The keywords used in the present study were obtained using Google Trends and Track My Hashtag tools. Google Trends provides the number of searches that have been carried out in relation to a term for a given

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period and a specific place in the world, allowing comparison between different terms (Arellano-Covarrubias et al., 2019). Conversely, the Track My Hashtag tool gives the total number of tweets obtained from the search of a specific term for a given period as well as the total number of Twitter users who wrote a tweet using that specific term (Forbes, 2020). Based on the prior search using Google Trends and Track my Hashtag tools, a set of 17 English terms related to food, fitness and gym use were proposed for studying nutritional needs and food products as relevant to Twitter users. The selected terms were the following: food and fitness, food and sport, food and gym, gym fitness, fitness life, eat and gym, healthy lifestyle, muscle growth, flexible diet, weightlifting, weight loss, balanced diet, calorie deficit, gain muscle, fitness supplement, muscle mass, nutrition coaching. From those 17 terms, *Food and Fitness* and *Gym and Fitness* were the most frequently mentioned.

1.2.2. Retrieval of Twitter data

Tweets containing the terms *Food and Fitness* and *Gym and Fitness* were retrieved from the social media firm Twitter using the *twitteR* package (Kearney, 2017) of R software (R Core Team, 2016). English was selected as the only language of interest for the tweets as the study was conducted globally. This package provides an interface to connect the Twitter web Application Programming Interface (API) with R software, in order to download the messages that Twitter users share about a particular topic by searching tweets with specific keywords. Tweets were downloaded by using the commands *openxlsx*, *xlsx*, *zipR* and *rtweet*. Since the Twitter server has limits on the number of tweets that can be retrieved in each search (Kearney, 2017), multiple searches were performed in order to obtain a representative sample size. Data collection was performed between February 1st and August 1st, 2022. A total of 62, 130 tweets were obtained with both terms (Table 1). Retweets and repeated tweets were removed afterwards to avoid utilization of repeated data.

Table 1. Record of searches performed to retrieve tweets containing the keywords Food and Fitness, and Gym and Fitness.

Keywords	Number of searches	Number of tweets retrieved	Number of tweets left after removal of repeated tweets and re-tweets
Food and Fitness	23	25986	24494
Gym and Fitness	23	36144	34514

1.2.3. Data cleaning

The data obtained from Twitter contained the users' text, along with other information, such as the date and time of that tweet, the username of the person who wrote it, the id number for each tweet, the location and country, the number of re-tweets and likes for the tweet, as well as the number of followers and friends, besides other information. However, since not all the tweets had the same information, due to missing information in many of them, the text body of the tweets was the only data used for the present study.

To prepare the text or tweet message for further analysis, data were cleaned, refined and extracted using the Orange Data Mining tool (Ljubljana-University, 2021), an open-source software. As a result, punctuation marks, emojis, numbers, accents, URL links (e.g., <https://linktoawebsite.com>), and mentions to other twitter accounts were removed. Furthermore, a method referred to as tokenization in the software was used to break the text into smaller components (words, sentences, bigrams), while stop words (e.g., *the*, *and* or *to*) were removed as they contain 3 or less characters that have little value for understanding the content of a sentence (Leskovec et al., 2014). Finally, all data were converted to lowercase since the same word written in uppercase and lowercase can be considered different by the software (e.g., Fitness and fitness).

1.2.4. Co-occurrence networks and data classification

Term co-occurrence networks were built using the VOSviewer software (Version 1.6.7, Centre for Science and Technology Studies, Leiden University, The Netherlands). VOSviewer software created a two-dimensional map based on the frequency of mentioned terms (occurrence) and on their co-occurrence within the same tweet (i.e., when both terms occur together in one tweet); the distance between terms can be interpreted as an indication of their relatedness. The co-occurrence map was based on text data using the binary counting method, which means that only the presence or the absence of a term in a document matters, and its frequency of mentioning. In this latter case, the threshold was set at 100.

Then, clusters and subclusters were built from the tweets retrieved using the previously selected terms (*Food and Fitness* and *Gym and Fitness*). Clusters were obtained by using the clustering tool from Orange Data Mining tool, which considers the data generated from the co-occurrence maps built via VOSviewer software. In the case of subclusters, tweets were manually analyzed according to the type of the terms and the meaning of the tweets. In particular, two researchers firstly read the tweet contents independently; after that, they reached an agreement regarding the most pertinent and often-used terms in the tweets. For example, the tweet “Vegan diet is a potential way to shrink your environmental footprint, fuel the highest fitness levels, and reduce the risk of diseases” _was coded into the subcluster: “vegan”, belonging to the “Diet objectives” _main cluster.

1.2.5. Relationship between tweets information and scientific knowledge

To assess the connection between the information from the tweets and the existing scientific knowledge, a bibliographic review was carried out using Web of Science. The keywords used to cover that scientific knowledge were *fitness*, *sport*, *gym*, *food* and *nutrition*. The information from the tweets, included in the clusters and subclusters, was compared with that obtained from the different research areas retrieved from the Web of Science. These

areas were *Food*, *Sports* or *Health*. The information provided by the Twitter users and the research outcomes was rated. A score from 1 to 5 (1 = very weak in red, 2 = weak in yellow, 3 = moderate in orange, 4 = strong in green, 5 = very strong in dark green) was assigned to each of the tweets depending on the strength of the correspondence between them and the scientific studies. Once all the tweets were scored, the average of the tweets scores, within each subcluster, was used to generate the heat map.

1.3. RESULTS

A preliminary study was conducted to identify the terms used by physically active Twitter users to describe their nutritional needs and gym workout. The terms with higher frequency of mentioning were used to retrieve the tweets. This preliminary activity showed that *Food and Fitness*, and *Gym and Fitness* were the most searched terms. Qualitative analysis, previously described in the data classification section, provided results that are presented below. Those results were compared to the extant literature describing athletes' nutritional needs and demands.

1.3.1. Co-occurrence map from pre-cleaned text

Figures 1 and 2 show the co-occurrence networks for the data obtained with both *Food and Fitness* and *Gym and Fitness* terms, and with the term *Gym and Fitness*, respectively. In this graphic (Figure 1 and 2), the items or words are represented by a node, whose size is determined by the weight of the item, or in other words, by the frequency of mentions. The higher the weight of an item, the larger the node of the item. Items are connected to each other by a line whose thickness depends on how many times both items have been mentioned together in a tweet. Finally, each item belongs to a specific cluster, with each cluster represented by a color. An item belonging to a specific cluster can be connected to items belonging to other clusters. In fact, Figure 1, which was obtained from the pre-cleaned text of the totality of tweets, shows how the different items are distributed throughout the map, some being more interconnected than others. The clustering of items of most importance provided seven clusters or communities of topics of conversation.

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Nevertheless, of the seven clusters obtained, three stand out. The terms “workout” along with the items “health”, “motivation” and “exercise” were the most frequently mentioned (bigger nodes).

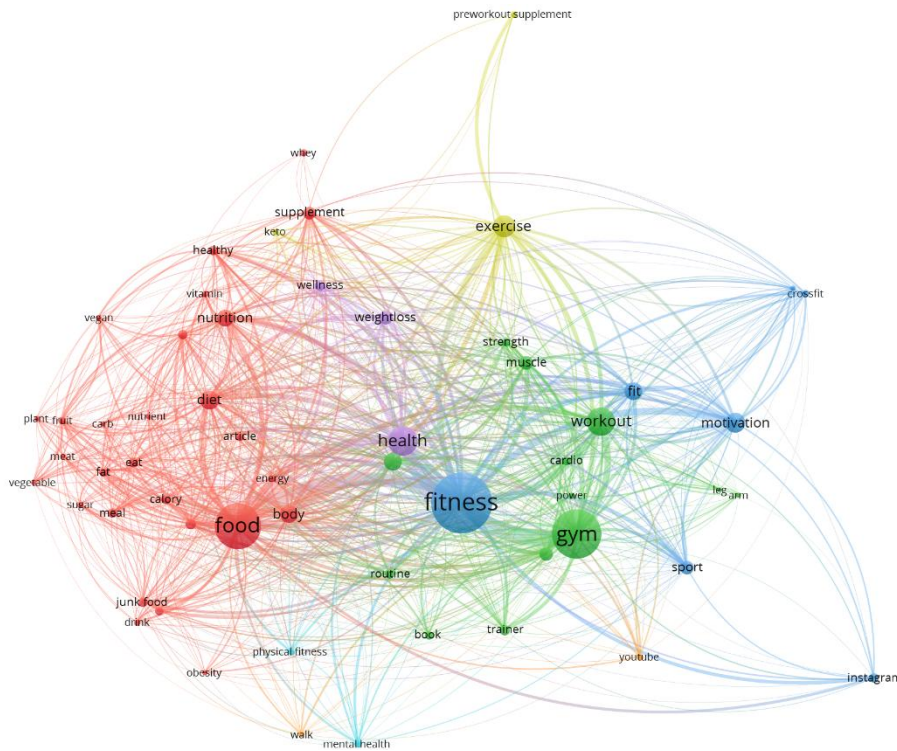


Figure 1. Co-occurrence network of Twitter information regarding both Gym and Fitness and Food and Fitness searches.

The first cluster (in blue) showed a great deal of items associated to different types of training and exercise with words such as “crossfit”, “bodybuilding” and “fitness”. Within the same cluster, interesting insights were obtained when observing the presence of the motivation node. Its interconnections between supplements and the different types of exercises seem to play an important role in the lives of physically active Twitter users.

The second cluster (in red) provided information related to food and nutrition as some of the items were “plant”, “nutrient”, “diet”, and “fruit”, among others. In the case of the third one (in green), this cluster was related to resistance exercises since words like “cardio”, “strength” and “power” appeared in the cluster. The other clusters were related generally to health, weight loss and supplementation.

Regarding Figure 2, the most frequent items were the words “workout”, “health” and “weight loss”. There were five different clusters. The first cluster (in blue) was loosely formed around the term “fitness” and was related to muscular exercise as it involved the items “muscle”, “body building”, “legs” and “arms”, among others. The second one (in yellow), was mainly related to energy expenditure since items such as “calory”, “workout” and “gym” were mentioned. Furthermore, the third cluster (in red) was formed around the term “weight loss” and it was mostly related to nutrition (“diet”, “ketogenic”, “vitamins”). As regards the fourth cluster (in purple), this was linked to a health theme, since it is formed around the word “health” and “healthy life”. Lastly, the fifth cluster (in green) was associated with high intensity exercises (“cardio”, “crossfit”, “strength”). In the case of Figure 2, it is interesting to point out that the topic searched *Gym and Fitness* was mainly connected to nutrition.

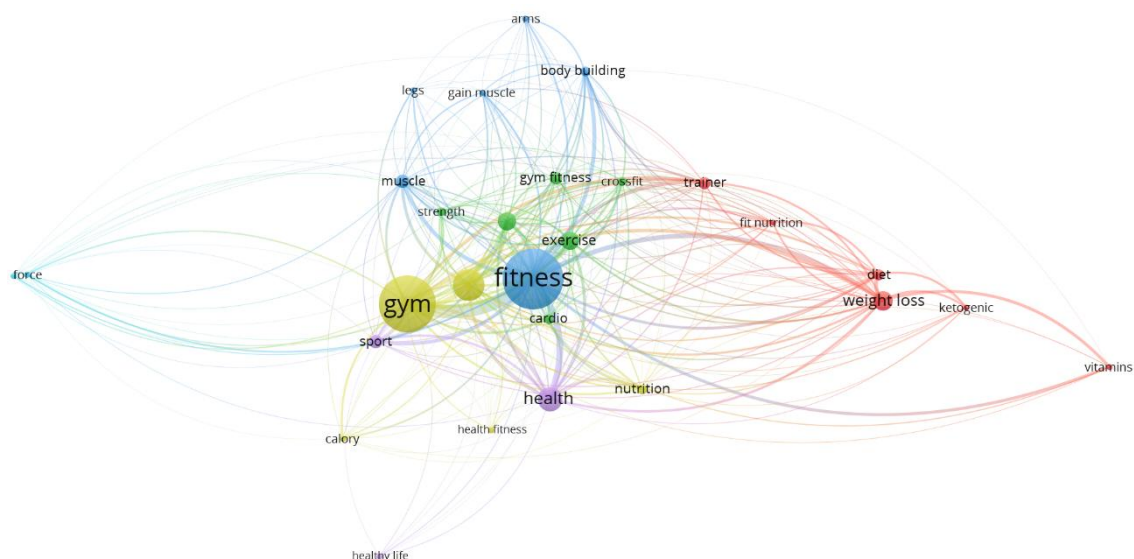


Figure 2. Co-occurrence network of Twitter information obtained from the Gym and Fitness search (B).

1.3.2. Classification of relevant concepts of Twitter raw data

The conceptual maps (Figure 3 and 4) were obtained from qualitative analysis of all the data once the cleaning process was done. From the *Gym and Fitness* search, three principal topics (physical objectives, type of training and supplements) were extracted (Figure 2). As shown in Figure 3, each of these topics had sub-clusters. Physical objectives were the category with the

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highest number (5) of subclusters: *gain muscle*, *maintain muscle*, *muscle definition*, *gain strength* and *gain resistance*. The most commented upon sub-cluster was *gain muscle*, which was the main objective for physically active Twitter users. This was correlated with the results obtained for the *type of training* cluster since *bodybuilding*, which was the most mentioned subcluster, is based on gaining muscle mass. Lastly, tweets referring to the cluster *supplements* were defined by the subclusters *pre- and post-workout*, with *pre-workout* being the most relevant for physically active Twitter users.

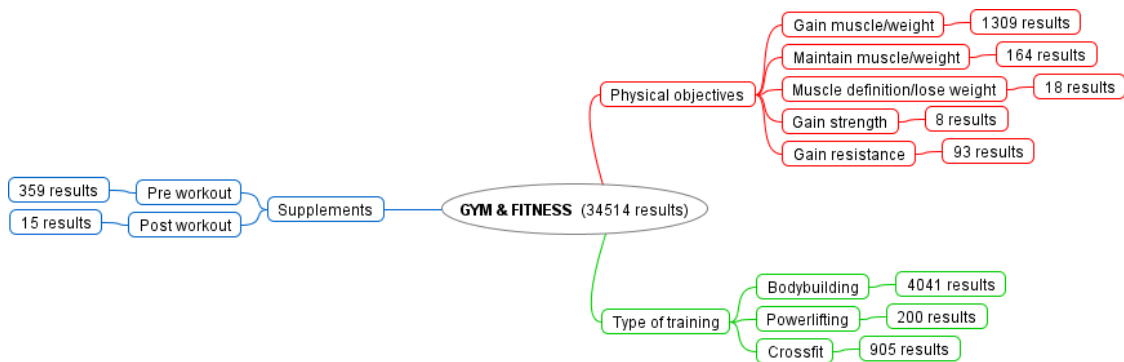


Figure 3. Clusters and subclusters obtained for the most highlighted words in the tweets containing Gym and Fitness with their number of mentions.

In the *Food and Fitness* conceptual map (Figure 4), tweets were mainly about *diet objectives*, *food*, *nutrients*, and *format*. *Food* was the cluster with the highest number of subclusters, followed by the cluster *format*. Within *food*, the subclusters *fruit*, *eggs*, and *rice* were the most mentioned in tweets. In addition, for the cluster *diet objectives*, *vegan* and *ketogenic* were primarily mentioned, with *carnivore* being the least mentioned. Within the cluster *nutrients*, *fat* was the most frequent subcluster. Finally, *supplements* within the subcluster *snack* were frequently mentioned in product *format*.

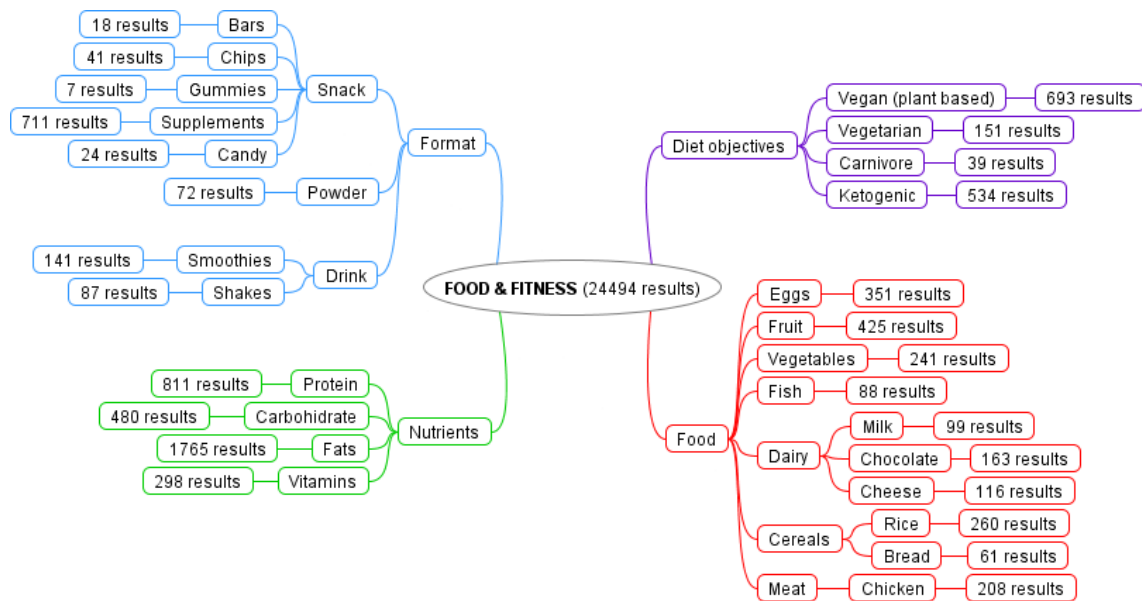


Figure 4. Clusters and subclusters obtained for the most highlighted words in the tweets containing Food and Fitness with their number of mentions.

1.4. DISCUSSION

1.4.1. Nutrition information in tweets of physically active Twitter users

For Twitter users, one of the main sources of information has been family and the Internet (Vazquez-Espino et al., 2022). Therefore, Twitter exploration using co-occurrence networks is a useful tool to gather knowledge about what is relevant to users (Puerta et al., 2020; Zarrabeitia-Bilbao et al., 2022). Our findings that health, weight loss and muscle gain were the major topics associated with Gym and Fitness agrees with findings for Swedish gym-goers, although the motive to build muscles was less important for training at a gym compared to the motive of improving strength (Soderstrom, 2021). Confirmation of our findings was also evident from a study conducted with students from different nationalities studying in the USA, where strength and endurance as well as weight management were the main objectives for most physically active individuals (Kilpatrick et al., 2005). Moreover, another study reported that the common motives for sport and physical activity of young Malaysian students was physical fitness as well as improving health (Lim et al., 2015).

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The most frequently mentioned diet related to *gym* was the plant-based one (Figure 4). Nevertheless, when searching *Gym and Fitness* and *Food and Fitness* together, plant based and ketogenic diets were linked to exercise and gym, but vegan diet was related only to the activities carried out at the gym (Fig. 1A). Therefore, on the basis of this study, a vegan diet is thought not to be able to provide the necessary nutrients for doing exercise. That assumption is in line with findings by Rogerson (2017), who indicated that some vegan diets promote only raw food consumption, leading to deficient macronutrient absorption and low energy density due to high fiber content, which in turn is a detriment to individuals seeking a high calorie diet. Nevertheless, vegan diet was linked with muscle, strength and weight loss, whereas keto diet was only associated with weight loss, and plant-based diet with muscle and weight loss (Figure 1). Therefore, even though there is scientific evidence of nutrient deficiencies in vegan diets, physically active Twitter users seem attracted to this diet to improve strength among other things. The limited knowledge of physically active individuals regarding nutritional needs, diets or supplementation in terms of sport has been previously reported (Tam et al., 2021).

Another interesting factor shown in Figure 2, was the motivation node. Some pre-workout supplements, containing the ingredients caffeine and L-theanine, have been related to a beneficial effect on mood states as they improved performance on speed and accuracy in attention-switching tasks 60–90 min after ingestion (Owen et al., 2008). In contrast, Ellerbroek & Antonio (2019) reported no significant changes in mood states 30 min after ingesting these pre-workout supplements.

1.4.2. How does Twitter information relate to peer-reviewed literature?

Figure 5 shows two heat maps illustrating the difference between the information that people shared on Twitter about Food and Fitness (A) and Gym and Fitness (B), compared to that reported in the scientific literature with respect to Food, Sport and Health. The greener the shade, the greater the

correspondence between Twitter information and the scientific literature. On the contrary, the redder the shade, the worse the correspondence to peer-reviewed literature.



Figure 5. Heat map comparing the knowledge of physically active Twitter users with the scientific literature (in the fields of food, sport and health) regarding the different clusters obtained with the Food and Fitness search (A) and the Gym and Fitness search (B). The green shades denote higher values, which means that the knowledge of athletes concurs with what has been extracted in research, while the red shades represent lower values, where the knowledge from Twitter differs with respect to the literature. Dark green represents the highest and dark red the lowest values.

In the case of the nutrients cluster, similarities were found in the subcluster fat and vitamins when compared with the Food and Sport research area, respectively. For example, physically active Twitter users shared that “Vitamins affect our bodies in many ways. They help with bone health and the immune system. They also help repair damage to our cells” and “Quality dietary fats are essential for a balance nutrition”. These statements are in line with research outcomes reported by Arenas-Jal et al. (2020). However, the contrary happened with the information from Twitter within the subclusters carbohydrate and fat when compared with the Sport research literature. In this case, tweets such as “Skipping carbohydrates intake after exercise is a quick technique to lose weight and help recovery” are contrary to research findings that energy consumption during exercise exceeds the one provided by endogenous carbohydrates. For this reason, a source of rapidly absorbed

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carbohydrate after exercise is necessary to promote glycogen resynthesis and maximize recovery, particularly when exercise sessions are less than eight hours apart (Reid, 2013). Moreover, according to the American College of Sports Medicine (2000) with the depletion of glycogen stores after exercise, consumption of 1.5 g per kilogram of body weight in carbohydrates within the first 30 min and repeating this intake every 2 h for a period of 4 to 6 h, will adequately restore glycogen levels.

Tweets related to the vegetarian and ketogenic subclusters of the diet objectives cluster compared well with research outcomes from the Food research area (Figure 5A). For instance, the tweets “a vegetarian diet for those who are training is unsustainable ... due to unbalanced meals and the feeling of being hungry all the time” or “Ketogenic diet is effective for weight loss and management, as well as for training athletes” were in line with descriptions by Paoli et al. (2021) and Rogerson (2017). In addition, the American College of Sports Medicine (2000) reported that vegetarian athletes might face challenges in maintaining adequate energy, protein, and micronutrient intake due to their reliance on low-energy-density foods and the exclusion of meat and dairy from their diet. However, these similarities were not found in the subclusters vegan and vegetarian with respect to the Sport and Health research areas, respectively. For example, some people tweeted that “Vegetarian diet is better than ketogenic diet to maintain physical shape and for training” and that “Vegan diet is a potential way to ... fuel the highest fitness levels and reduce the risk of diseases”. However, since managing energy balance is important for all physically active Twitter users, vegan diets are more likely to be a limiting factor as they are based on carbohydrate-rich foods that tend to promote early satiety and reduce appetite; thus, less food is consumed, leading to insufficient energy intake for training (Rogerson, 2017). In fact, plant-based protein sources are often incomplete, missing important essential amino acids. Also, both vegan and vegetarian diets tend to be lower in protein, fat, vitamin B12, riboflavin, vitamin D, calcium, iron and zinc when compared to omnivorous diets (Rogerson, 2017). Although the ketogenic diet does deliver considerable amounts of fat and adequate protein content, it has insufficient levels of carbohydrates for metabolic need. Nevertheless, it

appears to be a strategy to lose fat mass, without impairing strength, power and muscle mass (Paoli et al., 2021; Vargas et al., 2018).

There were more similarities for the food cluster compared to the other clusters (Figure 5A). The vegetables and fish subclusters showed less differences when compared with research outcomes. For instance, “Vegetables are low in calories and fat, and high in fiber and minerals, 4 essential ingredients for successful weight loss and recovery after exercise”. Fruits and vegetables are excellent sources of fiber, minerals and dietary antioxidants, which can help to accelerate recovery, reduce inflammation and protect the body against illness. In addition, vegetables such as potatoes are rich in potassium and magnesium, which play a critical role in muscular contraction as well as protecting against hypertension and improving bone health (Reid, 2013). On the other hand, when comparing Twitter information regarding Food with the Sport research area, the correspondence with the scientific literature was less evident, especially for the *cereals* subcluster. As an example, the tweet “Recent trends in fitness have made us think that eating rice makes you fat” is not compatible with cereals, such as rice, being a good source of carbohydrates, and this tends to promote early satiety and reduce food demand. Cereals are also beneficial for enhancing performance and maintaining energy levels since replacing carbohydrates rapidly is an urgent problem for athletes (Helms et al., 2014). In this regard, the American College of Sports Medicine (2000) stated that as energy requirements increase, athletes should prioritize consuming the maximum recommended servings from carbohydrate-rich food groups such as breads, cereals and grains, vegetables, and fruits.

For the *format* cluster, differences between the users’ tweets and the research in the *Health* area were greatest in the *powder* and *drink* subclusters. This is illustrated by tweets such as “Protein powder has negative impact on kidneys”. However, Devries et al. (2018) reported that the high consumption of protein in formats such as protein powder does not negatively affect kidney function.

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With respect to *physical objectives* cluster, comments and information shared on Twitter regarding *gain strength* and *gain resistance* subclusters were consistent with the scientific literature (Figure 5B). For example, tweets such as “A way to gain strength is with a resistance training” or “Regular exercise can help you to strengthen your muscles and increase your endurance” align with studies showing that sustained resistance training increases muscle strength and physical capacity (Knowles et al., 2018). Also, resistance exercise stimulates the release of hormones that promote protein synthesis, allowing for increases in muscle mass and strength (Knowles et al., 2018).

Finally, users' knowledge of *types of training (powerlifting)* and *supplements (post-workout)* was correlated with research outcomes in the *Food and Sport* areas. For example, physically active Twitter users shared comments such as “Powerlifting increases your physical strength and helps you lose weight”, “Carbohydrates are key players for powerlifters since they help with muscle growth and contraction” or “Taking a post workout recovery blend supplement helps you recover from muscle soreness quickly. It helps in muscle building by preventing you from lagging behind your routine”. These data are in line with what was described by Chiu (2007) and Maia et al. (2018) about the dietary intake of macro and micronutrients in powerlifting athletes and powerlifting for athletic performance, respectively.

1.5. CONCLUSION

The present study demonstrated the potential of Twitter to easily and rapidly provide massive amounts of information about the nutritional needs of physically active Twitter users and their knowledge in terms of nutrition and training. Moreover, through the use of the co-occurrence networks, as well as conceptual maps, it has been possible to obtain evidence about current trends and what would be possible future approaches when developing food products for physically active individuals. Furthermore, the study highlighted that the major physical objective for physically active Twitter users was to gain muscle, with bodybuilding being the principal type of training they follow. In addition, the diet that most physically active Twitter users tend to adhere to

was vegan, followed by ketogenic, due to the belief that the latter diet improves strength and helps with muscle mass. Utilization of heat maps showed that there were differences between people's knowledge about the various clusters and the scientific literature in different research areas. Generally, the average knowledge of physically active Twitter users in the field of food was in line with the outcomes of the scientific literature, especially in the case of diet and physical objectives clusters. On the contrary, in the case of regimens adopted for sport activities, the knowledge of physically active Twitter users regarding macronutrient composition and the use of supplements during the pre-workout stage was very far from the scientific evidence. Consequently, physically active Twitter users should be advised that before following a diet they should contact nutrition and dietetics professionals in order to develop optimal performance. This research demonstrates the wide potential of social media analyses for sport and nutrition researchers; through them researchers can better understand new trends in terms of sport activities, diets, eating habits, as well as assess the general knowledge degree of the Twitter users about those topics. In addition, the presented methodology may be successfully applied to quickly and easily analyze large amounts of information coming from social media in order to understand people's attitudes regarding ever-changing themes, such as food preferences and lifestyle habits.



CHAPTER 2

MAPPING THE GROWTH AND TRENDS OF ADAPTOGENS IN FOOD: A COMPREHENSIVE ANALYSIS OF LITERATURE AND MARKET INSIGHTS

2.1. INTRODUCTION

Adaptogens are harmless natural compounds present in certain plant species, and which are capable of increasing the ability of an organism to adapt and prevent damage from multiple stressors including physical, chemical and biological (Panossian, 2017). These substances are believed to help normalize the physiological functions without causing adverse effects (Rusu, 2018). Traditionally, adaptogens have been used for centuries in medical systems such as those in China, Japan, Russia, and India, primarily to reduce fatigue and improve resilience and longevity (Lavecchia et al., 2013; Rathore, 2010). In the mid-20th century, their application expanded for improving the performance and endurance of military personnel, astronauts, and athletes.

The chemical nature of adaptogens varies among plant species, but commonly includes phenylpropanoids, triterpenoid saponins, lignans, phytosterols and flavonoids (Todorova et al., 2021). These compounds are known to exhibit multiple targeted effects on the neuroendocrine-immune system, by interacting with the hypothalamic-pituitary-adrenal (HPA) axis,

thereby regulating stress hormones such as corticotropin and cortisol and, in turn, modulating cellular homeostasis (Kępińska-Pacelik & Biel, 2025).

Additionally, adaptogens can exert an effect on the lactate levels, blood glucose levels, hepatic enzymes and nitric oxide levels, among others (Todorova et al., 2021). Consequently, these natural compounds have been associated with a wide range of health benefits and therapeutic potential for diseases such as atherosclerosis, neurodegenerative cognitive impairment, metabolic disorders, cancer, and other aging-related diseases (Wal et al., 2019).

Furthermore, adaptogens have been used to manage mental illness and behavioral disorders, while improving mood and physical stamina (Panossian, 2017). Their capacity to improve physical performance, increase resistance to stress, and reduce fatigue is considered particularly relevant in athletic contexts, where these effects may contribute to enhanced endurance and fast recovery (Özdemir et al., 2018).

Most adaptogens are consumed in the form of dietary supplements containing the standardized herbal extracts from the roots, rhizomes or leaves. Accordingly, extraction methods have been extensively studied as they are considered a critical step; common techniques include solvent extraction using water, ethanol, or methanol, supercritical fluid extraction, and ultrasound or microwave-assisted extraction (Jamiol et al., 2023; Singirala et al., 2025). Recently, deep eutectic solvents have been applied to extract plant adaptogens (Stanisz et al., 2024).

Despite extensive research on the health benefits of adaptogens, there remains a notable gap in understanding their occurrence and application within food systems beyond dietary supplements. This study uniquely addresses this gap by systematically examining the presence of adaptogenic plants in commercially available food products and providing a critical analysis of market trends in comparison to current peer-reviewed scientific evidence. By bridging the divide between scientific research and market realities, this study offers valuable insights into the potential integration of adaptogenic plants within functional foods and highlights opportunities for future research and food product development.

2.2. MATERIALS AND METHOD

A comparative assessment of scientific literature and market data on food products containing adaptogens was performed. Scientific publications from 1945 to 2025 were included. Market data were collected from 2002 to 2025, as relevant product information was unavailable prior to 2002.

2.2.1. Scientific literature search

Bibliographic searches to compile the scientific documents were done using Web of Science and Scopus databases. A first search was carried out using the term *Adaptogen*, without specifying the date range. Therefore, all the results including the word *Adaptogen* within article title, abstract and keyword, were collected from both databases. Web of Science and Scopus were chosen due to their wide usage worldwide by the scientific community. Hence, this initial search was done to gather information on the most important adaptogens and select them for the second bibliometric analysis. In this second analysis, *Rhodiola rosea* AND *food*; *Eleutherococcus senticosus* AND *food*; *Panax ginseng* AND *food*; *Schisandra chinensis* AND *food*; and *Withania somnifera* AND *food* were chosen as terms within the article title, keyword and abstract. The results obtained from both databases, in each of the bibliometric analyses, were collected using the software Zotero (Zotero version 6, Virginia, USA). The collected documents were classified according to diverse aspects, including number of documents per year, and affiliation by region. In the second bibliometric analysis, the collected data was also classified by food subject categories.

All the scientific documents were manually analyzed in Zotero to remove duplicates. The results were represented with a co-occurrence network using the VOSViewer software (Version 1.6.7, Centre for Science and Technology Studies, Leiden University, The Netherlands). Thus, a two-dimensional map based on the frequency of mentioned terms (occurrence) and on their co-

occurrence within the same research paper was built considering a minimum number of 10 occurrences for each keyword.

2.2.2. Market search

The global presence of adaptogen-containing food products was evaluated using Innova (Innova Market Insights, Arnhem, The Netherlands). This database was selected for its extensive global product coverage, containing over 150M products from more than 90 countries. Keywords used were the same adaptogens as in the bibliometric study: *Rhodiola rosea*, *Eleutherococcus senticosus*, *Schisandra chinensis*, *Panax ginseng* and *Withania somnifera*. Each keyword was used independently, without specifying the date range and including all products.

Product information was exported to Microsoft Excel (Microsoft Office, Washington, WA, USA). Further data visualization and geographic representation were carried out using OriginPro 2024 (OriginLab Corporation, Northampton, Massachusetts, USA) and Map Chart tool, which is an open-source software.

