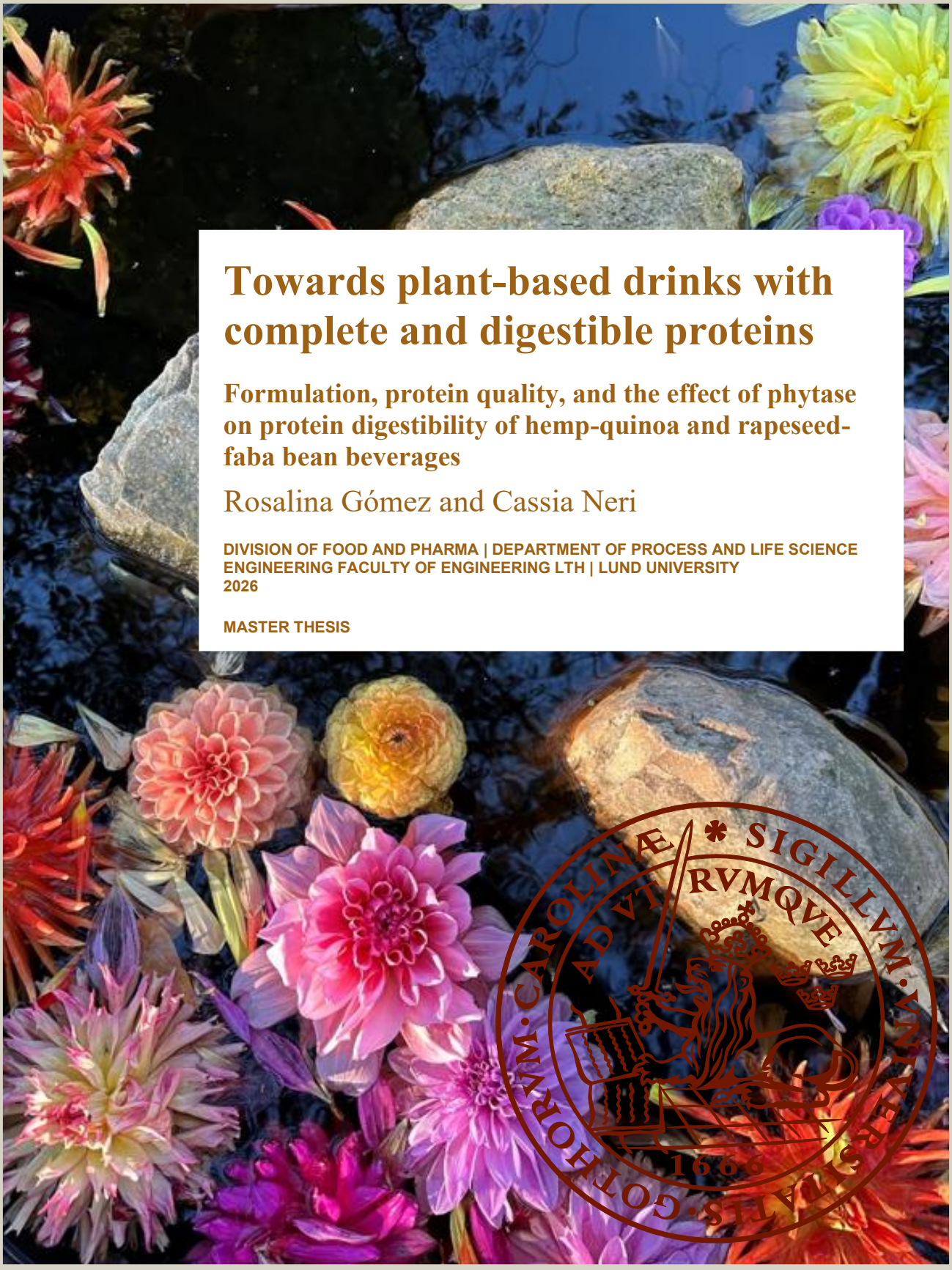

Towards plant-based drinks with complete and digestible proteins

Formulation, protein quality, and the effect of phytase on protein digestibility of hemp-quinoa and rapeseed-faba bean beverages

Rosalina Gómez and Cassia Neri

DIVISION OF FOOD AND PHARMA | DEPARTMENT OF PROCESS AND LIFE SCIENCE ENGINEERING FACULTY OF ENGINEERING LTH | LUND UNIVERSITY

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MASTER THESIS

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Popular science summary

Plant-based drinks with better protein: is it possible?

Yes. And we can (almost) prove it. Plant beverages can have all the essential amino acids (those our bodies cannot make on their own) in the right amounts, meaning they can be complete protein sources just like cow's milk, when foods such as beans and seeds are combined, as they complete each other's missing amino acids. We set out to make two drinks: hemp-quinoa team on one side, and rapeseed-faba bean isolates on the other, and the result is... that they are extremely close to being complete!

Both drinks have all 9 essential amino acids in almost the perfect ratio for adults, and by that we mean meeting 100% of our body's needs. By combining hemp and quinoa whole seeds, lysine is the only essential amino acid that does not fully reach the target, reaching 96% of the amount adults need. The rapeseed and faba bean isolates make a beverage that is missing a bit of leucine and valine, meeting 99% and 97% of our needs, respectively. These gaps are very small, and a few adjustments to the ingredient ratios and to the formulation could push both drinks toward becoming complete protein sources.

Still, both beverages are already far ahead of most plant milk-alternatives on the market, including oat, almond, and rice drinks. The rapeseed-faba bean drink's nutritional profile is similar to or even better than cow's milk. It has 3.4% protein, the same as cow's milk, but with lower levels of saturated fats and sugar. The quinoa-hemp beverage has less protein (1.1%), but this amount is still higher than almond and rice beverages, and it also has a low fat and sugar content.

Why does any of this matter? A growing number of people are choosing plant-based diets for environmental and ethical reasons, but also for personal health benefits. Sweden's plant-based dairy market alone is estimated at 140 million dollars. However, most plant-based milk alternatives are weak protein sources, with low protein content, incomplete amino acid profiles, and proteins that are difficult to digest. There is a real market gap for plant drinks that can actually compete with cow's milk on protein quality and quantity.

Better protein does not only mean having all the essential amino acids in the right amounts, but it also means making sure that the protein is easy to digest, so that the amino acids are released during digestion, then absorbed, and made available for the body to use. Plant proteins are usually more difficult to digest than animal proteins for a few reasons. One of them is that they contain antinutrients, such as phytic acid, which binds to minerals and proteins and blocks them from being properly digested. For this very reason, we decided to add an extra ingredient to our formulation: phytase, the enzyme that breaks down phytic acid. By breaking down phytic acid, the proteins and minerals are freed and can be digested and absorbed. By reducing phytic acid, we aimed to make the digestion of protein easier.

But how do we know if our plan actually worked? As scientists often do, we simulated the plant-based milk-alternatives' digestion in the lab, analysed the drinks before and after digestion with and without phytase, and then compared results. The enzymatic treatment did reduce phytic acid

in both beverages before digestion in moderate amounts, however, we found no clear evidence that the proteins were digested more easily. We think that not enough phytic acid was reduced, and that it would be interesting to explore if, by accommodating the enzyme to its preferred conditions (it works best in acidic environments, but our drinks are neutral), the phytic acid could be further reduced and perhaps lead to better-digested proteins.

For our beverages, we picked unconventional ingredient combinations, hemp + quinoa and rapeseed + faba bean, that do not exist as commercial products today. We also tried to choose our ingredients based on how sustainable they are. The rapeseed protein isolate (Vertis™ CanolaPRO®) is a certified upcycled plant protein obtained from rapeseed meal, oil extraction leftovers. Faba bean crops have low carbon footprints and fix nitrogen from the air, meaning less fertilisers needs to be used, while hemp is considered a highly sustainable crop because it absorbs and stores carbon dioxide.

This work provides a starting point for researchers and food developers interested in creating better plant-based milk alternatives. It shows that combining plant sources that complete each other's amino acid profiles is a powerful strategy for improving amino acid deficiencies and potentially reaching protein completeness, with careful considerations about the ingredients' ratio. It also provides knowledge on treating foods with phytase as a base for future research into improving plant protein digestibility.

Abstract

There is a market gap regarding diversity within plant-based beverages with a complete amino acid profile. For this reason, new ingredients that could be used for the formulation of two plant-based drinks were researched: one combining raw ingredients like seeds, and the other one using protein isolates. Once the final formulations were decided, the resulted drinks included a beverage made of quinoa and hemp hearts, and another one made from rapeseed and faba bean protein isolates.

Afterwards, the drinks were analysed following the Dumas method. Its total nitrogen and protein content were evaluated, and for the hemp-quinoa drink a 1.12% of protein was obtained, while for the rapeseed-faba drink, 3.36% protein content was reached.

Following the calculation of the amino acid scores during the formulation step, the protein digestibility-corrected amino acid score (PDCAAS) and the digestible indispensable amino acid score (DIAAS) were calculated for the hemp-quinoa (87%) and the rapeseed-faba (94%) beverages, respectively. In order to find out the actual amount of all the amino acids present in each of the drinks, samples were sent to Eurofins, where a total amino acid analysis was performed. The FAO/WHO amino acid requirements for adults were used as a reference. Using these values, for the hemp-quinoa beverage the only limiting amino acid was lysine (96%), while the estimated AAS presented leucine and lysine as the limiting amino acids (97.3% and 97.2%, respectively). As for the rapeseed-faba drink, while the estimated AAS was over 100%, the actual values presented two limiting amino acids: leucine (99%) and valine (97%).

Since both drinks were of plant-based origin, the study of the presence of different antinutrients was contemplated. Phytic acid was especially considered, as it can bind to proteins hindering their absorption rates. The use of food-grade phytase was applied to reduce the phytic acid content and, subsequently, the Bradford assay was used to evaluate if it led to an increase in the solubility of the proteins, and phytic acid content was also measured. The results concluded that even though the phytic acid was reduced after phytase treatment, this did not impact the protein solubility.

An *in vitro* enzymatic digestion was performed following the INFOGEST protocol in both beverages before and after the phytase treatment. The resulting products were analysed using a colourimetric assay (OPA) and SDS-PAGE to evaluate the effectiveness of the digestion and of the phytase treatment. The conclusion reached was that the digestion indeed worked, as the concentration of free amino acids increased and the band intensity in the gel was lower for the digested samples, but with no significant difference between phytase-treated and untreated samples.

The conclusion reached was that there is a challenge in finding the balance between making a plant-based beverage with a complete amino acid profile while keeping it palatable by consumers. Even with the limitations involved in this thesis like time and equipment, two beverages were formulated that almost met the amino acid score goal while achieving positive flavours and mouthfeel.

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First and foremost, we want to thank everyone at the department of Biotechnology and Applied Microbiology for allowing us to work there and use their facilities.

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Finally, we wanted to thank our friends for making this experience much more enriching. It's through their support and perspective that we were able to overcome all difficulties and complete this process that helped us become better professionals.

List of Abbreviations

AAS: amino acid score

ANF: antinutritional factor

ANOVA: One-Way Analysis of Variance

BSA: bovine serum albumin

$CaCl_2(H_2O)_2$: calcium chloride dihydrate

DIAAS: digestible indispensable amino acid score

FAO: Food and Agriculture Organisation

GI: glycaemic index

HCl: hydrochloric acid

HQB: hemp-quinoa beverage

H_2SO_4: sulphuric acid

IAA: indispensable amino acid

K_2HPO_4: dipotassium phosphate

kDa: kilodalton

M: molar

mM: millimolar

NaOH: sodium hydroxide

OPA: o-pthalaldehyde

PDCAAS: protein digestibility-corrected amino acid score

pI: isoelectric point

RFB: rapeseed-faba beverage

SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SGF: simulated gastric fluid

SID: standardised ileal digestibility

SIF: simulated intestinal fluid

SSF: simulated salivary fluid

TCA: trichloroacetic acid

TID: true ileal digestibility

V: volts

WHO: World Health Organisation

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1. Introduction

1.1 Background

As the world population is growing at a faster rate than what current food systems can support, transitioning towards plant-based diets has become increasingly important both for sustainability and food security (Sabaté & Soret, 2014). In the last decades, many people have decided to adjust their diets also based on health reasons and moral values. This has resulted in the food industry continuously adapting to new trends, including meat analogues and dairy alternatives that can offer the same nutritional benefits as their animal-based counterparts (McClements & Grossmann, 2022).

Alternatives to dairy products, especially, offer a solution not just for vegans, but for people with allergies or intolerances. Only in Sweden, the plant-based dairy market value is estimated to be around $140 million, as of latest reports (Sweden Plant-Based Dairy Market Report: Trends, Growth and Forecast (2026-2032), 2025). Even though some options already exist on the market, there is still a clear gap regarding complete protein options for drinks that are supposed to be an alternative to cow's milk. It is this problem that brought the need to investigate if it is possible to formulate a plant-based drink using new ingredients to achieve a complete amino acid profile.

The work was completed in collaboration between Tetra Pak and Lund University.

1.2 Aim

The aim of this master's thesis was to fill a market gap by formulating two stable plant-based beverages with a complete amino acid profile and good sensory properties. One of the drinks was to be formulated using raw, whole ingredients, like seeds, while the other consisted of protein isolates as the main ingredient. The reason behind this aim is that plant-based drinks usually do not offer a nutritionally comparable alternative to cow's milk, and that is why during this project, the combination of different ingredients to obtain a complete protein source was studied.

To achieve this aim specific objectives were decided:

- To study the amino acid scores and the protein quality of the beverages using PDCAAS and DIAAS, to reach the ratios that would confer the best amino acid profiles.
- To conduct a series of diverse analyses to study the stability of those drinks, like sedimentation rates, pH, protein solubility, starch content, and fat globule size.
- To evaluate whether the use of food-grade phytase could increase the bioavailability and digestibility of proteins using the Bradford assay and analysing the content of phytic acid in the drinks before and after treating them with phytase.
- To evaluate digestibility *in vitro* following the INFOGEST protocol.
- To analyse the protein profile of the drinks before digestion using the Dumas method, and before and after digestion using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as well as a colourimetric assay (OPA).
- To perform a sensory analysis to study the taste and mouthfeel of said beverages, as well as to evaluate possible consumer acceptability.

2. Theoretical Background

2.1 Plant Protein Quality

Diets rich in plant-based products are often connected to beneficial health effects, like a reduced risk of cardiovascular disease and obesity (Sharma et al., 2025). However, new plant-based products placed on the market as alternatives to traditionally animal-based foods, such as plant-based beverages as a replacement for cow's milk, should be nutritionally comparable to the products they are aiming to replace. There are several effects on health associated to protein quality, both in the short-term, with consequences such as stunting, a weakened immune system, or even a decline in mental performance, and in the long-term, with effects such as accelerated ageing and cognitive decline (FAO, 2013). Given this situation, it is essential to make sure that people are getting the right amount and quality of protein from their diet. The protein sources selected for the study were hemp and quinoa whole seeds, and rapeseed and faba bean protein isolates.

2.1.1 Protein quality

Proteins supply the human body with nitrogen and amino acids. Amino acids are required for making proteins and other molecules needed to support the body's typical functioning. There are 20 amino acids used for protein synthesis, and among these, nine are considered indispensable amino acids (IAA). These amino acids must be obtained through food since the human body is not able to produce them, and for this reason are classified as essential (Calvez et al., 2024). Protein quality refers to how well a protein source can supply nitrogen and all IAAs based on the body's requirements across various human conditions, including growth, maintenance, pregnancy, and lactation (Calvez et al., 2024).

2.1.2 Protein digestion

Just like with other macronutrients, protein digestion mainly consists of the breakdown of bigger structures into smaller molecules that are easier to be absorbed by the organism. In particular for proteins, during digestion, these molecules are broken into small peptides and free amino acids ready to be absorbed in the small intestine (Bhutta & Sadiq, 2013).

Physiologically, different organs take part in the digestion of proteins, and during each of these phases, different enzymes participate in the hydrolysis of these molecules. Proteins' digestion starts in the stomach, where pepsin breaks down big proteins into polypeptides and free amino acids.

Then, these new molecules reach the small intestine, where different pancreatic enzymes further break these polypeptides into smaller molecules. It is here, in the small intestine, that the end products will be absorbed. The residual non-digested proteins reach the colon, where some short-chain fatty acids are produced (Bhutta & Sadiq, 2013).

2.1.3 Protein quality assessment methods

The methods used to evaluate protein quality have changed throughout the years. With the amino acid score (AAS), the amino acid composition of a protein is compared to a reference pattern. This method provides information about amino acid composition, but it does not account for digestibility and bioavailability of the protein and the amino acids (FAO, 2013). A protein can have a complete amino acid composition, but if its digestibility is low, it may still not meet human nutritional needs.

The protein digestibility corrected amino acid score (PDCAAS), adopted by FAO/WHO in 1989, represented an important improvement as protein digestibility was taken into account in the assessment of protein quality. However, this method also had some limitations, including the use of a single crude protein digestibility value instead of individual amino acid values. Additionally, protein digestibility was determined at faecal level instead of ileal, and faecal measurements tend to be inaccurate (FAO, 2013).

In 2011, FAO suggested replacing PDCAAS with the digestible indispensable amino acid score (DIAAS) as the preferred method for assessing protein quality. DIAAS addressed the limitations of PDCAAS by using ileal digestibility values for each individual amino acid (FAO, 2013). A DIAAS below 75 represents protein with no claim to quality, 75-99 indicate high-quality protein, and for values above or equal to 100, the protein quality is considered to be excellent. Usually, plant proteins have a lower DIAAS than animal proteins, and this highlights the challenge of obtaining high-quality protein in plant-based beverages, especially if made from one only plant protein source (Kaur et al., 2022).

2.1.4 Plant proteins

The source of the protein determines its quality, and plant proteins generally have a lower protein quality compared to animal proteins. This is due to several reasons. First, many plant proteins do not contain the right amount of one or more IAAs, therefore have an incomplete amino acid profile and are unable to meet human requirements. Legume proteins are usually limiting in sulphur-containing amino acids (methionine and cysteine), while cereal proteins are limiting in lysine (Kaur et al., 2022). This means that when consumed alone, these foods do not provide all the IAAs in the required amounts. Moreover, plant proteins tend to be less digestible due to the proteins being trapped inside plant cell walls that are not digestible and for this reason they are less accessible to digestive enzymes (Calvez et al., 2024).

2.1.5 Factors that affect protein quality

Many different factors can affect how proteins are digested and to what extent they are absorbed and utilised by the organism. Some of these factors are related to how the food product is processed before consumption or even its physicochemical properties, while others rely on the presence or absence of antinutritional factors (ANFs). Antinutritional factors are substances that can form complexes with different nutrients and minerals, hindering their absorption rates (Thakur et al., 2019). To improve the bioavailability of nutrients, the reduction or elimination of these ANFs is recommended.

Since most antinutrients are mostly present in grains, beans, seeds and nuts, plant-based food products are the ones that present a bigger challenge regarding these molecules. One of these ANFs, known as phytic acid, is used as a phosphate storage unit for these ingredients, leading to lower absorption rates of minerals like iron, zinc or magnesium, as well as proteins. There are different methods used to reduce or eliminate this antinutrient, which include the soaking and germination of the raw ingredients, as well as fermentation or the use of enzymes (phytases), among others (Singh & Arora, 2023). The reduction of these ANFs would then result in an increase in the absorption of the nutrients, thus increasing their quality.

2.1.6 Methods to improve plant protein quality

2.1.6.1 Complementary strategy

Protein complementation is a nutritional strategy that can be used to improve the protein quality of a diet by combining protein sources that have complementary amino acid profiles in order to achieve a complete IAA pattern. An example is combining legumes, rich in lysine but limiting in methionine, with cereals, rich in methionine and limiting in lysine (Mariotti & Gardner, 2019). This strategy was used for the formulation of the two beverages in this study.

2.1.6.2 Enzymes

One of the ways to reduce ANFs and thus increase the absorption rates of different nutrients relies on the use of enzymes. This practice is not related just to antinutrients, as it can also be used to increase the bioavailability and absorption of big molecules like proteins, with the use of proteases.

Depending on the target molecule, different enzymes need to be used. One way would be to study which factor is hindering the absorption of proteins, whereas it is the presence of different antinutrients, or the lack of specific enzymes that can digest these within the human body. Then, based on the results, the specific needed enzymes would be chosen, like phytases for phytic acid.

2.2 Ingredients

To formulate the beverages, two different approaches were established: one based on raw ingredients, like seeds, and one based on protein isolates.

2.2.1 Raw ingredient beverage

The chosen ingredients presented some benefits in comparison with the usual ingredients used during the formulation of plant-based drinks, like better amino acid profiles, and a lower carbon footprint, as most of them could be sourced locally. In the end, the chosen ingredients were quinoa and hemp hearts, along with dates used as a sweetener, but yellow lupin and pumpkin seeds were also considered based on their complementary amino acid profiles.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal especially relevant in South America (Guo et al., 2025) that has gained popularity in Europe in recent years. Its nutritional content is the main

factor behind its increasing popularity, as it is considered a complete protein, meaning it contains all essential amino acids, something not so common among plant-based ingredients (Craine & Murphy, 2020). As for **hemp hearts** (*Cannabis sativa* L.), both its nutritional composition and its good digestibility rates are behind its growing popularity, along with its ability to absorb and store carbon dioxide, which compared with cereals can be around 15 times higher (Hemp – The Ideal Crop for Carbon Sequestration? n.d.). Hemp, as well as quinoa, is considered a complete protein, making it a good plant-based protein source (Chauhan, 2021).

Dates (*Phoenix dactylifera* L.) have a high percentage of carbohydrates, especially sugar, while it presents low quantities of other macronutrients like fat and protein (Al-Farsi & Lee, 2008). The reason why it was chosen as an ingredient is that not only it works as a sweetener, but it also presents a lower glycaemic index (GI) than table sugar, with an average GI of 42, which is considered low (Canadian Diabetes Association, 2021). Another reason behind why it was chosen as a sweetener, instead of, for example, cane sugar, is because it is considered as a more sustainable option. While intensive cultivation practices are usually related to non-sustainable standards, proper cultivation of climate-resistant crops as date palm trees align with the UN's Zero Hunger goal and helps preserve biodiversity in protected areas like Elche and Orihuela in the south of Spain (Sayas-Barberá et al., 2023).

Yellow lupin (*Lupinus luteus*) is a pulse usually consumed in the countries surrounding the Mediterranean Sea. Though it is more commonly used for animal feed, its nutritional content, specifically the proteins, has been an important factor behind why it has gained popularity in the recent years as a food ingredient for human consumption (Chukwuejim et al., 2024).

Pumpkin seeds are widely used worldwide based both on its organoleptic and nutritional properties (Hadidi et al., 2025). They were considered as an ingredient for the formulation based on its protein and fat content, as well as its amino acid profile, complementary to yellow lupin.

2.2.2 Protein isolate beverage

Four ingredients were initially considered: rapeseed protein isolate, faba bean protein isolate, potato protein isolate, and hemp protein concentrate. Their selection was based on their amino acid profile and protein quality. Sustainability of the ingredients was also taken into consideration. After the first two product development steps, rapeseed and faba bean isolates were chosen. Information about their amino acid profiles can be found in the Appendix (Table A 1).