2.2.3. Comparative analysis of market and scientific literature outcomes

For the comparison between market and scientific publications regarding the different food product categories and regions, the free software SRPlot was used. The balloon plot graphs were made by assigning a scale (0-10), being 10 the value given to the highest number of marketed products or scientific publications. This was done to compare each adaptogen in terms of market and scientific literature.

2.3. RESULTS AND DISCUSSION

2.3.1. Scientific literature mapping

2.3.1.1. General occurrence of adaptogens

The analysis of publication trends provides valuable insight into the evolving scientific interest in adaptogens. A literature search using the keyword adaptogen identified 1,722 research papers published between 1967 and 2025

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(Figure 6, black line). Three distinct phases can be observed. The initial period (1967s-1980s) was characterized by minimal research activity, with only a handful of papers published each year, indicating that adaptogens were a niche topic and not a major focus of scientific inquiry. Between 1980 and 1995, the number of publications on adaptogens began to rise steadily, likely reflecting emerging evidence of their therapeutic potential and the growing interest of the scientific community. This interest intensified in the late 1990s and accelerated sharply in 2007, when 33 papers on natural health products and complementary therapies were published (Mirjalili et al., 2009; Panossian & Wikman, 2009). A comparable surge was observed after 2019, reaching a peak of about 71 papers per year, possibly driven by the COVID-19 pandemic and heightened attention to the perceived benefits of botanical drugs and supplements, including adaptogens (Brendler et al., 2021).

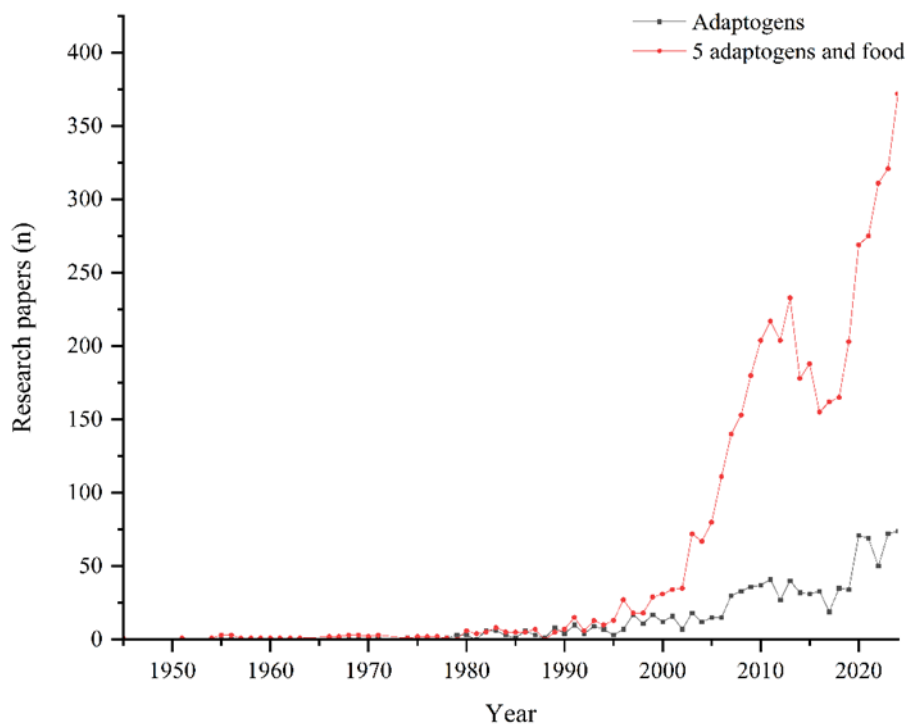


Figure 6. Time evolution of the number of research papers related to adaptogens and, to the five most important adaptogens and food bibliographic searches in WOS and SCOPUS.

Figure 7 provides insight into the thematic structure of this body of literature through a co-occurrence network analysis. Using data from the Web of Science and Scopus databases, a total of 741 keywords were retrieved from the 1722 scientific documents. However, only 69 keywords met the threshold, due to 10 being the minimum number of occurrences allowed for a keyword. As observed in this graphic, the keywords are represented by a node, whose size is determined by the frequency of mentions, and each node belongs to a cluster represented with a color. 5 different clusters were obtained based on these 69 keywords.

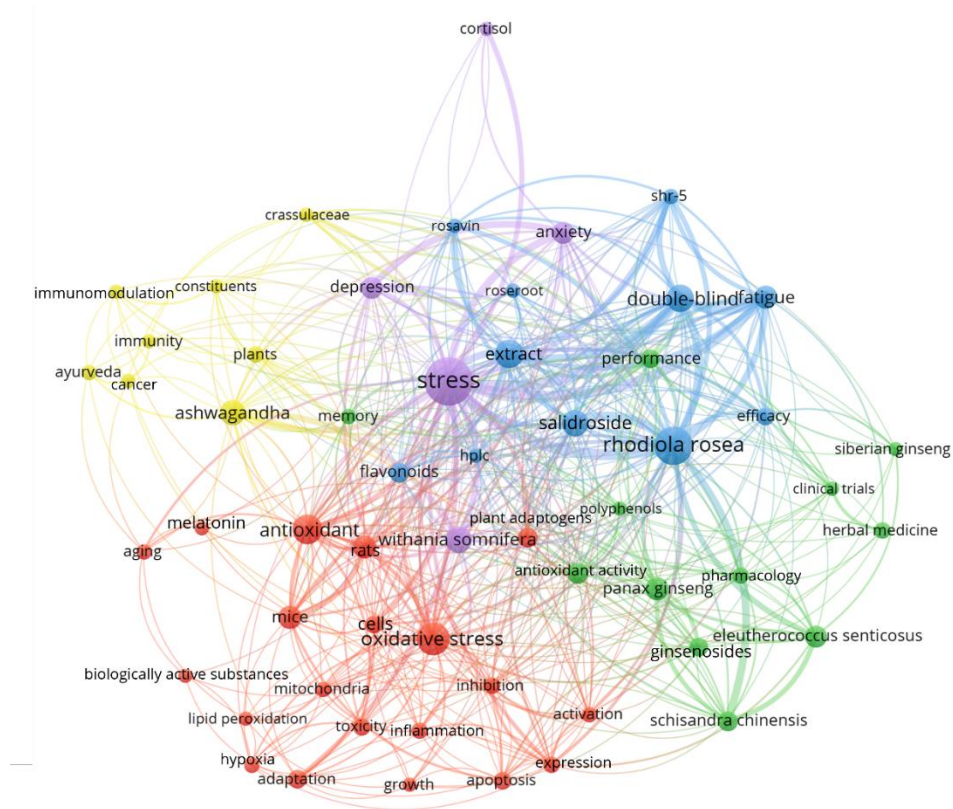


Figure 7. Co-occurrence network based on the bibliometric search using the keyword “adaptogen” in WOS and SCOPUS.

The blue cluster was centered on *Rhodiola rosea* and “stress,” which emerged as the most frequently studied terms in the network. The thick connecting line between them indicates a strong co-occurrence, consistent with the recognition that consumption of *Rhodiola rosea* is an effective strategy to normalize stress and boost energy levels by decreasing cortisol secretion (Toth-Meszaros et al., 2023). The cluster included its main bioactive compounds known as “salidroside”, “rosavin” and “flavonoids”, and their

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effects on the “efficacy” of reducing “fatigue” when consuming the commercial “extract shr-5”.

The red cluster (red color) was associated with the cellular and molecular mechanisms of the adaptogens as it included terms such as “antioxidant”, “oxidative stress”, “lipid peroxidation”, “inflammation” or “apoptosis”, among others (Figure 7). In this case, the effects were not related to a specific adaptogen, but with plant adaptogens in general.

The green cluster was connected to Chinese herbs and herbal medicine as it contained keywords such as “*Eleutherococcus senticosus*” (known as Siberian ginseng), “*Panax ginseng*” and “*Schisandra chinensis*”. In addition, the term “performance” and “memory” were linked to these medicinal herbs, meaning that scientific researchers have investigated their efficacy in the improvement of memory and performance. The bioactive compounds ginsenosides and polyphenols were also included in this cluster.

The yellow cluster focused on “ashwagandha”, “cancer”, “immunity” and “ayurveda”, suggesting research interest in the adaptogenic plant, ashwagandha, and its potential anticancer effects through immunomodulation.

The purple cluster focused on terms such as “cortisol”, “depression”, and “anxiety”, suggesting a strong research emphasis on the role of adaptogens in stress modulation and mental health. This cluster also included *Withania somnifera* (ashwagandha), which has been widely studied for its ability to normalize cortisol levels and alleviate symptoms of depression and anxiety. Overall, the co-occurrence network revealed five adaptogenic plants as the most prominent in the literature: “*Rhodiola rosea*”, “*Eleutherococcus senticosus*”, “*Panax ginseng*”, “*Withania somnifera*” and “*Schisandra chinensis*” (Figure 7). These results are in line with Kępińska-Pacelik & Biel (2025), who reported these five plants as the most widely known adaptogenic plants.

2.3.1.2. *Food-related scientific articles investigating adaptogens*

This section further narrows the focus on the five most important adaptogenic plants and their relationship with food. As observed in Figure 6 (red line), the trend closely follows that of the overall adaptogens curve (black line). During the initial period (1940s-1980s), publications were extremely rare, with fewer than 10 papers per year, indicating that research in this area was virtually nonexistent. From the 1980s onward, the number of publications grew steadily, reaching around 20 papers per year by the late 1990s. The most significant growth occurred from 2000 onwards, with annual publications surging from under 50 to over 350 papers, driven by growing interest in functional foods, herbal supplements, and natural health products. A total of four regions contributed to the retrieved publications, with Asia leading (2525 publications), followed by North America (384). At the country level, *Rhodiola rosea* research was led by China (86 publications) and the USA (22). For *Schisandra chinensis*, most publications originated from China (199) and South Korea (112). In the case of *Eleutherococcus senticosus* and *Panax ginseng*, publications were largely concentrated in South Korea, with 30 and 962 outputs, respectively. Interestingly, *Whitania somnifera* showed the highest activity in India (141) and USA (41).

From 4756 scientific documents, a total of 1889 keywords were collected, of which 101 met the inclusion threshold. These 101 terms were represented in the co-occurrence map (Figure 8), with the majority relating to medicine and health effects. Overall, *Panax ginseng*, together with *Withania somnifera* and *Schisandra chinensis*, appear to be the most extensively studied plants in relation to food applications, whereas *Rhodiola rosea* and *Eleutherococcus senticosus* have received comparatively less research attention. Five different clusters were obtained as observed in Figure 8. From these five clusters, two stand out.

increase of physical performance, endurance, aerobic capacity and exercise duration for athletes (Guo & Rezaei, 2024; G. Tinsley et al., 2024).

The green cluster was mostly connected to "*Schisandra chinensis*" and *Eleutherococcus senticosus*, which appeared by the name "*Acanthopanax senticosus*". Moreover, "antioxidant" and "antioxidant activity" appear to be of great importance as observed by the size of the nodes. These traits are associated with their secondary metabolites such as lignans, flavonoids and polysaccharides, which according to the results in Figure 8, appear to be predominantly located in the "leaves" and "fruits". This is in line with Amir et al. (2023), who described the abundance of bioactive substances in the fruits of *Schisandra chinensis*, with smaller amounts being in the leaves, stems, and seed. In contrast, Bocharova et al. (2022) described the high content of different classes of chemical compounds when analyzing rhizomes and roots belonging to *Eleutherococcus senticosus*. It is important to highlight that the terms "functional food" and "nutraceuticals" were present in this cluster, meaning that scientific research has been done in terms of incorporating these two plants in food matrices and nutraceuticals.

As for the other groups, the blue cluster was characterized by analytical methodologies ("performance liquid-chromatography", "mass-spectrometry", "metabolomics") used to identify the chemical compounds and metabolites present in the roots. The yellow cluster was associated with "*Panax ginseng*", its bioactive compounds and conversion mechanisms ("biotransformation", "bioconversion" or "fermentation") that transform major ginsenosides into smaller and more absorbable forms such as "compound K" (Figure 8). In this regard, "ginsenosides", which are a class of saponins, appear to be the bioactive compounds from *Panax ginseng*, primarily studied for their importance in pharmacology (Ratan et al., 2021). In addition, the terms "food" and "*Panax ginseng*" were found in the same cluster, indicating that there has been some investigation in this regard.

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2.3.1.3. Food categories in scientific articles investigating adaptogens

In this section, a deeper evaluation was conducted on the food products containing adaptogens, based on the research papers obtained from the second co-occurrence network. Figure 9 shows the distribution of product categories and the number of products in each category associated with *Rhodiola rosea*, *Eleutherococcus senticosus*, *Schisandra chinensis*, *Panax ginseng*, and *Withania somnifera*.

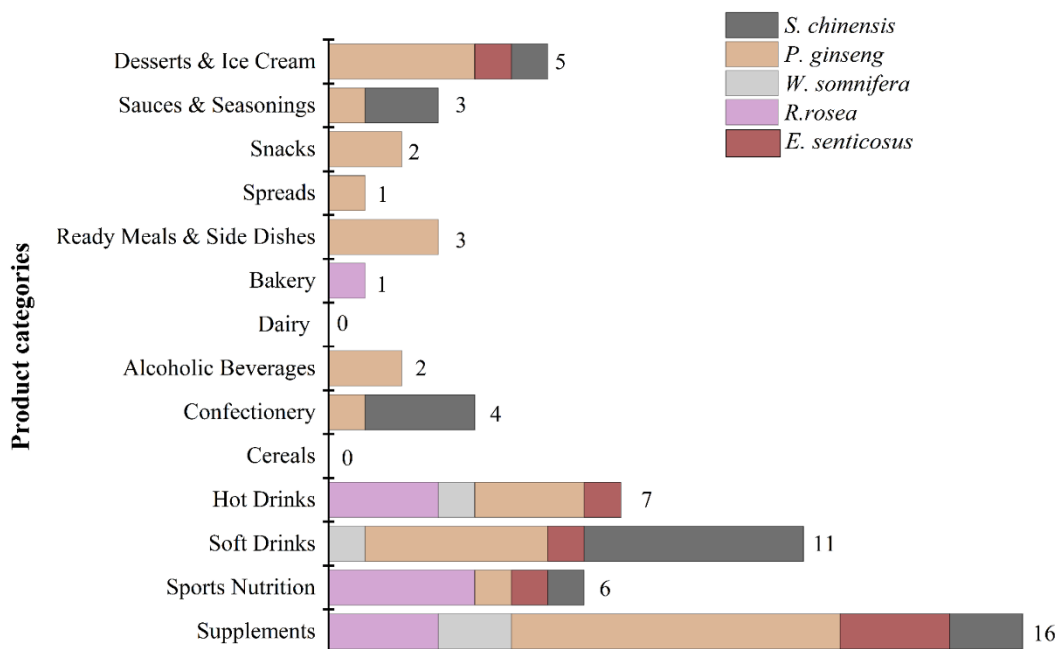


Figure 9. Product categories obtained when searching *Rhodiola rosea*, *Eleutherococcus senticosus*, *Schisandra chinensis*, *Panax ginseng*, *Withania somnifera* and Food in WOS and SCOPUS databases from 1900 to 2025.

The major categories were supplements followed by soft drinks, with 16 and 11 products, respectively. This is in accordance with Tinsley et al. (2024), who stated that adaptogens are experiencing increased popularity in the dietary supplement industry. *Panax ginseng* was the predominant adaptogenic plant in the supplements category, followed by *Rhodiola rosea*. As a matter of

fact, *Panax ginseng* was the most studied for all the product categories, except for soft drinks and sport nutrition (Figure 9). Furthermore, a notable number of studies related to the incorporation of adaptogens into soft drinks (n = 11), hot drinks (n = 7) and sport nutrition (n = 6) was observed. In the sport nutrition case, *Rhodiola rosea* was the most examined as compared to the rest of adaptogenic plants, likely due to its potential ergogenic effects on exercise performance and recovery, among others (Wal et al., 2019). In addition, the inclusion of *Panax ginseng* and *Schisandra chinensis* was greater in the confectionery (n = 4) and sauces and seasonings categories (n = 3). In the case of *Schisandra chinensis*, the confectionery products were chewing gum and chocolate made with extracts of *Schisandra chinensis*, while the seasoning was fermented vinegar using the seeds (Kim et al., 2024; Vahram G & Anna P, 2008; Wang F, 2009). The novelty of these products was associated with sleep promotion and therapeutic and prophylactic effects when consuming the chocolate and chewing gums, respectively. As for *Panax ginseng*, the reported confectionery products included chewing gum and chocolate formulated with root powder. On the other hand, in the sauces and seasonings category, a vinegar-processed preparation contained *Panax ginseng* extract. (Chung SH et al., 2007; Ishida Y et al., 1987; Nie C & Zhang Z, 2009).

Interestingly, no studies have focused on the incorporation of these adaptogenic plants into bakery products, except for *Rhodiola rosea* (n = 1). The relative patent was about a highland barley biscuit which comprised barley and *Rhodiola rosea* root powder as the main ingredients (Zhuoma, 2009). It is worth noting that adaptogen-containing dairy and cereal products showed no records, indicating that scientific research did not yet explore these major food categories (Figure 9). A possible explanation for this gap could be the technical challenges associated with incorporating adaptogens into liquid or highly processed matrices, which may affect their stability, bioavailability, or sensory properties. Additionally, the regulatory landscape and consumer acceptance in these food categories might also limit research and development efforts.

2.3.2. Market insights into adaptogens

2.3.2.1. Evolution of marketed adaptogen-containing food products

Figure 10 represents the temporal evolution (2002–2025) of marketed food products containing *Rhodiola rosea*, *Panax ginseng* and *Eleutherococcus senticosus*, *Withania somnifera* and *Schisandra chinensis*. The data indicates a steady increase in product launches for *Panax ginseng* and *Eleutherococcus senticosus* (or Siberian ginseng) from 2002, with a marked acceleration after 2018 and a peak around 2023, with more than 1700 products. As both plants share similar common names, Innova database was unable to differentiate between these species. Consequently, the information obtained included both adaptogenic plants.

On the contrary, *Withania somnifera* showed a rapid growth beginning in the late 2010s, reflecting its recent surge in popularity. In fact, from 2017 to 2022, 1091 new products containing *Withania somnifera* or its extracts were commercialized. This growth is fueled by increasing consumer awareness and demand for functional food that offers health benefits beyond basic nutrition (Özdemir et al., 2018). It is noteworthy to mention that the increase from 2020 onwards might be associated with raising interest toward health and nutrition during and after the coronavirus pandemic, which in turn, led to a rising demand for functional foods and dietary supplements (Hamulka et al., 2021).

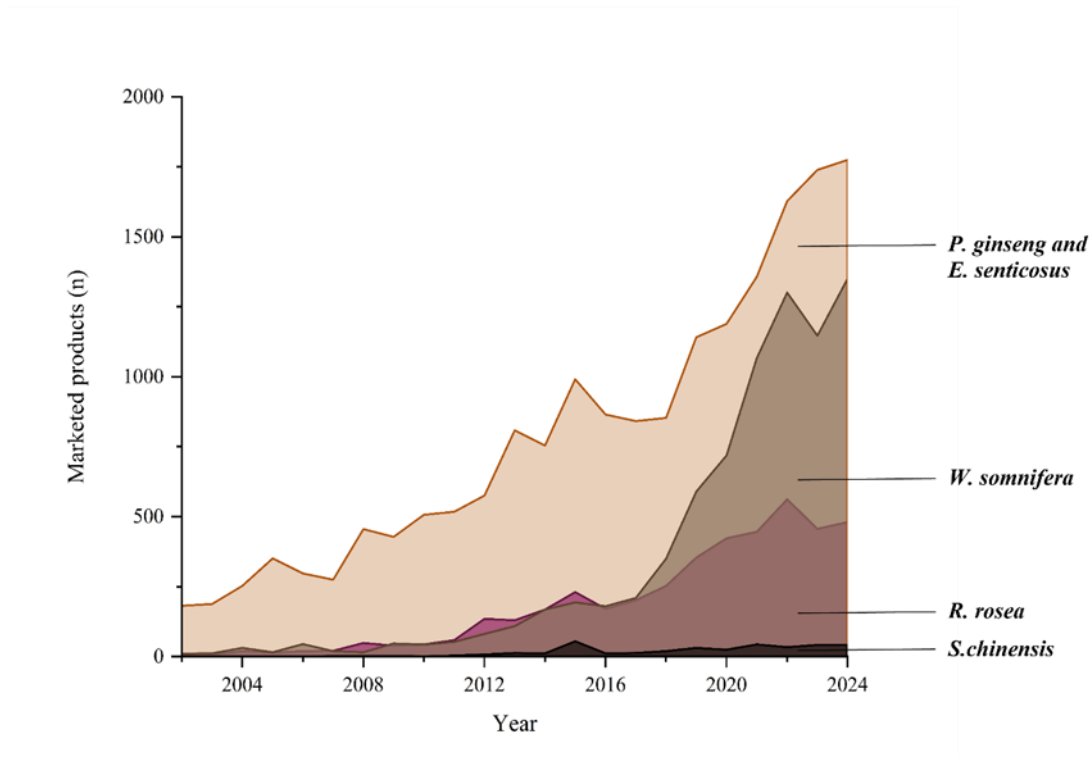


Figure 10. Time evolution of number (n) of marketed products containing *Rhodiola rosea*, *Panax ginseng* / *Eleutherococcus senticosus*, *Withania somnifera* and *Schisandra chinensis*.

A similar trend was observed for *Rhodiola rosea*, in Figure 10. However, from 2015 onwards the growth of marketed products was moderate compared to the other mentioned adaptogens with the exception of *Schisandra chinensis*, which has remained consistently low throughout the period (Figure 10). Overall, the trend analysis highlighted a strong dominance of *Panax ginseng* and *Eleutherococcus senticosus* in the market, with *Withania somnifera* emerging as a significant contender in the last decade. Global Market Insights (2024), on the other hand, reported that a significant increase from 2025 to 2032 is expected in the market size of products incorporating *Rhodiola rosea* and *Whitania somnifera*, due to their stress-relieving and energy-boosting properties.

2.3.2.2. Geographical overview of marketed adaptogen-containing food products

The global distribution of marketed food products from 2002 to 2025 containing *Schisandra chinensis*, *Rhodiola rosea*, *Panax ginseng* and

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Eleutherococcus senticosus, and *Withania somnifera* is shown in Figure 11. *Schisandra chinensis* (Figure 11A) showed limited market penetration, with the highest presence in North America (n = 138), followed by Western Europe (n = 92) and Asia (n = 79), and minimal representation in other regions. In contrast, *Rhodiola rosea* displayed a stronger market presence, particularly in North America (n = 2200) and Western Europe (n = 1369), with smaller but notable numbers in Eastern Europe (n = 415) and Oceania (n = 162) (Figure 11B). In this context, the Asia Pacific region is known to have a sizable share of the adaptogens market due to its rich history of traditional medicine, where adaptogenic plants have been used for centuries (Global Market Insights, 2024). However, these results demonstrate that there has been a transition from a historical dominance to a current market led by Europe and North America, where consumer demand for functional and wellness-oriented products has surged in recent years.

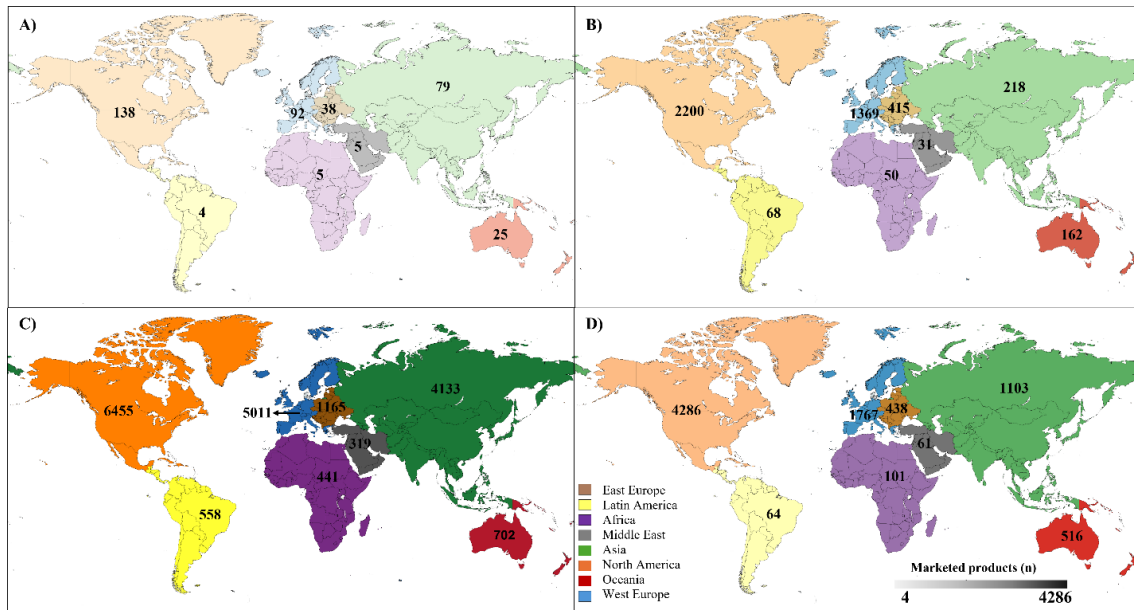


Figure 11. Number of marketed products from 2002 to 2025 in the different regions, containing A) *Schisandra chinensis*, B) *Rhodiola rosea*, C) *Panax ginseng* / *Eleutherococcus senticosus* D) *Withania somnifera*. Color intensity is correlated with the number of marketed products.

Among the five adaptogens, *Panax ginseng* and *Eleutherococcus senticosus* seemed to dominate the global market as shown by the high color intensity, with North America (n = 6455) and Western Europe (n = 5011) leading, followed by Asia (n = 4133) and Eastern Europe (n = 1165) (Figure 11C). This could be attributed to its global distribution, which occurs primarily in the northern hemisphere. In this case, all regions showed substantial representation as compared to the rest of the adaptogenic plants. As for *Whitania somnifera*, a stronger presence was observed in North America (n = 4286), West Europe (n = 1767), and Asia (n = 1103) (Figure 11D). This is in accordance with the results reported by Mukherjee et al. (2021), who mentioned a higher predominance of products containing *Whitania somnifera* in Asia, North America and Western Europe.

2.3.2.3. Categories of marketed food products containing adaptogens

Figure 12 presents the distribution of marketed food and beverage products containing the selected five adaptogenic plants, classified by product category. The results revealed that supplements constitute the largest

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category (n = 14,065), with 5709 and 5632 supplements containing *Panax ginseng* / *Eleutherococcus senticosus*, and *Whitania somnifera*, respectively. Moreover, sports nutrition (n = 7,892), along with non alcoholic beverages (soft drinks and hot drinks), were the most demanded products. Global Market Insights (2024) also stated an increasing demand for dietary and sport supplements. This outlines the importance of the adaptogens for athletes and physically active individuals. Among the other food categories, confectionery (n = 301), soups (n = 279), and sauces & seasonings (n = 262) stand out, with *Panax ginseng* / *Eleutherococcus senticosus* being the main adaptogenic plant. New delivery formats such as adaptogen-infused gummies and dissolvable powders are being launched due to their convenience and versatility. However, there is still a niche in the snack and bakery categories, where the only marketed products are made with *Whitania somnifera* (Figure 12).

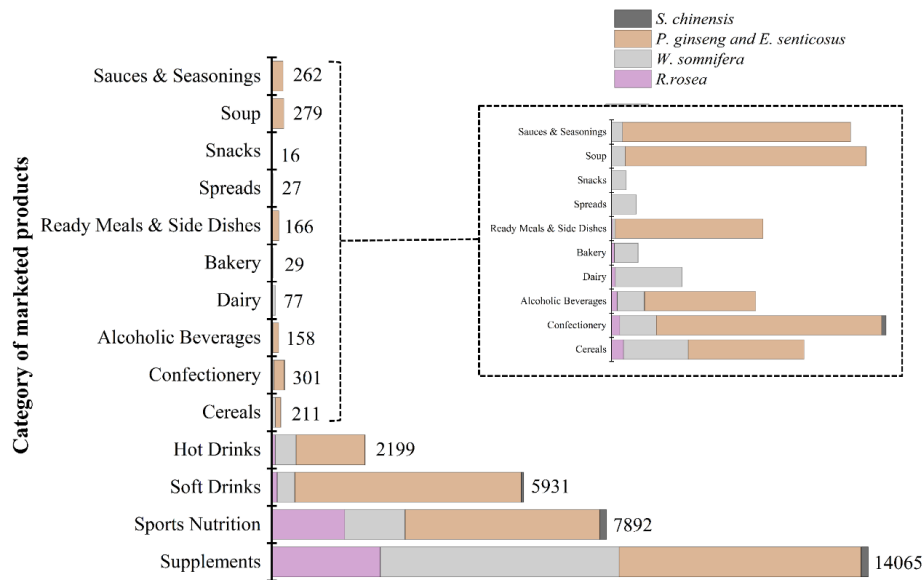


Figure 12. Categories of marketed products as for 2025, containing *Schisandra chinensis*, *Panax ginseng* / *Eleutherococcus senticosus*, *Whitania somnifera* and *Rhodiola rosea*.

2.3.3. Alignment of adaptogenic literature research and market trends

Figure 13 illustrates the relationship of adaptogenic plants in the food market and in the scientific literature. Color intensity and bubble size represented the frequency of appearance of the items (0–10 scale) within each category, both in the market and scientific literature on adaptogens.

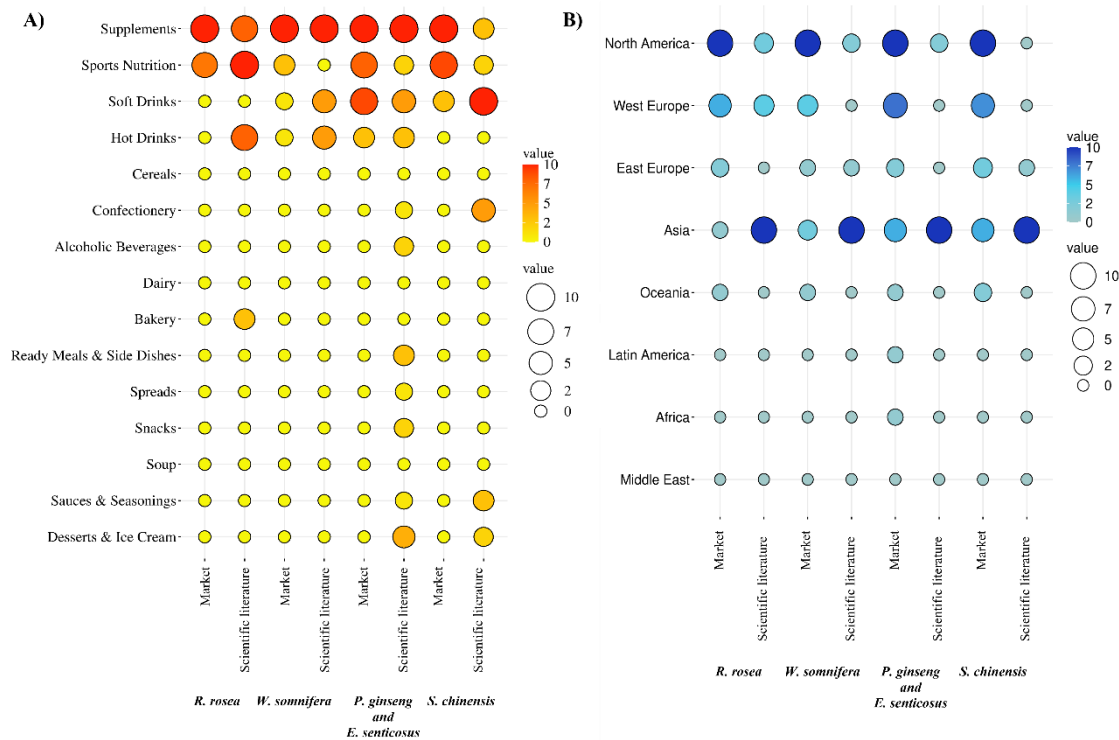


Figure 13. Frequency of each adaptogen appearance in scientific literature and market insights by a) food category and b) region. A scale from 0-10 has been assigned to each balloon plot, being 10 the value given to those who have the highest number of marketed products or scientific publications.

Supplements, sports nutrition and soft drinks dominate the market offerings and scientific publications. In both the market and scientific literature, the bubbles for the supplements category across all adaptogens (except *Schisandra chinensis*) displayed the same size and color intensity, suggesting that scientific findings are being consistently translated into marketed products (Figure 13A). However, in the case of *Schisandra chinensis*, there are proportionately more products launched than scientific documents related its use in the supplement category. Hence, there is a lack of scientific base for the supplements containing *Schisandra chinensis*.

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Interestingly, in the sports nutrition products containing *Rhodiola rosea*, prominence in the scientific literature has overtaken market interest. The same happens with soft drinks made with the different adaptogens, except for *Rhodiola rosea*. Furthermore, hot drinks seem to hold particular importance from a scientific standpoint, likely due to research investigating the stability of the bioactive compounds present in the adaptogenic plants. Sauces and seasonings, as well as desserts and ice cream, have also attracted attention. This may be related to the minimal processing involved in manufacturing these food products (sauces, seasonings, desserts and ice cream), which tends to better preserve the bioactive compounds (Figure 13A). As for the rest of the categories, there is minimal representation in the market or in the scientific field, as shown by the yellow light color intensity. Hence, the incorporation of the adaptogens in these food categories (cereals, bakery, alcoholic beverages, snacks, dairy, spreads, soup and ready meals) remains unexplored by the scientific community and by food companies.

According to Figure 13B, Asia and North America emerge as the most prominent regions for both market presence and scientific research, followed by Western Europe. This alignment suggests a close correlation between research output and market presence in these regions, likely due to the cultivation, availability, and long-standing traditional use of adaptogenic plants in the former, as well as well-established functional food markets. Western Europe, however, has focused more on the market perspective rather than the scientific research as shown by the difference in the bubble size (except for *Rhodiola rosea*). As for the other regions (Oceania, Latin America, Africa, and the Middle East), it could be observed that there is minimal activity in both domains. This uneven distribution may be attributed to factors such as limited local production of adaptogens, lower consumer awareness, reduced research funding, and less established functional food markets, which together constrain both scientific investigation and commercial adoption in these areas (Global Market Insights, 2024).

2.4. CONCLUSION

This study provides a comprehensive mapping of scientific literature and market trends related to key adaptogenic plants, *Rhodiola rosea*, *Panax ginseng*, *Eleutherococcus senticosus*, *Withania somnifera*, and *Schisandra chinensis*. The bibliometric analysis revealed that research predominantly focuses on the physiological and molecular mechanisms underlying adaptogenic effects, with particular emphasis on stress reduction, cognitive enhancement, and metabolic health. Among these, *Rhodiola rosea* and *Withania somnifera* stand out for their strong associations with stress and metabolic regulation. Food-related scientific literature analysis revealed that *Rhodiola rosea* and *Eleutherococcus senticosus* were the least studied adaptogens. Nevertheless, the incorporation of the adaptogenic plants into food products remains limited to dietary supplements and beverages. This highlights a clear opportunity for innovation in food formulation, particularly in categories such as snacks and bakery products, to broaden consumer access to adaptogens.

Market research indicates a growing global consumer interest in adaptogen-containing products, especially in North America and Europe, driven by increasing awareness of health and wellness benefits. This has been proven by the strong concordance between scientific research and market trends for adaptogenic plants, with supplements, sports nutrition, and soft drinks emerging as the dominant categories, while most other food sectors remain largely unexplored. Asia, North America, and Western Europe were identified as regions with the highest levels of research and market activity, whereas other regions remain relatively underdeveloped in this space. Overall, this integrative approach underscores the promising role of adaptogens as both health-promoting agents and functional food ingredients. However, further research is needed to overcome challenges related to nature of the ingredients (extract or powdered plant material), bioactive compound stability, regulatory frameworks, and consumer acceptance to fully harness their potential in diverse food matrices.



CHAPTER 3

CHEMICAL PROFILING AND PROCESSING EFFECTS ON BIOACTIVE COMPOUNDS IN *RHODIOLA ROSEA* AND *ELEUTHEROCOCCUS SENTICOSUS*

3.1. INTRODUCTION

Modern lifestyles are characterized by constant mental and physical stresses that can lead to health issues that include hypertension, heart disease and depression. Herbal remedies have been used worldwide for therapeutic purposes because of their multiple effects on health, with demonstrated anti-inflammatory, antioxidant and antibiotic effects, among others (Masoud et al., 2019). Particularly, the consumption of herbal tea has increased worldwide and is expected to double its market value during the forecast period from 2023 to 2033 (Future Market Insights, 2023). The reason behind this fact is the numerous benefits of herbal teas.

In this regard, among the most common methods used to alleviate stress-related symptoms, medicinal herbs with adaptogenic effects in the form of herbal tea, have shown promising results (Winston, 2019). However, only a few medicinal herbs have been classified as adaptogenic due to proven capability to help the organism adapt to stress conditions: *Schisandra chinensis*, *Rhodiola rosea*, *Eleutherococcus senticosus* and *Panax ginseng* have been

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recognized as adaptogens (Todorova et al., 2022). On the other hand, these plants have also garnered attention for their potential ergogenic properties, which could aid individuals in coping with the physical and mental demands of an active life, while minimizing adverse effects such as fatigue, dehydration, and loss of physical skills (Todorova et al., 2022). In this particular context, the utilization of *R. rosea* and *E. senticosus* as herbal teas has regained attention over the last years (Amir et al., 2023).

R. rosea also known as “golden root”, or “roseroot” is an herbaceous plant of the Crassulaceae family. It grows in the cold mountainous regions of Europe, North America, and Asia where it was used as a traditional medicine against hysteria, depression, infections or gastrointestinal diseases (Coors et al., 2019). It has been characterized as housing more than 40 biologically active compounds, including phenylethanoids (salidroside or rhodiololide, p-tyrosol), monoterpenes (rosiridin, rosiridol) and phenylpropanoid glycosides (rosarin, rosavin, rosin) (Ma et al., 2011). This last group is referred to with the term rosavins. Rosavins, along with salidroside, are considered as the main bioactive compounds and pharmaceutical quality markers of this herb (Seczyk et al., 2023). The efficient ratio of rosavins:salidroside used in human clinical studies as well as in animal-based experiments is 3:1, as these bioactive compounds are naturally present in the root of *R. rosea* in this proportion (Ma et al., 2011a).

E. senticosus also known as “siberian ginseng”, is a perennial plant belonging to the *Araliaceae* family. Its natural habitat is East Russia (Sakhalin), Korea, China, and Japan (Lucio-Gutiérrez et al., 2011). Its secondary metabolites include saponins and their glycosides (known as eleutherosides), phenylpropanoids, phenolic acids, coumarins and lignans. Although all of them have an important effect on resistance to fatigue, inflammation and immunological improvement, eleutherosides B and E have been recognized as the key molecules responsible for the adaptogenic and ergogenic properties of *E. senticosus* (Arouca and Grassi-Kassisse, 2013). These bioactive

compounds are found mostly in roots, and they have been consumed in different forms such as liquid extracts or powders for teas.

Recently, the demand for both medicinal herbs has increased not only for pharmaceutical preparations, but also for their incorporation into dietary supplements and beverages offered worldwide (Seczyk et al., 2023). However, given their primary usage as herbal teas, most existing products on the market are in the forms of powders for brewing tea and capsules. In relation to this, the tea procedure used to brew tea has become an important factor as it can affect the extraction efficiency of the bioactive compounds, and thereby, their potential effects. Although tea is usually consumed after an infusion in water between 95 and 100°C, different conditions have been explored in existing studies for other roots. These include brewing temperatures ranging from 25 to 100°C, brewing times spanning 3 to 720 minutes, and pH levels from 3 to 9 (Cheng et al., 2023; Musa Özcan et al., 2008; da Silva Port's et al., 2013; Vinci et al., 2022). In these studies, authors have stated that the optimal infusion conditions for the extraction of phenolic compounds and the obtention of a mineral-rich tea from other herbal roots are 10 minutes at 100°C. Moreover, acidic solutions were found to be more effective for polyphenol extraction rather than basic solutions, since low pH can stabilize polyphenols by reducing their oxidation.

In the case of *R. rosea* and *E. senticosus*, previous studies have focused on the methods of extraction and quantification of the bioactive compounds (Ma et al., 2011; Tsvetov et al., 2023), as well as health effects (anticancer, anti-inflammatory, improvement of muscular endurance, diabetes, etc.) associated with the consumption of extracts in the form of supplements and herbal teas or infusions (Kim et al., 2018; Liu et al., 2023; Masi et al., 2023; Sęczyk et al., 2023).

To date, no studies in the scientific literature have comprehensively analyzed the nutritional composition of the roots of *R. rosea* and *E. senticosus*, particularly in the context of their potential applications in food product development. Furthermore, there is a lack of information on the stability of their bioactive compounds under varying temperature and pH conditions, which is essential for optimizing their use in food processing and ensuring

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their functional efficacy. Therefore, the aims of the present study were to determine the chemical characterization of two root powders primarily used as herbal teas which are obtained from *R. rosea* and *E. senticosus*. In addition, we investigated how variations in pH and temperature affect the availability of their bioactive compounds.

3.2. MATERIALS AND METHOD

3.2.1. Plant materials

Five samples each of dried *R. rosea* (R) and dried *E. senticosus* (E) roots (Figure 14) were purchased from different Canadian suppliers (Richters Herbs Co., Ontario, Canada (Supplier 1); Spirit of the Boreal Botanicals, Manitoba, Canada (Supplier 2); Rhodiola Growers of Canada, Alberta, Canada (Supplier 3)). The reference standards of tyrosol, salidroside, rosarin, rosin, rosavin, eleutheroside E and eleutheroside B were purchased from ChromaDex (California, USA). All reagent grade chemicals used were obtained from Thermo Fisher Scientific (Waltham, USA).



Figure 14. Raw samples of R and E roots from different suppliers (R1 and R2 from Supplier 2, R3 and R4 from Supplier 1, R5 from Supplier 3, E1 and E2 from Supplier 2, E3, E4 and E5 from Supplier 1).

3.2.2. Chemical composition

Each sample from the dried roots was milled with a Udy Cyclone mill (Udy Corp., Fort Collins, Colorado, USA). A 0.5 mm screen was used to obtain a particle size smaller than 1 mm as described by Iheozor-Ejiofor & Dey (2009)

and Kurkin & Ryazanova (2022) (Figure 15). The powder obtained was used for the following analyses. Moisture, lipid, protein, and ash content were determined using the AACC standard methods 44-15.02, 30-25.01, 46-12.01 and 08-01.01, respectively (AACC, 2010). In addition, total dietary fiber content was carried out using method 2 of the Total Dietary Fiber Assay Megazyme Kit (Megazyme International Ireland Co. Ltd., Wicklow, Ireland) based on AACC method 32-05.01 and AOAC Method 985.29. Total carbohydrates were obtained by difference. All tests were done in triplicate for each one of the five samples of R and E roots.



Figure 15. Ground samples of R and E roots from different suppliers (R1 and R2 from Supplier 2, R3 and R4 from Supplier 1, R5 from Supplier 3, E1 and E2 from Supplier 2, E3, E4 and E5 from Supplier 1).

3.2.3. Color

The color of the root powders was analyzed using the LC 100 Spectrocolorimeter Lovibond (Burlington, Canada). The CIE coordinates (L^* , a^* , b^*) as well as the chroma (C_{ab}^*) were obtained on a white background, using the illuminant D65 and the 10° observer as a reference, with a measurement window of 30 mm in diameter. The a^* coordinate refers to the red-green range, while the b^* refers to the blue-yellow range, and L^* indicates the lightness to darkness range. Chroma (C_{ab}^*) expresses the degree of saturation (intensity of the color) for an area viewed. Three replicates were measured for each sample.

3.2.4. Determination of bioactive compounds in root powder

The extraction of the root powder and preparation of standard solutions were carried out following the procedure described by Ma et al. (2011). Root powders ($0.100 \text{ g} \pm 0.001 \text{ g}$) were extracted for 15 minutes with a solution of methanol:

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water at a ratio of 75:25 (v:v) using an ultrasonic bath set at 37°C, and the supernatant was filtered through a 0.20 µm membrane filter. The standards eleutheroside B and E were prepared at a concentration of 0.1 mg/mL, while the rest of the standards were prepared at a final concentration of 1 mg/mL in methanol. Sample extracts and standard solutions were kept at 4°C for further analysis.

The identification and quantification of the bioactive compounds was performed by following the method of Ma et al. (2011) with slight modifications. HPLC (Acquity Arc, Waters, Massachusetts, USA) equipped with a photodiode array detector and an LC Gemini C18 5 µm i.d. column, 150 mm × 4.6 mm (Phenomenex, California, USA) was used. The mobile phase was purified water (A) and acetonitrile (B) at a flow rate of 1.0 mL/min. The linear gradient was: 0-15 min, 6-17% B; 15-18 min, 17-19.7% B; 18-22 min, 19.7% B, 22-25 min 19.7-100% B. The detection wavelength was 205 nm for eleutheroside E, rosavin, rosarin and rosin, and 220 nm for eleutheroside B, salidroside and tyrosol. Column temperature was kept at 40°C, and the injection volume was 10 µL. Values are the mean of three replicates. Data acquisition and processing were done using the Empower 3 software (Version 3.8.0, Waters, Massachusetts, USA).

3.2.5. Effect of pH and temperature on the aqueous extraction of bioactive compounds

To evaluate the influence of pH and temperature on the bioactive compounds of both roots, the sample found to have the highest content of bioactive compounds was selected for each root. The pH range considered for the study was from 3 to 9, based on the findings reported by Vinci et al. (2022), as it represents the pH values found in most commercial beverages. Buffers at pH 3 and 5 were prepared using solutions of 0.1 M of sodium acetate and sufficient quantity of glacial acetic acid. For buffers at pH 7 and 9, 0.01 M sodium borate and a sufficient quantity of 0.1 M NaOH solution were used. To simulate the effects of temperature on the infusion of bioactives from the roots, as in the

creation of an herbal tea, samples were infused for 10 min and then frozen. Distilled water at four different temperatures (25, 50, 75 and 100°C) were used as per Cheng et al. (2023).

A sample of 0.1 g was mixed with 10 mL of water at the corresponding infusion temperature. An identical procedure was performed for each of the buffer solutions conducted at room temperature for 10 min and then frozen. All infusions were lyophilized after freezing (VirTis Genesis 25L, New York, USA) and extracted and quantified as above described in section 3.2.4. Values are the mean of three replicates.

3.2.6. *Statistical analysis*

Statistical analysis was carried out using STATGRAPHICS CENTURION XVII software (Version 17.2, Virginia, USA). Data plotting was carried out using OriginPro 2020 (OriginLab, Northampton, Massachusetts, USA). The analysis of variance (ANOVA) was used to describe significant differences ($p \leq 0.05$) between the variables. Fisher's least significant differences test was employed to determine significant variations between experimental mean values, with a confidence level of 95%. In addition, a Pearson's matrix was applied to establish correlations between the parameters obtained for the different samples of both roots separately. A principal component analysis (PCA) was built to explain the variability of the data for the properties measured with samples from both roots. All the data were expressed as the mean \pm standard deviation.

3.3. RESULTS AND DISCUSSION

3.3.1. *Chemical composition*

The proximate composition of the five different samples of each root, *Rhodiola rosea* (R) and *Eleutherococcus senticosus* (E), are shown in Table 2, where values of nutrients are reported on a dry weight basis. Significant differences ($P \leq 0.05$) in the nutrients were obtained between both roots depending on their source, especially for fiber and moisture content.

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Table 2. Chemical composition of the 5 different samples of *Rhodiola rosea* (R) and *Eleutherococcus senticosus* (E), expressed on a dry weight basis.

Sample	Moisture (g/100 g)	Ash (g/100 g)	Fat (g/100g)	Protein (g/100 g)	Fiber (g/100g)	Carbohydrates (g/100 g)*
R1	5.73 ± 0.03 ^b	5.54 ± 0.28 ^b	1.40 ± 0.15 ^{ab}	1.56 ± 0.05 ^a	42.86 ± 2.07 ^{ab}	48.64
R2	5.19 ± 0.35 ^a	6.83 ± 0.14 ^c	1.54 ± 0.19 ^b	1.79 ± 0.06 ^b	59.06 ± 1.22 ^d	30.78
R3	5.89 ± 0.04 ^b	4.50 ± 0.02 ^a	1.17 ± 0.07 ^a	3.06 ± 0.04 ^c	40.74 ± 0.56 ^a	50.53
R4	5.82 ± 0.11 ^b	4.54 ± 0.01 ^a	2.01 ± 0.10 ^c	3.24 ± 0.07 ^d	50.75 ± 1.50 ^c	39.46
R5	6.90 ± 0.36 ^c	5.37 ± 0.17 ^b	2.06 ± 0.14 ^c	4.16 ± 0.11 ^e	44.61 ± 1.62 ^b	43.80
E1	3.61 ± 0.26 ^{bc}	4.83 ± 0.25 ^d	2.11 ± 0.14 ^a	2.08 ± 0.09 ^a	73.52 ± 3.34 ^{ab}	17.46
E2	3.86 ± 0.12 ^c	4.14 ± 0.13 ^c	2.21 ± 0.19 ^a	2.36 ± 0.15 ^{bc}	77.96 ± 2.64 ^c	13.33
E3	3.07 ± 0.10 ^a	2.28 ± 0.25 ^a	2.22 ± 0.16 ^a	1.96 ± 0.05 ^a	76.11 ± 2.87 ^{bc}	17.43
E4	3.65 ± 0.19 ^{bc}	3.13 ± 0.09 ^b	2.95 ± 0.12 ^b	2.16 ± 0.03 ^{ab}	70.89 ± 1.30 ^a	20.87
E5	3.50 ± 0.35 ^{ab}	3.33 ± 0.18 ^b	2.44 ± 0.07 ^a	2.46 ± 0.14 ^c	74.11 ± 1.62 ^{abc}	17.66

Values correspond to means ± standard deviation obtained from three independent replicates on which are based the statistical analysis. Different superscript letters within a column for each root indicate significant differences (P -value ≤ 0.05).

*Carbohydrates were calculated by difference.

In the case of R root, the moisture content of the different samples was similar, albeit R5 presented a higher value (6.90 g/100 g). Significant differences were found in the protein content, being higher in sample R5 (4.2 g/100 g). Moreover, fibre and carbohydrates emerged as the predominant macro components across all R samples, with values ranging from 41 to 59 g/100 g and 31 to 51 g/100 g, respectively. Sample R2 exhibited the highest fibre content (59.1 g/100 g), which could be attributed to the duration of crop growth, as reported in another root crop (*Withania somnifera*). In that study, authors stated that the fibre content in *Withania somnifera* roots increased with the length of crop growth (Ramesh Kumar et al., 2011). Anilakumar et al. (2006) reported a higher content of protein (15 g/100 g), fat (11 g/100 g) and ash (18 g/100 g) for R root in comparison with the results obtained in the present study. Differences may be due to the geographical and environmental conditions during cultivation or the genetic variation inherent in different cultivars.

Significant variations ($P \leq 0.05$) were also found for all the nutrients between the different sourced samples of E root (Table 2). In this regard, percentage differences were higher for ash and protein content, which ranged from 2.3 to 4.8 g/100 g and from 2.0 g to 2.5 g/100 g, respectively. Samples E2 and E3 presented higher values in terms of fiber, being these 78 g and 76 g/100 g, respectively, compared to a low value of 71 g/100 g (E4) between the different sourced samples of E root. Moreover, fat content was found to be highest in sample E4 (3.0 g/100 g). Similar results for protein content (2.0 g/100 g) were obtained for *Anethum sowa* L. root, which belongs to the same order as E root (Saleh-e-In et al., 2017). However, the fibre (54.09 g/100 g) value reported by Saleh-e-In et al. (2017) was lower than the present study (71-78 g/100 g), which might be due to the differences between roots.

3.3.2. Color

Significant differences ($P \leq 0.05$) were observed between the color of samples of both roots, with the parameter a^* being generally more predominant in the case of R samples (Table 3). In general, samples from E root presented higher results for the L^* parameter when compared to R root, meaning that this root was brighter. Since both types of samples were

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milled to the same particle size, differences in granularity are not a factor in the lightness values (Figure 15). In the case of R root, for the parameter L^* , higher values were shown in samples R3 (65.1) and R4 (61.2), respectively, meaning these samples were brighter than the others (Table 2). Regarding the a^* and b^* parameters, it was observed that R samples fell within 5.0-11.0 and 15.0-21.6, respectively. R2 presented the highest values for the coordinate a^* (11.0) while for the parameter b^* , R1 showed higher results (21.6) (Table 3). Moreover, chromatic C_{ab}^* , which is defined by the saturation, was higher in sample R1 (24.1), compared to the other samples.

Table 3. Color of the 5 different sources of *R. rosea* (R) and *E. senticosus* (E).

Sample	L^*	a^*	b^*	C_{ab}^*
R1	47.87 ± 0.46 ^a	10.70 ± 0.26 ^c	21.57 ± 0.35 ^e	24.10 ± 0.46 ^d
R2	49.00 ± 1.15 ^a	10.97 ± 0.45 ^c	17.67 ± 0.47 ^b	20.80 ± 0.56 ^c
R3	65.13 ± 0.64 ^d	5.03 ± 0.06 ^a	18.63 ± 0.21 ^c	19.30 ± 0.17 ^b
R4	61.23 ± 0.31 ^c	5.63 ± 0.12 ^b	19.57 ± 0.5 ^d	20.37 ± 0.49 ^c
R5	58.27 ± 0.38 ^b	5.07 ± 0.06 ^a	15.03 ± 0.21 ^a	15.90 ± 0.20 ^a
E1	64.03 ± 0.21 ^b	4.53 ± 0.15 ^c	20.40 ± 0.10 ^b	20.90 ± 0.17 ^b
E2	61.03 ± 0.87 ^a	5.00 ± 0.17 ^d	20.40 ± 0.40 ^b	21.00 ± 0.40 ^b
E3	69.97 ± 0.61 ^c	3.47 ± 0.12 ^a	19.07 ± 0.70 ^a	19.40 ± 0.66 ^a
E4	64.00 ± 0.66 ^b	3.87 ± 0.06 ^b	18.80 ± 0.26 ^a	19.20 ± 0.26 ^a
E5	62.93 ± 0.97 ^b	3.73 ± 0.21 ^{ab}	19.17 ± 0.25 ^a	19.50 ± 0.20 ^a

Values correspond to means ± standard deviation obtained from three independent replicates. The superscript letters indicate significant differences (P -value ≤ 0.05) among values within columns.

Significant differences (P ≤ 0.05) were also found between E samples, being higher the values obtained for sample E3 (70.0) and E2 (5.0) in terms of luminosity and redness, respectively (Table 3). In this regard, E4 seemed to be brighter than E3. However, luminosity was higher in E3 (70.0), compared to the rest of samples (S1B). Furthermore, sample E1 and E2 showed a less yellowish color as b^* coordinate was higher (20.4). Higher values of C_{ab}^* were also found in samples E2 (21.0) and E1 (20.9), which could be due to the

growing conditions, the land or the geographical area. In general, differences were higher within the R samples compared to E samples in terms of parameters C_{ab}^* , L^* and b^* . Therefore, R samples were more heterogeneous. Hitherto, no studies have reported the color of the R and E roots.

3.3.3. Determination of bioactive compounds in powdered roots

The R and E root samples were evaluated in terms of the content of the following substances: tyrosol derivatives (salidroside and its aglycon tyrosol) as well as rosavins (rosavin, rosarin, rosin) in the case of R root, and the lignan compounds, eleutheroside B (syringin) and eleutheroside E [(-)-syringaresinol-di-O-13-Dglucoside], for E root samples. The results for the five different samples of R and E roots are shown in Table 4 and 5, respectively.

Significant differences ($P \leq 0.05$) were obtained for all the bioactive compounds of R root. The amounts of salidroside and rosavin, the main bioactive components of R root, were more abundant in the R3 sample in comparison to the other samples (Table 4). It should be highlighted that the values for R1 (1.4 mg/g root) and R2 (4.7 mg/g root) were significantly lower for rosavin than the rest of the samples, which could be attributed to the origin of the root or the growing conditions. In this context, a positive correlation was observed between protein content and rosavin ($r = 0.86$, $P \leq 0.05$).

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Table 4. Contents of bioactive compounds in 5 different samples of *R. rosea* roots (R).

Sample	Salidroside (mg/g)	Tyrosol (mg/g)	Rosarin (mg/g)	Rosavin (mg/g)	Rosin (mg/g)
R1	47.36 ± 0.98 ^c	14.15 ± 0.28 ^e	6.87 ± 0.17 ^a	1.43 ± 0.13 ^a	0.50 ± 0.00 ^c
R2	12.15 ± 0.07 ^a	5.89 ± 0.04 ^b	6.08 ± 0.48 ^a	4.65 ± 0.05 ^b	0.13 ± 0.00 ^a
R3	68.71 ± 0.50 ^e	7.97 ± 0.27 ^c	28.32 ± 0.13 ^b	68.33 ± 0.81 ^e	0.21 ± 0.01 ^b
R4	50.24 ± 0.55 ^d	9.82 ± 0.11 ^d	32.01 ± 0.31 ^c	61.60 ± 1.73 ^d	0.20 ± 0.00 ^b
R5	24.76 ± 0.32 ^b	3.46 ± 0.12 ^a	44.33 ± 0.84 ^d	54.88 ± 0.43 ^c	0.56 ± 0.01 ^d

Values correspond to means ± standard deviation obtained from three independent replicates. Different superscript letters within a column indicate significant differences (P -value ≤ 0.05).

The content of salidroside ranged from 12.2 to 68.7 mg/g of root, which is within the wide range reported by Ma et al. (2011), being the range between 3 to 132 mg/g root extracts. However, the results obtained for rosavin, ranged from 1.4 to 68.3 mg/100 g, and these are considerably higher than those obtained by Ma et al. (2011), with values between 0.33 to 27.12 mg/g root extract. Probably cultivar differences or due to the fact that those authors used standardized *Rhodiola* extracts might explain the divergence. Moreover, positive strong correlations between L^* and the bioactive compounds rosavin ($r = 0.97$, $P \leq 0.05$), salidroside ($r = 0.80$ $P \leq 0.05$) and rosarin ($r = 0.74$ $P \leq 0.05$) were obtained.

The contents of tyrosol (3.5-14.2 mg/g root) and rosarin (6.1-44.3 mg/g root) were also higher when compared to the results stated by Ma et al. (2011), being 0.52-9.35 mg/g root extract and 0.09-10.86 mg/g root extract, respectively. A very strong correlation between protein content and rosarin ($r = 0.99$ $P \leq 0.05$) was obtained. This outcome could be interesting to find possible markers for rosarin content prediction in R roots. It should be noted that negative correlations have been found between a^* and the bioactive compounds rosarin ($r = -0.94$ $P \leq 0.05$) and rosavin ($r = -0.98$ $P \leq 0.05$). In addition, tyrosol presented a positive strong correlation with b^* parameter ($r = 0.98$ $P \leq 0.05$).

Table 5 summarizes the contents of eleutheroside B and eleutheroside E in five samples of E root. The contents of eleutheroside B and E were more abundant in sample E5 and lower in sample E2. This could be explained with the higher content of fibre in sample E2 (78 g/100 g) which might limit the release of these bioactive compounds, as a negative correlation was obtained between eleutheroside B and fiber ($r = -0.81$, $P \leq 0.05$). The contents of eleutheroside B were lower in this study (0.51-1.49 mg/g root) to the ones reported by Ma et al. (2011), 2.1-3.1 mg/g of root extract. In contrast, the quantities of eleutheroside E were higher in the present study (10.0-14.7 mg/g root). Differences may be due to the use of standardized extracts of *E. senticosus* in the study done by Ma et al. (2011). Yat et al. (1998) also reported lower results of eleutheroside E (0.15-6.93 mg/g root) and a wide range of results for eleutheroside B (0.06-10.50 mg/g root) within whose range are those obtained in the present study. The different extraction method used in the mentioned study could explain the differences with eleutheroside E contents, as contents range between 10-15 mg/g root. Another study described lower contents of eleutheroside E (0.561 mg/g dried root) and B (0.445 mg/g dried root) when analyzing the roots (Kang et al., 2001). According to Jung et al. (2014), the concentration of pharmacologically active compounds can vary depending on factors such as plant age or the different parts of the plants. Negative correlations were obtained between the eleutheroside E ($r = -0.90$, $P \leq 0.05$) and B ($r = -0.72$, $P \leq 0.05$) with the α^* parameter. Thus, the degree of greenness in E samples could discriminate higher contents of E's bioactive components.

Table 5. Contents of bioactive compounds in five different samples of *E. senticosus* roots (E).

Sample	Eleutheroside E (mg/g)	Eleutheroside B (mg/g)
E1	11.36 ± 0.03 ^b	1.28 ± 0.00 ^c
E2	10.09 ± 0.05 ^a	0.51 ± 0.01 ^a
E3	14.33 ± 0.08 ^d	1.15 ± 0.01 ^b
E4	11.93 ± 0.33 ^c	1.33 ± 0.00 ^d
E5	14.70 ± 0.10 ^e	1.49 ± 0.00 ^e

Values correspond to means ± standard deviation obtained from three independent replicates. Different superscript letters within a column indicate significant differences (P -value ≤ 0.05).

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3.3.4. Principal component analysis

In order to have a global view of the different analyses carried out in the present study, and to discriminate among the different samples in terms of proximate composition and bioactive compounds, a Principal Component Analysis (PCA) was carried out. The five samples were differentiated from each other according to the factors studied, which were distributed throughout the two-dimensional plane defined by two components. These components were able to explain 79% of the variability observed among samples from R root (Figure 16). Component 1 explained 51% whereas Component 2 described 28% of the variance. In this regard, a high correlation was shown between the bioactive compounds rosarin and rosavin with protein, as they appear very close to each other and by the positive x-axis. Therefore, it can be said that sample R5 is characterized by a high content of rosavin, rosarin and protein. In addition, the salidroside compound showed a proximity with sugar and sample R3, meaning that a higher content of salidroside, could be directly related to a higher content of sugar. As shown in S2, it should be highlighted that tyrosol and the parameter b^* showed strong correlations as they are in the same quadrant. Thereby, sample R1 could be defined by a high content of tyrosol and b^* parameter.

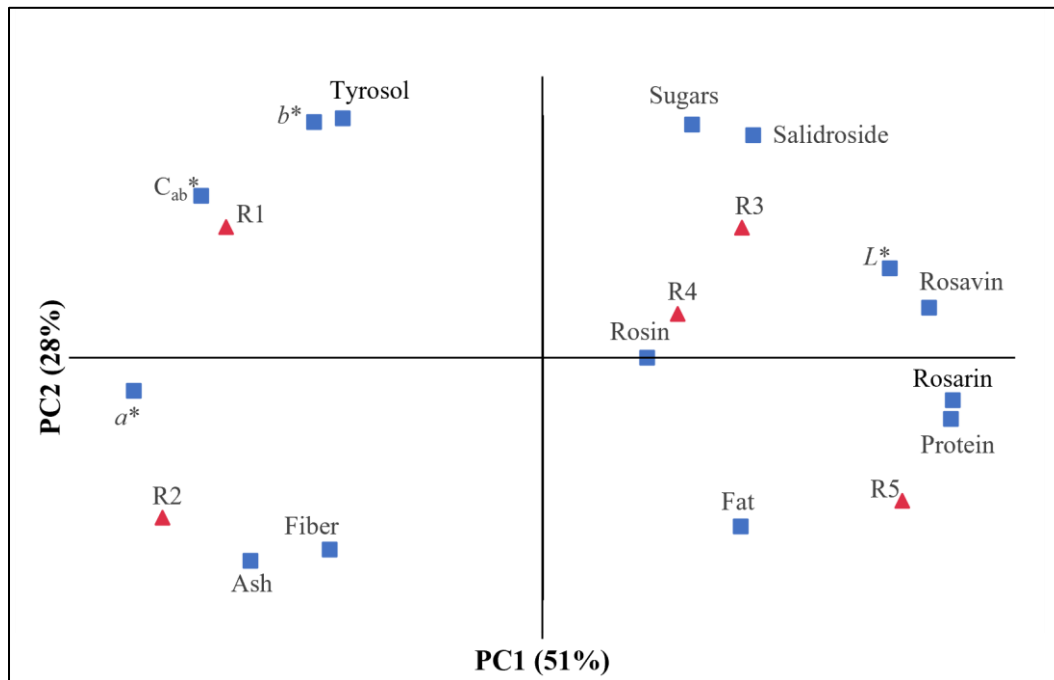


Figure 16. PCA of the different samples of *Rhodiola rosea* (Rx), its bioactive compounds and the analytical parameters (L^* : brightness; a^* : green-red coordinates; b^* : blue-yellow coordinates and C_{ab}^* : chroma).

In the case of E root, Figure 17 revealed that with two main components, 82% of the differences between samples could be explained. While the first component explained 61% of the variance among the samples, the second component was able to clarify up to 21% of the differences along the y-axis. Eleutheroside B was highly correlated with sugar content as well as samples E5 and E4. Hence, these two samples presented higher content of eleutheroside B and sugar. Furthermore, the compound eleutheroside E presented a closer proximity with the a^* parameter but not with any of the macronutrients (Figure 17).

Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

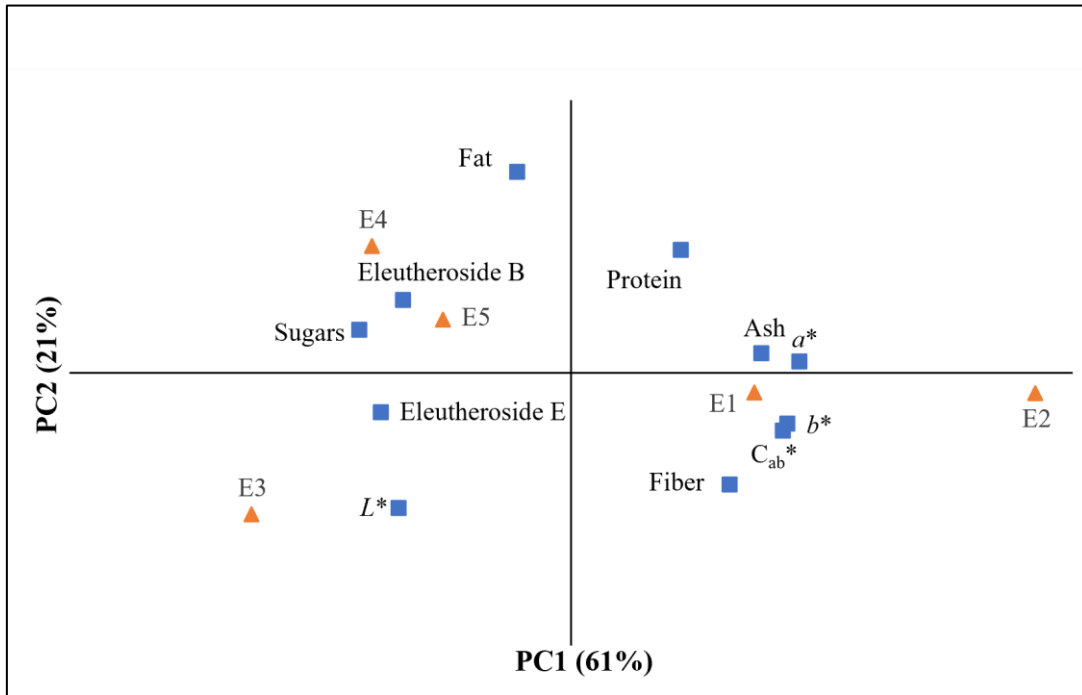


Figure 17. PCA of the different samples of *Eleutherococcus senticosus* (Ex), its bioactive compounds and the analytical parameters (L^* ; brightness; a^* : green-red coordinates; b^* : blue-yellow coordinates and C_{ab}^* : chroma).

3.3.5. Effect of pH and temperature on the bioactive compounds

Rhodiola rosea and *Eleutherococcus senticosus* roots are traditionally consumed as tea (Amsterdam and Panossian, 2016). However, the temperature and time for preparing tea can vary depending on factors such as individual preferences, country of origin and culture, and all these factors could affect the stability of the bioactive compounds. The temperatures and times that have been reported in several studies range from 25 to 100°C, and from 3 to 720 minutes (Cheng et al., 2023; Musa Özcan et al., 2008; da Silva Port's et al., 2013). Nevertheless, the optimal infusion time to prepare a good quality tea beverage with higher extraction of phenolic compounds was 10 minutes, as reported by da Silva Port's et al. (2013) and Musa Özcan et al. (2008). Therefore 10 minutes was selected as the infusion time for this study. Moreover, pH levels from 3 to 9 were

considered in the present study as it reflects the pH values of most commercial beverages (Vinci et al., 2022). Therefore, the effect of temperature and pH on the bioactive compounds was studied to investigate the stability of the bioactive compounds. The samples that contained higher content of these compounds, R3 and E5 (Table 4 and 5), were selected for studying the pH and temperature impact.

Eleutheroside E was found to be more affected by pH than eleutheroside B (Figure 18). In fact, a trend was observed for eleutheroside E, indicating a decreasing content with increasing pH, which could be due to the presence of OH groups of the tetrahydrofurans and tetrahydropyrans that behave as weak proton acceptors (Kurkin, 2003) (Figure 19). Eleutheroside E and B, have pKas values of 12.4 and 12.2, respectively. In the case of eleutheroside B, the content in the infusion was higher at pH 5 (0.64 mg/g root), decreasing at pH 7 (0.41 mg/g root) and increasing at pH 9 (0.57 mg/g root). A possible explanation for the increase of solubility at pH 9 could be attributed to the presence of an allylic OH group in eleutheroside B, which is more acidic than alcoholic OH groups found in sugars, and which begins to deprotonate at pH 9, thereby increasing solubility.

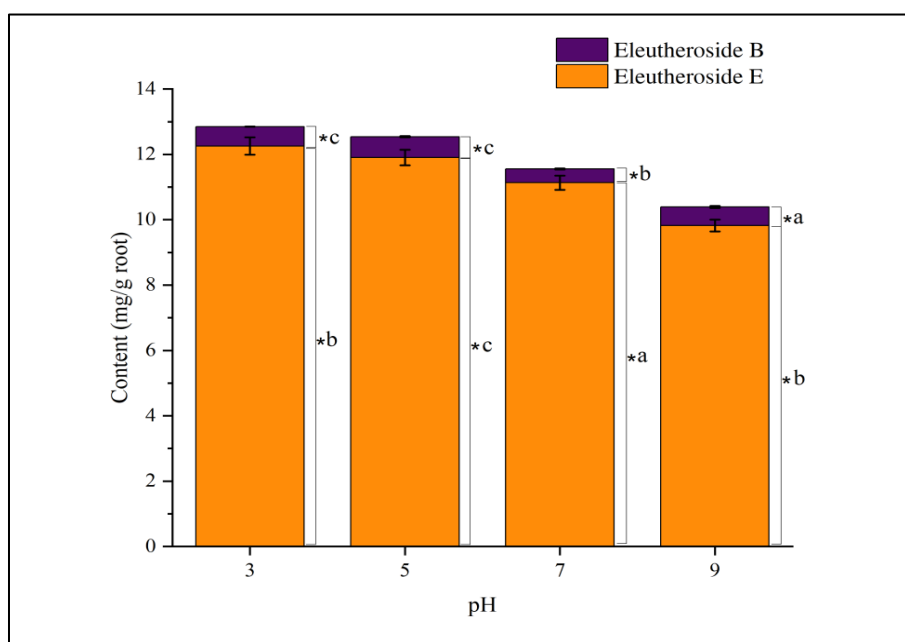


Figure 18. Effect of pH on the bioactive compounds of sample E5. Different letters above the bars indicate significant differences within the pHs for each bioactive compound ($P \leq 0.05$).

Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

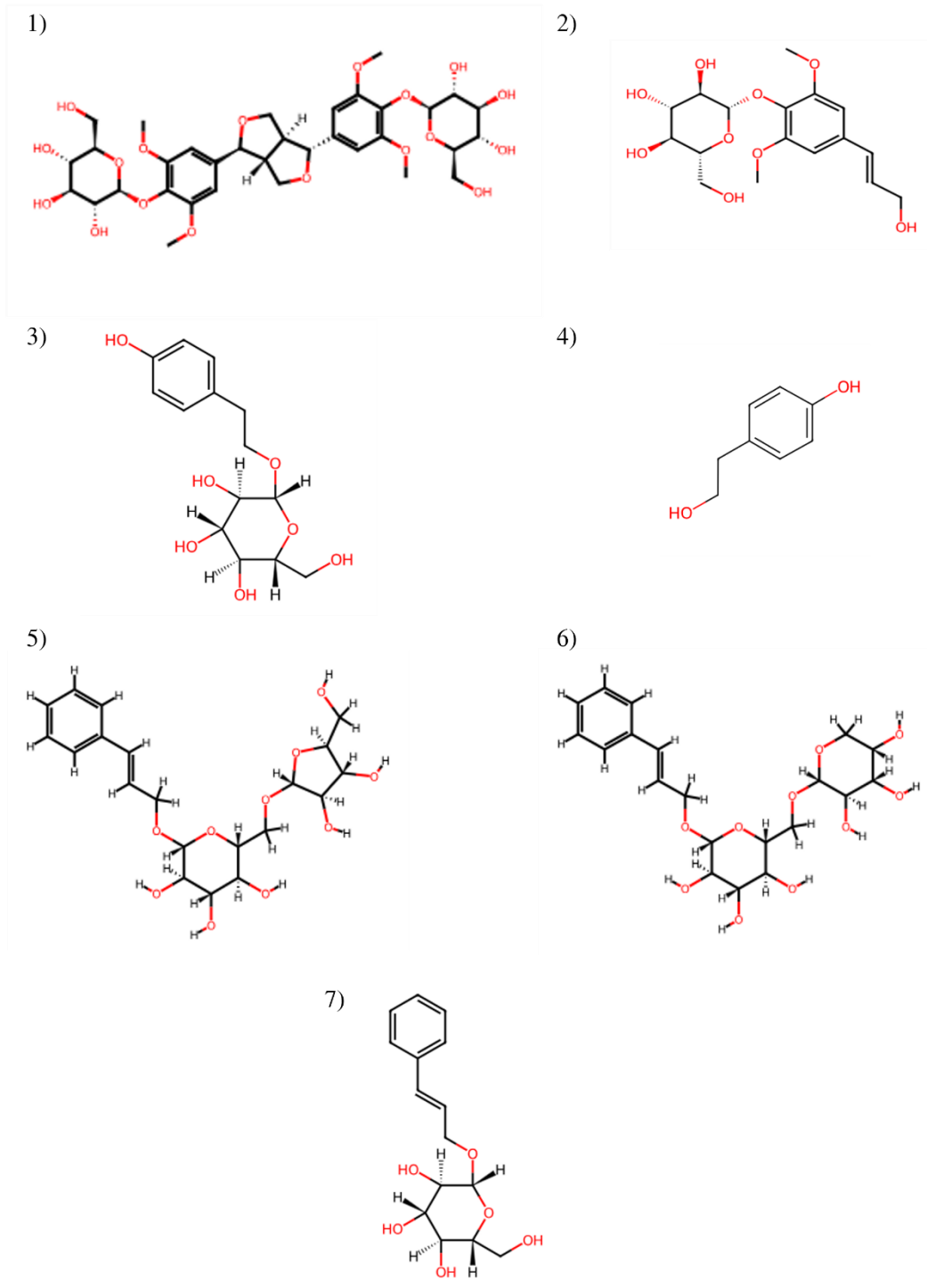


Figure 19. Chemical structure of the bioactive compounds from *R. rosea* and *E. senticosus*: 1) Eleutheroside E, 2) Eleutheroside B, 3) Salidroside, 4) Tyrosol, 5) Rosarin, 6) Rosavin and 7) Rosin.

Figure 20 represents the effect of temperature on these compounds, where significant differences ($P \leq 0.05$) were found for eleutheroside E. Higher content was observed at 50 and 100°C likely due to the enhanced solubility of the solvent at higher temperature. The increased solubility facilitates better penetration of the solvent inside the solid matrix, thereby enhancing the diffusivity and leading to a higher yield and extraction rate (Izadifar and Baik, 2008). However, this effect is not shown at 75°C for the eleutheroside E (11.3 mg/g root), probably due to changes in the structure of the matrix that could influence the diffusion of the solvent and subsequently affect extraction efficiency.

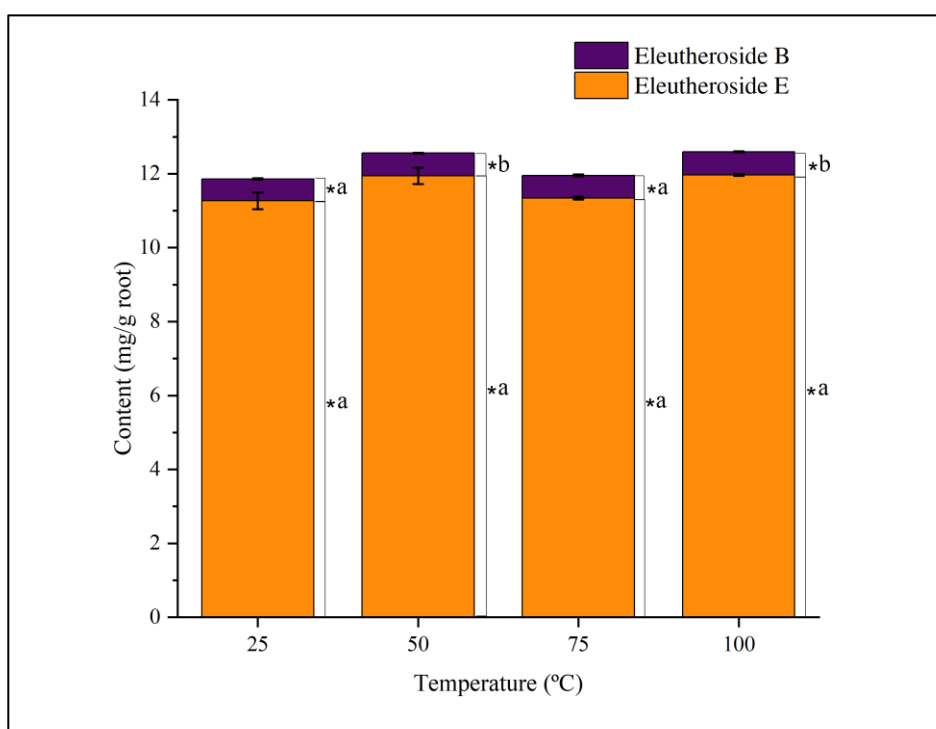


Figure 20. Effect of temperature on the bioactive compounds of sample E5. Different letters above the bars indicate significant differences within the temperatures for each bioactive compound ($P \leq 0.05$).

The extraction of pharmacologically active compounds from R root was dependent on pH (Figure 21). Significant differences ($P \leq 0.05$) were observed with changes in pH for all the bioactive compounds. In general, all compounds have greater solubility at acidic pH (3 and 5) due to the presence of OH groups that interact with H^+ , facilitating ionization and increasing solubility (Figure 19). The solubility of salidroside and tyrosol at the different pHs showed significant differences, but not quantitatively

Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

important possibly due to the presence in both compounds of a phenolic group which gives them an equal pKa, 10.2. Although rosarin, rosavin and rosin have similar chemical structures (phenylpropanoids glycosides) with pKas of 12.89, 12.89 and 12.91, respectively, the behaviour of these compounds is different with pH changes. The infusion extraction rate was higher at pH 5 in the case of rosarin and rosin, but at pH 3 in the case of rosavin. The different behaviour at pH 3 may be attributed to the superior proton-accepting capability of the two tetrahydropyran groups, compared to the presence of one tetrahydropyran group in rosin and one tetrahydroxy pyran group plus one tetrahydrofuran in rosarin (Figure 19).

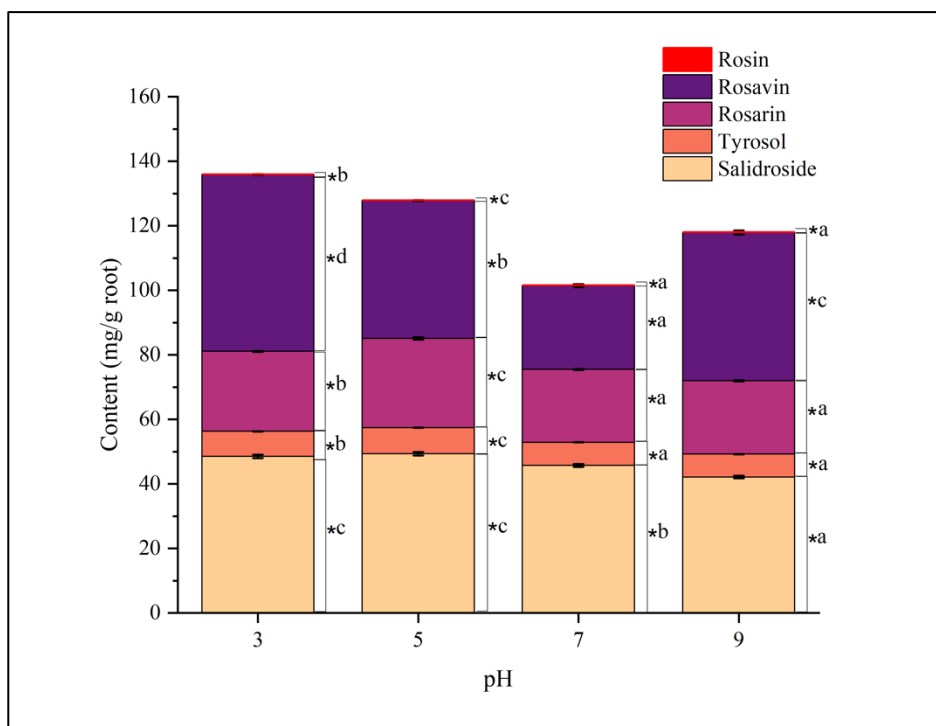


Figure 21. Effect of pH on the bioactive compounds of sample R3. Different letters above the bars indicate significant differences within the pHs for each bioactive compound ($P \leq 0.05$).

The temperature of the root infusion significantly ($P \leq 0.05$) affected the number of bioactive compounds extracted. As shown in Figure 22, the extraction rates for salidroside and rosavin were higher at 75 and 100°C, which can be related to favourable migration of these bioactive compounds when the temperature rises. Factors such as solubility and

mass transfer rate (diffusion coefficient) of the bioactive compounds are influenced by the temperature (Mohamad et al., 2013). However, these effects were not seen with rosarin and rosin as their infusion content was higher at 25 and 50°C. A possible justification for these findings is that at temperatures higher than 50°C, the target compounds may decompose due to structural damage, as high temperatures can also decrease the cell barrier by weakening integrity of the cell wall and membrane, as described by Mohamad et al. (2013). Conversely, tyrosol extraction was higher at 75°C (8 mg/g root) and 50°C (7.9 mg/g root), meaning that lower and higher temperatures did not favor the extraction of this bioactive compound (Figure 22).

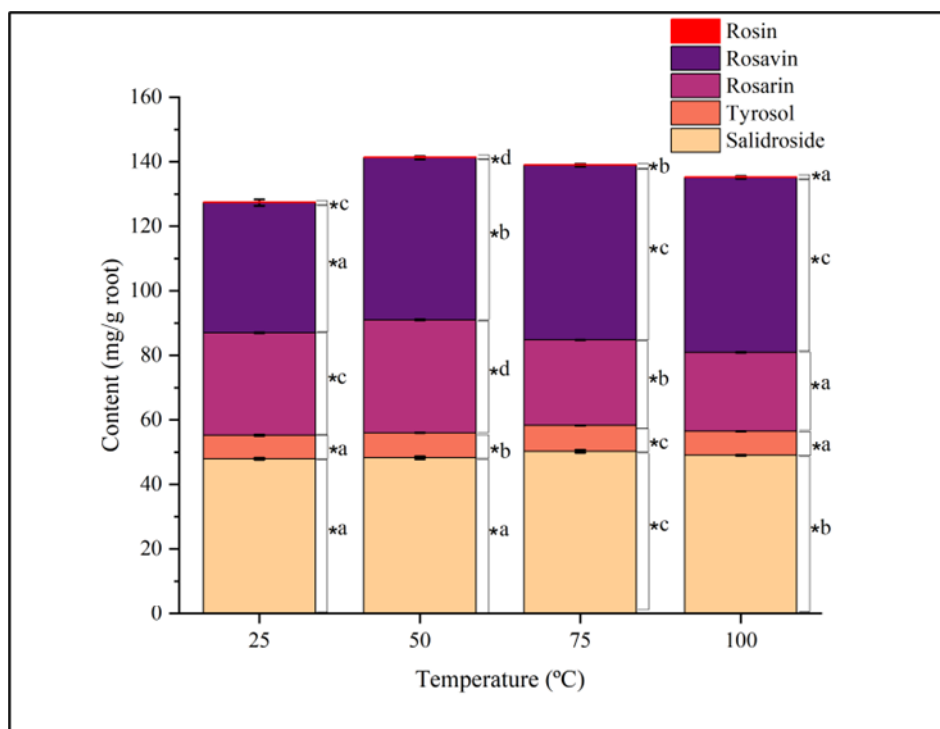


Figure 22. Effect of temperature on the bioactive compounds of sample R3. Different letters above the bars indicate significant differences within the temperatures for each bioactive compound ($P \leq 0.05$).

3.4. CONCLUSION

Rhodiola rosea and *Eleutherococcus senticosus* have been investigated as adaptogens in numerous pharmacological and clinical studies, primarily in the form of herbal tea or supplement containing the extract from different

Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

parts of the herbs. This study, however, represents the first comprehensive analysis of the proximate composition of the raw material rather than the extract, along with the investigation of their bioactive compounds' stability under food processing conditions, specifically across varying temperatures and pH levels. The results demonstrate that both roots are excellent source of fiber, especially *E. senticosus*, which showed a remarkably higher content. Furthermore, levels of key bioactive compounds such as rosavin, tyrosol, rosarin, and eleutheroside E were found to exceed those reported in studies employing standardized extracts, suggesting that the raw powdered roots may serve as viable sources of these compounds.

Higher extraction rates for most bioactive compounds were identified as pH levels between 3 and 5, and a temperature of 50°C, although specific compounds like rosavin and salidroside showed better yields at 75°C. The bioactive compounds in *E. senticosus* showed better stability at pH 5 and 3, while for temperature, eleutheroside E demonstrated a higher stability at 50 and 100°C. In the case of eleutheroside B, it was stable across the full temperature range studied. These findings highlight the potential of using the powdered roots as functional ingredients in food product development, offering both nutritional and health benefits. Unlike extracts, the powdered roots contain additional dietary fibre and other valuable nutrients, making them an attractive option for developing novel food products with added value.

Nonetheless, seasonal variations in both roots should be considered for future potential studies, as well as the optimization of the extraction conditions to obtain food ingredients rich in bioactive compounds. Future research should also investigate the synergistic effects of these compounds in food matrices, their bioavailability, and their long-term stability during storage and processing.



CHAPTER 4

DEVELOPMENT OF HIGH-PROTEIN FUNCTIONAL CRACKERS ENRICHED WITH ADAPTOGENIC COMPOUNDS

4.1. INTRODUCTION

Contemporary lifestyles, along with evolving dietary preferences, are driving the demand for convenient and healthy on-the-go snacks. In this context, crackers have emerged as a versatile snack category, presenting significant opportunities for innovation in aspects such as texture, flavor, and color (Innsai Monitor, 2024). According to recent projections, the global cracker market is expected to grow at an annual rate of 6.2% between 2025 and 2030 (Statista, 2025b). However, from a nutritional point of view, crackers do not necessarily provide nutrient profiles that appeal to all consumers. In particular, crackers are low in protein, ranging from 7-8% (Han et al., 2010).

As many consumers now seek for high protein snacks, the enrichment of crackers with protein is of pivotal importance. Proteins can be derived from different sources such as plant and animal. Although animal proteins, especially whey, are considered excellent sources of essential amino acids, plant proteins are gaining more attention for their high protein content, including a relatively balanced amino acid profile and a moderate digestibility

(Anyiam et al., 2025). Moreover, their increased consumption is largely driven by their lower environmental footprint, particularly in terms of greenhouse gas emissions, and their potential health benefits related to the reduction of cardiovascular disease risk (Rout & Srivastav, 2025). In this context, faba bean (*Vicia faba* L.) is considered a high protein and high fiber source, while low in fats (Gasparre et al., 2024). This makes it ideal for producing protein-rich ingredient fractions such as faba bean protein isolate. Faba bean protein isolate has been used for producing meat analogues, bread, pasta, biscuits and extruded snacks (Hsu et al., 1982; Martin et al., 2022). However, little research has been done regarding the use of faba bean protein isolates in baked snacks such as crackers. Hence, crackers offer a promising opportunity to promote wellness by delivering health-promoting ingredients.

Medicinal herbs are recognized for their health benefits including their effects on the central nervous system due to the presence of secondary metabolites that are present in plants. Among the various medicinal herbs, a limited group of them exhibit adaptogenic properties, traits that are related to the capacity of the body to respond to different emotional, mental, and physical stressors (Tóth-Mészáros et al., 2023). In this context, *Rhodiola rosea* and *Eleutherococcus senticosus* have gained significant popularity worldwide and are among the most popular plants used in Europe and Asia for health and wellness reasons. These two medicinal herbs have been widely consumed in different forms such as teas, beverages, and pharmaceutical forms alone or in combination with other medicinal herbs (AAFC, 2016; Lee et al., 1999; Ma et al., 2011).

In recent years, some food products containing *R. rosea* and *E. senticosus*, such as biscuits, teas coffee and chewing gums, have been launched in the global market (AAFC, 2016; Haworth & Haskell-Ramsay, 2024). However, most of these products are formulated from extracts that have been derived from either powders of *R. rosea* or *E. senticosus*. Accordingly, *R. rosea* extracts are normally standardized to contain 3% rosavins (rosavin, rosarin and rosin) and 1% salidroside, as these root-derived bioactive compounds are responsible for the adaptogenic effects (Sist et al., 2018). In contrast, although eleutherosides E and B are recognized as the primary bioactive compounds in *E. senticosus*,

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the extracts are not standardized (Ma et al., 2011). Therefore, combining faba bean protein isolate with powders of *R. rosea* or *E. senticosus* roots at different levels presents the possibility of developing high-protein crackers enriched with bioactive compounds that possess adaptogenic properties.

This study aimed to develop high-protein crackers with potential adaptogenic properties from root powders and examine the root powders' impact on the physicochemical properties of crackers. In addition, because the stability of the bioactive compounds in the high protein cracker is critical to consumer appeal, the study also evaluates the effect of processing.

4.2. MATERIALS AND METHOD

4.2.1. Materials

Commercial faba bean (*Vicia faba L.*) protein isolate (FBPI) PUREPRO FABA 85S was purchased from Univar Solutions (Winnipeg, Manitoba, Canada). FBPI contained on a dry basis, 88.0 g of protein, 8.2 g of carbohydrates, 0.6 g of fat, and 3.2 g of ash per 100 g. Wheat flour (*Triticum aestivum*) was kindly supplied by Cereals Canada (Winnipeg, Manitoba, Canada), and contained on dry basis, protein 17.5 g, carbohydrate 80.9 g, fat 1 g and ash 0.6 g per 100 g. Other baking ingredients, sugar, canola oil and salt, were purchased from a local grocery store.

The dried roots of *Rhodiola rosea* and *Eleutherococcus senticosus* were purchased from Rhodiola Growers of Canada (Mountain View County, Alberta, Canada) and Richters Herbs Co (Goodwood, Ontario, Canada), respectively. These two dried roots were milled with a Udy Cyclone mill (Udy Corp., Fort Collins, Colorado, USA) equipped with a 1 mm screen, as described by Iheozor-Ejiofor & Dey (2009).

4.2.2. Preparation of crackers

A basic un-yeasted cracker formulation, adapted from Ujong et al. (2023), was used as the control with minor modifications. The cracker formulation included the following ingredients: 55.22 g of FBPI, 44.78 g of wheat flour, 60

g of water, canola oil 7.14 g, salt 2.14 g and sugar 1 g. Considering the Daily Value (DV) for protein is 50 g/day, and that a product must provide at least 20% of the Daily Reference Value (DRV) to qualify for a high-protein claim (FDA, 2024), the cracker formulation was devised to meet these criteria, resulting in a high-protein product.

For the crackers enriched with dried roots of *Rhodiola rosea* (RR) or *Eleutherococcus senticosus* (ES), wheat flour was partially substituted with varying levels of the dried roots at 0.3% (low), 1.7% (medium), and 3.3% (high), as detailed in Table 6. To assure the same dough consistency across all samples, Mixolab® (Chopin, Tripette et Renaud, Paris, France) was used to optimize the quantity of water (Figure 23). Once the optimal water level was determined, all the ingredients were mixed using a KitchenAid KSM150PSEB (Benton Harbor, Michigan, USA) mixer machine for 15 min, achieving the desired dough consistency (Figure 23). The dough was sheeted to a uniform thickness of 2 mm using a pasta sheet roller and then cut into squares measuring 3.5 × 3.5 cm using a square cutter. A stainless-steel dough docker was used to prick the surface of the crackers before baking them at 180°C for 6 min in a forced-air convection oven (Blodgett, Essex Junction, Vermont, USA). Preliminary baking trials indicated that 6 min baking under these conditions sufficed to attain a crunchy cracker texture.

Table 6. Formulation of the control crackers and the crackers containing different levels of RR or ES.

Ingredients	Formulations						
	Control	RR low	RR medium	RR high	ES low	ES medium	ES high
Wheat flour (g)	44.78	44.45	43.11	41.45	44.45	43.11	41.45
FBPI (g)	55.22	55.22	55.22	55.22	55.22	55.22	55.22
RR root (g)	-	0.33	1.67	3.33	-	-	-
ES root (g)	-	-	-	-	0.33	1.67	3.33
Sugar (g)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (g)	2.14	2.14	2.14	2.14	2.14	2.14	2.14
Canola oil (mL)	7.14	7.14	7.14	7.14	7.14	7.14	7.14
Water (mL)	60.00	60.00	60.00	59.00	60.00	59.00	59.00

Development of high-protein functional crackers enriched with adaptogenic compounds

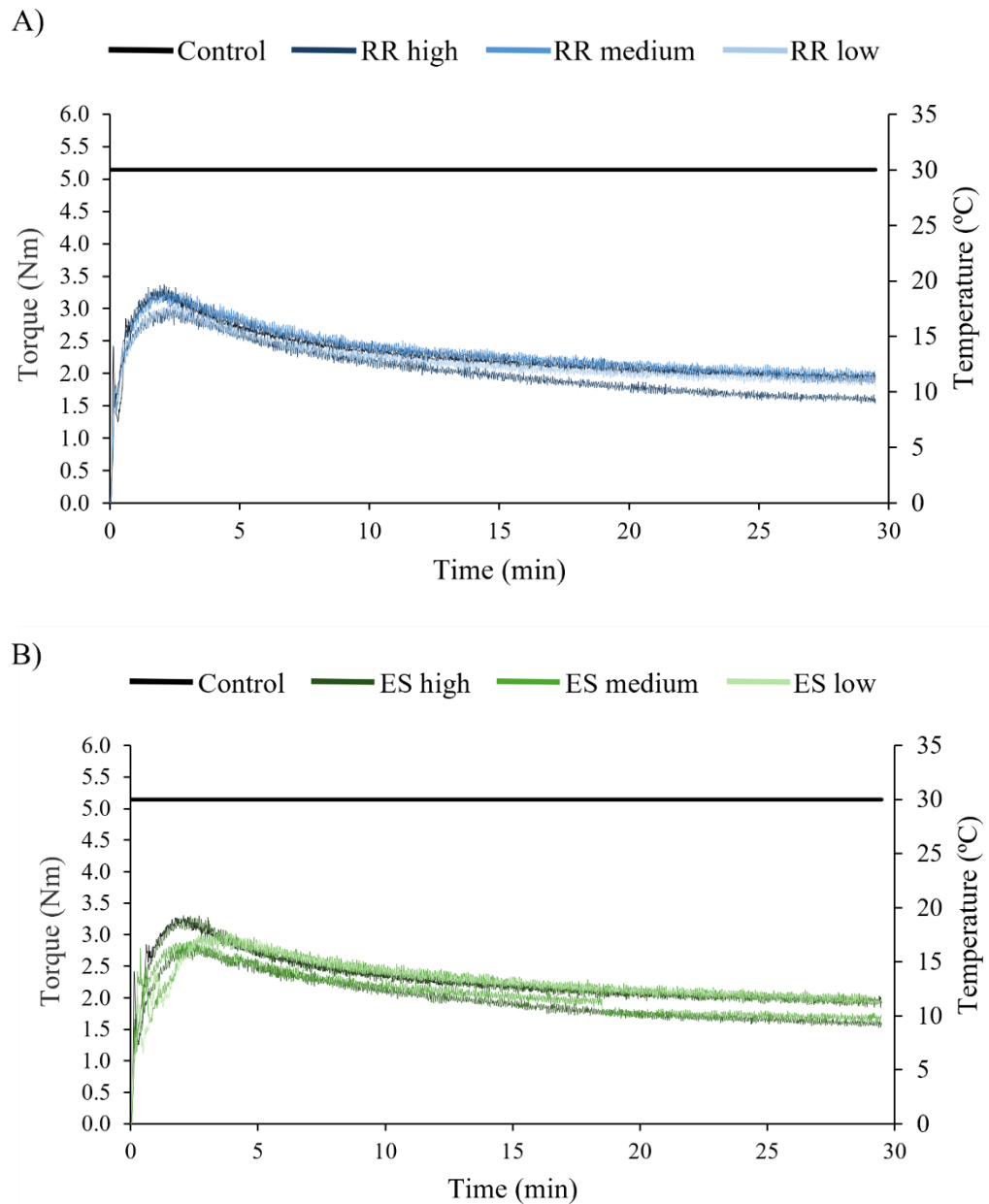


Figure 23. Mixolab curves representing the rheological behavior of the dough crackers for the different formulations with RR (A) and ES (B).

4.2.3. Proximate composition of the crackers

Crackers were milled with a Udy Cyclone mill (Udy Corp., Fort Collins, Colorado, USA) using a 0.5 mm screen. The obtained powder was used to carry out the following analyses for the control cracker. Moisture, lipid, protein, and ash

content were determined using the AACC standard methods 44-15.02, 30-25.01, 46-12.01 and 08-01.01, respectively (AACC, 2010). Total carbohydrates were obtained by difference. For the crackers containing the dried roots, only the moisture content was determined. Cellulose, hemicellulose and lignin contents in the roots were estimated using the neutral and acid detergent method (AOAC Official method 973.18).

4.2.4. Color of the crackers

The color of the crackers was analyzed using an LC 100 Spectrocolorimeter Lovibond (Burlington, Canada). The CIE coordinates (L^* , a^* , b^*) were obtained on a white background, using the illuminant D65 and the 10° observer as a reference, with a measurement window of 30 mm in diameter.