Rapeseed (*Brassica napus* L.) is an oilseed with a complete amino acid profile. DIAAS values range from 76-83 for the protein isolate, and 100-110 for the heat-treated protein isolate, placing it in the high or even excellent protein quality category. Rapeseed also contains antinutrients, including erucic acid, phytic acid, and glucosinolates. Glucosinolates are linked to bitterness, though they also have antimicrobial and anticarcinogenic characteristics. As erucic acid has been shown to be toxic to animals in high amounts, the rapeseed varieties used for food production are grown to contain low amounts (Axentii & Codină, 2024). From a sustainability perspective, rapeseed protein isolate can be obtained from the byproduct of oil production, which is rapeseed meal, making its use an example of valorising food waste (Di Lena et al., 2023).

Faba bean (*Vicia faba* L.) is a pulse with high protein levels (20-41%). It has high amounts of lysine but is limited in methionine and cysteine. Faba bean contains bioactive peptides, with anti-inflammatory and antioxidant properties, but also antinutrients, specifically phytic acid, vicine and convicine. These last two ANFs are responsible for haemolytic anaemia (favism) in certain individuals with a genetic condition characterised by the lack of a specific enzyme, but breeding strategies can reduce these compounds (Dhull et al., 2022; Martineau-Côté et al., 2022). The DIAAS for whole faba beans is below 75 (Herreman et al., 2020); however, when extracting protein from its native plant structure to make an isolate, the protein quality is known to increase (Matthews et al., 2025). From an agronomic perspective, faba bean is a nitrogen-fixing crop, meaning the need for fertilisers is reduced, contributing to the sustainability aspects of the ingredient (Martineau-Côté et al., 2022).

Potato (*Solanum tuberosum* L.) has a low protein content (1-1.5% fresh weight), but potato protein can be extracted from potato fruit juice, which is what is obtained after potato starch production (Day, 2013; Etzbach et al., 2025). This aligns with circular economy principles by valorising a stream that would otherwise not be used. Potato protein has a complete amino acid profile with a total IAA content of 37%, even higher than what is found in animal proteins such as casein (34%) and eggs (32%) (Sarathy et al., 2025). Its DIAAS value is in the excellent protein quality category (≥100%) (Herreman et al., 2020).

Hemp (*Cannabis sativa* L.) is a seed with a complete amino acid profile and a high protein content (31.6 g per 100 g). It is rich in arginine, an amino acid linked with promoting cardiovascular health. However, its protein digestibility values are quite low, falling in the category that cannot declare any protein quality. This is due to antinutrients in the seeds, mainly phytic acid and trypsin inhibitors (Axentii & Codină, 2024). In any case, as mentioned previously, the protein quality value tends to increase when the protein is extracted to make a concentrate or isolate. As for rapeseed, hemp protein can also be obtained from hemp press cake, which is what is left over after oil production from hemp seeds, supporting a sustainable approach to valorising side-streams in food systems (Helstad et al., 2022).

3. Materials and methods

3.1 Product development

3.1.1 Raw ingredient beverage

Different raw ingredients were intended as the main constituents of the beverage. These were decided based on their amino acid profiles, in order to achieve the formulation of a complete protein source, and they consisted of tricolour quinoa, hemp hearts, and pumpkin seeds, all sourced from Rawfoods shop, as well as yellow sweet lupin purchased from Nordisk Råvara.

Other ingredients included dates, sourced from ICA, which were used as a sweetener, and tap water, used as the main solvent. Dipotassium phosphate was used to adjust the pH, and food-grade phytase (Enzymes.bio) was used in order to improve the digestibility and availability of the proteins present in the drink.

Equipment used to reach the final product included a thermomixer (Thermomix® TM5), used to blend all the ingredients together, and a sieve (Vegan Milker® - ChufaMix), used to remove the pulp and big particles. Food-grade thermometers and different kitchen utensils were also used during the production of the drink.

The formulation of the drink included different steps. During this process, different trials were performed, which are shown in detail in the flow charts provided in the Appendix (Figure A 1 and Figure A 2). Each of the trials resulted in drinks with specific parameters that led to adjustments, like excessive particles in the drink, unpleasant taste or mouthfeel, or fast creaming rates. Hereunder, each step followed during the formulation of the product will be explained in detail.

Step 1: Selection of main ingredients

To begin, based on the amino acid profiles of each of the main ingredients, along with its sensory properties (colour, taste, aroma), the different combinations were decided. Two combinations were established as a way to optimize time and resources: yellow lupin and pumpkin seeds for one of them, and quinoa with hemp hearts for the other. Different ratios among these ingredients were decided based on their combined amino acid score, as well as the desired protein content of the final drink.

Step 2: Blend composition

In order to establish the ratios for each of the drinks, the analysis of the amino acid profiles of the ingredients was performed. Different trials were performed for each of the combinations. In the end, the protein digestibility-corrected amino acid score (PDCAAS) was calculated for the final formulation, and it can be found in the results (4.2.4 Protein quality assessment). The sensory properties of each of the blends is presented in Table A 2 in the Appendix.

Step 3: Pre-treatments

The soaking times and temperatures were decided upon optimal taste and mouthfeel through trial and error. As a way to avoid an excessive leaching of nutrients into the soaking water, and following the recommendation from other recipes, room temperature water was used to soak the ingredients.

The cooking times for both quinoa and yellow lupin were based and decided upon the average denaturation temperature of the protein present in these ingredients.

Since yellow lupin's proteins' average denaturation temperature is around 95ºC (Beyer et al., 2024), one of the trials consisted of the production of the yellow lupin-pumpkin seeds milk using raw lupin, to avoid long cooking times. Based on the obtained results, the temperatures and times were adjusted in order to make sure the ingredient was fully cooked. The final conditions were established following the manufacture's recommendations (Nordisk Råvara).

In the case of quinoa, a first trial using raw quinoa was designed to avoid excessive loss of nutrients. Based on taste and mouthfeel, and since the average denaturation temperature of its proteins landed around 90.6 °C (Sargolzaee et al., 2024), the cooking temperature was in the end set to 80-90 °C. As temperature was lowered, the cooking time increased from 15 to 30 minutes.

Step 4: Processing

Different steps were involved during the actual production of the drink, including blending the ingredients together, using a sieve to get rid of the residual pulp, adjusting pH, and finalizing with pasteurisation. In order to choose the combination that could result in a better result, at first only blending and sieving were used for comparison among both drinks, as well as the overall organoleptic properties.

After soaking and cooking the ingredients, the ingredients were added to the Thermomixer according to the decided ratios. The speed was set to maximum velocity, and the time was set to 2 minutes. This allowed for a homogeneous blend to be formed. In order to remove the pulp, a sieve was used following the instructions of the manufacturer. Afterwards, the pH of the blend was measured for future reference.

As the natural pH of the drink was around 6.4-6.45, pH had to be adjusted to around 7.1 by the addition of 0.5g of dipotassium phosphate. This change in pH allowed for a better stability of the drink during the pasteurisation, as it reduced the probabilities of the proteins to aggregate (Duggan et al., 2024).

Pasteurisation time and temperature were decided based on the denaturation temperature of the proteins present both in quinoa (90.6 °C) and hemp hearts (95 °C). According to these, and taking into account equipment limitations, the final parameters were decided to be 65 °C for 15 minutes ± 2 minutes. As soon as the pasteurisation was done, the drink was transferred to 180 ml capacity aseptic containers and placed in an ice bath until it reached 4 °C. Afterwards, these containers were transferred to the cold room (4 °C), where they were kept for further analysis.

3.1.2 Protein isolate beverage

The main protein sources considered for the protein isolate beverage were rapeseed protein isolate (DSM-Firmenich), faba bean protein isolate (Green Goddess), potato protein isolate (Saporepuro), and hemp protein concentrate (Rawfoodshop), as it was not possible to source hemp isolate. The rapeseed isolate used was Vertis™ CanolaPRO®, a certified upcycled plant protein extracted from rapeseed meal.

Additional ingredients included rapeseed oil, agave syrup, salt, cocoa powder, vanilla essence, cinnamon powder, cardamom powder, turmeric powder, black pepper, and honey (ICA). Sunflower lecithin powder, high acyl gellan gum, and inulin (chicory root) were provided by Tetra Pak. Dipotassium phosphate (K_2HPO_4; 30% w/v solution), HCl (1 M), and NaOH (1 M) were used to adjust pH. Food-grade phytase (Enzymes.bio) was used for enzymatic treatment.

The equipment used included a thermomixer (Thermomix® TM5), a bench-scale homogeniser (IKA® T18 digital Ultra Turrax), a pH-meter (Metrohm 914 pH/conductometer), a food-grade thermometer, a digital balance, and plastic storage containers.

The formulation was developed through five steps. Throughout the process, pH was kept within a target range of 6.5-6.9, chosen to produce a neutral beverage and to remain below the isoelectric point of rapeseed protein (pI 7-10) above which solubility decreases, as the other ingredients had a pI below the target range (DSM-Firmenich, 2026). A protein target of 3.4% (w/w) was set to match the average protein content of cow's milk, ensuring nutritional comparability (Tetra Pak, n.d.-a). The stability assessments were based on visual observation, and this is a recognised limitation.

Step 1: Characterisation of main ingredients

Each protein source was characterised from a formulation perspective. The objective was to assess sensory properties, solubility, thermal stability, and emulsifying behaviour to identify which ingredients were compatible with a plant-based beverage, and which presented challenges.

Sensory and dissolution assessment

Solutions were prepared by dissolving 1 g of each ingredient in 50 g of warm water (50°C) to make a 2% (w/w) solution, and assessed for appearance, taste, aftertaste, and dissolution.

Soluble protein content

The Bradford assay was conducted to quantify the soluble protein fraction of each ingredient at its natural pH and at pH 6.5-6.9, to assess which ingredient was more soluble. The full Bradford assay method is described in Section 3.3.2 Soluble protein content. For this step, 2% (w/w) solutions were prepared and pH adjusted, except for faba bean, as its pH was already within the target range.

Thermal stability

To assess the effect of pasteurisation on protein stability, 2% (w/w) solutions of each isolate and concentrate were adjusted to pH 6.5-6.9 and heated to 65°C for 16 minutes in the thermomixer, based on the conditions chosen for the final product. Low-temperature long time (LTLT)

pasteurisation was selected to avoid heating the proteins to higher temperatures and the parameters were adjusted based on equipment limitations. Changes in appearance during heating and after cooling were recorded.

Emulsifying behaviour

To assess the emulsifying behaviour of each protein source, 2% (w/w) solutions adjusted to pH 6.5-6.9 were blended with rapeseed oil at 2.5% (w/w) at high shear speed for 5 minutes. Appearance was observed over 1 hour.

Step 2: Blend composition

The second step involved selecting the optimal combination and ratio of protein sources, evaluated from a sensory but also from a nutritional perspective.

Sensory and stability assessment

Four combinations of protein isolate blends were prepared as 2% (w/w) solutions and assessed for appearance, taste, and sedimentation over 24 hours (refrigerated, 4°C).

Optimal blend ratios: AAS, DIAAS, and flavour

The final ratio of the beverage was decided after calculating the AAS and DIAAS for four rapeseed/faba bean blend ratios, while also taking flavour into consideration. The AAS was estimated to ensure that the amino acid profile for the beverage would be complete. The method is described in section 3.2.4 Amino acid profile. The DIAAS was calculated to identify the combination with the best balance between protein quality and taste (FAO, 2013). The method is described in section 3.2.5 Protein quality assessment.

Step 3: Processing

The third step involved assessing the effects of homogenisation and pasteurisation on a base formula of rapeseed and faba bean isolates (3.8% w/w to achieve 3.4% w/w protein target, in a 56/44 proportion) blended with water and rapeseed oil (2.5% w/w), in order to determine whether stabilising additives were still required and if these processing steps created new stability issues.

Homogenisation

Rapeseed and faba bean isolates were blended with warm water for 5 minutes at medium shear speed. Rapeseed oil was added and blended for another 5 minutes. The blend was homogenised using the Ultra Turrax at full speed (25,000 rpm) for 2 minutes. The homogenised sample was visually compared to a non-homogenised control after 24 hours of refrigerated storage (4°C). Mouthfeel was also assessed.

Pasteurisation

The same base formula was also assessed for thermal stability by repeating the process above but without the homogenisation step. The blend was pasteurised at 65 °C for 16 minutes in the thermomixer, cooled to 4°C on ice, and compared to a non-pasteurised sample.

Step 4: Stability

Three more ingredients were considered to improve the drink's stability: lecithin as an emulsifier to reduce creaming, gellan gum as a stabiliser to reduce phase separation, and inulin to increase viscosity. Gellan gum and inulin were also used to improve the mouthfeel of the beverage and make its viscosity more similar to other plant-based beverages. Each ingredient was tested following the full processing procedure used in Step 3: Processing.

Sunflower lecithin

Three concentrations were tested: 0.25%, 0.35%, and 0.45% (w/w), following recommendations for dairy alternatives (Cargill, 2019; DSM-Firmenich, 2026). Lecithin powder was dissolved in rapeseed oil, then added to the water-isolate blend and mixed at high shear for 5 minutes at room temperature. The samples were observed over 24 hours and compared to a control without lecithin.

Gellan gum

High acyl gellan gum was hydrated in a small portion of the beverage by heating it to 80 °C for a few minutes, in order to avoid heating the entire batch to a high temperature. Then, that portion was cooled on ice while continuously stirring, added back to the rest of the beverage and stirred at room temperature for 20 minutes to break up the gel as it formed during cooling. Three concentrations were tested: 0.01%, 0.03%, and 0.05% (w/w) (Cinogel, 2025). Lecithin was not included to observe the effect of gellan gum alone. Appearance was monitored over 24 hours and compared to a control not containing gellan gum. Mouthfeel was also assessed.

Inulin

Inulin was added at 2.5% (w/w), based on a study that showed increased viscosity and reduced sedimentation already at 2% (w/w) in chocolate milk (Homayouni Rad et al., 2012). To assess the combined effect with the already tested additives, three samples were compared: the full combination (inulin, lecithin, and gellan gum), a first control containing lecithin and gellan gum without inulin, and a second control with no additives. All samples were assessed for flavour, mouthfeel, and stability.

Step 5: Flavouring

Even though inulin improved the flavour profile, a final formulation step was needed to further mask the off flavours, which were bitterness and astringency given by rapeseed isolate. Three flavours were assessed: chocolate, "cinnamon bun", and turmeric latte.

3.2 Characterisation of plant-based beverages

3.2.1 Stability assessment

The raw ingredient beverage was tested for stability after completing the formulation, while the protein isolate beverage was not further analysed as stability had been improved during formulation.

3.2.1.1 Starch analysis

The iodine test was used to determine whether starch was present in the final drink. As a qualitative analysis, it was used as reference and to determine whether the use of food-grade amylases was necessary.

The test consisted of the addition of 2-3 drops of a Lugol solution to each of the samples. The shift of colour towards black would indicate the presence of starch in the sample, while the presence of yellow colour would indicate the absence of this substance. A positive and negative controls were used as a way to validate the method; the negative control consisted of milli-Q-water, and the positive control consisted of 500 mg of pure starch (powder).

The samples were prepared on two different ways, and per each triplicate were performed:

- No treatment: 2 ml of the final drink.
- Centrifugation: 2 ml samples were centrifuged before the analysis for 10 minutes at 10000 rpm and room temperature. The analysis was then completed using the supernatant.

3.2.1.2 Homogenisation and microscopic assay

A homogenisation step was performed to reduce the size of the fat globules present in the drink. As a way to measure if the homogenisation was effective, a microscopic comparison was performed using samples that had not being homogenised, samples that were homogenised using the high-pressure homogeniser, and samples that were emulsified using the Ultra Turrax equipment. All three samples were visualized using a microscope (Olympus, BX50) at 40x.The homogenisation was performed during 10 minutes at a pressure of 200 bar (He & Xu, 2024), while the emulsification was performed for 10 minutes at a speed of 25,000 rpm, both at room temperature.

3.2.2 Sensory analysis

A sensory analysis was performed through the participation of a focus group made up of 6 people as a preliminary way of understanding consumer acceptability. The list of ingredients was given to avoid risks of allergic reactions due to the presence of possible allergens, such as dates, rapeseed and faba bean protein isolates, which can pose a cross-reactivity with pollen (Kwaasi et al., 2002), mustard (EFSA (2013)) and legumes (Mastrorilli et al., 2024), respectively. The participants were asked to describe the aroma, colour, taste, and mouthfeel of the drink, and the results were annotated for future reference. It is recognised that a sensory analysis conducted with trained panellists could provide more accurate information on consumer acceptability.

3.2.3 Nutritional composition

For the hemp-quinoa beverage, the nutritional content of the drink was calculated following the guide provided by the European Food Information Resource (EuroFIR) for food businesses (Guideline for Calculating Nutrient Content for Nutrition Declaration as Indicated in the Regulation (EU) No 1169/2011 on the Provision of Food Information to Consumers). A comparison with two other plant-based options available in the market was performed using the data available at Livsmedelsverket.

The nutritional composition of the rapeseed-faba bean beverage was estimated by summing each nutrient contribution from all ingredients as shown in Equation 1, where *i* stands for each ingredient and *n* for the total number of ingredients:

Equation 1. Nutritional composition

$$Nutrient_{blend} = \sum_{i=1}^{n} \left(nutrient\ content_{ingredient} \times proportion_{ingredient} \right) \qquad (1)$$

Nutrient content is expressed as g per 100 g of ingredient and proportion is the mass fraction of each ingredient in the final drink. Results were expressed as g nutrient per 100 g of beverage and compared with the nutritional profiles of soy drink and whole cow's milk using data from Livsmedelsverket.

3.2.4 Amino acid profile

To analyse the amino acid profile of each of the drinks, samples of both drinks without phytase and before digestion were freeze-dried using a 7L freeze dry system (Lyph lock 18 - Ninolab) and sent to Eurofins, where a total amino acid test was performed. The IAA content was converted from g/100 g freeze-dried beverage to mg/g protein by using the dry base protein content obtained from the Dumas analysis (Section 3.2.7 in page 14). The AAS was then calculated for each beverage by dividing the content of each IAA with the adult requirement reference pattern (FAO, mg/g reference protein) and multiplied by 100. The lowest AAS obtained determined the overall score for the beverages. The AAS obtained was then compared to the estimated values for each beverage.

To calculate the estimated AAS for the protein isolate blend, for each isolate, the IAA content (mg/g protein) was multiplied by the isolate's proportion in the blend. The results for each IAA from each isolate were summed to obtain the estimated IAA content in the beverage, then divided by the adult requirement pattern (mg/g reference protein) and multiplied by 100. The lowest AAS determined the overall score for the blend ratio.

For the hemp-quinoa beverage, the IAA content (mg/g protein) of white quinoa and hemp were multiplied by the proportion of total quinoa and hemp present in the drink. These values were then compared with adult requirements, with the lowest AAS determining the score for the whole blend.

3.2.5 Protein quality assessment

For the hemp-quinoa beverage, following the final formulation (Table 1), the PDCAAS was calculated using the estimated AAS of the mixture, and the recipe protein digestibility (i.e. the protein digestibility of the formulation taking into account the percentage of each ingredient present in the final blend). The protein digestibility of the drink was calculated taking into account the percentage of quinoa and hemp protein in the recipe, along with their digestibility values. The digestibility of quinoa protein was found to be 92% (Ruales & Nair, 1992), while the one in hemp ranged from 84.1% to 86.2% (House et al., 2010). For hemp, the lowest range of its protein digestibility was used as a way to avoid an overestimation of the values.

For the rapeseed-faba bean beverage, the DIAAS was calculated in two steps. First, the digestible content of each IAA in the blend was calculated and expressed as mg of IAA per g of blend protein. For each isolate, the content of each IAA (mg/g protein) was multiplied by its ileal digestibility value and by the isolate's proportion in the blend. The result for each IAA in each isolate was then summed to obtain the total digestible IAA content for the blend. Standardised amino acid ileal digestibility (SID) values were used for rapeseed isolate (Bailey & Stein, n.d.). For faba bean isolate, true amino acid ileal digestibility (TID) values from cooked, dehulled faba beans were used as the closest available approximation (Itkonen et al., 2024). Second, the DIAAS for each IAA was obtained by dividing its digestible content in the blend by the FAO adult reference requirements, as seen in Equation 2:

Equation 2. DIAAS calculation

$$DIAAS\ \% = 100 \times \left(\frac{mg\ digestible\ IAA\ in\ 1\ g\ test\ protein}{mg\ IAA\ in\ 1\ g\ reference\ protein}\right) \quad (2)$$

The overall DIAAS for the blend was determined by the lowest individual amino acid DIAAS value, which identifies the first limiting amino acid (FAO, 2013).

3.2.6 Dry matter content

This analysis consisted of measuring the dry matter content of the chosen drinks as a way to test that the percentage was within the usual range for plant-based drinks. In order to perform this analysis, around 3 g of sample was measured. The analysis was performed in triplicates, and the samples were placed in an oven (Termaks, B-8023) at 105 °C for 24 h, until they were completely dry. The weight of the samples was measured before and after the drying treatment.

3.2.7 Total protein content

The analysis was performed using freeze dried samples from both drinks, without phytase and before digestion. The equipment used for the analysis was the nitrogen/protein analyser FlashEA 1112 Series (Thermo Electron Corporation), and the samples were freeze dried using the 7L freeze dry system (Lyph lock 18 - Ninolab). Aspartic acid was used for calibration purposes.

Before placing the samples in the protein analyser, these were first weighed and placed in a vessel made with aluminium foil. These capsules were then pressed and placed in a carrousel. Apart from

the samples from the drinks, of around 25 mg each, three standards of either 23-27 mg, or 48-52 mg of aspartic acid were prepared, following the instructions from the manufacturer. Two empty capsules were placed into the carrousel to act as the blank and the bypass.

In order to calculate the protein content in the original drinks, Equation 3 was used.

Equation 3. Protein content in the drinks (%)

$$Original\ protein\ (\%) = Dry\ base\ protein\ (\%)\ x\ \frac{Dry\ matter\ content\ (\%)}{100} \quad (3)$$

where:

- Original protein corresponds to the protein content in the drinks before freeze drying.
- Dry base protein is the value obtained from the protein analyser from the freeze-dried samples.

3.3 Protein digestibility

3.3.1 Phytase treatment

In order to increase the bioavailability and absorption rates of the proteins present in the beverages, food-grade phytase was added to the drink following the maximum dosage recommended by the manufacturer (0.1 mg/g dry solids). Based on this, 0.7 mg and 1.24 mg of phytase were added per 100 g of the hemp-quinoa drink and rapeseed-faba bean drink, respectively. The beverages were incubated for 1 hour at 37°C, following the manufacturer's recommendation (*Phytase Enzyme for Sale – Bulk Supplier*, n.d.). However, pH was not adjusted (recommended: pH 4-6) to avoid destabilising the beverages. The drinks were then assessed for taste, aroma, and stability, to check whether the enzyme treatment had changed these properties. The treated samples were kept frozen for further analysis. The calculation of the dry matter content is presented in Table A 10 (hemp-quinoa) and Table A 11 (rapeseed-faba bean) in the Appendix.