4.2.5. Dough characterization

4.2.5.1. Rheological measurements

The dynamic rheological properties of the dough samples were analyzed as described by Sun et al. (2020), using an AR 2000 Advanced Rheometer (TA instruments, Delaware, USA) with a Peltier plate temperature-controlled system. Briefly, 5 g of dough were weighed and loaded onto the bottom of the 4 cm diameter smooth parallel steel plate configuration. Then, the top plate was lowered to a gap of 2 mm, and the excess dough was removed from the outer edge of the top and bottom plate geometry. Dimethicone was then added to the outer edge of the dough to prevent drying during measurement. The temperature of the plate was set at 30°C, and the dough was allowed to rest for a 10 min equilibration period.

For each dough sample, a dynamic oscillatory frequency sweep was conducted at a constant strain amplitude ($\gamma = 0.1\%$) and an angular frequency (ω) from 0.628 to 628 rad s⁻¹, to measure the linear viscoelastic behavior of the dough samples, namely the loss modulus (G'' , Pa), storage modulus (G' , Pa). Both moduli allowed determination of the $\tan \delta$ (G''/G').

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4.2.5.2. Intermolecular forces

The different chemical interactions of the dough (control, ES high and RR high) were quantified based on the procedure described by Li et al. (2021a), with slight modifications. The reagents used for the chemical interactions were the following: NaCl (0.05 M) (PA), NaCl (0.6 M) (PB), NaCl (0.6 M) + Urea (1.5 M) (PC), NaCl (0.6 M) + Urea (8.0 M) (PD), and NaCl (0.6 M) + Urea (8.0 M) + DTT (50 mM) (PE). All reagents were prepared in phosphate buffer (0.05 M, pH = 7.5). Doughs were freeze-dried and 10 mg of freeze-dried dough powder was weighed, to which 1 mL of each buffer (PA, PB, PC, PD and PE) was added in separate centrifuge tubes. Samples were mixed thoroughly with vortex shaking. After that, samples were incubated in a water bath at 25°C for 2 h, with gentle oscillation to promote interactions between the solvent and the powder-enriched dough samples for each reagent.

After incubation, samples were centrifuged at $10,000 \times g$ for 20 min at 4°C, and 20 μL of the supernatant were transferred into a new centrifuge tube and mixed vigorously with 1 mL of Coomassie Brilliant Blue G-250 dye. After that, 200 μL of the mixture were transferred into a microplate and the absorbance was determined at the wavelength of 595 nm using a UV spectrophotometer (BioTek Epoch 2, Thermo Fisher Scientific, Massachusetts, USA). Results were expressed as mg soluble protein/g sample. At least 3 measurements per dough were performed for the control, RR high and ES high samples.

A standard curve was prepared by dissolving bovine serum albumin (BSA) in the PA buffer solution, expressed as mg soluble protein/g sample. The difference in terms of soluble protein content between PB and PA was defined as the ionic bonds; the difference between PC and PB as the hydrogen bonds; the difference between PD and PC was identified as the hydrophobic interactions and the difference between PE and PD was related to covalent bonds (Li et al., 2021b).

4.2.6. *Pasting properties*

The pasting properties of the crackers were recorded using a Rapid Visco Analyzer (RVA 4800 Perkin Elmer, Massachusetts, USA). 3.32 g of each formulation (Table 6) was mixed with 21 g of distilled water. Standard 1 RVA profile was used. Hence, samples were subjected to the following heating and cooling cycle: heating step from 50 to 95°C in 288 s, held at 95°C for 150 s, followed by a cooling step from 95 to 50°C in 228 s and held at 50°C for 60 s. The pasting characteristics, including onset temperature (To), peak viscosity (PV), peak time, trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV), were recorded.

4.2.7. *Texture*

Texture of the crackers was measured using a texture analyzer (TA.XTplus, Stable Micro Systems, Surrey, UK) equipped with a 30 kgF load cell and a crisp fracture support. A penetration test was performed using the following parameters: pre-test speed 10 mm/s, test speed 10 mm/s, and post-test speed 10 mm/s. The test measured hardness (N), defined as the maximum force required to break the sample; distance to break (mm), the distance at the breaking point; and stiffness (N/mm), calculated as the slope of the force-distance curve. Ten replicates were conducted for each sample to ensure statistical reliability.

4.2.8. *Quantification of bioactive compounds*

Based on previous work (Molina et al., 2025), the quantity of the bioactive compounds in the crackers was performed using HPLC (Acquity Arc, Waters, Massachusetts, USA). Eleutheroside B and E standards were prepared at 0.1 mg/mL in methanol, while rosavin, rosarin, rosin, salidroside and tyrosol were prepared at 1 mg/mL in methanol. Standard solutions were stored at 4°C until analysis.

Targeted bioactive compounds from crackers were extracted using microwave-assisted extraction (MAE) with a Monowave 400 microwave reactor (Anton Paar, Styria, Austria). The optimal conditions for extraction of the bioactive compounds from both roots were achieved by carrying out a

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preliminary trial using the temperatures and solvents described by Molina et al. (2025) and Kirusnaruban et al. (2025). Hence, 170 and 50°C, along with the solvents (water and methanol: water solution (75:25 v/v)) were chosen to carry out the preliminary trial. Based on the results, for crackers containing *Rhodiola rosea* (RR), 1.0 g ± 0.001 g of sample was extracted with water at 170°C for 10 min. On the other hand, for crackers containing *Eleutherococcus senticosus* (ES), extraction was done using a methanol: water solution (75:25 v/v) at 50°C for 10 min.

After extraction, samples were centrifuged at 20,000 × g for 10 min at 4°C. The supernatant was transferred to Falcon tubes and concentrated using a Vacufuge Plus centrifuge concentrator (Eppendorf, Connecticut, USA) for 16 hours at 45°C. The concentrated extracts were reconstituted in 3 mL of 75% methanol and homogenized with an Ultra-Turrax® T 18 (IKA, Baden-Württemberg, Germany). Samples were filtered through a 0.45 µm membrane filter and transferred into vials for analysis.

Bioactive compounds were identified and quantified using an HPLC system with purified water (A) and acetonitrile (B) as the mobile phases at a flow rate of 1.0 mL/min and a column temperature of 40°C. The linear gradient program was as follows: 0-15 min, 6-17% B; 15-18 min, 17-19.7% B; 18-22 min, 19.7% B; and 22-25 min, 19.7-100% B (Molina et al., 2025). The injection volume was 50 µL. Data acquisition and processing were performed using Empower 3 software (Version 3.8.0, Waters, Massachusetts, USA).

4.2.9. Statistical analysis

All the experiments were conducted in two independent batches and analysis was done in triplicates. Statistical analysis was carried out using STATGRAPHICS CENTURION XVII software (Version 17.2, Virginia, USA). Analysis of variance (ANOVA) was used to describe significant differences ($p \leq 0.05$) between variables. Fisher's least significant differences test was employed to determine significant variations between experimental mean values, with a confidence level of 95%. In addition, a Pearson correlations matrix was applied to establish correlations between the parameters

obtained for the different levels of both roots and the control. All data were expressed as the mean \pm standard deviation.

4.3. RESULTS AND DISCUSSION

4.3.1. *Proximate composition of the crackers*

The control was characterized by a moisture content of 9.5 g/ 100 g, which is higher than that in the crackers with the root powders. Interestingly, the replacement with RR had less effect on the decrease of moisture content compared to substitution with ES. On the other hand, a trend was observed for ES samples, with moisture decreasing with the increase in root substitution.

For crackers, USDA reports a target moisture content between 2.0 to 3.5 g/ 100 g (USDA, 2017). Nevertheless, the moisture content reported for crackers ranged from 3.0-4.3 g/ 100 g observed with the inclusion of modified cassava starch in crackers (Otero-Guzmán et al., 2023) to 10.6 g/ 100 g to 18.3 g/ 100 g reported when replacing wheat flour with different pulse flours (faba bean and pea) (Gangola et al., 2022; Millar et al., 2017).

Protein and fat content for the control cracker was 57 g/ 100 g and 3.9 g/ 100 g, respectively. Gangola et al. (2022) reported a lower protein content (43 g/ 100 g) but higher fat content (4.8 g/ 100 g) for faba bean protein isolate supplemented cracker. The difference may be due to the lower amount of protein isolate used for the crackers (40%), as compared to the present study. Ash and carbohydrates content, values were 2.3 g/ 100 g and 37 g/ 100 g, respectively.

4.3.2. *Color of the crackers*

Color is a crucial factor in sensory evaluation as it directly impacts consumer acceptance of a product. Statistically significant differences ($P \leq 0.05$) among the samples were obtained for color parameters (Table 7). For the luminosity (L^*), higher values were observed for ES low (59.8) followed by ES medium (58.9) and control (58.2). In general, L^* was lower in the RR crackers due to the darkness of the RR root compared to the ES root. Meanwhile, samples RR high (6.12) and medium (5.62) presented the highest values for a^* , being lowest in

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the control (3.55). These results are attributable to the redness color of the RR root as reported by Molina et al. (2025) with a^* values ranging from 10.97 to 5.07 for RR root, while for ES root the values were 3.47-5.00. Furthermore, differences could also be related to a higher extent of browning reactions due to the presence of more sugars in RR root as compared to ES root (Millar et al., 2017; Molina et al., 2025).

Table 7. Color of the 5 different crackers.

Sample	L^*	a^*	b^*
Control	58.2 ± 0.5e	3.55 ± 0.28a	19.67 ± 0.16d
RR low	56.3 ± 0.5d	4.36 ± 0.13c	18.37 ± 0.13c
RR medium	51.9 ± 0.7b	5.62 ± 0.03e	16.69 ± 0.59b
RR high	50.0 ± 0.4a	6.12 ± 0.06f	15.18 ± 0.28a
ES low	59.8 ± 0.2f	3.59 ± 0.20a	21.12 ± 0.24f
ES medium	58.9 ± 0.1e	3.99 ± 0.16b	21.46 ± 0.20f
ES high	55.1 ± 0.1c	4.97 ± 0.06d	20.52 ± 0.08e

Values correspond to means ± standard deviation obtained from three independent replicates. The letters indicate significant differences ($P \leq 0.05$) between values within columns.

In addition, the b^* parameter (blue-yellow range) was higher in ES samples (20.52-21.46) compared to RR samples (15.18-18.37) and control (19.67), indicating an enhanced golden color (Table 7). Different values for L^* , a^* , and b^* were reported for wheat-faba bean crackers, which may be due to their lower substitution of wheat flour (40%) as compared to the present study (55%) (Millar et al., 2017).

4.3.3. Dough characterization

4.3.3.1. Rheology

Figure 24 shows the viscoelastic behavior and loss tangent ($\tan \delta$) of dough samples with the different substitution levels of RR and ES root compared to the control. The storage modulus (G') reflects the elastic component, representing solid-like behavior, while the loss modulus (G'') represents the viscous component of the viscoelastic characteristics of the doughs.

Generally, all the cracker doughs exhibited a viscoelastic behavior in the examined angular frequency range, with both moduli increasing in a power law fashion with a fractional exponent as the angular frequency increased. Moreover, values of the storage modulus were higher compared to the loss modulus in all samples, indicating a solid-like character of the cracker doughs as reported by other authors for wheat flour doughs (En Tay et al., 2022; Sun et al., 2020). In doughs formulated with RR root (Figure 24A), values for both the storage (G') and loss (G'') moduli were lower than those of the control dough. A decreasing trend was observed for both moduli with increasing RR levels, with RR-high dough exhibiting the lowest values. This suggests that RR powder had a disruptive effect on the network structure of the dough. In this regard, En Tay et al. (2022) reported similar effects when substituting whey protein isolate into a wheat flour dough. The observed decrease in G' could be due to the replacement of wheat flour with protein isolate, leading to a weaker gluten network as stated by En Tay et al. (2022).

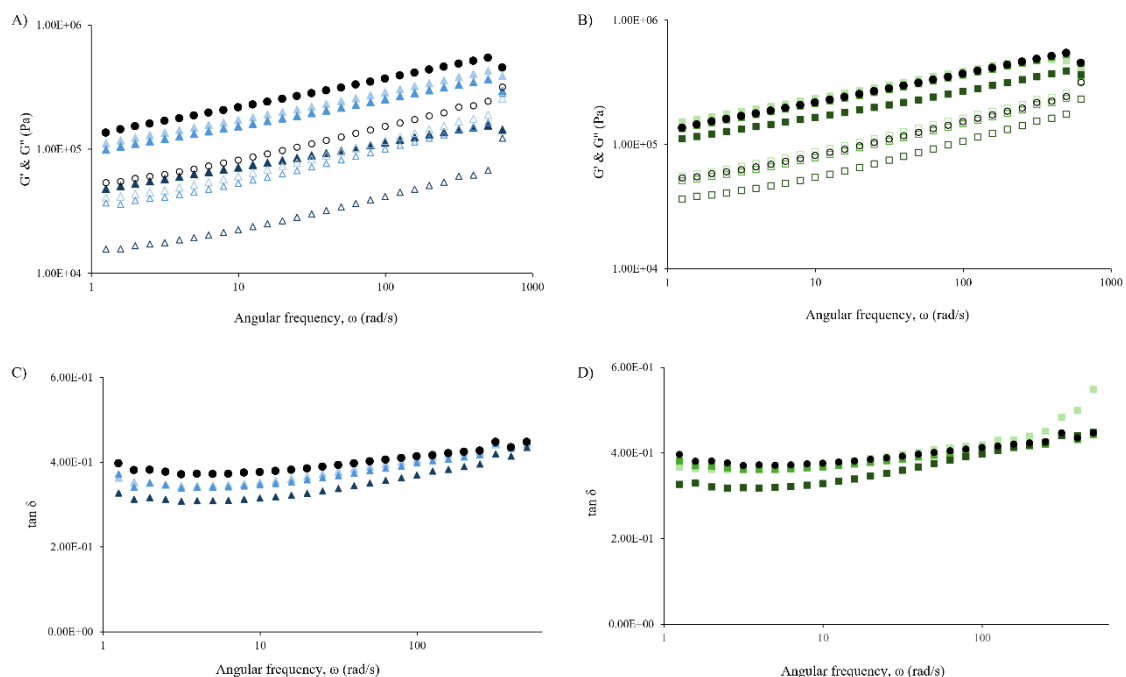


Figure 24. The storage (G' , filled symbols) and loss (G'' , unfilled symbols) shear modulus and $\tan \delta$ as a function of angular frequency (ω) for the different doughs. A and C) Dough cracker with RR dried root at the different levels (RR low (light blue), RR high (dark blue)). B and D) Dough cracker with ES dried root at the different levels (ES low (light green), ES medium (green), ES high (dark green)). Control corresponds to the cracker without dried roots (black).

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Contrasting viscoelastic behavior was observed for ES samples (Figure 24B). For the storage modulus, no significant differences ($P \leq 0.05$) were obtained between samples, except for ES high, which showed a drop in G' values. Similar results were obtained for the loss modulus. In addition, when comparing samples from both roots, ES high sample showed a higher G'' than RR high. ES root had a higher amount of cellulose (37.3%), lignin (12.6%) and hemicellulose (29.2%) as compared to RR root, which only had hemicellulose (19.7%) and lignin (11.2%). Therefore, ES root's hemicellulose probably contains more water-soluble fractions that soften the dough matrix (Aydogdu et al., 2018).

Another indicator for the viscoelastic behavior of the dough is $\tan \delta$, which is the tangent of the phase angle expressed by the ratio of viscous modulus (G'') to elastic modulus (G') (Figure 24C and 24D). As illustrated in Figure 24C and 24D, all samples had $\tan \delta$ values less than unity. Hence, samples were more elastic than viscous, indicating elastic-dominant behavior. Similar values were obtained for doughs for wheat crackers (0.44), suggesting that the substitution with both roots did not lead to differences in $\tan \delta$ (Nammakuna et al., 2016).

4.3.3.2. Intermolecular forces in dough

Figure 25 illustrates the ionic, hydrogen, hydrophobic and disulfide interactions between soluble proteins within the different samples. These interactions were analyzed only for the control and the high-level root substitution. Notably, ionic interactions were absent in all samples except for RR-high. These findings align with those reported by Li et al. (2021a) for wheat flour dough, who reported that ionic bonds contributed the least to the protein network. They found that hydrophobic interactions were the main force that maintained the protein network. However, in the present study, the primary forces in the control sample were hydrogen bond interactions followed by disulfide bonds. This difference from the result reported by Li et

al. (2021a) may be due to the replacement of wheat flour with faba bean protein isolate.

The amino acid profiles of wheat and faba bean proteins are pivotal in determining their interaction mechanisms. Wheat proteins, rich in non-polar amino acid residues like proline and leucine, tend to form hydrophobic interactions. In contrast, faba bean proteins, with a higher content of polar and charged amino acid, including glutamic and aspartic acid, are more predisposed to establish hydrogen bonds (Multari et al., 2015).

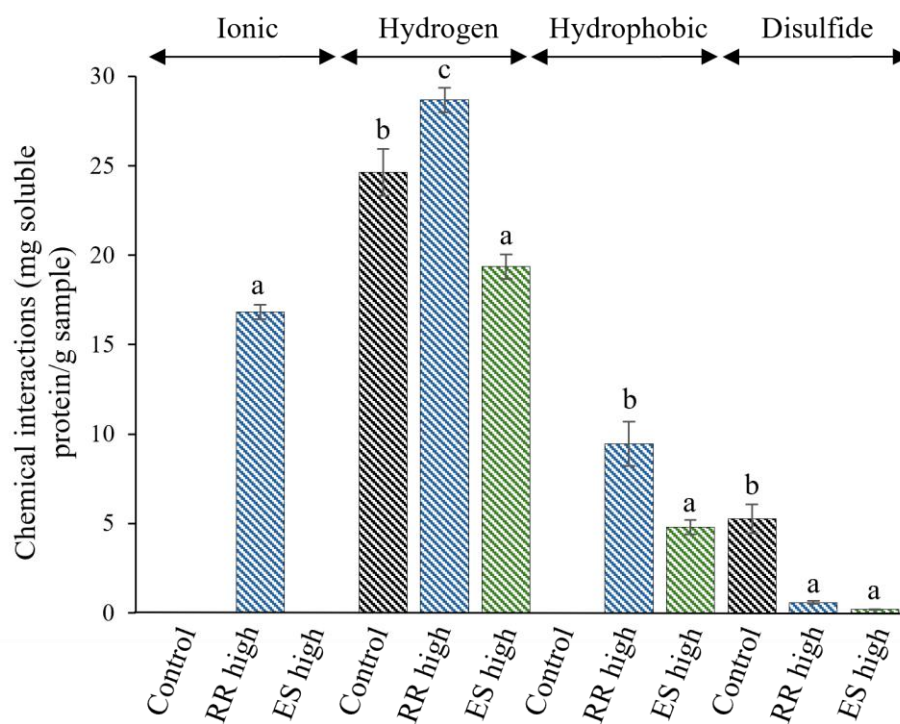


Figure 25. Contributions of chemical interactions between soluble proteins in cracker doughs. Different superscript letters for each sample indicate significant differences ($P \leq 0.05$).

Significant differences were found among samples ($P \leq 0.05$). RR-high exhibited a higher content of hydrogen bonds and hydrophobic interactions compared to ES-high (Figure 25). In this regard, negative correlations were found between the loss modulus (G'') and hydrophobic interactions within the dough ($r = -0.9988$, $P \leq 0.05$). Similar results were reported by Li et al. (2021b) in which G'' increased, while hydrophobic interactions decreased in wheat doughs made with increasing glutenin: gliadin ratios. In contrast, no significant differences were observed in disulfide bonds content between the

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root samples ($P \leq 0.05$). As expected, the control sample had the highest disulfide bond content, likely due to its higher gluten protein concentration, which facilitates network formation through cysteine residue crosslinking (Gasparre & Rosell, 2023).

4.3.4. Pasting properties

The apparent viscosity changes during the heating-cooling cycle are presented in Table 8, showing significant differences among the samples ($P \leq 0.05$). The substitution with both RR and ES roots at different levels resulted in a significant decrease in onset temperature and an increase in peak viscosity and breakdown viscosity compared to the control. The presence of root-derived components therefore alters the starch pasting properties. Specifically, the onset temperature decreased in all root-substituted samples, indicating that starch gelatinization required less thermal energy, in agreement with Yildiz et al. (2013), who reported a decrease in pasting temperature with increasing fiber content in wheat starch/fiber blends.

Table 8. Pasting properties of cracker doughs with different formulations.

Sample	Onset temperature (°C)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)
Control	89.1 ± 0.5d	419 ± 5a	388 ± 8ab	31 ± 3a	779 ± 36e	392 ± 44c
RR low	84.5 ± 0.9c	652 ± 31f	452 ± 25e	260 ± 54e	702 ± 28d	210 ± 28b
RR medium	83.7 ± 0.5abc	638 ± 25d	439 ± 13cd	199 ± 11bcd	622 ± 18c	184 ± 7ab
RR high	84.5 ± 0.5c	590 ± 6c	408 ± 2bc	182 ± 5bc	569 ± 7b	161 ± 6a
ES low	83.1 ± 0.0a	687 ± 9e	470 ± 6de	217 ± 3cd	670 ± 4d	200 ± 4b
ES medium	83.4 ± 0.5ab	634 ± 52de	434 ± 34c	226 ± 18de	621 ± 46c	187 ± 12ab
ES high	84.2 ± 0.5bc	516 ± 23b	359 ± 15a	157 ± 10b	513 ± 12a	154 ± 3a

Values correspond to means ± standard deviation obtained from three independent replicates (basis for the statistical analysis). Different letters within a column for each root indicate significant differences ($P \leq 0.05$).

When root powders were included into the mixtures, peak viscosity was enhanced compared to the control (Table 8). This is probably due to the presence of insoluble dietary fibers that may have reduced the water availability for the starch granules. Hence, the friction between insoluble dietary fiber and starch granules increased during the gelatinization process, leading to an increase in the peak viscosity (Li et al., 2022). A strong negative correlation was observed between peak viscosity and disulfide bonds ($r = -0.87$, $P \leq 0.05$), which could explain the lower peak viscosity for the control sample. This is mainly because a more cross-linked gluten network can restrict the swelling of starch granules during heating, leading to a lower peak viscosity (Gasparre & Rosell, 2023). Hence, the lower peak viscosity for the control sample was probably driven by the formation of a more stabilized and rigid protein network that may have restricted water uptake by starch granules (Li, 2022).

On the other hand, when comparing peak viscosities among root samples, values decreased as more of both root samples were added (Table 8). At higher substitution levels, the dilution of starch became significant enough to mask the impact of the fiber (Tudorică et al., 2002). Additionally, the higher contents of cellulose (37.3%) and lignin (12.6%) in ES probably reduced water availability for starch swelling, leading to a lower peak viscosity compared to RR high, which had a lower lignin content (11.3%) and no cellulose.

In the case of the breakdown parameter, an indicator of starch stability under shear and heat, significantly lower values were obtained for the control (31 cP) compared to the samples containing root powder (ranging from 157 cP to 260 cP). This suggests that the control dough had greater resistance to thermal and mechanical stress, while the substitution with root powder compromised the stability and shear resistance of the starch. Indeed, this could be explained by the negative correlation observed between disulfide bonds and breakdown viscosity ($r = -0.97$, $P \leq 0.05$).

Meanwhile, the final viscosity, reflecting amylose reassociation during the cooling stage, was highest in the control (779 cP), while fiber-rich samples showed lower values (from 513 cP to 702 cP). In this case, the presence of fiber interferes with starch recrystallization, as observed by Betoret & Rosell (2020)

when using *Brassica napobrassica* leaves with rice and maize starch. A significant positive correlation between final viscosity and disulfide interactions ($r = 0.9914$, $P \leq 0.05$) confirms that the formation of a stronger protein network protected the starch granules from excessive rupture, resulting in less damaged amylose and contributing to the higher final viscosities.

Setback viscosity, an indicator of starch retrogradation, was also highest in the control (392 cP) (Table 8). However, among the substituted samples, no significant difference was observed between different levels of substitution, suggesting that the mere presence of the roots, rather than their concentration, affected retrogradation behavior. This contrasts with Yildiz et al. (2013), who reported an increase in setback viscosity for a starch/lemon fiber blend. In their study, the higher soluble fiber content (20%) in the blend appeared to limit starch swelling while protecting the starch from thermal and mechanical degradation, thereby maintaining a higher viscosity. In this study, samples with the highest root substitution rate showed the lowest setback values. This could be attributed to the higher hydrophobic interactions between proteins when adding the root (hydrophobic interactions, $r = -0.86$, $P \leq 0.05$). These interactions may have reduced the mobility of amylose chains needed for reassociation (Zhu et al., 2009), suggesting lower starch retrogradation and syneresis, which could enhance the long-term texture and quality (Yildiz et al., 2013).

4.3.5. Cracker texture

The textural properties of crackers, such as hardness, stiffness, and distance to break, are crucial quality parameters. Figure 26 presents the texture attributes of crackers resulting from substituting wheat flour with varying levels of root powder. Significant differences were observed among the samples for all parameters ($P \leq 0.05$).

In general, the control sample exhibited larger values for hardness, stiffness, and distance to break. Substituting wheat flour with both roots led to a reduction in hardness (Figure 26A). As anticipated, RR low (22 N) was harder compared to the other RR root levels. In contrast, ES high (22 N) significantly

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increased the hardness of the cracker in the ES root sample set. This phenomenon with ES is probably linked to its fiber content as observed by Millar et al. (2017), who reported a positive correlation between hardness and total fiber ($r = 0.753$). Similar results were stated by Han et al. (2010) when comparing the hardness of crackers made from yellow pea flour (10.87 N), pea protein (10.99 N) and fiber isolates crackers (13.67 N). However, this effect was only shown with ES. No significant differences ($P \leq 0.05$) were found between the rest of the ES and RR root levels. The results for the hardness of the control sample (39 N) were surprising as it had the highest moisture content (9.51%), which has been negatively correlated with hardness (Otero-Guzmán et al., 2023). This finding could be explained by the higher disulfide bonds content associated with the gluten network structure of the control samples making them stiffer, as it has been positively correlated with the hardness ($r = 0.94$, $P \leq 0.05$) (Figure 26A). In contrast, Waglay & Karboune (2020) observed a slight increase in the hardness of cookies with the addition of potato protein isolate when compared to wheat flour. Similarly, Nammakuna et al. (2016) reported a higher hardness when 2.5 to 10% of pea or whey protein isolates were added to a blend of rice flour and tapioca starch. Thus, these differences highlight that different proteins interact uniquely with other components, affecting dough structure and final product texture.

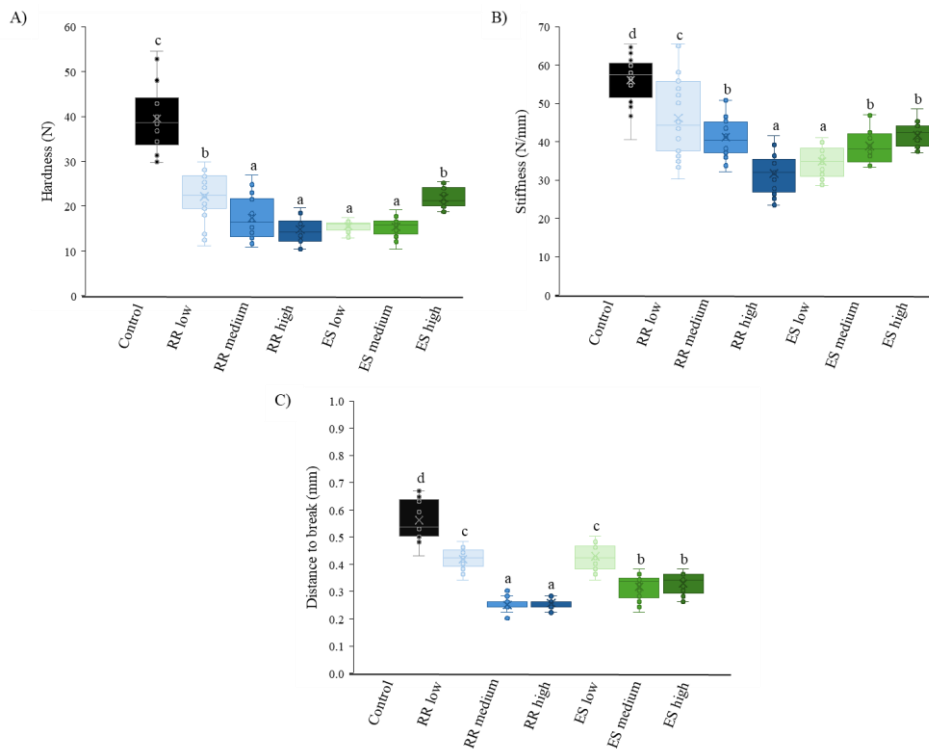


Figure 26. Texture properties of crackers with different formulation. A) Hardness of the crackers. B) Stiffness of the crackers. C) Distance to break of the crackers. Control corresponds to the cracker without dried roots. Different superscript letters for each sample indicate significant differences ($P \leq 0.05$).

Similar results were also observed for the texture parameter stiffness (Figure 26B). The control crackers (56 N/mm) exhibited a higher stiffness compared to the rest of samples, which may be due to the disulfide bonds that reinforce the gluten network making it more difficult to deform ($r = 0.88$, $P \leq 0.05$).

Distance to break had a greater variation among samples (Figure 26C). A greater distance indicates higher flexibility, meaning that crackers that break at shorter distances have higher fracturability. A higher distance to break was shown for control (0.53 mm), ES low (0.42 mm) and RR low (0.42 mm). In general, samples containing the roots were easier to break. These could be attributed to the greater fiber content which may lead to a more brittle structure in RR and ES high as compared to control. These findings are consistent with the results reported by Waglay & Karboune (2020), as wheat control cookies exhibited a higher distance to break compared to the samples made with 20% addition of potato protein isolate. The strong positive

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correlation ($r = 0.81$, $P \leq 0.05$) between the distance to break and the final apparent viscosity, as well as with setback viscosity ($r = 0.82$, $P \leq 0.05$) indicates that a stronger gel structure with more extensive amylose recrystallization was more prone to fracture under applied force.

4.3.6. Quantification of bioactive compounds

Table 9 shows the content of bioactive compounds in the crackers containing the root powders. Significant differences ($P \leq 0.05$) were observed among the samples for both roots. For ES, a clear trend was observed with the increase in eleutheroside E content as the substitution levels of ES increased. However, no significant differences were detected between the low and medium levels of ES substitution for eleutheroside B. This could be attributed to the limit of detection of the equipment. For RR, similar results were observed for tyrosol and rosin, with no significant differences ($P \leq 0.05$) between the low and medium RR substitution levels.

Table 9. Contents of bioactive compounds in crackers containing RR or ES dried roots at the different levels.

Sample	Eleutheroside B (mg/g)	Eleutheroside E (mg/g)	Salidroside (mg/g)	Tyrosol (mg/g)	Rosarin (mg/g)	Rosavin (mg/g)	Rosin (mg/g)
RR low	-	-	0.02 ± 0.00a	0.02 ± 0.00a	0.00 ± 0.00a	0.01 ± 0.00a	0.00 ± 0.00a
RR medium	-	-	0.18 ± 0.03b	0.03 ± 0.01a	0.09 ± 0.00b	0.03 ± 0.00b	0.00 ± 0.00a
RR high	-	-	0.39 ± 0.05c	0.19 ± 0.01b	0.36 ± 0.05c	0.06 ± 0.01c	0.02 ± 0.00b
ES low	0.01 ± 0.00a	0.02 ± 0.00a	-	-	-	-	-
ES medium	0.01 ± 0.00a	0.21 ± 0.03b	-	-	-	-	-
ES high	0.04 ± 0.01b	0.33 ± 0.01c	-	-	-	-	-

Values correspond to means ± standard deviation obtained from three independent replicates on which are based the statistical analysis. Different superscript letters within a column for each root indicate significant differences ($P \leq 0.05$).

4.4. CONCLUSION

The present study examined the effect of incorporating RR and ES roots in protein-enriched crackers. Overall, the addition of both root powders had a notable impact on the dough rheology and apparent viscosity, increasing peak viscosity while reducing tendency of amylose to retrograde. Crackers with root powder presented lower hardness and higher fracturability. The differences observed were linked to the fiber content in the powders, which was higher in ES, and to chemical interactions between the proteins and the roots (hydrogen bonding and hydrophobic interactions). Furthermore, the quantification of bioactive compounds in the crackers revealed that higher root levels corresponded to increased concentrations of eleutheroside E, salidroside, rosarin, and tyrosol. These findings highlight the potential functional and structural advantages of incorporating RR and ES roots into cracker formulations while improving the overall quality of the snacks. Considering the interest in boosting protein content and the health benefits of bioactive compounds, this offers promising opportunities for the development of nutritionally enriched and texturally optimized functional crackers. Future research should focus on understanding the mechanisms governing interactions between the bioactive compounds in the roots and proteins/carbohydrates to ensure their release during digestion.