3.3.2 Soluble protein content

The Bradford spectrophotometric assay was conducted to quantify soluble protein in both beverages before and after phytase treatment, to assess whether the enzymatic treatment was able to improve protein digestibility by increasing the soluble protein content.

Materials used included Bradford Dye reagent (Bio-Rad), bovine serum albumin (BSA, Sigma Aldrich, used as standard), and milli-Q water. Equipment included 2 mL tubes, a 96-well microplate, a centrifuge (Eppendorf 5425), and a spectrophotometer (Thermo Scientific Multiskan GO) set to 595 nm.

Beverage samples were diluted 1:10 in milli-Q water and centrifuged at 10,000 x g for 10 minutes to separate the insoluble particles. The supernatant containing soluble protein was collected for analysis. A BSA standard curve was prepared at six concentrations ranging from 0 to 2 mg/mL,

prepared in milli-Q water. Milli-Q water was also used as the blank. Twenty microliters of blank, standard, or sample were transferred to 2 mL tubes, to which 1 mL of Bradford Dye Reagent was added. The tubes were quickly vortexed, then 300 µL from each were transferred to a 96-well microplate and incubated at room temperature for 5 minutes. Absorbance was read at 595 nm. All measurements were performed in triplicate. Protein concentration was calculated from the BSA standard curve equation ($R^2 = 0.99$), multiplied by 10 considering the dilution, and expressed as mg of protein per mL of beverage. The soluble fraction was calculated as a percentage of total protein in the drink based on Dumas results. Raw data and the standard calibration curve can be found in the Appendix (Table A 15 and Figure A 4).

3.3.3 *In vitro* digestion simulation

To analyse if the phytase treatment had improved protein digestibility in the drinks, an *in vitro* digestion was simulated on both drinks, with and without phytase. Reagents included HCl (1M) and NaOH (1 M) to adjust the pH according to the protocol, casein powder, used as a reference, and trichloroacetic acid (TCA) in a 5% concentration. TCA was used to precipitate the residual proteins and the enzymes that were not digested to be excluded from further analyses. The digestion was divided in three phases (oral, gastric, and intestinal), each of which included the use of a variety of reagents, enzymes, and simulated fluids, all measured and used following the instructions provided by the INFOGEST protocol (Brodkorb et al., 2019). Some acknowledged limitations of this analysis include the assumption that the information regarding enzyme activity provided by the manufacturers was factual, and the fact that the digestion was performed only once.

Oral phase

The materials needed included the drinks with and without phytase, casein, simulated salivary fluid (SSF), HCl, NaOH, and 1.5 mmol/L $CaCl_2(H_2O)_2$. The pH of the mixture was adjusted to 7.

Gastric phase

For this phase, apart from the simulated gastric fluid (SGF), porcine pepsin (Sigma Aldrich, P7012) was used to simulate the conditions in the stomach during digestion, along with HCl, NaOH, used to adjust pH to 3, and 0.15 mmol/L $CaCl_2(H_2O)_2$.

Intestinal phase

For this last phase, simulated intestinal fluid (SIF) was used to mix with the gastric chyme, and to prepare the pancreatin and bile salts dilution. Other reagents included 0.6 mmol/L $CaCl_2(H_2O)_2$, bile salts (Sigma Aldrich, 48305-50G-F) and porcine pancreatin (Sigma Aldrich, P7545), as well as HCl and NaOH to adjust pH to 7.

In order to simulate the movements and temperature during all the digestion phases, a shaking incubator (Labwit ZWY-103D) was used during the completion of the analysis. A pH-meter (SI-Analytics, Lab 845) was used to measure and adjust the pH. Falcon tubes with a 50 ml capacity were used to contain the samples during the experiment.

3.3.4 Digestion studies

3.3.4.1 SDS-PAGE

To verify the effectiveness of the simulated *in vitro* digestion, SDS-PAGE was used to compare the intensity of the band patterns before and after digestion. This analysis was also used to observe if the phytase-treated samples showed an improved digestibility compared to the untreated ones.

Materials used included: protein sample loading buffer (Laemmli Sample Buffer with 2-mercaptoethanol added, Bio-Rad); running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, Bio-Rad); precision plus protein unstained standards (10-250 kDa, 10 bands, Bio-Rad); casein from bovine milk as a protein reference (GermanMicell, MCC 8000 Premium); and milli-Q water.

Equipment included 1.5 ml tubes, Mini-PROTEAN TGX Stain-Free Precast Gels (4-15%, Bio-Rad), a microcentrifuge (VWR MiniStar), a thermomixer (Starlab Mixer HC), a vertical electrophoresis system (Mini-PROTEAN Tetra cell, Bio-Rad), a power supply (PowerPac™ Basic Power Supply, Bio-Rad), a gel imaging system (GelDoc Go Gel, Bio-Rad) loaded with the UV and stain-free sample tray. A 7L freeze-drier (Lyph lock 18 - Ninolab) was additionally used to concentrate the hemp-quinoa beverage samples.

The hemp-quinoa beverage samples were freeze-dried and reconstituted at 16 mg/mL, as initial trials showed barely visible band proteins at the original beverage concentration. The rapeseed-faba bean beverage samples before digestion were diluted eight times in milli-Q water to achieve the same protein concentration as in the digested samples. Casein (3.4% w/w in milli-Q water, also diluted eight times) was included as a reference. The samples were centrifuged (10,000 x g, 10 minutes) and the supernatant collected.

For all samples, 16 µL were combined with 4 µL of loading buffer in 1.5 mL tubes, quickly mixed by pulse centrifugation and then heated at 100 °C for 10 minutes in the thermal mixer. After heating, samples were pulse-centrifuged again for a few second, and then 8 µL were loaded per well. Two wells were loaded with 4 µL of protein molecular weight standards. Separation was performed at 80 V for 10 minutes followed by 160 V for 45 minutes. The gels were then imaged.

3.3.4.2 Free amino acid content

The o-phthaladehyde (OPA) colourimetric assay was used to measure the concentration of free amino acids in both beverages before and after digestion to confirm the effectiveness of the simulated digestion, and to compare protein digestibility between the phytase-treated and untreated samples, to see whether it had improved in the enzymatically treated samples.

Materials used included sodium borate buffer (100 mM, pH 9.5), 2% (w/v) sodium dodecyl sulphate (SDS) solution (Sigma-Aldrich), o-phthalaldehyde (OPA, Sigma-Aldrich), methanol (Sigma-Aldrich), β-mercaptoethanol (Bio-Rad), TCA (Sigma-Aldrich), glycine used as a standard (Sigma-Aldrich), and milli-Q water. Equipment included a centrifuge (Eppendorf 5425), a spectrophotometer (VWR Biowave II), 2 ml tubes, and plastic cuvettes (Starstedt).

A glycine standard curve was prepared at five concentrations in the range of 0 to 2.00 mM in milli-Q water. Milli-Q water was used as the blank. From each sample, 1 mL was transferred to a 2 mL tube and mixed with 1 mL of 5% (w/v) TCA to precipitate proteins and have only free amino acids in solution. Samples were kept on ice for 10 minutes, then centrifuged at 10,000 x g for 10 minutes, and then the supernatant was collected. The OPA reagent was prepared immediately before using it in a fume hood by combining 25 mL of sodium borate buffer, 2.5 mL of 2% (w/v) SDS, 40 mg of OPA dissolved in 1 mL of methanol, and 100 µL of β-mercaptoethanol. Lastly, the solution was brought to 100 mL with milli-Q water.

Twenty µL of blank, standard, or sample were transferred to plastic cuvettes, to which 2 mL of OPA reagent were added. After exactly 2 minutes of reaction at room temperature, absorbance was read at 340 nm. All measurements were done in triplicate. For the standards, only the average absorbance was recorded, therefore the standard deviation could not be calculated, and this is a recognised limitation. Free amino acid concentration was calculated from the glycine standard curve equation ($R^2 = 0.99$), corrected for the dilution factor (2), and expressed as mmol glycine equivalents per L of solution (mM). The percentage increase was also calculated. Raw data and the standard calibration curve can be found in the Appendix (Figure A 5 and Table A 16).

3.3.4.3 Phytic acid content

A kit from Megazyme (International Ireland Ltd., Wicklow, Ireland; reference code: K-PHYT; SKU: 700004327) was used to measure phytic acid content. Following the instructions of the manufacturer, the contents of all bottles, except bottle 6, were used as supplied. Other reagents needed for the experiment included ascorbic acid (10% w/v), sulphuric acid (1M), ammonium molybdate (5% w/v), TCA (50% w/v), HCl (0.66 M), NaOH (0.75M), and milli-Q water.

As for the equipment, usual laboratory utensils were used, including: glass beakers, 2 ml microtubes, 15 ml Falcon tubes, plastic semi-micro cuvettes, micro-pipettors, analytical balance, and vortex. Other equipment included a spectrophotometer (WPA, Biowave II) set to 655 nm, an incubator (Star Lab, Smart Instruments, Mixer HC) set to 40 °C, and a centrifuge (Eppendorf centrifuge 5425) set to 13,000 rpm.

The methodology followed in order to perform this analysis was based on the assay protocol provided by Megazyme (Megazyme, 2025), and the concentration of phytic acid per sample was calculated using the formulas provided by the protocol.

3.4 Statistical analysis

Microsoft Excel was used to do the statistical analysis of the data. One-way ANOVA was used to evaluate statistical differences, and the Tukey Post Hoc test was done for inter-group comparisons. A statistical paired two-tailed Welch's t-test was used to compare differences between untreated and phytase-treated samples in the Bradford assay and the free amino acid OPA colorimetric assay. The confidence interval was set to 95% ($\alpha = 0.05$) for all tests.

4. Results and discussion

4.1 Product development

4.1.1 Raw ingredient beverage

The final formulation for the hemp-quinoa beverage is presented in Table 1 below. All ingredient proportions are expressed as g per 100 g of final product. The formulation was decided to find the balance between the best AAS and the overall sensory properties of the drink. As the version that could have resulted in a higher score ended up having an unpleasant flavour and mouthfeel, the recipe had to be adjusted so that the drink could be palatable, even though this led to a lower AAS.

Table 1. Final formulation ratios for the drink made of raw ingredients

Ingredient	g per 100 g product
Water	68.87
Quinoa	26.17
Hemp hearts	3.58
Dates	1.38

4.1.2 Protein isolate beverage

Step 1: Characterisation of main ingredients

Sensory and dissolution assessment

Results for each ingredient are presented in Table 2. Faba bean isolate displayed the most favourable sensory profile, showing to be ideal for a plant-based beverage. Rapeseed, potato, and hemp had an unpleasant flavour which would require flavourings. All ingredients dissolved rapidly in water except hemp, which left hard undissolved particles, making its use challenging for a plant drink. Rapeseed and potato isolates made stable foams when blended, with potato foam remaining visible for a few hours, and this may imply a processing challenge for larger scale production.

Table 2. Sensory and dissolution assessment of each protein isolate and concentrate (2% w/w solution, 50 °C).

Ingredient	Appearance	Taste	Aftertaste	Dissolution	pH
Rapeseed	Dark yellow, stable foam	Bitter, liquorice, unpleasant	Bitter, astringent	Complete	7.5

Potato	Pale yellow, stable foam	Salty, unpleasant	None	Complete	3.1
Faba bean	Milky white, no foam	Sweet, beany, pleasant	None	Complete	6.5
Hemp	Dark green, no foam	Earthy, unpleasant	Hemp flavour	Not complete, hard particles remain	7.0

Soluble protein content

Results for the soluble protein concentration of each ingredient are shown in Figure 1. At natural pH, potato had the highest soluble protein concentration (1.82 mg/mL), followed by rapeseed (1.55 mg/mL), faba bean (0.59 mg/mL), and hemp (0.11 mg/mL). At pH 6.5-6.9, rapeseed showed the highest soluble protein concentration (1.71 mg/mL), and this increase was expected as the pH moved away from the protein's isoelectric points of 7-10 (DSM-Firmenich, 2026). Potato solubility nearly halved (0.99 mg/mL) when the pH was adjusted. The reason is that protease inhibitors, the second most abundant group that make up potato proteins, have an isoelectric point between 5-8, and as the adjusted pH falls within this range, solubility decreased (Andlinger et al., 2021). Hemp remained poorly soluble at both pH values.

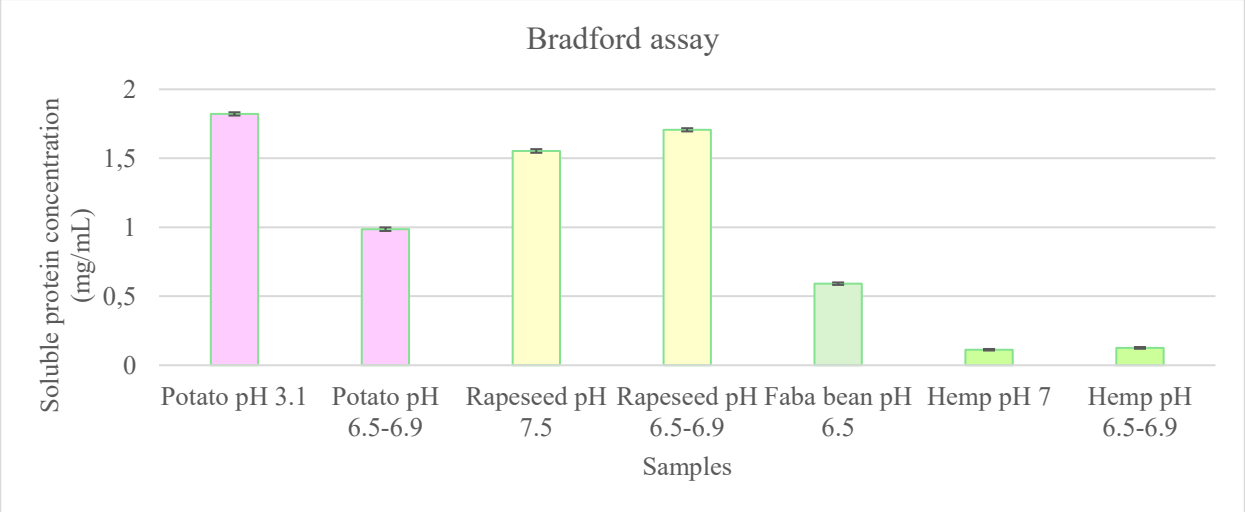


Figure 1. Bradford assay results for individual protein isolates and concentrate at natural pH and at the target beverage pH

The main findings from this step were that rapeseed isolate proved to be a favourable option for a plant-based beverage, as it had the highest concentration of proteins in solution at the target pH, while hemp remained poorly soluble at the target pH, proving not to be ideal for a plant beverage.

Thermal stability

For rapeseed and potato isolates, protein precipitation was visible during heating. The precipitation observed for potato isolate is consistent with its denaturation temperature of 50-70°C (Masijn et al., 2025). The precipitation observed for rapeseed was expected since cruciferin, one of the two major protein fractions, has a denaturation temperature of 65°C (DSM-Firmenich, 2026). This means that from a formulation perspective, when using rapeseed or potato isolate, stabilising strategies will be needed to prevent precipitation. DSM-Firmenich suggested that combining rapeseed isolate with a second protein isolate and using hydrocolloids can mitigate this effect. Faba bean isolate and hemp concentrate showed no visible changes after heating and cooling, which is in line with their denaturation temperature being high (>84°C for faba bean, 80°C for hemp) (Badjona et al., 2024; Raikos et al., 2015). These two ingredients can be considered heat stable under the chosen pasteurisation conditions, and their use is favourable in the beverage.

Emulsifying behaviour

The oil content of 2.5% was chosen based on the fat content in semi-skimmed cow milk (~2%) (Tetra Pak, n.d.-b). Faba bean and hemp produced more stable emulsions, as they remained opaque even after 1 hour, indicating that the oil droplets stayed dispersed instead of coalescing. Rapeseed and potato showed rapid phase separation, with a clear phase appearing within minutes. A top layer was observed in all samples after 1 h which could be due to creaming, an emulsion instability. Since none of the ingredients produced a fully stable emulsion, these results showed that an emulsifier would be required if homogenisation would not solve the problem.

Ingredient selection

Based on all the results of the first step, hemp concentrate was excluded from further development due to its strong, unpleasant flavour, failure to dissolve completely, and low soluble protein concentration at the target pH, even though it proved to be heat stable and had good emulsifying behaviour. From a nutritional perspective, hemp also had the weakest amino acid profile among the four ingredients considered based on the FAO reference pattern for adults (Table A 1). Faba bean isolate was considered promising as one of the main ingredients, given its mild and pleasant flavour, heat stability, and good emulsifying behaviour. Rapeseed isolate was also promising as a main ingredient due to its high protein solubility at the target pH, together with its excellent amino acid profile (Table A 1); however, its bitter and astringent sensory profile would require masking with flavourings, and its thermal instability would require corrective strategies. At this step, potato isolate was selected to be used in the formulation only in small amounts, due to the intense foaming, acidic pH, and unpleasant taste, as it had the strongest amino acid profile (Table A 1). The findings from this step also suggest that an emulsifier and hydrocolloid would be needed to stabilise the beverage, if the instability issues were not to be resolved in the following steps.

Step 2: Blend composition

Sensory and stability assessment

Results of the sensory and stability assessment for the four protein isolate blends are presented in Table 3. The blends containing both faba bean and potato had a large amount of sediment, and this could be due to the pH being close to faba bean protein's isoelectric point of 4-5 (Badjona et al., 2025). All blends containing potato also had enhanced bitterness and astringency. Although potato isolate had the strongest amino acid profile (Table A 1), the negative impact on taste in all blends containing it led to its exclusion at this stage from further development. The rapeseed/faba bean blend had the most acceptable sensory profile, with only a mild bitterness and astringency due to the rapeseed isolate and was therefore chosen for the final formulation.

Table 3. Sensory and stability assessment of four protein isolate blends (2% w/w solutions) over 24 hours at 4℃

Protein blend	Taste	Sediment (24 h)	pH
Rapeseed/faba bean	Mild bitterness and astringency	Small amount present	6.7
Rapeseed/potato	Astringent, unpleasant, enhanced bitterness	Very little to none	4.4
Faba bean/potato	Astringent, unpleasant, gritty	Large amount	4.2
Rapeseed/faba bean/potato	Bitter, astringent	Large amount	4.7

Optimal blend ratios: AAS, DIAAS, and flavour

AAS and DIAAS values for the four blend ratios are shown in Table 4 and Table 5. Valie was identified as the first limiting amino acid for all ratios in both calculations. The tables with the AAS and DIAAS for each IAA can be found in the Appendix (Table A 3 and Table A 4). Data used to calculate DIAAS can be found in the Appendix (Table A 9). In both calculations, the IAA content for each isolate was based on data provided by the companies from which the protein isolates were obtained, DSM-Firmenich and Green Goddess, and can be found in Table A 1.

Table 4. Estimated AAS for four rapeseed/faba bean isolate blends, calculated relative to FAO/WHO adult requirements

	Rapeseed/faba bean blends			
	AAS ratio A (50/50) (%)	AAS ratio B (60/40) (%)	AAS ratio C (70/30) (%)	AAS ratio D (56/44) (%)
AAS (%)	**117***	**120***	**122***	**119***

*Valine is the first limiting amino acid for all ratios.

Table 5. Estimated DIAAS values for four rapeseed/faba bean isolate blends, calculated relative to FAO/WHO adult requirement

Rapeseed/faba bean blends

	DIAAS ratio A (50/50) (%)	DIAAS ratio B (60/40) (%)	DIAAS ratio C (70/30) (%)	DIAAS ratio D (56/44) (%)
DIAAS (%)	**94***	**94***	**94***	**94***

*Valine is the first limiting amino acid for all ratios.

As valine's DIAAS remained constant for all the ratios considered, the ratio was selected based on the AAS and flavour. Ratio D (56% rapeseed / 44% faba bean) was chosen as the optimal compromise, achieving an AAS of 119% while maximising faba bean content for its pleasant taste. Moving towards a ratio with 70% rapeseed would increase the AAS to 122%, as rapeseed has more valine, but faba bean has a higher ileal digestibility value for this IAA (Table A 9). Increasing rapeseed proportion would produce a less favourable sensory profile due to rapeseed's bitterness.

It should be noted that the DIAAS value reported here was calculated using ileal digestibility values for unheated rapeseed protein isolate. However, moderate heat treatment of rapeseed protein has been shown to improve digestibility, suggesting that the true DIAAS of the final heated beverage may be higher than the values presented here (Bailey & Stein, n.d.).

Step 3: Processing

Homogenisation

Both the homogenised and non-homogenised samples formed a visible top layer, consistent with creaming, and showed phase separation after 24 h. Slight grittiness was also present in both samples. The presence of a top layer due to creaming and in both homogenised and non-homogenised samples showed that homogenisation alone was not sufficient to stabilise the emulsion, confirming the need for an emulsifier. Phase separation still took place in the homogenised sample, indicating the need for a stabiliser to slow down particle sedimentation. The homogenisation step was also not sufficient to solve the grittiness issue. However, the equipment here used replicated homogenisation at bench scale, so the result may be different with a high-pressure homogeniser.

Pasteurisation

No visible change in stability could be seen between the pasteurised and unpasteurised samples, suggesting that pasteurisation at 65°C for 16 minutes does not affect the stability of the drink. Since no precipitation took place, as opposed to when rapeseed was pasteurised alone, combining the isolate with another one can actually prevent precipitation. However, phase separation still took place, indicating that a hydrocolloid would still be necessary to stabilise the beverage.

Step 4: Stability

Sunflower lecithin

The addition of lecithin delayed the start of the phase separation in all samples, indicating better emulsion stability. As the samples with lecithin were more stable compared to the control, it was decided to use lecithin in the final formulation. Since there were no evident differences amongst the concentrations, the lower one was selected (0.25%). However, as phase separation was still observed after 24 hours in all samples, lecithin alone was insufficient for full beverage stability.

Gellan gum

The addition of gellan gum improved stability, by slowing down phase separation, and mouthfeel, by increasing viscosity, in all samples containing it, demonstrating its importance in the formulation; however, the beverage still felt too thin compared to commercial plant-based beverages. As the phase separation was less evident in the sample containing 0.05% gellan gum, this concentration was chosen for the final formulation.

Inulin

Results of the combined additive assessment are presented in Table 6. The full combination of lecithin, gellan gum, and inulin produced the best results: improved flavour profile with mild sweetness, creaminess, and a fuller mouthfeel, and reduced phase separation compared to the other samples. Therefore, inulin was selected as an ingredient in the final formulation. Even though reduced, slight bitterness and astringency remained, requiring the addition of flavouring.