CHAPTER 5

PROTEIN MATRIX INTERACTIONS AND ADAPTOGENIC BIOACTIVE RELEASE IN HIGH-PROTEIN CRACKER SYSTEMS ENRICHED WITH *RHODIOLA ROSEA* AND *ELEUTHEROCOCCUS SENTICOSUS*

5.1. INTRODUCTION

Bioactive compounds are phytochemicals found in plants and certain foods. In the case of plants, roots are a typical matrix from which bioactive compounds can be extracted. Phenylpropanoids and phenylethanoids are two groups of bioactive compounds which are particularly notable for their antioxidant and adaptogenic properties. These groups of bioactive compounds are distributed in medicinal plants, such as *Rhodiola rosea* and *Eleutherococcus senticosus*. The primary application of these medicinal herbs is in dietary supplements where the bioactive compounds are extracted from the root powder. The root powders themselves are largely limited to incorporation into herbal infusions, energy drinks and coffee (AAFC, 2016; EMA, 2016).

To expand the use of adaptogenic compounds beyond beverages, solid food matrices, like crackers, present a promising delivery platform that will broaden consumer appeal. Crackers are widely consumed as snacks for their

convenience and versatility, but they can also be a flexible and innovative platform to meet evolving consumer demands such as high protein food products (Huamaní-Perales et al., 2024).

Pulse protein isolates have emerged as cornerstone ingredients in new product formulations for high-protein foods, due to consumer preferences and to their relatively low cost, compared to animal-derived proteins (Nogueira et al., 2021). Canada is one of the countries leading the global production of pulses (World Milling and Pulses Technologies, 2025). Beans, lentils, chickpeas, peas and lupins are among the most known and consumed pulses worldwide (Ferreira et al., 2021). Studies involving the incorporation of pulse protein isolates into crackers have primarily focused on the rheological properties of the dough, and the texture, microstructure, in vitro enzymatic hydrolysis, antioxidant activity and sensory evaluation of the crackers (Gangola et al., 2022; Han et al., 2010; Madhavan & Sreekantaiah, 2025; Morales-Polanco et al., 2017). In these studies, 2.5-46.2% of pea or faba bean protein isolate was used to replace wheat flour. However, some other authors replaced 100% of the wheat flour with pea protein isolate to obtain gluten-free crackers (Han et al., 2010).

However, the techno-functional properties of pulse proteins as well as their interaction with other food matrix components can vary considerably (Gravel & Doyen, 2023). Indeed, the bioaccessibility of bioactive compounds during digestion can change considerably as a result of degradation during food processing or interactions with macronutrients in the food matrix (Sęczyk et al., 2023). Therefore, in formulating foods with adaptogenic properties, the bioaccessibility of the bioactive compounds is critical because it determines their subsequent bioavailability and the possibility of the food to exert biological effects. To date, no studies have evaluated how pulse protein isolates interact with adaptogen root powders in crackers to alter the quality of protein-enriched crackers, especially not with a focus on effects on the bioaccessibility of bioactive compounds.

For this reason, the present study aimed to develop functional crackers built on a high protein matrix by incorporating different pulse protein isolates along with root powders from *R. rosea* and *E. senticosus*. The study further evaluated how these dual-fortified formulations influence the crackers'

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physicochemical properties and the *in vitro* bioaccessibility of their bioactive compounds.

5.2. MATERIALS AND METHOD

5.2.1. Materials

Commercial faba bean (*Vicia faba*) protein isolate (FBPI) PUREPRO FABA 85S (88 g of protein, 8.2 g of carbohydrates, 0.6 g of fat, and 3.2 g of ash per 100 g d.b) was purchased from Univar Solutions (Winnipeg, Manitoba, Canada). Commercial lupin (*Lupinus albus*) protein isolate (LuPI) (81 g of protein, 11.0 g of carbohydrates, 4.0 g of fat, and 4.0 g of ash per 100 g d.b) was kindly supplied by Lupin Platform Inc (Calgary, Alberta, Canada). In addition, pea (*Pisum sativum*) protein isolate (PPI) (84 g of protein, 8.9 g of carbohydrates, 0.5 g of fat, and 6.5 g of ash per 100 g d.b) and lentil protein isolate (LPI) LTVCP-80C (86 g of protein, 8.6 g of carbohydrates, 0.4 g of fat, and 5.0 g of ash per 100 g d.b) were obtained from AGT Foods (Regina, SK, Canada). Wheat flour (*Triticum aestivum*) was kindly supplied by Cereals Canada (Winnipeg, Manitoba, Canada). It contained 17.5 g of protein, 80.9 g of carbohydrate, 1 g of fat and 0.6 g of ash per 100 g d.b. Other baking ingredients, which included sugar, canola oil and salt, were purchased from a local grocery store.

The dried roots of *R. rosea* and *E. senticosus* were purchased from Rhodiola Growers of Canada (Mountain View County, Alberta, Canada) and Richters Herbs Co (Goodwood, Ontario, Canada), respectively. These two dried roots were milled with a Udy Cyclone mill (Udy Corp., Fort Collins, Colorado, USA) equipped with a 1 mm screen, as described by Molina et al. (2025b).

5.2.2. Dough characterization

5.2.2.1. Thermomechanical properties

The thermomechanical properties of each dough were assessed using the standard Chopin S protocol from the Mixolab® (Chopin, Tripette et Renaud,

Paris, France). First, the water holding capacity (WHC) of each protein isolate was determined by the method of Shi & Nickerson (2022). WHC values (g water per g protein isolate) were: pea protein isolate (PPI) 6.40; lentil protein isolate (LPI) 5.14; faba bean protein isolate (FBPI) 4.13; and lupin protein isolate (LuPI) 2.12. These WHC results, together with preliminary machinability trials, where dough was hand-stretched and water added incrementally, were used to set the water content for each protein-isolate formulation. When root powders were included, water addition was adjusted so that each dough matched the dough handling properties of its corresponding control. Once water levels were optimized, 75 g of each flour blend (including protein isolate, wheat flour, and, where applicable, root powder) was mixed in the Mixolab® at 30°C and 80 rpm for 30 min. For isolate-only formulations, protein isolate comprised 55.2 g and wheat flour 44.8 g. In root-fortified formulations, 3.33 g of wheat flour was replaced with root powder. All formulations were prepared in triplicate. The following Mixolab® parameters were recorded for each run: maximum consistency (Nm), water absorption (%), development time (min) and stability (min).

5.2.2.2. Protein aggregation behavior

Protein aggregation properties of the different blends were analyzed using the GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany). In addition, the effect of the incorporation of the root powder in the protein isolate/flour blends was investigated. According to AACC 82-23-01 standard method for wheat flour with minor modifications. The selected solid (ingredient blends) to water ratio was 1:1.8, to ensure paste formation. The temperature was maintained at 30°C for 7 min while the paddle was set to rotate at 3000 rpm. The parameters analyzed were the maximum torque expressed in Brabender units (BU), which corresponds to the peak occurring as protein aggregates; and the peak maximum time expressed in min and related to the time of mixing required to reach peak torque. All measurements were performed in triplicate.

5.2.3. Preparation of crackers

Crackers were prepared according to the method and formulation proposed by Molina et al. (2025b). Briefly, the cracker formulation included 55.2 g of

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

protein isolate, 44.8 g of wheat flour, canola oil 7.1 g, salt 2.1 g and sugar 1 g. In the case of crackers containing the dried roots of *Rhodiola rosea* (RR) or *Eleutherococcus senticosus* (ES), the quantity of wheat flour was partially reduced to substitute with 3.3 g of root. The quantity of water added for each of the protein isolates formulation was: 60 g/ 100 g for FBPI, 38 g/ 100 g LuPI, 92 g/ 100 g for LPI and 106 g/ 100 g for PPI. After mixing for 15 min, the dough was sheeted using a pasta sheet roller (KitchenAid KSM150PSER, Benton Harbor, Michigan, USA) to achieve a 2 mm thickness and cut into squares (3.5 × 3.5 cm). Lastly, the dough was pricked using a dough docker and baked for 6 min at 180°C in a forced-air convection oven (Blodgett, Essex Junction, Vermont, USA).

5.2.4. Proximate composition and color of crackers

Crackers were milled with a Udy Cyclone mill (Udy Corp., Fort Collins, Colorado, USA) using a 0.5 mm screen. The obtained powder was used to analyze the moisture, lipid, protein, and ash content using AACC standard methods 44-15.02, 30-25.01, 46-12.01 and 08-01.01, respectively (AACC, 2010). Total carbohydrates were obtained by difference.

The color of the crackers was analyzed using an LC 100 Spectrocolorimeter Lovibond (Burlington, Canada). The CIE coordinates (L^* , a^* , b^*) were obtained on a white background, using the illuminant D65 and the 10° observer as a reference, with a measurement window of 30 mm in diameter. Samples were analyzed in triplicate.

5.2.5. Texture of crackers

Texture of the crackers was measured using a texture analyzer (TA.XTplus, Stable Micro Systems, Surrey, UK) equipped with a 30 kgF load cell and a crisp fracture support (HDP/ CFS). A penetration test was performed at room temperature, using the following parameters: pre-test speed 10 mm/s, test speed 10 mm/s, and post-test speed 10 mm/s. The test measured hardness (N),

defined as the maximum force required to break the sample; distance to break (mm), the distance at the breaking point; and stiffness (N/mm), calculated as the slope of the force-distance curve. Ten replicates were conducted for each formulation.

5.2.6. *Fourier Transform Infrared spectroscopy*

To study molecular interactions between the root powders and the different pulse protein isolates, the changes in the secondary structure of the protein were characterized for each cracker using a Fourier transform infrared spectroscopy (FTIR) instrument with an attenuated total reflectance cell (Bruker Invenio S, Bruker, Leipzig, Germany). The ground samples of crackers were measured in the 400 to 4000 cm^{-1} spectral range at a resolution of 4 cm^{-1} . The spectra for each sample were analyzed with Origin software (OriginPro2024, OriginLab Corporation, Massachusetts, USA). The spectra data was normalized, and a second derivative curve was generated applying the Savitzky-Golay method with eight smoothing points, followed by curve fitting to identify secondary structure components. Samples were analyzed in duplicate. The quantitative estimation of protein secondary structure in the amide I region of dough was based on the second-derivative spectra. The spectral regions were assigned as 1609-1615 for protein aggregates, 1618–1638 and 1680-1688 for β -sheets, 1642–1645 for random coil, 1650–1660 for α -helix, 1660–1680 cm^{-1} for β -turn and, 1690-1695 for protein aggregates (β -type structures) (Shevkani et al., 2019). Thus, the second derivative area for each secondary structural region was divided by the total area of the amide I region.

5.2.7. *Microstructure of crackers*

To evaluate the effect of the root powders on the microstructure of crackers, a confocal laser scanning microscopy (CLSM) ZEISS LSM 900 (Zeiss, Oberkochen, Germany) was used. A mixture of rhodamine B and fluorescein 5-isothiocyanate (FITC) was prepared following the procedure described by Hu et al. (2022). Briefly, 10 μL of the mixture (0.025% rhodamine B (w/v) and 0.25% FITC (w/v)) was added to stain the sample for 10 min. After that, excess dye was removed with acetone. The excitation wavelengths were 488 nm for FITC and 555 nm for rhodamine B. FITC stained the starch (green) while rhodamine B stained the protein (red). Each sample was analyzed in duplicate.

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CLSM micrographs were obtained using a 10× objective at constant z-position. The Zeiss Zen software version 3.11 (Zeiss, Oberkochen, Germany) was used to visualize the samples.

5.2.8. Quantification of bioactive compounds in crackers

The bioactive compounds from *R. rosea* (rosin, rosarin, rosavin, salidroside and tyrosol) and *E. senticosus* (eleutheroside E and B) in crackers were quantified using HPLC (Acquity Arc, Waters, Massachusetts, USA) using the conditions and method described by Molina et al. (2025b). Eleutheroside B and E standards were prepared at 0.1 mg/mL in methanol, while rosavin, rosarin, rosin, salidroside and tyrosol were prepared at 1 mg/mL in methanol. Standard solutions were stored at 4°C until analysis.

HPLC quantification was carried out using miliQ water (A) and acetonitrile (B) as the mobile phases at a flow rate of 1 mL/min, injection volume of 50 µL and a column temperature of 40°C. The linear gradient program was 0-15 min, 6-17% B; 15-18 min, 17-19.7% B; 18-22 min, 19.7% B; and 22-25 min, 19.7-100% B (Molina et al., 2025). Data processing was conducted using Empower 3 software (Version 3.8.0, Waters, Massachusetts, USA).

5.2.9. Bioaccessibility of bioactive compounds in crackers

An in vitro gastrointestinal digestion procedure was carried out using the method described by Minekus et al. (2014). α -Amylase (A1031), pepsin (P7000), pancreatin (P7545, 8 X USP) and bile extract bovine (70168) were obtained from Sigma-Aldrich Chemical Co. Stock solutions (simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF)) were prepared according to Minekus et al. (2014). 1 g of each cracker was mixed with 0.785 mL SSF, 0.015 mL of α -amylase solution (1500 U/mL in SSF), 0.195 mL distilled water and 0.005 mL calcium chloride (0.3 M). The oral mix was vortexed and incubated in a shaking water bath for 2 min at 37°C. For the gastric digestion, the oral bolus obtained from the oral phase, was mixed with

1.57 mL SGF, 0.250 mL pepsin solution (25,000 U/mL in SGF), 0.001 mL calcium chloride (0.3 M), 0.139 mL distilled water and 0.04 mL hydrochloric acid (1M). The gastric solution at was adjusted to pH 3 and then placed in a water bath for 2 h at 37°C, with agitation. Finally, to the gastric chyme, resulted from the gastric phase, 1.8 mL SIF, 1 mL pancreatin solution (800 U/mL in SIF), 0.5 mL of bile (0.38g/mL in SIF), 0.008 mL calcium chloride (0.3 M), 0.03 mL sodium hydroxide (1 M) and 0.262 mL distilled water were added. The intestinal solution was left 2 h at 37°C to complete the intestinal digestion. All the digesta were centrifuged at 4°C and 15000 rpm, for 10 min. After that, the supernatants were analyzed using the HPLC with the conditions described in section 2.8 to analyze the bioactive compounds after in vitro gastrointestinal digestion. Samples were analyzed in triplicate. The bioaccessibility of each bioactive compound in each sample was calculated using the formula: Bioaccessibility (%) = (analyzed amount of bioactive compound in the intestinal digest / theoretical value) x 100 (Günel-Köroğlu et al., 2023). The theoretical value corresponded to the expected content of the bioactive compound, calculated based on the amount of root powder included in the cracker formulation.

5.2.10. *Statistical analysis*

Statistical analysis was carried out using STATGRAPHICS CENTURION XVII software (Version 17.2, Virginia, USA). Analysis of variance (ANOVA) was employed to determine significant differences ($P \leq 0.05$) between the experimental values of each parameter. Moreover, Fisher's least significant difference test was used to discriminate between samples within a confidence level of 95%. In addition, to explore relationships among physicochemical, textural, and bioaccessibility parameters, Pearson correlation coefficients were calculated. FTIR and texture data were plotted and further analyzed in Origin Pro 2024 (OriginLab Corporation, Massachusetts, USA). All data were expressed as the mean \pm standard deviation.

5.3. RESULTS AND DISCUSSION

5.3.1. *Thermomechanical properties and protein aggregation behavior*

Table 10 summarizes the thermomechanical behavior of the cracker doughs. In general, LuPI_control and FBPI_control had the highest consistency, with values of 3.3 and 3.2 Nm, respectively. The addition of ES and RR roots had a significant effect ($P \leq 0.05$) on the consistency of LuPI, LPI and FBPI doughs. In general, the incorporation of ES root resulted in higher consistency values compared to RR, except in the case of FBPI. This increase in consistency may be attributed to the higher insoluble fiber content of ES relative to RR, as reported by Molina et al. (2025b). The presence of insoluble fiber may have interfered with gluten hydration as a result of formation of fiber-gluten interactions, which reduced the amount of water available for gluten development. This limited hydration may have increased the dough's resistance to mixing, resulting in higher consistency values (Zhou et al., 2021).

Table 10. Mixolab parameters representing the mechanical behavior of the protein-enriched cracker doughs containing adaptogenic root powders.

Samples	Max consistency (Nm)			Water absorption (%)			Development time (min)			Stability (min)		
PPI_control	1.9	± 0.1	aA	106.4	± 0.0	dA	2.40	± 0.47	bA	3.81	± 0.46	bA
PPI_ES	1.9	± 0.0	aA	108.5	± 0.0	dC	2.59	± 0.01	cA	3.08	± 0.12	cA
PPI_RR	1.9	± 0.1	aA	107.5	± 0.0	dB	1.28	± 0.70	abA	3.24	± 0.04	cA
LPI_control	2.9	± 0.0	bB	92.0	± 0.0	cA	1.73	± 0.04	aA	2.14	± 0.18	aA
LPI_ES	3.0	± 0.0	bC	94.0	± 0.0	cC	1.77	± 0.19	bA	1.94	± 0.15	bA
LPI_RR	2.8	± 0.0	bA	93.0	± 0.0	cB	1.89	± 0.25	abA	2.79	± 0.01	bB
LuPI_control	3.3	± 0.1	cA	38.0	± 0.0	aA	2.40	± 0.05	bB	1.54	± 0.20	aA
LuPI_ES	3.5	± 0.0	dB	38.0	± 0.0	aA	1.09	± 0.01	aA	1.27	± 0.00	aA
LuPI_RR	3.2	± 0.0	cA	40.8	± 0.0	aC	1.09	± 0.08	aA	1.45	± 0.02	aA
FBPI_control	3.2	± 0.0	cA	60.0	± 0.0	bB	2.01	± 0.01	abA	2.00	± 0.00	aC
FBPI_ES	3.3	± 0.0	cB	59.0	± 0.0	bA	2.08	± 0.01	cA	1.37	± 0.00	aA
FBPI_RR	3.4	± 0.0	dC	59.0	± 0.0	bA	2.11	± 0.01	bA	1.63	± 0.00	aB

Values correspond to means ± standard deviation obtained from three independent replicates (basis for the statistical analysis). Different lowercase letters within a column refer to significant differences between protein isolates while different uppercase letters within a column represent significant differences between samples for a given protein isolate ($P \leq 0.05$). *Control refers to the sample without root powder.

Samples containing PPI showed the highest water absorption (106.4%), while LuPI the lowest (38%). Madhavan & Sreekantaiah (2025) reported lower water absorption (60.2%), for PPI dough formulated with a similar ratio of wheat flour (53.8%) and pea protein isolate (46.2%). The discrepancy may be attributed to their PPI having a lower protein content (51 g/ 100 g), which would reduce water binding capacity. Additionally, differences in the protein isolate extraction method may have contributed, as extraction conditions can significantly alter surface properties and functional characteristics (Higa et al., 2024). Although the addition of root powders affected water absorption, the water content in each formulation was adjusted to achieve a max consistency comparable to that of the corresponding control samples (Table 10).

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Furthermore, lower development time was found in the LPI_control (1.73 min), while PPI_control (2.40 min) and LuPI_control (2.40 min) presented the highest. Hence, PPI_control and LuPI_control needed more time for dough development as compared to the other samples (Table 10). In contrast, Madhavan & Sreekantaiah (2025) reported lower development time (1.5 min) for PPI control doughs. Statistically significant differences ($P \leq 0.05$) in development time were observed for LuPI doughs upon incorporation of both roots, with values decreasing to 1.09 min. This reduction suggests a faster formation of the dough structure. The effect may be attributed to the high insoluble fiber content in the root powders, as previously reported by Molina et al. (2025b), where dilution of the gluten network can accelerate dough development by limiting gluten hydration and matrix formation (Zhou et al., 2021). No significant effect was observed for stability, except for the PPI_control, which presented the highest value (3.81 min). Madhavan & Sreekantaiah (2025) obtained lower values for PPI dough stability (1.8 min). Differences in the stability may be due to the strong potential of the charged amino acid side chains (in higher protein content samples) to bind water molecules, as stated by Ma et al. (2022).

The results obtained for the protein aggregation behavior of the different dough samples are shown in Table 11. During the high-speed rotating test, the sample undergoes mechanical stress, initially causing an increase in torque, which is later followed by a decrease as the dough network breaks down due to overmixing. Significantly different ($P \leq 0.05$) maximum torques, and peak maximum times were obtained. Maximum torque, which corresponds to the peak that occurs as protein aggregates, was higher for PPI_control (41.3 BU) as compared to the rest of the protein isolate blends. Hoehnel et al. (2019) also observed the effect on gluten aggregation when replacing wheat flour with different pulse protein isolates. In their study, the maximum torque values for PPI (72 BU) and LuPI (59 BU) were higher than those observed in the present study. This difference may be due to the lower extent of wheat flour

substitution in their formulations (15%), resulting in reduced dilution of wheat gluten and starch. Significant positive correlations were obtained between maximum torque and the water absorption ($r = 0.90$, $P \leq 0.001$), as well as between maximum torque and dough stability ($r = 0.86$, $P \leq 0.001$). These strong positive correlations indicate that, in mixed-protein systems containing both wheat flour and plant protein isolate, higher water absorption results in more effective hydration of both wheat and plant proteins. This results in the formation of a denser, more resistant protein matrix during mixing. Consequently, samples with greater water absorption display higher maximum torque (reflecting greater resistance to mixing) and enhanced dough stability (reflecting prolonged resistance to mechanical breakdown).

Table 11. Protein aggregation behavior of the protein-enriched cracker doughs containing adaptogenic root powders.

Samples	Maximum torque (BU)			Peak maximum time (s)		
PPI_control	41.3	± 1.4	dA	18	± 0.0	bB
PPI_ES	37.9	± 3.4	cA	18	± 0.0	aB
PPI_RR	39.5	± 2.1	cA	6	± 0.0	aA
LPI_control	27.3	± 0.2	cA	54	± 0.0	cC
LPI_ES	22.9	± 2.8	bA	18	± 0.0	aB
LPI_RR	24.6	± 0.3	bA	12	± 0.0	bA
LuPI_control	9.3	± 0.3	aA	12	± 0.0	bA
LuPI_ES	9.5	± 0.0	aA	12	± 0.0	aA
LuPI_RR	9.6	± 0.1	aA	12	± 0.0	bcA
FBPI_control	19.1	± 0.1	bA	18	± 0.0	bAB
FBPI_ES	23.5	± 0.0	bB	18	± 0.0	aB
FBPI_RR	27.2	± 0.0	bC	18	± 0.0	cA

Values correspond to means ± standard deviation obtained from three independent replicates (basis for the statistical analysis). Different uppercase letters within a column indicate significant differences between samples for each protein isolate, while different lowercase letters within a column refer to significant differences between protein isolates ($P \leq 0.05$).

*Control refers to the sample without root powder.

Regarding the peak maximum time, values ranged from 18 to 54 s for control samples (Table 11). Slightly higher values were reported for protein isolate/flour

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blends by Hoehnel et al. (2019), being 25 s for PPI and 33 s for LuPI. According to Goldstein et al. (2010), wheat flours with a fast build-up of gluten-network within a short period of time, are considered weak flours, as strong flours need longer time to allow for a fully developed and more stable gluten-network. Based on this criterion, the LPI_control sample in the present study formed a stronger gluten network than the other samples. However, a shorter peak maximum time does not always indicate weaker dough properties; it may also reflect rapid network formation involving interactions between non-wheat proteins and gluten. Hoehnel et al. (2019) reported that protein solubility and the abundance of charged amino acids (such as in our high protein samples) can accelerate gluten aggregation by increasing the number of available interactive sites.

As for the effect of the addition of the roots, statistically significant difference ($P \leq 0.05$), were shown in all control blends, except for LuPI_control. In this regard, RR root decreased the peak maximum time in all the other protein isolate/flour blend samples. Meanwhile, ES only had a reducing effect on LPI sample. The effect of RR could be related to the lower content of insoluble fiber and higher amount of soluble fiber as compared to ES, thereby, leading to a rapid aggregation of proteins as shown by Zhang et al (2021), when mixing apple pectin with wheat flour.

5.3.2. Proximate composition and color of crackers

Proximate composition and color of the crackers are shown in Table 12. In general, moisture content varied between 3.0 and 9.5 g/ 100 g, with LPI_control cracker the lowest and FBPI_control the highest. The moisture content was decreased with the addition of ES (4.9 g / 100 g) and RR (8.1 g/ 100 g) in FBPI crackers. LuPI_RR cracker presented a higher moisture content (8.5 g/ 100 g), while PPI_RR cracker (3.0 g/ 100 g) had lower moisture content compared to control samples. The protein content was similar for all samples, except for PPI_control cracker which was slightly lower (49 g/ 100 g). Similar

results (50 g/ 100 g) were obtained by Madhavan & Sreekantaiah (2025) for high protein crackers made with PPI. Gangola et al. (2022) reported a lower protein content (43 g/ 100 g) in FBPI crackers which appears to be due to the lower wheat replacement (40%) compared to the one in this study (~45%). Significant correlations were found between protein content and maximum consistency ($r = 0.75$, $P \leq 0.05$), as well as maximum torque ($r = -0.71$, $P \leq 0.01$). These results suggest that samples with higher protein content tend to exhibit greater dough max consistency, indicating that more force is required to form a well-structured protein network. In this regard, no statistically significant differences ($P \leq 0.05$) were observed with the addition of the root powders, except for PPI_RR cracker (Table 12).

Although lipids are typically removed during the extraction of protein isolates (Johnston et al., 2015), LuPI_control cracker still showed a relatively high fat content (8.4 g/ 100 g). This was also reflected in LuPI_ES (8.2 g/ 100 g) and LuPI_RR (8.1 g/ 100 g) crackers. Regarding ash content, FBPI_control cracker showed lower values compared to the other control samples (Table 12). The addition of roots generally increased ash content in most samples, except for LPI_ES.

Table 12. Chemical composition and color of the protein-enriched crackers containing adaptogenic root powders, expressed on a wet weight basis.

Samples	Moisture (g/100 g)			Protein (g/100 g w.b.)			Fat (g/100 g w.b.)			Ash (g/100 g w.b.)			*Carbohydrates (g/100 g w.b.)	<i>L*</i>			<i>a*</i>			<i>b*</i>									
PPI_control	4.7	±	0.5	bB	49	±	1	aB	5.4	±	0.4	cA	3.0	±	0.1	bA	38	67.1	±	1.1	bB	8.77	±	0.99	cA	27.37	±	1.16	bB
PPI_ES	4.6	±	0.2	bB	46	±	2	aAB	6.3	±	0.1	cB	4.3	±	0.1	cB	39	65.5	±	1.2	bB	8.50	±	0.26	cA	26.20	±	1.82	bB
PPI_RR	3.0	±	0.8	bA	42	±	2	aA	5.7	±	0.0	bA	4.1	±	0.2	bcB	45	51.9	±	0.9	bA	7.50	±	0.70	bA	17.50	±	2.60	aA
LPI_control	3.0	±	0.9	aA	51	±	2	bA	4.4	±	0.0	bA	3.4	±	0.1	cB	38	73.1	±	2.7	cB	7.63	±	0.38	bcB	27.63	±	0.12	bB
LPI_ES	2.5	±	0.3	aA	51	±	1	bA	4.0	±	0.4	bA	3.2	±	0.1	aA	39	70.5	±	0.6	dB	6.27	±	0.64	bA	24.70	±	0.26	bA
LPI_RR	2.0	±	0.4	aA	52	±	2	bA	4.2	±	0.0	aA	4.3	±	0.1	cC	37	52.6	±	0.3	bA	7.23	±	0.12	bB	24.27	±	0.46	bA
LuPI_control	7.4	±	0.1	cA	52	±	1	bA	8.4	±	0.1	dA	3.7	±	0.2	cA	28	73.3	±	0.7	cC	6.50	±	0.20	bA	28.23	±	0.50	bB
LuPI_ES	7.2	±	0.3	cA	51	±	1	bA	8.2	±	0.1	dA	3.8	±	0.0	bAB	30	68.4	±	1.0	cB	8.00	±	0.10	cB	28.87	±	0.31	cB
LuPI_RR	8.5	±	0.1	dB	52	±	2	bA	8.1	±	0.2	cA	3.9	±	0.0	bB	27	63.7	±	0.2	cA	7.83	±	0.23	bB	22.37	±	0.31	bA
FBPI_control	9.5	±	0.4	dC	51	±	1	bA	3.5	±	0.0	aA	2.3	±	0.2	aA	34	58.2	±	0.4	aC	3.55	±	0.28	aA	19.67	±	0.16	aB
FBPI_ES	4.9	±	0.3	bA	50	±	0	bA	3.3	±	0.2	aA	3.3	±	0.1	aB	38	55.1	±	0.1	aB	4.97	±	0.06	aB	20.52	±	0.08	aC
FBPI_RR	8.1	±	0.0	cB	51	±	0	bA	4.5	±	0.3	aB	3.2	±	0.0	aB	33	50.0	±	0.4	aA	6.12	±	0.06	aC	15.18	±	0.28	aA

Values correspond to means ± standard deviation obtained from three independent replicates on which are based the statistical analysis. Different uppercase letters within a column indicate significant differences between samples for each protein isolate, while different lowercase letters within a column refer to significant differences between protein isolates ($P \leq 0.05$). *Carbohydrates were calculated by difference.

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The color of the crackers was also evaluated (Table 12). Statistically significant differences were found between L^* , a^* and b^* parameters. In general, higher lightness (L^*) values were observed for LPI_control and LuPI_control crackers. The addition of RR root decreased lightness across all formulations, probably due to the darker color of RR root powder (Molina et al., 2025). Regarding the a^* coordinate (green-red range), PPI_control cracker showed the highest value (8.77) among the control samples. This difference may be related to the Maillard browning reactions promoted by the high content of lysine in PPI (K. K. Ma et al., 2022).

Upon root addition, a^* values increased particularly in FBPI and LuPI crackers. No significant differences were observed among control samples for the b^* coordinate (blue-yellow range). Han et al. (2010) reported lower values of a^* (5.21) and higher values of b^* (31.24) for PPI crackers. However, their formulation was based solely on pea protein isolate, without the inclusion of wheat flour as in the present study. The incorporation of ES root notably increased the b^* value in FBPI crackers, reaching 20.52, suggesting an enhanced golden hue. This may be attributed to the polyphenolic compounds in ES, which can undergo oxidation during processing and contribute to yellowish pigment formation (Gębalski et al., 2025).

5.3.3. Texture of crackers

Figure 27 shows the texture characteristics of crackers. FBPI_control cracker displayed the highest hardness (39 N) when compared to the other crackers. Despite their low moisture content, PPI_control and LPI_control crackers exhibited the lowest hardness as compared to FBPI_control cracker, which had the highest moisture content (9.5 g/ 100 g). In this regard, a significant positive correlation between hardness and moisture content ($r = 0.73$, $P \leq 0.01$) was found. Nogueira et al. (2021) reported slightly higher hardness (17.65 N) for PPI crackers when replacing 30% of wheat flour. Interestingly, a significant negative correlation ($r = -0.72$, $P \leq 0.01$) was found between hardness and a^* parameter of color, indicating that samples with a greater redness tended to exhibit lower hardness values. This correlation is opposite to the results of

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Zhou et al. (2013), who found a positive correlation between the development of brown color and the changes in texture of nutritional bars made with whey protein isolate. Their study indicated that the Maillard reaction promoted the formation of high-molecular-weight protein polymers through non-disulfide cross-links, which they stated contributed to a firmer texture.

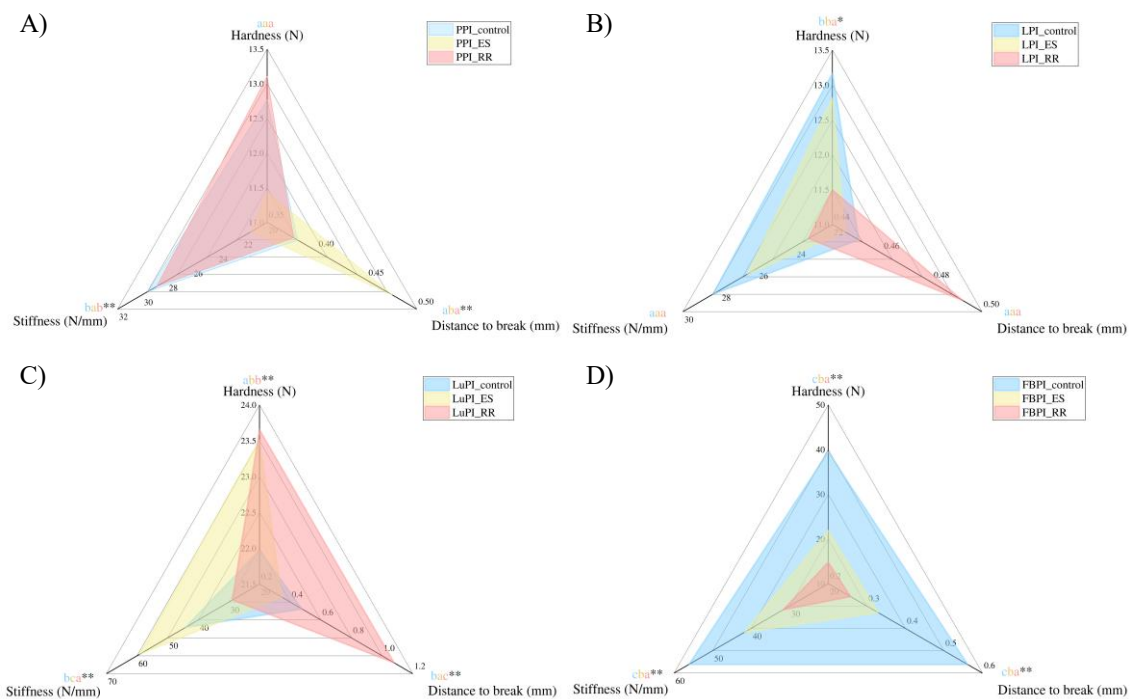


Figure 27. Texture parameters of crackers formulated with different protein isolates containing adaptogenic root powders: A) PPI, B) LPI, C) LuPI and D) FBPI. Different lowercase letters refer to significant differences between samples (*: $P < 0.05$, **: $P < 0.01$).