Table 6. Sensory and stability results for the three samples in the inulin assessment

Sample	Taste	Mouthfeel	Stability (24 h)
Inulin + lecithin + gellan gum	Mild sweetness, bitterness and astringency	Smoother, more body and creamier, slight grittiness	Slight phase separation
Control 1 (lecithin + gellan gum)	Mild bitterness and astringency	Smoother, more body, slight grittiness	Medium phase separation
Control 2 (no additives)	Bitter and astringent	Thin, slight grittiness	Evident phase separation

Step 5: Flavouring

Flavour assessment results are presented in effectively covering the bitterness of rapeseed, even though slight astringency was still present. The chocolate flavour was therefore selected for the final formulation.. The cinnamon bun flavour had a very gritty and unpleasant mouthfeel caused by the cinnamon and cardamom powders not dissolving completely and was therefore excluded. The turmeric latte had a pleasant flavour; however, the chocolate flavour was evaluated as the one with the most acceptable sensory profile, effectively covering the bitterness of rapeseed, even

though slight astringency was still present. The chocolate flavour was therefore selected for the final formulation.

Table 7. Flavour assessment results for the three flavour options for the rapeseed-faba bean beverage

Flavouring	Ingredients	Taste/mouthfeel
Chocolate	Cocoa powder, agave syrup, vanilla essence, salt	Pleasant, slight astringency and grittiness
Cinnamon bun	Cinnamon powder, cardamom powder, agave syrup, vanilla essence, salt	Unpleasant, slight astringency, extremely gritty
Turmeric latte	Turmeric powder, honey, black pepper, salt	Pleasant, slight astringency and grittiness

Final formulation and production process

The final formulation for the chocolate rapeseed-faba bean beverage is presented in Table 8. All ingredient proportions are expressed as g per 100 g of final product.

Table 8. Final formulation of the rapeseed-faba bean beverage

Ingredient	g per 100 g product
Water	86.55
Rapeseed isolate	2.13
Faba bean isolate	1.67
Agave syrup	2.5
Rapeseed oil	2.5
Inulin	2.5
Cocoa powder	1.0
Vanilla essence	0.8
Lecithin	0.25
Gellan gum	0.05
Salt	0.05

The final production process is shown schematically in Figure 2.

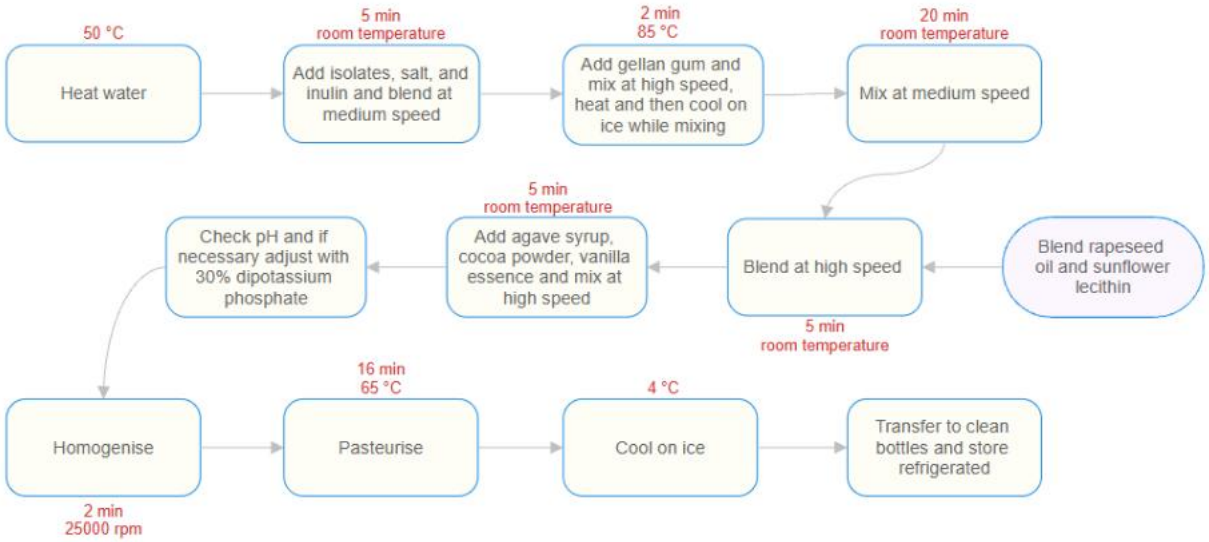


Figure 2. Flow chart of the production process for the rapeseed-faba bean beverage

4.2 Characterisation of plant-based beverages

4.2.1 Stability assessment

4.2.1.1 Starch analysis

The results are presented in Table 9 below, and the photos of the samples are presented in Figure A 3 in the Appendix.

The iodine test resulted confirmed the presence of starch in the final drink. The positive and negative controls presented the expected results. The negative control presented yellow tones due to the lack of starch; meanwhile, the positive control presented black tones due to the reaction between the pure starch and the Lugol solution. Based on this, a depending on the starch concentration present in the rest of the samples, different colours were obtained.

Following the Cornell scale (G-D. Blanpied & Kenneth J. Silsby, 1914) and looking at the obtained results (Figure A 3 in the Appendix), the drink could potentially present moderate levels of starch content, as the colour shifted towards purple. Compared to the positive control, pure starch, which colour shifted towards black, an indicator of high concentrations of starch.

This test was performed to have an idea of the concentration of starch present in the final drink, as high amounts could lead to the formation of gels during pasteurization, hindering the overall mouthfeel of the drink, as well as de-stabilize the emulsion (Xie et al., 2023). If high concentrations were to be present, the use of amylases would have been needed to avoid these issues.

Table 9. Results obtained from the starch analysis

Sample	Colour	Result	*Starch concentration
Negative control	Yellow	-	Low/Negative
Positive control	Black	+	High
No treatment	Light purple	+	Moderate
Centrifugated	Light purple	+	Moderate

*Based on the Cornell scale

4.2.1.2 Microscope assay

The results obtained from the comparison between the sample that was not homogenised (a), the sample that was treated with the high-pressure homogeniser (b), and the one that was treated with the Ultra Turrax equipment (c) are presented in Figure 3 below. Figure (a) presented bigger molecules compared to (b) and (c), and this is because this sample was not treated with any kind of homogeniser; meanwhile, (b) and (c) present similar results even though they were treated using different equipment (homogeniser and Ultra Turrax). These both samples presented smaller and more dispersed molecules compared to (a).

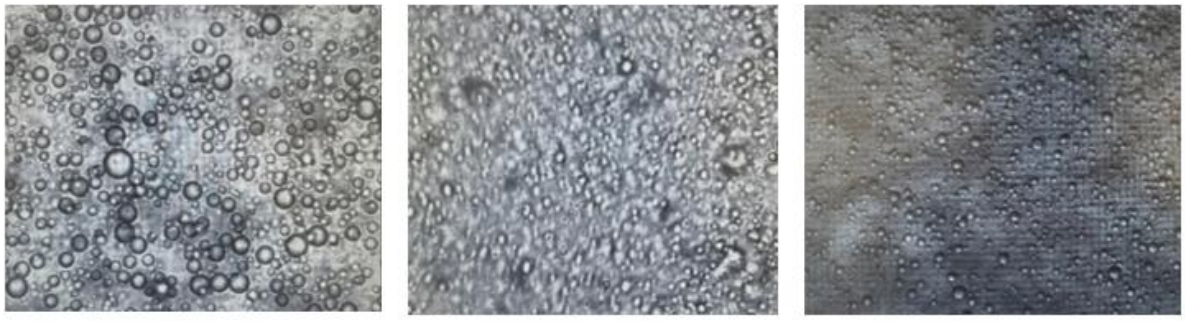


Figure 3. Microscope views of the a) non-homogenised sample, b) homogenised sample, c) sample treated with Ultra Turrax, respectively.

When comparing the results, Figure 3 (b) and (c), there is not a clear difference in the fat globule size among them, which could arguably mean that at kitchen scale, the Ultra Turrax equipment could lead to similar stability results. If further research were to be performed, the use of the Mastersizer equipment could be useful.

4.2.2 Sensory analysis

The focus group described the hemp-quinoa drink as a white blend, with an earthy aroma, and neutral taste with hempy undertones. The mouthfeel was described as creamy, and the drink was

said to present a good body. Perhaps the use of amylases could have further improve the mouthfeel of the drink.

For the rapeseed-faba bean beverage, the focus group described the aroma as pleasant, with a clearly perceptible cocoa and vanilla odour. The taste was considered insufficiently chocolate flavoured, with a correct level of sweetness, no bitterness, slight astringency, and a mild grittiness. The mouthfeel was described as intermediate between water and a commercial plant-based drink.

As the drink was not perceived as bitter, the flavouring step was successful in masking rapeseed's off flavour. However, the focus group found the drink lacking in chocolate flavour, therefore the cocoa powder content should be increased. Slight astringency could still be detected and strategies to reduce it involve including sweeteners, fats, or thickening agents (Rollinat et al., 2025). This had already been done during the formulation, and astringency was reduced throughout the process, but further adjustments could help reduce it even more. The remaining grittiness is due to insufficient homogenisation achieved at bench scale, and high-pressure homogenisation may solve this issue. The considerations on mouthfeel indicate that viscosity was improved by inulin and gellan gum, but not enough to reach the thickness expected by consumers. Increasing these ingredients further may improve body and mouthfeel.

4.2.3 Nutritional composition

The results of the estimated nutritional composition of the hemp-quinoa drink are presented in Table 10, along with the values from other plant-based drinks already on the market. These last values were obtained from Livsmedelsverket (Search for Nutrients - Livsmedelsverket, n.d.).

Table 10. Nutritional composition of the drink made from raw ingredients, along with the values from Livsmedelsverket

Nutrient (unit)	**Quinoa-Hemp milk**	***Soy drink**	***Oat drink (1.5% fat fortified)**
Weight standard (g)	100	100	100
Energy (kJ)	50	141	199
Energy (kcal)	23	34	48
Fat, total (g)	0.4	1.5	1.5
Saturated fat (g)	0.04	0.2	0.2
Carbohydrates (g)	3.7	1.9	7.1
Sugar (g)	0.1	0.5	3.4
Protein (g)	1	3.1	1

Fiber (g)	0.5	0	0.8
Salt (g)	0	0.1	0.1

*Data obtained from Livsmedelsverket

The results from Table 10 show that the hemp-quinoa drink differs a bit from the usual values found on the market. Since oat drink was use a reference for the protein content, according to the estimated values this goal was achieved, while comparing it with the soy drink is a bit lower, but this was acknowledged. Also, the hemp-quinoa drink has a lower fat content compared with the soy and oat drinks (0.4 g/100g HQB compared to 1.5 g/100g for the soy and oat drinks). On the other hand, the carbohydrates fall within the range, even presenting lower quantities than the most oat drink. As for the sugar and saturated fats, both values are lower than those of the drinks found on Livsmedelsverket, something that could be interesting for consumers.

Overall, these values could serve as a base for the reasoning behind why this drink could serve as a good alternative. For example, when compared to the oat drink found through Livsmedelsverket, the hemp-quinoa beverage could be a source of the same amount of protein, while offering a lower caloric intake, which could be an important factor for prospect consumers.

The estimated nutritional composition of the chocolate protein isolate beverage is presented in Table 11, along with the values for soy drink and whole cow's milk as reference products (Livsmedelsverket). Data used for the calculations can be found in the Appendix (Table A 5). The protein isolate beverage was estimated to have a higher protein content (3.8%) than cow's milk (3.6%) and soy drink (3.1%), with lower levels of saturated fat (0.36%) and sugar (2.2%) compared to cow's milk (1.9% and 4.6%, respectively).