Overall, the impact of root powder addition varied significantly between crackers. Incorporation in FBPI crackers resulted in a reduction in hardness with the most pronounced effect observed in the RR sample (14 N). In contrast, RR led to an increase in the hardness of PPI and LuPI crackers, with values of 13.1 N and 23.7 N, respectively. A positive correlation ($r = 0.753$) has been reported by Millar et al. (2017) between the fiber content and the hardness of

crackers made with faba bean flour. In line with this, Molina et al. (2025) found that ES roots contained a higher fiber content (71-78g / 100 g) compared to RR roots (43-59g/100 g). However, no significant differences ($P \leq 0.05$) were shown between the control and the addition of RR and ES root on cracker hardness, except for LuPI crackers (Figure 27).

Regarding the distance to break, which is directly related to the fracturability of the cracker structure, LuPI_control cracker showed the highest values (1.08 mm), followed by FBPI_control (0.55 mm). Therefore, FBPI and LuPI crackers were less brittle and crisp, which may be due to fewer air pockets (Nammakuna et al., 2016). Interestingly, the addition of ES root in LuPI cracker made it more brittle. The opposite effect was found on PPI_ES and FBPI_ES crackers. As for stiffness, it was highest for FBPI_control cracker (54 N/mm) and lowest for LPI_control crackers (28 N/mm). An opposite effect was observed upon incorporation of ES in LuPI crackers, as compared to RR addition (Figure 27). In contrast, in FBPI crackers, the addition of both root powders decreased stiffness. A significant negative correlation was observed between stiffness and water absorption of dough ($r = -0.70$, $P \leq 0.01$), meaning that doughs with higher water absorption led to a softer or more flexible cracker texture.

5.3.4. FTIR spectra of formulated crackers

FTIR spectroscopy enabled the investigation of how the addition of root powders altered the crackers' molecular structure. All spectra showed a series of characteristic bands typical of starch domain ($1200-800\text{ cm}^{-1}$), protein ($1700-1239\text{ cm}^{-1}$) and lipids ($3000-2800\text{ cm}^{-1}$), as shown in Figure 28.

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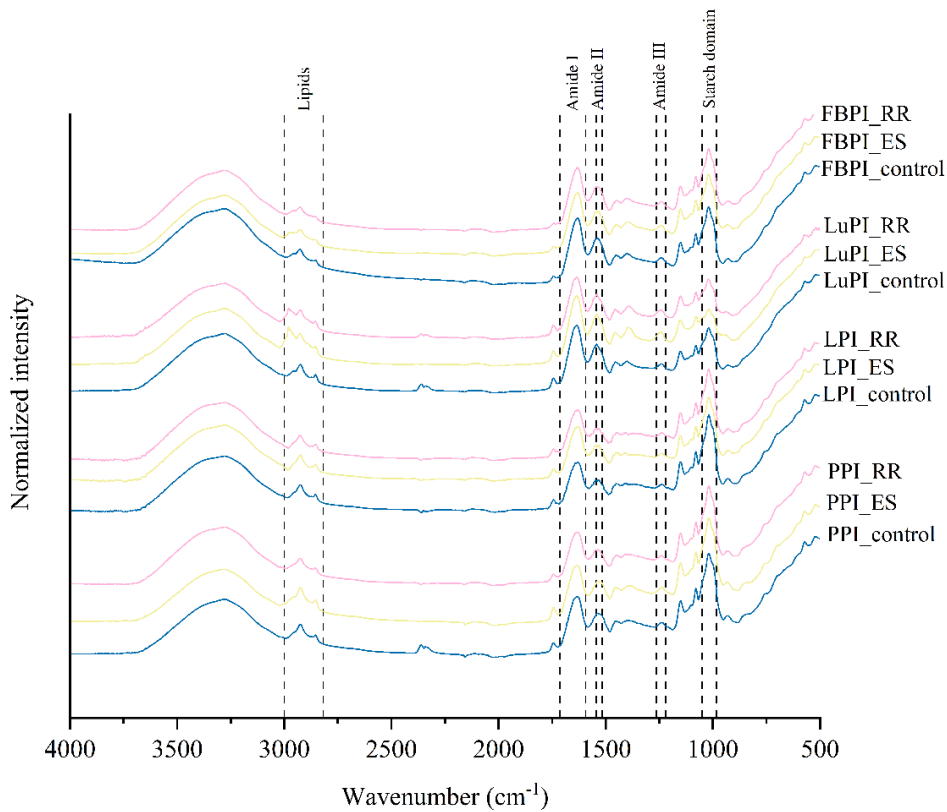


Figure 28. FTIR spectra of protein-enriched crackers containing adaptogenic root powders.

Detailed analysis was done only on the protein secondary structure of the crackers and, more specifically, in the amide I band, which is known to be a good indicator of secondary structure changes (Table 13). The amide I band, which appears within the wavenumber range of 1700 to 1600 cm⁻¹, is associated with stretching of the C=O bonds. Furthermore, it consists of α -helix, β -sheet, random coil, β -turns and protein aggregates (Shevkani et al., 2019). Relative proportions of the mentioned components for all the samples are shown in Table 13.

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Table 13. Relative proportion of secondary structures of proteins in protein-enriched crackers containing adaptogenic root powders. Secondary structure components and their relative proportions were analyzed from the Amide I peak.

Samples	Protein aggregates		α -helix		β -turns		β -sheet		Protein aggregates (β -type structures)		Random coil	
	Peak center value (cm ⁻¹)	Relative content (%)	Peak center value (cm ⁻¹)	Relative content (%)	Peak center value (cm ⁻¹)	Relative content (%)	Peak center value (cm ⁻¹)	Relative content (%)	Peak center value (cm ⁻¹)	Relative content (%)	Peak center value (cm ⁻¹)	Relative content (%)
PPI_control	-	-	1650	23.0 ± 0.3	1661, 1669	17.7 ± 0.2	1625, 1682	46.4 ± 1.4	1695	12.9 ± 1.1	-	-
PPI_ES	1609	1.4 ± 0.0	1650	16.6 ± 0.1	1659, 1663	9.8 ± 0.8	1625, 1680	52.0 ± 0.9	1694	11.2 ± 0.4	1642	8.9 ± 0.0
PPI_RR	1610	2.8 ± 0.1	1650	10.6 ± 0.6	1660, 1669	24.9 ± 0.0	1620, 1627, 1630, 1681	46.6 ± 2.1	1694	10.3 ± 0.2	1643	4.8 ± 0.1
LPI_control	1611	6.5 ± 0.6	1650, 1659	14.9 ± 1.7	1665, 1679	21.4 ± 2.2	1620, 1630	34.7 ± 3.6	1692	10.5 ± 1.1	1642	12.0 ± 0.8
LPI_ES	1611	6.6 ± 0.2	1650, 1659	16.0 ± 0.3	1665, 1678	19.8 ± 0.0	1620, 1630	34.5 ± 0.1	1692	11.2 ± 0.4	1642	12.0 ± 0.1
LPI_RR	1611	7.2 ± 0.6	1650, 1659	16.2 ± 0.9	1665, 1678	18.8 ± 1.0	1620, 1630	34.0 ± 1.2	1692	11.3 ± 0.7	1642	12.5 ± 0.4
LuPI_control	1609	2.5 ± 0.1	1651	17.6 ± 0.3	1661, 1669	15.4 ± 0.9	1619, 1627, 1635, 1682	54.9 ± 3.4	1695	6.3 ± 0.2	1644	3.3 ± 0.1
LuPI_ES	1610	2.3 ± 0.2	1651	17.9 ± 0.5	1660, 1668	14.8 ± 0.9	1618, 1630, 1681	55.8 ± 2.6	1693	7.0 ± 0.5	1643	2.2 ± 0.0
LuPI_RR	1608	3.0 ± 0.1	1650	22.8 ± 2.0	1662, 1670	13.3 ± 0.8	1618, 1626, 1635	53.9 ± 5.5	1697	7.1 ± 0.9	-	-
FBPI_control	1610	3.8 ± 0.1	1650	11.0 ± 0.7	1660, 1667	17.3 ± 1.2	1619, 1630, 1681	52.3 ± 5.0	1693	8.7 ± 0.6	1643	6.8 ± 0.2
FBPI_ES	1610	4.4 ± 0.0	1650	10.0 ± 0.1	1660, 1666	17.8 ± 0.3	1619, 1630, 1680	49.9 ± 1.1	1693	9.0 ± 0.1	1642	8.9 ± 0.2
FBPI_RR	1610	4.6 ± 0.2	1650	9.7 ± 0.8	1660, 1666	17.5 ± 0.1	1629, 1630, 1680	49.6 ± 1.9	1692	9.1 ± 0.0	1642	9.5 ± 0.7

LPI_control cracker showed the highest content of protein aggregates (6.5%), while none were present in the PPI_control cracker. However, with the incorporation of roots, the aggregation of proteins occurred in PPI crackers. Overall, aggregates were more prominent with the addition of RR in all the crackers. Moreover, the α -helix structure appeared to be higher in the PPI_control cracker (23%) as compared to the other samples (Table 13). In contrast, Li et al. (2024) reported a higher proportion of α -helices in FBPI (26.9%) as compared to PPI (9.1%). Differences may be related to the effect of baking, indicating that PPI had a higher thermal resistance while the protein of FBPI lost native helical segments. In this case, the incorporation of the root powder affected the α -helices differently. A reduction in α -helix content was noticed in PPI and FBPI crackers with the addition of both root powders. Ma et al. (2024) found that the addition of insoluble fiber from mung bean skin produced an increase in the α -helices due to the influence of the insoluble fiber on the charge of the protein and the formation and stabilization of the α -structure. Their study analyzed the changes in the structure of the gluten proteins. Hence, charges of the amino acids conforming the proteins from pulses could be influencing the results in distinct ways.

LPI_control (21.4%) was characterized by a higher content of β -turn structures, while similar results were shown between PPI_control (17.7%) and FBPI_control (17.3%). Lower amounts were observed with the addition of both roots in the LPI and LuPI crackers, such that insoluble fiber could be interacting with the amino acid residues from the β -turn such as proline and glycine. These interactions could promote protein aggregation or the formation of a more rigid network, reducing the presence or detectability of β -turns (Shevkani et al., 2019).

As for β -sheet, LuPI_control cracker (54.9%), and FBPI_control cracker (52.3%) had the highest amounts (Table 13). ES incorporation caused an increase in β -sheet structures in the PPI and LuPI crackers, with relative contents of 52% and 55.8%, respectively. These findings suggested that the addition of ES strengthened the hydrogen bonding within the β -sheet structure, contributing to a more stable secondary conformation. It has been reported that a high content of β -sheets can hinder the access of proteolytic enzymes, potentially reducing protein digestibility (Aryee & Boye, 2017).

In the case of protein aggregates due to β -type structures, greater amounts were present in PPI_control compared to the other crackers (Table 13). A higher amount was found in all crackers, except in PPI, when both roots were added. Interestingly, a strong negative correlation was obtained between protein aggregates (β -type structures) and hardness ($r = -0.95$, $P \leq 0.05$), and stiffness ($r = -0.9895$, $P \leq 0.01$). This suggests that protein aggregates may interfere with proper cross-linking within the protein–starch matrix, resulting in a cracker that is less hard and more fragile. In addition, a higher content of random coil was shown in LPI_control, but no differences were demonstrated in LPI crackers with the addition of the roots. An increase was found in FBPI crackers with addition of RR and ES root powders, indicating that the internal structure of gluten and FBPI proteins evolved to be looser, leading to an additionally unfolded protein structure (Ma et al., 2024).

5.3.5. Microstructure of crackers

The confocal micrographs of the crackers when stained with FITC (green) and rhodamine B (red) are shown in Figure 29. Overall, the sample microstructures indicated physical changes to varying extents, depending on protein isolate and addition of the RR and ES root powders. While protein was represented in red, starch was labelled in green.

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

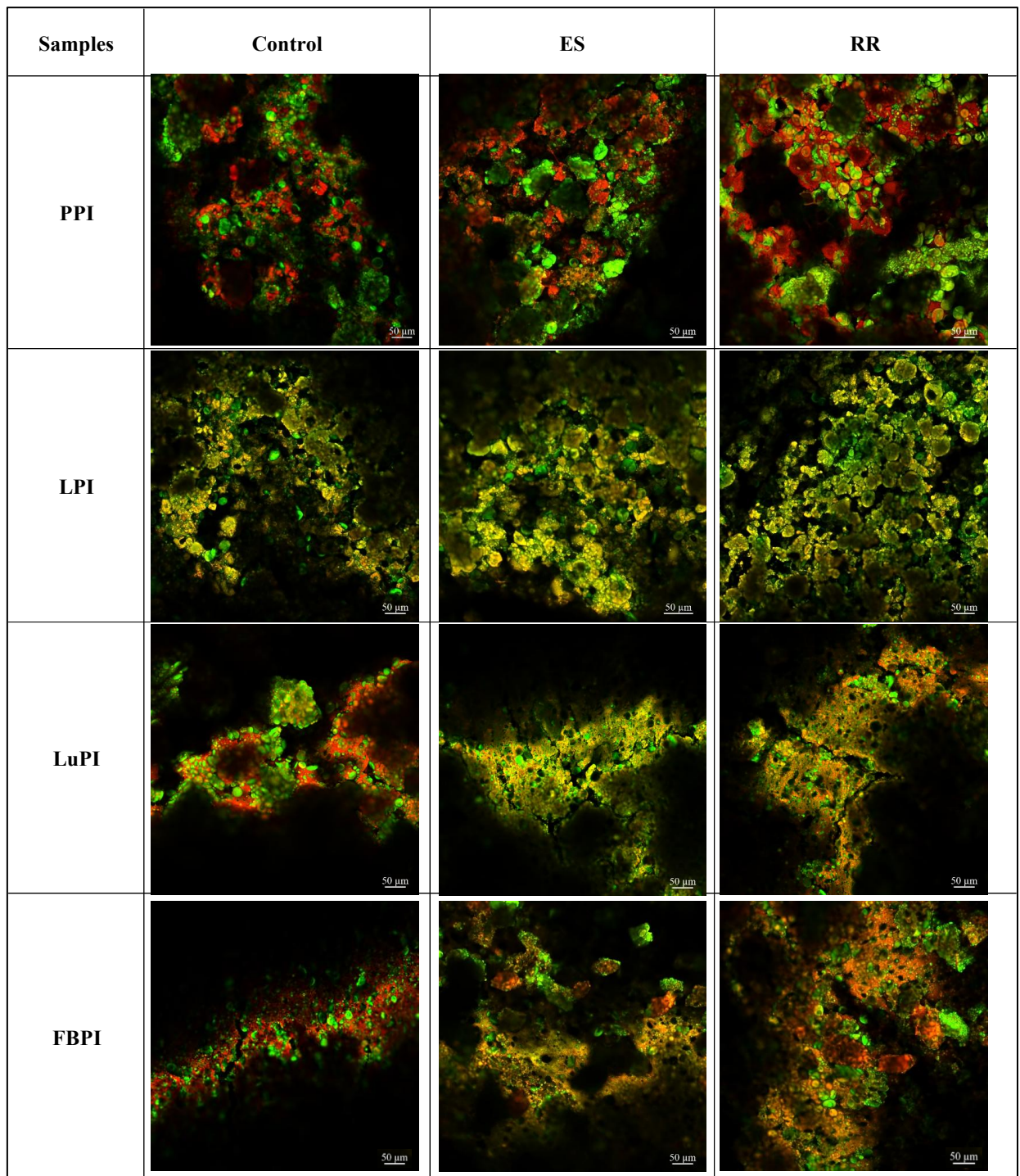


Figure 29. Confocal laser scanning micrographs of protein-enriched crackers containing adaptogenic root powders.

PPI_control cracker presented a heterogenous and less uniform structure characterized by the formation of a protein network enveloping starch granules. Overall, due to the protein network formation, limited interaction between protein and starch was observed in this sample, indicating a less integrated matrix. In this regard, the addition of ES in PPI crackers led to higher number of starch aggregates along with greater exposure of the starch granules. Furthermore, the structure appeared less cohesive and with a more clustered protein network as compared to PPI_control cracker, likely due to the higher quantity of insoluble fiber from ES root (Molina et al., 2025b). The observed effect was also described by Martins et al. (2017), when analyzing the different replacement levels of wheat flour using fiber enriched extracts. In addition, the high fiber content in ES may have reduced water availability and disrupted starch–protein interactions, as reported by Ma et al. (2024). Although the addition of RR reduced the number of starch aggregates, a less compact microstructure was obtained as compared to PPI_control cracker. In this regard, PPI_RR cracker presented significant fractures which could be observed as black voids (air pockets) within the matrix.

LPI_control cracker was characterized by a more continuous and integrated structure than the PPI_control cracker. As indicated by the overlapping green and red fluorescence, starch and protein are more closely associated, therefore, suggesting stronger interactions and a more cohesive matrix. In the LPI_ES cracker, a denser microstructure with less air pockets and less fragmentation was found, as compared to LPI_control. Moreover, with the addition of ES, the starch and protein phases remained better integrated compared to the PPI_ES cracker. On the contrary, a more fragmented yet still integrated structure was shown with the incorporation of RR root (Figure 29). Thus, lentil protein matrix may be more resilient to structural disruption by the addition of fibrous root powders.

LuPI_control cracker had a less integrated protein and starch structure comparable to that of PPI_control cracker. Nevertheless, the protein network seemed to be more continuous and compact. Native starch granules, primarily derived from wheat flour, were more prominently detected in this case (Figure 29). The addition of root powders produced clear structural changes, especially in the ES-containing sample. Its microstructure was

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

denser and more integrated, with starch granules more fully embedded within the protein-starch network. This may result from the physical action of fiber, which redistributed moisture throughout the dough matrix, increasing local moisture around starch granules and thereby facilitating their gelatinization. However, the same fiber can also disrupt gluten network formation, introducing weak points that manifest as fractures in the structure as also seen in the LuPI_RR cracker.

The microstructure of FBPI_control cracker was comparable to that of LuPI_control cracker, although a more continuous and denser matrix was observed in the FBPI cracker. Both FBPI_ES and FBPI_RR crackers exhibited a level of starch-protein integration similar to that seen in LuPI_RR cracker. However, in the FBPI crackers, some ungelatinized starch granules appeared to surround the protein-starch network, whereas in LuPI_RR, the granules were predominantly embedded within it. Overall, the incorporation of both root powders in the FBPI crackers may have promoted a more homogeneous network between starch and protein components.

5.3.6. Quantification of bioactive compounds and in vitro bioaccessibility in crackers

The content of the bioactive compounds from RR and ES present in the different crackers is shown in Table 14. In the case of RR, statistically significant differences ($P < 0.05$) were obtained for all the bioactive compounds. Higher amounts of salidroside were found in LPI_RR and PPI_RR crackers. FBPI_RR cracker was characterized by the highest content of tyrosol and the lowest of rosinarin, with values of 0.19 and 0.36 mg/g cracker, respectively. In addition, an increased amount of rosavin was found in the LuPI_RR cracker. As for ES root, FBPI cracker presented a higher content of both bioactive compounds, eleutheroside B and E. The differences in the amounts of these bioactive compounds in crackers could be due to the interaction of these compounds with the proteins, and this can differ depending on protein size,

secondary/tertiary structure, and amino acid composition (Günel-Köroğlu et al., 2023). As indicated by the FTIR results, the incorporation of roots increased the random coil content in FBPI crackers, resulting in a more unfolded protein structure that may have facilitated the release of eleutherosides (Ma et al., 2024).

A highly significant negative correlation was found between salidroside and protein aggregates (β -type structure) ($r = -0.9996$, $P \leq 0.01$), indicating that a higher number of protein aggregates would restrict salidroside release. Protein aggregates, especially those with β -sheet characteristics, tend to form dense, ordered structures that can physically entrap small molecules, reducing their solubility during digestion. In addition, negative correlations were obtained between eleutheroside E and α -helix ($r = -0.97$, $P \leq 0.05$) and eleutheroside B and α -helix ($r = -0.98$, $P \leq 0.01$). Therefore, by destabilizing the flexible α -helices structure, eleutherosides may have made the protein matrix more open, facilitating their release from the food matrix during digestion.

Table 14. Contents of bioactive compounds in protein-enriched crackers containing adaptogenic root powders.

Samples	Salidroside (mg/g)		Tyrosol (mg/g)		Rosarin (mg/g)		Rosavin (mg/g)		Rosin (mg/g)		Eleutheroside B (mg/g)		Eleutheroside E (mg/g)	
PPI_ES	-	-	-	-	-	-	-	-	-	-	0.00 ± 0.00	a	0.06 ± 0.00	ab
PPI_RR	0.51 ± 0.06	c	0.08 ± 0.00	a	0.90 ± 0.04	b	0.08 ± 0.01	b	0.00 ± 0.00	a	-	-	-	-
LPI_ES	-	-	-	-	-	-	-	-	-	-	0.00 ± 0.00	a	0.07 ± 0.00	b
LPI_RR	0.62 ± 0.03	c	0.09 ± 0.01	a	0.91 ± 0.04	b	0.00 ± 0.00	a	0.00 ± 0.00	a	-	-	-	-
LuPI_ES	-	-	-	-	-	-	-	-	-	-	0.00 ± 0.00	a	0.05 ± 0.00	a
LuPI_RR	0.12 ± 0.03	a	0.11 ± 0.00	a	0.97 ± 0.04	b	0.14 ± 0.01	c	0.00 ± 0.00	a	-	-	-	-
FBPI_ES	-	-	-	-	-	-	-	-	-	-	0.04 ± 0.00	b	0.33 ± 0.01	c
FBPI_RR	0.39 ± 0.05	b	0.19 ± 0.01	b	0.36 ± 0.00	a	0.06 ± 0.01	b	0.02 ± 0.00	b	-	-	-	-

Values correspond to means ± standard deviation obtained from three independent replicates on which are based the statistical analysis. Different lowercase letters within a column refer to significant differences between protein isolates ($P \leq 0.05$).

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

For bioactive compounds to exert their physiological effects, they must first be bioaccessible. This means that they need to be effectively released from the food matrix during gastrointestinal digestion, making them available for subsequent intestinal absorption. Table 15 shows the bioaccessibility of the bioactive compounds from RR and ES after the in vitro gastrointestinal digestion of the crackers. A high reduction of salidroside's bioaccessibility was observed in all the crackers. A similar effect was found for rosavin. In fact, their bioaccessibility was less than 50%. In the case of rosavin, Sęczyk et al. (2023) also reported the lowest bioaccessibility (40%), when analyzing powdered plant material (root and rhizome) of RR. Probably rosavin may have undergone hydrolysis into glycosidic components in the acidic environment of the digestive tract, as reported by Zhang et al. (2019). Another contributing factor could be the significant negative correlation obtained between rosavin content and protein aggregates ($r = -0.9984$, $P \leq 0.05$), suggesting that rosavin may become entrapped or bound within aggregated protein structures, further limiting its release during digestion.

As for salidroside, it is noteworthy that Wang et al. (2019) described an inhibition of starch hydrolysis followed by a transglycosylation reaction of salidroside, mediated by the presence of α -amylase and starch. This reaction was shown to reduce free salidroside levels. In their study, a higher concentration of salidroside derivatives was detected at pH 7.5, which closely resembles the pH of the oral cavity (approximately pH 7.0). This suggests that enzymatic modification of salidroside may have occurred early during digestion, potentially reducing its stability and contributing to its low bioaccessibility in all cracker formulations (Table 15).

Table 15. Bioaccessibility of bioactive compounds after in vitro gastrointestinal digestion of the protein-enriched crackers containing adaptogenic root powders.

Samples	Salidroside (%)	Tyrosol (%)	Rosarin (%)	Rosavin (%)	Rosin (%)	Eleutheroside B (%)	Eleutheroside E (%)
PPI_ES	-	-	-	-	-	0 ± 0 a	15 ± 0 ab
PPI_RR	14 ± 1 a	68 ± 10 b	45 ± 0 a	4 ± 0 b	0 ± 0 a	-	-
LPI_ES	-	-	-	-	-	0 ± 0 a	12 ± 1 a
LPI_RR	20 ± 1 b	25 ± 4 a	58 ± 4 b	0 ± 0 a	0 ± 0 a	-	-
LuPI_ES	-	-	-	-	-	0 ± 0 a	22 ± 1 d
LuPI_RR	17 ± 2 b	18 ± 0 a	56 ± 1 b	16 ± 2 c	0 ± 0 a	-	-
FBPI_ES	-	-	-	-	-	7 ± 0 b	17 ± 2 c
FBPI_RR	32 ± 1 c	100 ± 0 c	53 ± 3 ab	2 ± 0 ab	26 ± 1 b	-	-

Values correspond to means ± standard deviation obtained from three independent replicates on which are based the statistical analysis.

Different lowercase letters within a column refer to significant differences between protein isolates ($P \leq 0.05$).

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

FBPI_RR cracker achieved complete tyrosol bioaccessibility (100%), whereas the other RR-containing crackers displayed lower tyrosol recovery. According to Ma et al. (2022), FBPI contained the highest proportion of charged and polar amino acids when compared to lentil and pea protein isolates. This composition may explain the improved tyrosol release, as the phenolic hydroxyl groups of tyrosol can form hydrogen bonds and other weak interactions with the abundant polar residues in the faba bean protein matrix, facilitating its release during digestion. In the case of rosin, 58% and 56% was recovered after gastrointestinal digestion for LPI_RR and LuPI_RR crackers, respectively.

No statistically significant differences ($P < 0.05$) were observed for rosin bioaccessibility within samples, except for FBPI_RR cracker. However, a highly significant negative correlation was observed between rosin bioaccessibility and β -sheet content ($r = -0.9998$, $P \leq 0.01$) and a positive correlation between rosin and α -helix ($r = 0.9994$, $P \leq 0.05$), indicating that denser, β -sheet-rich protein networks may hinder rosin release, while more flexible, α -helix-rich regions facilitate its diffusion from the cracker matrix.

In contrast, no clear trend was observed for the bioaccessibility of the bioactive compounds from ES root. Notably, eleutheroside B and eleutheroside E exhibited higher bioaccessibility in FBPI (7%) and LuPI (22%) crackers, respectively (Table 15). The observed reduction in the bioaccessibility of these bioactive compounds may also be attributed to the formation of complexes with other macronutrients such as proteins or fiber.

5.4. CONCLUSION

The present study demonstrated that the incorporation of bioactive root powders (RR and ES) into pulse protein-based crackers significantly influenced the physicochemical properties and bioaccessibility of bioactive compounds. ES increased dough consistency and promoted denser microstructures, often enhancing β -sheet formation but limiting water

Protein matrix dynamics and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

availability and gluten hydration. On the other hand, RR, tended to accelerate protein aggregation and modify color while producing less cohesive microstructures. These structural changes altered texture outcomes, ranging from softer matrices in FBPI crackers to increased hardness in LuPI crackers, and directly impacted the release of bioactive compounds. In this regard, both roots promoted protein aggregation and secondary structural rearrangements, which in turn limited the bioaccessibility of salidroside and rosin, when increasing the β -sheet and protein aggregates. In contrast, other bioactive compounds including eleutheroside B and E and tyrosol exhibited higher release during digestion due to favorable protein–bioactive compound interactions.

Overall, this study highlighted how strategic selection of pulse protein isolates and adaptogenic root powders can be leveraged to fine-tune both the structural and functional attributes of high-protein crackers. By elucidating the relationships between dough thermomechanical properties, texture, protein secondary structure and bioactive compound release, this research demonstrated a clear pathway for designing clean-label, plant-based snacks with targeted nutrient delivery and tailored texture profiles.



GENERAL DISCUSSION

Regular exercise is one of the most important determinants of human health and well-being. Scientific evidence has consistently demonstrated that maintaining an active lifestyle reduces the risk of developing chronic diseases including diabetes, obesity, cardiovascular disorders and certain types of cancer (Padulo, 2025). Besides disease prevention, exercise also plays a key role in reducing mental health symptoms (depression, stress, anxiety, etc.), promoting longevity, and improving immune function and sleep (World Health Organization, 2024). Accordingly, international organizations such as the World Health Organization (WHO) highlight the importance of doing regular exercise as a cornerstone for enhancing quality of life and lowering the risk of chronic diseases. The last report from the European Commission highlighted that only 44% of the EU population practised physical activity at least once a week in 2019 (Eurostat, 2022). However, over the past decades, the importance of exercise has led to a significant increase of the number of individuals who exercise. As an example, 242 million Americans (78.8% of the population) participated in a physical activity one or more times in 2023 (SFIA, 2024). Also, 63.7% of the adult population in England met the WHO recommendations of doing at least 150 minutes of moderate intensity physical activity a week (Sport England, 2025). This trend is particularly strong among younger generations. In fact, the global gym and fitness industry revenue reached US \$124.7 billion in 2024 (Fortune Business Insights, 2025).

The sociocultural shift toward a more active population underscores the need for food products that support the nutritional requirements of physically active individuals. In the last decade, one of the main sources of information has been social media. Its increased use has made Twitter (X after July 2023) a useful tool for individuals to gather information about different topics related to nutrition and food choices (Puerta et al., 2020; Zarrabeitia-Bilbao et al., 2022). Twitter, along with other social media platforms, is considered a successful tool because it avoids the bias of asking people questions, such as surveys, by capturing spontaneous and naturally occurring user expressions and behaviors. In this present research study (Chapter 1), the total period of research was six months because it allowed coverage of seasonal variation of consumer food preferences. Although the common motives for doing physical activity have been evident, physically active individuals have shown limited knowledge regarding sport nutritional needs, diets or supplementation. As shown in the present research study (Chapter 1), physically active individuals knew the importance of vitamins and minerals intake and their consumption in foods such as vegetables and fish for a faster recovery (Arenas-Jal et al., 2020). However, contradictions were found with respect to carbohydrates, because people thought skipping carbohydrates' intake after exercise is a quick technique to lose weight and help recovery. Nevertheless, when it comes to carbohydrates, simple sugars are needed to restore glycogen levels and maximize recovery (Reid, 2013).

With the shift towards plant-based food consumption and vegan diets, physically active individuals seemed to believe that these diets can lead to an improvement in strength (Wirnitzer et al., 2025). Conversely, vegan diets containing low energy-density foods are actually detrimental to physically active individuals due to insufficient energy intake (Rogerson, 2017). In this context, the food industry has shown a great interest in the development of functional products that not only meet the nutrition and energy demands of these individuals, but which will also contribute to athletic performance and recovery, among others. For instance, companies such as Haskalife have developed haskap berry powder stick packs, which increase cognitive function along with athletic performance (Food in Canada, 2025). Another example is Vital Pursuit, a brand from Nestle, a company which has designed

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meals that are high in protein and other essential nutrients for consumers focused on weight management (Nestle, 2024). Nevertheless, dietary supplements are considered the most consumed food products by physically active individuals, as reported in several studies (Domínguez et al., 2025; Lotti et al., 2025). Some of these supplements contain ingredients like caffeine and L-theanine, which have been related to a beneficial effect on the mood states of physically active individuals (Owen et al., 2008). Among these dietary supplements, vitamins and minerals appeared to be the most commonly used, followed by botanical products (Lotti et al., 2025).

In this context, medicinal herbs, and more specifically a subset called adaptogenic plants, have become important due to their potential effect on mood state (Rathore et al., 2022). In fact, a major barrier to engaging in physical exercise is insufficient motivation, which is strongly modulated by mood, defined as a persistent affective state (Portela-Pino et al., 2017). The most popular adaptogenic plants were found to be *Rhodiola rosea*, *Schisandra chinensis*, *Panax ginseng*, *Whitania somnifera* and *Eleutherococcus senticosus* (Chapter 2). In the early 2020s, growing scientific interest in this group of medicinal plants was driven by evidence of their therapeutic properties, attributed to the presence of bioactive compounds. The specific effects, however, varied among different adaptogenic plants. In the case of *Rhodiola rosea*, its consumption was related to its capacity to boost energy levels and reduce stress, as well as reduce fatigue (Toth-Mezzaros et al., 2023). The improvements of memory and performance were some of the recognized effects of *Eleutherococcus senticosus* (known as Siberian ginseng), *Panax ginseng* and *Schisandra chinensis*. As for *Whitania somnifera*, its use was linked to its ability to normalize cortisol levels and alleviate symptoms of depression and anxiety (Kępińska-Pacelik & Biel, 2025). Overall, *Panax ginseng*, together with *Whitania somnifera* and *Schisandra chinensis*, appear to be the most extensively studied adaptogenic plants in relation to food applications, whereas *Rhodiola rosea* and *Eleutherococcus senticosus* have received comparatively less research attention as stated in Chapter 2. As previously discussed, the major food categories containing these

adaptogenic plants were supplements, followed by drinks. In this regard, a strong dominance of *Panax ginseng* and *Eleutherococcus senticosus*, along with *Withania somnifera* has emerged in the market during the last decade. Similarly, a significant increase, but somewhat lower, has been seen in food products containing *Rhodiola rosea*. Indeed, its presence in the market is expected to rise from 2025 to 2032, particularly in North America and Western Europe (Global Market Insights, 2024). New delivery formats such as adaptogen-infused gummies are being launched due to their convenience and versatility. However, there is still a niche in the snack and bakery categories, which remain unexplored by the scientific community and food companies, as shown by the findings in Chapter 2.

Currently, the use of *Rhodiola rosea* and *Eleutherococcus senticosus* in supplements and food products has been primarily in the form of extracts, where the content of the bioactive compounds responsible for the mentioned effects, tend to be standardized (Seczyk et al., 2023). Hence, the potential use of certain parts of these adaptogenic plants as ingredients has not been investigated. As the root is considered to be the richest source of these bioactive compounds, it could be considered a key ingredient in the formulation of food products for physically active individuals. Fiber (41-78 g/100 g) emerged as the predominant macronutrients in the roots of both adaptogenic plants, with the highest content of fiber in ES root (71-78 g/100 g) (Chapter 3).

From the main bioactive compounds in *Rhodiola rosea*, which are rosavins (rosavin, rosarin and rosin), salidroside and tyrosol, the highest content was found for salidroside (12.15-68.71 mg/g) and rosavin (1.43-68.33 mg/g). For *Eleutherococcus senticosus*, although both eleutheroside E and B are recognized as key bioactive compounds contributing to its adaptogenic effects, eleutheroside E was found in higher concentrations (10.09–14.70 mg/g). However, these concentrations in the root powder do not necessarily translate into bioactives delivered to consumers. In fact, the bioaccessibility of bioactive compounds during digestion can change considerably as a result of degradation during food processing or interactions with macronutrients in the food matrix (Sęczyk et al., 2023). Thus, to elicit any benefits, bioactive

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compounds must be present in the end food products. Therefore, evaluating the effect of temperature and pH are key variables for controlling the stability of these bioactive compounds during processing (Vinci et al., 2022).

Chapter 3 demonstrated that at lower pHs (3-5), the extraction rates were higher for all the bioactive compounds in both adaptogenic plant roots, due to the presence of OH groups that interact with H⁺, leading to an increased solubility. In addition, higher temperatures (50 and 100°C) favored the extraction of both eleutherosides and rosavin and salidroside, while 25-50°C was more effective for rosarin and rosin. These powdered roots can thus be used as functional ingredients even when processing involves high temperatures.

Translating the nutritional and health benefits of those functional ingredients into consumer products is especially promising. Therefore, snacks, and more specifically crackers, were selected as the food matrix, as they represent a relatively underexplored food category for the incorporation of adaptogens (Chapter 2). Although most bioactive compounds from *Rhodiola rosea* and *Eleutherococcus senticosus* were more efficiently extracted at acidic pH values (3 and 5), crackers, with an approximate pH of 6, were deliberately chosen to evaluate how these roots behave within a more complex food matrix, given that their primary mode of consumption is typically in teas and beverages. Thus, crackers represent a promising vehicle for delivering functional ingredients, aligning with consumer demand for convenient, protein-enriched products that are particularly valuable for physically active individuals. However, their naturally low protein content (when made solely from wheat flour) necessitates enrichment.

Protein can be derived from different sources, with pulse proteins gaining more attention nowadays because of their lower environmental footprint and their high protein content (Anyiam et al., 2025). Faba bean protein isolate (FBPI) is particularly suitable, as its incorporation at high replacement levels (52 g/ 100 g) substantially increased protein content (Chapter 4), compared with earlier cracker formulations using 40 g/ 100 g (Gangola et al., 2022) and 20 g/ 100 g substitution levels (Morales-Polanco et al., 2017). Mechanistically,

this high level of replacement weakened the gluten network (reduced G'), while promoting hydrogen bonding within the protein network. Conversely, higher disulfide bonding in the control restricted starch swelling during gelatinization, explaining the lower peak viscosity for the dough, in line with results obtained by Gasparre & Rosell (2023). The strong negative correlation ($r = -0.87$, $P \leq 0.05$) between peak viscosity and disulfide bonds supports this mechanism.

The addition of *Rhodiola rosea* (RR) and *Eleutherococcus senticosus* (ES) root powders further modified dough functionality. Both reduced viscoelastic moduli (G' , G''), with ES showing higher G'' , likely linked to its higher hemicellulose fraction (Aydogdu et al., 2018), which contributed to dough softening. RR doughs, instead, exhibited stronger hydrogen and hydrophobic interactions, as confirmed by their negative correlation ($r = -0.9988$, $P \leq 0.05$) with loss modulus (G''). Root powders also affected pasting behavior: insoluble dietary fibers reduced water availability, increased friction with starch granules during gelatinization, and thereby raised peak viscosity (Li, 2022). Reduced setback viscosity further indicated a reduction in starch retrogradation, with hydrophobic interactions likely hindering amylose reassociation (Zhu et al., 2009). Quality attributes of the final product were also impacted. Root powders darkened crackers (lower L^* , b^* , higher a^*), and decreased hardness, particularly with addition of RR, consistent with its lower fiber content (Millar et al., 2017). In this regard, chroma (C_{ab}^*) was only analyzed in the roots and not in crackers, because $L^*a^*b^*$ parameters were sufficient to discriminate between samples. Greater brittleness was observed with the addition of root powders, likely due to fiber disrupting the matrix. Importantly, bioactive compounds central to adaptogenic properties—including eleutheroside B, salidroside, and rosarin—were detected in the enriched crackers, suggesting protein–matrix interactions influenced their release and availability.

Therefore, as chemical interactions between proteins were affected with root incorporation, different pulse protein isolates were incorporated in the present work (Chapter 5). In fact, previous studies have highlighted that the protein source is a key determinant of bioactive release, as protein–compound

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interactions can strongly influence both physicochemical properties and bioaccessibility (Sęczyk et al., 2023). In the present work (Chapter 5), texture and structure differences were observed among crackers made with the different pulse protein isolates from faba bean (FBPI), lentil (LPI), pea (PPI) and lupin (LuPI). FBPI and LuPI crackers showed higher hardness and higher proportions of protein in the β -sheet conformation, although LPI contained more β -turns and PPI more α -helix conformations. Therefore, the higher presence of β -sheet protein conformation, which is a more ordered structure, in FBPI and LuPI crackers, increased the hardness of the crackers.

The incorporation of adaptogenic roots further modified protein conformations and cracker microstructure. ES, rich in insoluble fiber, generally increased dough consistency and promoted β -sheet formation, whereas RR reduced peak development times and increased protein aggregation. Both roots decreased α -helix content in PPI and FBPI, indicating destabilization of ordered structures. In FBPI, a rise in random coil suggested protein unfolding, which may facilitate bioactive release. In fact, microstructural analysis supported these findings. ES tended to form denser, integrated matrices, while RR produced looser or less continuous networks depending on the protein isolate. Such modifications are consistent with previous evidence that fiber–protein interactions can limit hydration (Zhou et al., 2021) and that higher β -sheet content may enhance hydrogen bonding but reduce digestibility (Aryee & Boye, 2017).

Although these physicochemical parameters are important for obtaining a high-quality product, achieving an adaptogenic effect requires not only the presence of bioactive compounds in the final product but also their bioaccessibility after digestion. In terms of bioaccessibility, specific protein–bioactive relationships have been identified. For RR, salidroside release was negatively correlated with β -type aggregates, while tyrosol reached complete bioaccessibility in FBPI, likely facilitated by its high proportion of charged/polar amino acids (Ma et al., 2022). In contrast, ES-derived eleutherosides B and E negatively correlated with α -helix disruption, suggesting that loosening of the protein matrix enabled their release.

Nonetheless, overall bioaccessibility of some compounds (salidroside and rosavin) remained below 50% after digestion, emphasizing the restrictive role of certain protein conformations on release of bioactive molecules.

In summary, the pulse protein source interacted differently with *E. senticosus* (ES) and *R. rosea* (RR) root powders, leading to distinct structural and functional outcomes. In the case of faba bean protein isolate (FBPI) (Chapter 4), the incorporation of root powders resulted in a reduction of disulfide bonds, primary interactions for structure formation in gluten-based systems, while simultaneously increasing hydrophobic and hydrogen bonding interactions. These effects were more pronounced in RR-containing doughs, likely due to the higher soluble fiber content of RR.

The dominance of hydrophobic interactions altered the viscoelastic behavior of the dough, particularly increasing the loss modulus (G''), indicative of a weakened protein network with enhanced flowability. Additionally, these interactions reduced starch retrogradation, as evidenced by lower setback and final viscosity values in the RVA test. This behavior is attributed to restricted amylose chain mobility, limiting amylose's ability to reassociate during cooling.

From a textural perspective, FBPI crackers containing root powders exhibited lower hardness compared to the control, as hydrophobic interactions are weaker than disulfide bonds. However, this shift also resulted in a more brittle cracker structure. These observations were consistent with the findings in Chapter 5, where FBPI crackers with ES or RR showed an increase in random coil protein structures. The increase of these protein structures, likely led to the exposure of hydrophobic regions, which promoted protein-protein interactions and aggregation, reducing protein solubility and ultimately contributing to higher hardness values in the crackers relative to crackers formulated with other protein isolates.

In addition, the higher proportion of β -sheet structures in FBPI crackers restricted the release of certain bioactive compounds, such as salidroside and rosavin, due to the ordered and compact nature of the protein structures. Conversely, this structural arrangement enhanced the release and bioaccessibility of other compounds, such as tyrosol.

General discussion

In contrast, pea protein isolate (PPI) and lentil protein isolate (LPI) exhibited higher maximum torque and greater water absorption during mixing. Therefore, the higher hydration of these pulse protein isolates as well as the gluten proteins, possibly led to the formation of a denser protein network, that had a higher resistance during mixing. These rheological findings from Mixolab and GlutoPeak analyses were supported by protein secondary structure data, which showed a higher prevalence of protein aggregates (β -type structures) in PPI and LPI systems. The presence of these protein aggregates were also evident in the cracker microstructure and were associated with a reduction in cracker hardness.

Overall, these results demonstrate that the protein matrix plays a dual role, not only defining cracker texture but also governing adaptogen release. This control is mediated through protein secondary structure, aggregation behavior, and microstructural organization, underscoring the importance of protein selection in designing functional baked products with targeted textural and bioaccessibility outcomes.



CONCLUSIONS, LIMITATIONS AND FUTURE RESEARCH

The conclusions of the present research study are the following:

- This study demonstrated that social media can be a valuable tool for identifying current food trends and consumer demands, enabling the development of products that are better accepted by individuals while also reducing food waste and supporting sustainability.
- Twitter allowed me to gather information about the nutritional needs and food product demands of physically active individuals, as well as their knowledge of these matters. The knowledge of users regarding nutritional diets aligned with the scientific literature, but major gaps were found in macronutrient balance and supplement use during pre-workout. In addition, an association between motivation and pre-workout supplements and exercise was found.
- The most studied adaptogenic plants were found to be *Rhodiola rosea*, *Panax ginseng*, *Eleutherococcus senticosus*, *Withania somnifera*, and *Schisandra chinensis*. However, the incorporation of *E. senticosus* and *R. rosea* into mainstream foods like snacks and bakery products remains limited, with supplements and functional beverages dominating.
- The stability of most bioactive compounds under acidic and high-temperature conditions suggests that dried *R. rosea* and *E. senticosus*

powdered roots have potential to be used as functional ingredients due to their high fiber content and their content of bioactive compounds.

- The incorporation of *R. rosea* and *E. senticosus* powdered roots into protein-enriched crackers with faba bean protein isolate modified dough functionality and cracker texture, while protein–matrix interactions influenced the release and availability of bioactive compounds.
- The use of different pulse protein isolates, along with the addition of root powders from *R. rosea* and *E. senticosus*, significantly modified physicochemical properties and bioactive compound bioaccessibility in crackers. Both roots promoted protein aggregation and structural rearrangements, which restricted the release of some compounds (salidroside and rosin) while favoring others (eleutherosides B and E and tyrosol), depending on the protein isolate source.

In summary, the findings from this research study showed that the bioactive compounds are more bioaccessible depending on the pulse protein source used to develop the protein-enriched crackers. However, there are a few limitations:

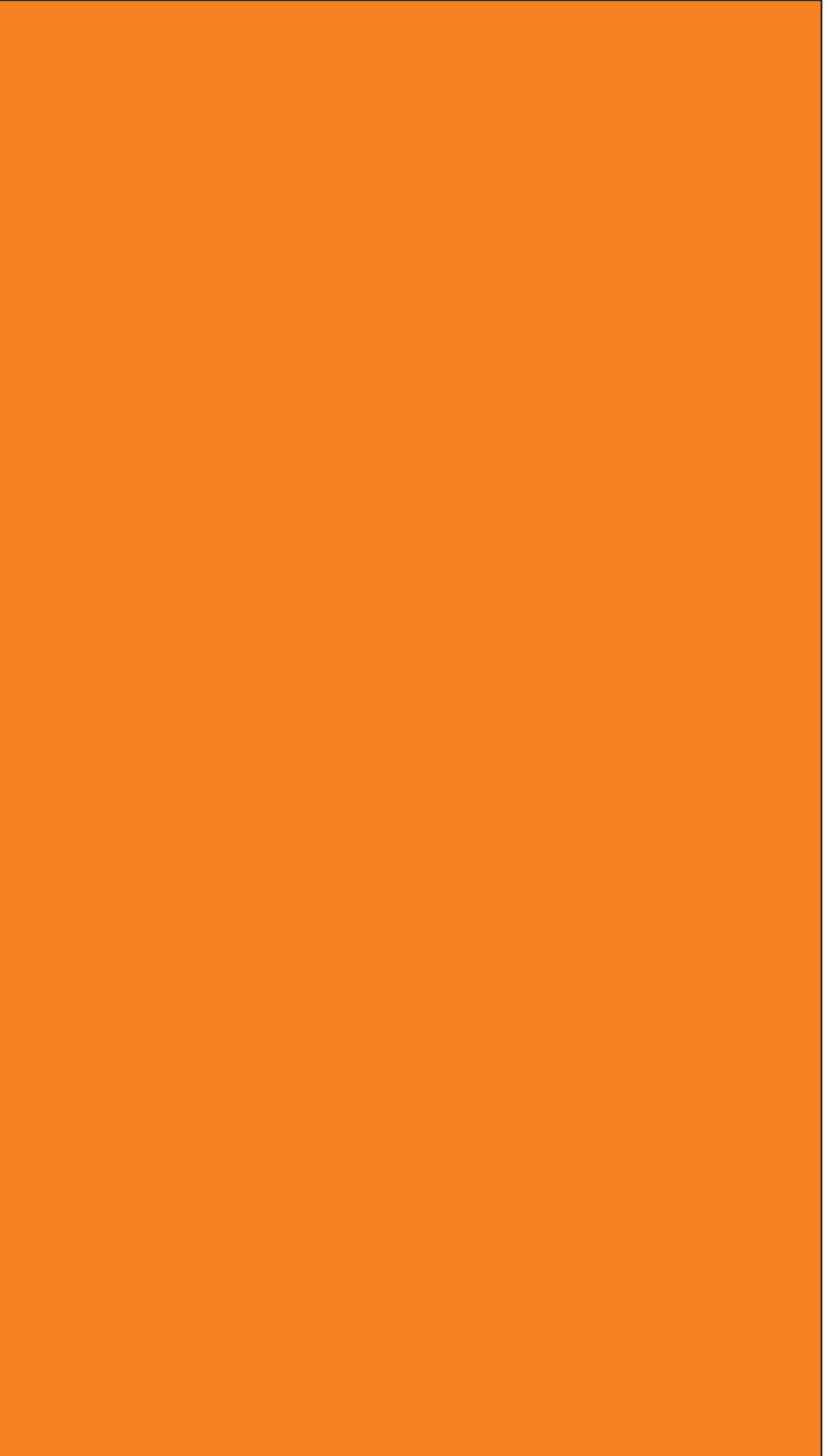
- Bioaccessibility was evaluated only *in vitro*: No *in vivo* confirmation of adaptogen absorption or physiological effects was conducted and this limits translation of research results to human health outcomes.
- Limited consumer perspective: No sensory evaluation or consumer acceptance testing was performed, and these are critical for market relevance.
- Stability of bioactive compounds: Other variables such as storage and shelf life have not been studied. Hence, the extent of degradation of bioactive compounds beyond immediate processing is unknown.
- Protein focus: Because of the structural role of protein in bakery systems, protein–bioactive interactions have been emphasized. Other macronutrient interactions (lipids, polysaccharides) have not been explored.

Conclusion and future research

Building upon these findings and limitations, several avenues for future research should be considered.

- The observed interactions between proteins and bioactive compounds indicate that the type of protein and its secondary structure can influence compound release. However, further research is needed to investigate potential interactions with other macronutrients, such as carbohydrates, which are also integral components of the cracker matrix.
- Given the high moisture content of crackers formulated with FBPI and LuPI, investigating their shelf life is essential. Moreover, the results obtained when assessing the effects of processing conditions (temperature and pH) on the bioactive compounds showed that these factors differentially influence their stability. Therefore, future studies should also address the stability of bioactive compounds during storage.
- Scientific literature reports that pulse protein isolates are often associated with bitterness and beany flavors. As shown in the present study, they also alter textural properties. Therefore, sensory evaluations are necessary to determine consumer acceptance with respect to flavor, texture, and appearance.
- Based on the bioaccessibility results of the compounds present in the pulse protein-isolate-enriched crackers, I can confirm that these compounds are bioaccessible, an essential aspect since it enables their absorption by the body. However, a controlled human intervention study is needed to evaluate the efficacy of these bioactive compounds when consumed in the developed crackers, and their effects on both mood and physical performance. This could be designed as a randomized placebo-controlled trial with physically active individuals as participants, including two treatment groups (crackers enriched with *Rhodiola rosea* or *Eleutherococcus senticosus*) and one control group (crackers without adaptogenic roots). Mood states could be assessed using the Profile of Mood States (POMS) questionnaire, which

is widely recognized for its efficacy in evaluating psychological responses in athletes.



CONTRIBUTIONS OF AUTHORS

Publication 1: Exploring consumers' perceptions and biases on eating behaviors and sport nutrition: A twitter perspective

Carla N. Molina: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Nicola Gasparre:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Raquel Garzon:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Martin G. Scanlon:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Jordi Mañes Vinuesa:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Publication 2: Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

Carla N. Molina: Writing—original draft, Investigation, Data curation, Formal analysis, Methodology. **Nicola Gasparre:** Conceptualization, Supervision, Formal analysis, Writing—review and editing. **Jordi Mañes Vinuesa:** Conceptualization, Investigation, Supervision, Writing—review & editing. **Martin G. Scanlon:** Conceptualization, Investigation, Supervision, Writing—review & editing, Resources, Project administration.

Publication 3. Development of high-protein functional crackers enriched with adaptogenic compounds

Carla N. Molina: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Nicola Gasparre:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Jordi Mañes Vinuesa:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Martin G. Scanlon:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Publication 4. Protein matrix interactions and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*.

Carla N. Molina: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Nicola Gasparre:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Nandika Bandara:** Writing – review & editing, Validation. **Jordi Mañes Vinuesa:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Martin G. Scanlon:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Publication 5. Mapping the growth and trends of plant adaptogens in food: A comprehensive analysis of literature and market insights

Carla N. Molina: Writing – original draft; investigation; data curation; formal analysis; methodology. **Fatma Boukid:** Conceptualization; supervision; formal analysis; writing – review and editing. **Jordi Mañes Vinuesa:** Conceptualization; investigation; supervision; writing – review and editing. **Martin G. Scanlon:** Conceptualization; investigation; supervision; writing – review and editing; resources; project administration.

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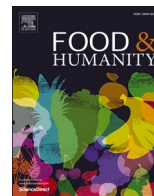
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APPENDIX 1: PUBLISHED RESEARCH PAPERS



Exploring consumers' perceptions and biases on eating behaviors and sport nutrition: A twitter perspective

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ABSTRACT

Sports and endurance activities have become a growing practice as people adopt a more active lifestyle. However, most physically active Twitter users seem to have limited knowledge about nutritional needs or supplementation. This study aimed to mine Twitter data to provide insight into the consumers' perceptions related to food and nutrient needs in physical activities. Using the search terms *Food and Fitness*, and *Gym and Fitness*, a total of 62,130 tweets were retrieved, filtered, and analyzed to obtain co-occurrence networks. Data showed that the primary goal for physically active users on Twitter was found to be muscle gain, primarily through bodybuilding exercises. Most of these users tend to follow either a vegan or ketogenic diet. In addition, the study highlighted disparities between people's understanding of various fitness clusters and the scientific literature. Generally, the knowledge level of physically active Twitter users matched well with the clusters related to *diet* and *physical objectives*. However, significant gaps were observed particularly in understanding macronutrient composition and the usage of supplements before workouts, where their knowledge deviated significantly from scientific consensus. Therefore, this study represents a cost-effective technique to identify the main sport activities and food categories used by physically active Twitter users, as well as to investigate the general levels of nutritional knowledge.

1. Introduction

Setting goals for healthier lifestyles is becoming a growing practice worldwide. In fact, sports and endurance activities are already part of the daily lives of many individuals who are pursuing an active lifestyle. Evidence of that is the increase in the health and fitness market in recent years, which generating an estimated revenue of \$96.7 billion worldwide (2019), with a prediction of \$136.6 billion by the end of the 2024 (Statista, 2021). Activities such as physical activity, exercise, physical fitness and sport are included in definitions for that market, despite their different meanings. While physical activity is defined by the World Health Organization (WHO) as "any bodily movement produced by skeletal muscles that requires energy expenditure", exercise is used to describe "any physical activity that is planned, structured, repetitive, and purposive in the sense that improvement or maintenance of one or more components of physical fitness is an objective" (World Health Organization, 2020). As far as physical fitness and sport are concerned,

the former is defined as a set of attributes/components such as muscular endurance and body composition that are either health- or skill-related, while the latter term is related to a subset of exercises undertaken individually or in a team.

The main reason behind practicing a sport is its significant health benefits, mainly associated with blood pressure reduction, maintenance of a healthy body weight, lowering of total- and low-density lipoprotein levels, improvement of cardiorespiratory and muscular fitness, as well as an increase in the amount of high-density lipoprotein (Kapoor et al., 2022).

Some studies have reported the greater understanding of physically active individuals and athletes regarding general nutrition compared to sports nutrition concepts (Devlin et al., 2017; Tam et al., 2021). As a result, they often consume an inadequate diet to support their energy requirements and/or nutritional needs (Tam et al., 2021). Therefore, moved by the need for covering their sport nutrition knowledge, physically active individuals commonly resort to different sources, such as

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Chemical profiling and processing effects on bioactive compounds in *Rhodiola rosea* and *Eleutherococcus senticosus*

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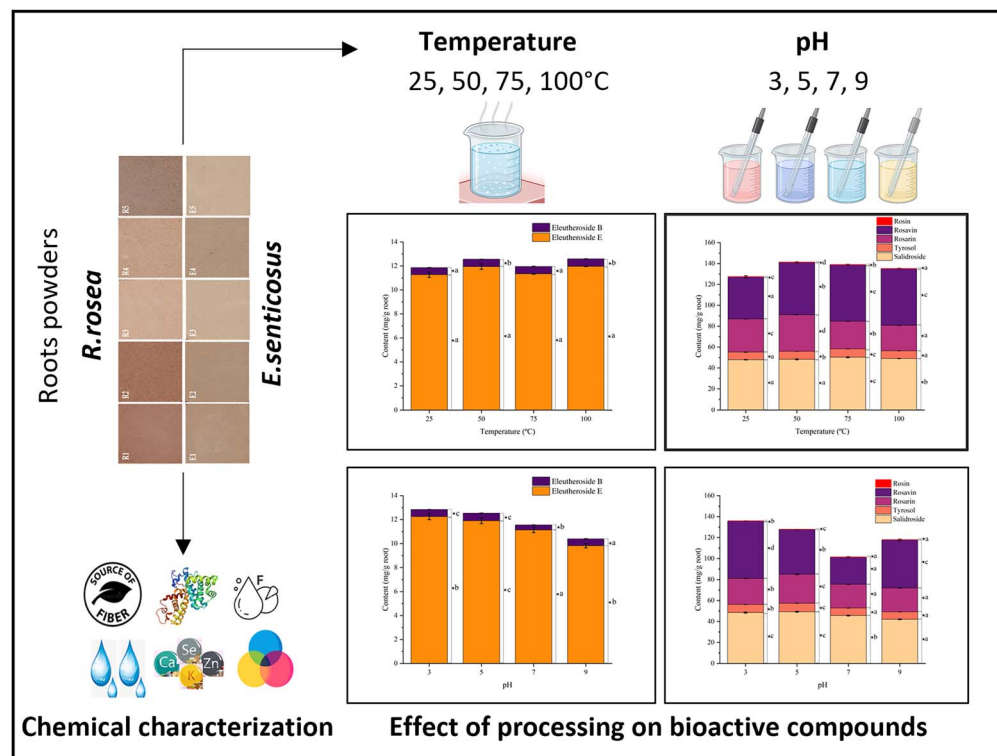
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Abstract

Rhodiola rosea and *Eleutherococcus senticosus* are widely studied adaptogens, often consumed as herbal tea or supplements. However, further insights into their roots are needed to expand their applications. The aim of this study was to examine their proximate composition and the impact of processing conditions (pH and temperature) on their bioactive compounds. The results revealed that both roots are abundant sources of fibre, particularly *E. senticosus* (70.89 to 77.96 g/100 g). Moreover, rosavin and salidroside were highest in samples R3-R5, with concentrations ranging from 55 to 68 and 25 to 69 mg/g root, respectively. As for *E. senticosus*, eleutheroside E showed similar contents within samples (10–15 mg/g root). Optimal extraction occurred at pH 3–5 and 50 °C, except for rosavin and salidroside. The stability of most bioactive compounds under acidic and high-temperature conditions suggests that dried *R. rosea* and *E. senticosus* roots have potential for functional food development.

Keywords: degradation, extraction/separation, health foods, processing effects, solubility

Graphical abstract



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Development of high-protein functional crackers enriched with adaptogenic compounds

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ABSTRACT

Consumers increasingly seek convenient, healthy snacks with innovative flavors and strong nutritional profiles. Traditional crackers are versatile but low in protein. Adding faba bean protein isolate offers a plant-based way to enhance protein content. Incorporating powdered roots from *Rhodiola rosea* (RR) and *Eleutherococcus senticosus* (ES) can further provide functional health benefits. This study aimed to develop high-protein crackers enriched with root powders and examined their impact on dough rheology, pasting properties, cracker texture, and bioactive compound stability after processing. RR and ES powders significantly reduced both G' and G'' moduli of the dough and increased apparent peak and final viscosity. Cracker hardness and distance to break decreased with root addition. Quantification of bioactive compounds confirmed that higher RR and ES levels led to increased salidroside, tyrosol, rosarin and eleutheroside E contents. These findings highlight the functional and structural advantages of incorporating *Rhodiola rosea* and *Eleutherococcus senticosus* into cracker formulations.

1. Introduction

Modern lifestyles, along with evolving dietary preferences, are driving the demand for convenient and healthy on-the-go snacks. In this context, crackers have emerged as a versatile snack category, presenting significant opportunities for innovation in aspects such as texture, flavor, and color (Innsai Monitor, 2024). According to recent projections, the global cracker market is expected to grow at an annual rate of 6.2 % between 2025 and 2030 (Statista, 2025). However, from a nutritional point of view, crackers do not necessarily provide nutrient profiles that appeal to all consumers. In particular, crackers are low in protein, ranging from 7 to 8 % (Han et al., 2010).

As many consumers now seek for high protein snacks, the enrichment of crackers with protein is of pivotal importance. Proteins can be derived from different sources such as plant and animal. Although animal proteins, especially whey, are considered excellent sources of essential amino acids, plant proteins are gaining more attention for their high nutritional value, including a relatively balanced amino acid profile and a moderate digestibility (Anyiam et al., 2025). Moreover, their increased consumption is largely driven by their lower environmental footprint, particularly in terms of greenhouse gas emissions, and their potential health benefits related to the reduction of cardiovascular

disease risk (Rout & Srivastav, 2025). In this context, faba bean (*Vicia faba* L.) is considered a high protein and high fiber source, while low in fats (Gasparre et al., 2024). This makes it ideal for producing protein-rich ingredient fractions such as faba bean protein isolate. Faba bean protein isolate has been used for producing meat analogues, bread, pasta, biscuits and extruded snacks (Hsu et al., 1982; Ferawati et al., 2021; Martin et al., 2022). However, little research has been done regarding the use of faba bean protein isolates in baked snacks such as crackers. Hence, crackers offer a promising opportunity to promote wellness by delivering health-promoting ingredients.

Medicinal herbs are recognized for their health benefits including their effects on the central nervous system due to the presence of secondary metabolites that are present in plants. Among the various medicinal herbs, a limited group of them exhibit adaptogenic properties, traits that are related to the capacity of the body to respond to different emotional, mental, and physical stressors (Tóth-Mészáros et al., 2023). In this context, *Rhodiola rosea* and *Eleutherococcus senticosus* have gained significant popularity worldwide and are among the most popular plants used in Europe and Asia for health and wellness reasons. These two medicinal herbs have been widely consumed in different forms such as teas, beverages, and pharmaceutical forms alone or in combination with other medicinal herbs (AAFC, 2016; Lee et al., 1999; Ma et al., 2011). In

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

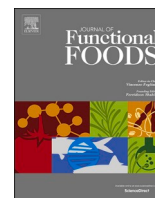
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.118257>.

Data availability

Data will be made available on request.

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Protein matrix interactions and adaptogenic bioactive release in high-protein cracker systems enriched with *Rhodiola rosea* and *Eleutherococcus senticosus*

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Bioaccessibility

ABSTRACT

Bioactive compounds like phenylpropanoids and phenylethanoids, found in adaptogenic roots *Rhodiola rosea* (RR) and *Eleutherococcus senticosus* (ES), are commonly used in supplements. Crackers made with pulse protein isolates represent a vehicle to deliver these compounds. This study investigated interactions of RR and ES root powders with protein isolates (pea (PPI), faba bean (FBPI), lentil (LPI) and lupin (LuPI)) and their impact on dough and cracker physicochemical properties. In addition, *in vitro* bioaccessibility was studied. Root powders significantly increased dough consistency, with RR promoted protein aggregation. In the crackers, a reduced lightness and increased yellowness was shown, particularly with RR. FBPI control crackers were the hardest and stiffest, while root addition generally softened texture. FTIR revealed that secondary structure changes influenced *in vitro* bioaccessibility, with β -sheets and protein aggregates limiting and random coil enhancing release of bioactives. Combining adaptogenic roots with pulse proteins is a promising strategy for producing functional high-protein crackers.

1. Introduction

Bioactive compounds are phytochemicals found in plants and certain foods. In the case of plants, roots are a typical matrix from which bioactive compounds can be extracted. Phenylpropanoids and phenylethanoids are two groups of bioactive compounds which are particularly notable for their antioxidant and adaptogenic properties (Esmaelzadeh et al., 2022). These groups of bioactive compounds are distributed in medicinal plants, such as *Rhodiola rosea* and *Eleutherococcus senticosus* (Tóth-Mészáros et al., 2023). These medicinal plants have been used as potential remedies for various diseases such as cancer and arthritis, among others (Rashid et al., 2024). The primary application of these medicinal herbs is in dietary supplements where the bioactive compounds are extracted from the root powder. The root powders themselves are largely limited to incorporation into herbal infusions, energy drinks and coffee (AAFC, 2016). To expand the use of adaptogenic compounds beyond beverages, solid food matrices, like crackers, present a promising delivery platform that will broaden consumer appeal. Crackers, a form of crisp bread characterized by low fat and sugar content, are widely consumed for their convenience and versatility.

Moreover, they can also represent a flexible and innovative platform to meet evolving consumer demands such as high protein food products (Huamání-Perales et al., 2024).

Pulse protein isolates have emerged as cornerstone ingredients in new product formulations for high-protein foods, due to consumer preferences and to their relatively low cost, compared to animal-derived proteins (Nogueira et al., 2021). Canada is one of the countries leading the global production of pulses (World Milling and Pulses Technologies, 2025). Beans, lentils, chickpeas, peas and lupins are among the most known and consumed pulses worldwide (Ferreira et al., 2021). Studies involving the incorporation of pulse protein isolates into crackers have primarily focused on the rheological properties of the dough, and the texture, microstructure, *in vitro* enzymatic hydrolysis, antioxidant activity and sensory evaluation of the crackers (Gangola et al., 2022; Han et al., 2010; Madhavan & Sreekantaiah, 2025; Morales-Polanco et al., 2017). In these studies, 2.5–46.2 % of pea or faba bean protein isolate was used to replace wheat flour. However, some other authors replaced 100 % of the wheat flour with pea protein isolate to obtain gluten-free crackers (Han et al., 2010).

However, the techno-functional properties of pulse proteins as well

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jff.2025.107086>.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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