Table 11. Nutritional composition of the rapeseed-faba bean beverage, soy drink, and cow milk 3% fat fortified

Nutrient (unit)	**Protein isolates beverage**	***Soy drink**	***Cow's milk 3% fat fortified**
Weight standard (g)	100	100	100
Energy (kcal)	58	34	60
Fat, total (g)	3.0	1.5	3.0
Saturated fat (g)	0.36	0.2	1.9
Carbohydrates (g)	2.4	1.9	4.6
Sugar (g)	2.2	0.5	4.6
Protein (g)	3.8	3.1	3.6

Fiber (g)	2.3	0.0	0.0
Salt (g)	0.1	0.1	0.1

*Data obtained from Livsmedelsverket

The estimated protein content was higher than the initial formulation target. This is due to the protein contribution of other ingredients, mainly cocoa powder (24% protein, w/w), and to a clarification on rapeseed isolate nutritional data after finishing the formulation: the product label indicated >90% protein, but the manufacturer's detailed nutritional specifications confirmed a content of 97% (w/w). In any case, the final estimated protein content is higher than both soy drink and cow's milk, supporting the beverage as an optimal plant-based alternative.

The fibre content of the drink was 2.3% due to the addition of inulin, a prebiotic fibre that can confer health benefits by supporting intestinal and immune functions and is therefore considered a functional ingredient. It can also impact protein digestibility; however, its addition in the beverage was essential for improving taste, mouthfeel, and texture (Partanen et al., 2025; Sheng et al., 2023). Fibre was not present in the reference products.

In terms of energy and fat content, the rapeseed-faba bean beverage was comparable to cow's milk. However, the saturated fat content was much lower due to rapeseed oil being the main fat source in the formulation, making it a more favourable than cow's milk in this aspect. The rapeseed-faba bean drink had higher energy and fat content compared to soy drink. Sugar content (2.4 g/100 g) was higher in the rapeseed-faba bean beverage than in the soy drink (0.5 g/100 g) due to the added sugar needed to balance cocoa powder. It was below the sugar content of cow milk (4.6 g/100 g), making it a more balanced option.

Overall, based on these estimations, the rapeseed-faba bean beverage is a favourable option compared to both soy drink and cow's milk in terms of protein content and dietary fibre. It must be noted that all values are estimated based on ingredient compositional data and may not fully reflect the real nutritional composition.

4.2.4 Protein quality assessment

The results of the PDCAAS calculations are presented in Table 12 below, and the data in detail used to calculate these values is presented in Table A 7 and Table A 8 in the Appendix.

Looking at PDCAAS of the hemp-quinoa drink (0.87), it is clear that even though this drink could not be considered as a complete protein source (PDCAAS = 1.0), it would still fall under the good protein quality category. When comparing this value with the scores from other plan-based drinks more available in the market, it can be discussed that this drink could be a better protein source than some of these drinks, like oat, coconut or almond milk, with a PDCAAS of 0.59, 0.72, and 0.43 respectively (Asif et al., 2026). Regarding the AAS, this drink presented two limiting amino acids: leucine and lysine, with AAS of 97.3% and 97.2% respectively. The average among these two values is presented below.

Table 12. Digestibility percentage, AAS and PDCAAS of the drink made out of raw ingredients

Combination	Recipe protein digestibility	AAS	PDCAAS
Hemp-Quinoa	90.22%	97.20%	0.87

Meanwhile, the calculated DIAAS and AAS for the final formulation of the rapeseed-faba bean chocolate beverage is presented in Table 13 below.

Based on the estimated DIAAS of 94% for the rapeseed-faba bean beverage, the drink classifies as a high-quality protein source (DIAAS > 75%). If compared to the DIAAS for oat, soy, and cow's milk, the score for the protein isolate beverage is higher than both oat (57%) and soy (83%) drinks, but lower than cow's milk (116%) (Hammer et al., 2024; Yu et al., 2023). This means that the rapeseed-faba bean drink is a more favourable option compared to other plant-based beverages in terms of protein quality but still doesn't quite reach the protein digestibility of cow's milk.

Table 13. Estimated DIAAS and AAS for the final rapeseed-faba bean chocolate beverage

Ingredients and ratio	AAS (%)	DIAAS (%)
56% rapeseed and 44% faba bean isolates	119	94

4.2.5 Amino acid profile

The results obtained from the analysis performed by Eurofins on both drinks are presented in Table 14 and Table 15 below, along with the actual AAS and the estimated AAS for both drinks.

The results presented in Table 14 showed that the hemp-quinoa drink did not have a complete amino acid profile, when comparing the values with the FAO/WHO requirements for adults, having lysine as the only limiting amino acid with a AAS of 96.2%, instead of leucine and lysine as previously estimated. The discrepancy between the values could be due to the fact that the beverage was made with tricolour quinoa (white, red and black), while the AAS was estimated using just the values from white quinoa.

On the other hand, the results from Table 15 showed that even though the estimated AAS presented no limiting amino acids, the real score exhibited two limiting amino acids: leucine (98.8%) and valine (97.4%). The discrepancy could be due to incorrect initial IAA content values, as these amounts can vary according to cultivar and growing conditions.

In any case, the measured AASs for both beverages were very close to 100% and answers the question if the combination of ingredients with complementary amino acid profiles could reach a complete protein source, since with some adjustments to the ingredient ratio and formulation, both beverages could potentially become a source of complete proteins.

Table 14. Indispensable amino acid profile of the hemp-quinoa drink

IAA	AA content (g/100g powder)	Uncertainty (%)	AA content (mg/g protein)	Adult requirements (mg/g reference protein)	AAS (%)	Estimated AAS (%)
Histidine	0.448	± 14	27.6	15	184	177.8
Isoleucine	0.619	± 14	38.1	30	127	118.9
Leucine	1.06	± 14	65.3	59	110.7	97.3
Lysine	0.702	± 14	43.3	45	**96.2**	97.2
*SAA	0.617	± 14	38.0	22	172.7	161.5
*AAA	1.234	± 14	76.0	38	200	161.9
Threonine	0.550	± 14	33.9	23	147.4	131.4
Tryptophan	0.211	± 10	13	6.0	216.7	251.4
Valine	0.800	± 14	49.3	39	126.4	106.7

*SAA: sulphur amino acids // AAA: aromatic amino acids

Table 15. Indispensable amino acid profile of the rapeseed-faba drink

IAA	AA content (g/100g powder)	Uncertainty (%)	AA content (mg/g protein)	Adult requirements (mg/g protein)	AAS (%)	Estimated AAS (%)
Histidine	0.582	± 14	21.5	15	143	207
Isoleucine	0.841	± 14	31.0	30	103	149
Leucine	1.58	± 14	58.3	59	**98.8**	144
Lysine	1.26	± 14	46.5	45	103	154
SAA	0.770	± 14	28.4	22	129	173
AAA	1.40	± 14	51.7	38	136	206
Threonine	0.765	± 14	28.2	23	123	171
Tryptophan	0.265	± 14	9.78	6.0	163	223

Valine	1.03	± 14	38.0	39	**97.4**	119

Note: SAA, sulphur amino acids; AAA, aromatic amino acids.

4.2.6 Dry matter content

The average dry matter content of the final hemp-quinoa beverage formulation was 6.9% ± 0.12, and the data used to calculate it is presented in Table A 10 in the Appendix. The rapeseed-faba bean beverage had an average dry matter content of 12.4% ± 0.44. Full calculation data can be found in Table A 11 in the Appendix.

The values for both drinks fall within the usual range already available in the market, which goes from 2.52% for almond milk to 13.4% for rice milk (Awad & Mortas, 2026). They are also lower than the values found for cow's milk, typically around 13% (Tetra Pak, n.d.-a).

4.2.7 Total protein content

The data obtained from the protein analyser (total nitrogen) is presented in Table 16 below, and the raw data is presented in Table A 12 in the Appendix. The dry base and original protein content were calculated as described in Section 3.2.7 Total protein (page 14), using Equation 3. The chosen conversion factor, 6.25, is used as an average when multiple ingredients form a complex matrix like a plant-based beverage (*Analyzing Plant-Based Milk*, n.d.).

The results obtained following the Dumas method were consistent with the estimated values of the hemp-quinoa beverage, in terms of protein content, while for the rapeseed-faba bean beverage, the obtained protein content (3.36%) was very close to the target content (3.40%), but slightly lower than the estimated one (3.80%). This result suggests that the formulation was accurate and that the values used for calculating the nutrient content were not entirely precise.

The results from Table 16 and the estimated nutritional composition of both drinks (Table 10 and Table 11) show that the use of protein isolates can lead to higher protein concentrations compared to the use of whole ingredients.

Table 16. Results obtained from the total protein content analysis

Sample	Protein factor	Total nitrogen	Dry base protein (%)	Original protein (%)
HQB	6.25	2.59 ± 0.06	16.2 ± 0.37	1.12 ± 0.026
RFB	6.25	4.33 ± 0.01	27.1 ± 0.06	3.36 ± 0.007

4.3 Protein digestibility

4.3.1 Phytase treatment

The addition of food-grade phytase did not affect the taste, aroma, or mouthfeel of the beverages, however, it affected the stability of the drinks. When compared to the untreated control after 24 hours in refrigerated conditions, the phytase-treated samples showed visible phase separation, with a large clear layer at the top that was not present in the controls. This is consistent with findings from a study on soy drink, in which complete degradation of phytic acid by phytase caused protein aggregation and instability in the beverage. This means that phytate is important for the physical stability of soy drink and a moderate rather than complete degradation of phytate is considered better (Chen et al., 2025). If its use proves to be helpful in improving protein digestibility, the phytase dosage or incubation conditions should be optimised to keep the drinks stable.

4.3.2 Soluble protein content

The results of the Bradford assay are presented in Table 17 below. While, unexpectedly, the phytase treated samples showed a slightly lower soluble protein concentration in both beverages, this difference was not statistically significant ($p = 0.17$ for HQB; $p = 0.31$ for RFB).

Table 17. Soluble protein concentration and percentage of total protein content for both beverages

Sample	Concentration (mg/mL)	Soluble protein (% of total protein)
HQB untreated	0.710 ± 0.19	6.3%
HQB phytase treated	0.560 ± 0.08	5.0%
RFB untreated	5.71 ± 0.42	17%
RFB phytase treated	5.14 ± 0.65	15%

Note: HQB, hemp-quinoa beverage; RFB, rapeseed-faba bean beverage.

The Bradford assay showed that the rapeseed-faba bean beverage contained around 10 times more absolute soluble protein content than the hemp-quinoa beverage, while the soluble protein fraction was around 3 times higher. The difference between the two beverages could have been expected, as protein isolates are purified proteins from which most of the original plant matrix has been removed, while the quinoa-hemp beverage was made from whole ingredients where protein can remain trapped in plant cell structures, limiting protein solubility (Holland et al., 2020).

In contrast to the expected outcome, phytase treatment did not increase soluble protein fraction in either beverage. A slight decrease was observed in both cases, though the difference was not statistically significant. Since phytate binds strongly with proteins often forming insoluble complexes, phytase hydrolysis of phytate was expected to release proteins from the complexes and increase protein solubility.

A possible explanation for solubility not increasing is that the incubation was carried out at the beverages' final pH (6.5-7.1) rather than the enzyme's optimal range suggested by the manufacturer (4-6), therefore the enzyme may not have had the correct conditions for an efficient activity. A previous study on rapeseed protein found that phytase significantly increased protein solubility at an acidic pH, but had limited effect under neutral conditions (Lihme et al., 2026). Also, as discussed in Section 3.3.1 Phytase treatment, phytate may play a role in the beverages' stability and its reduction may have caused protein aggregation. This means that even though protein was released from the complexes, it may have aggregated and not changed the soluble protein content.

Based on these results, it is not possible to say whether the enzymatic treatment influenced protein digestibility. Future trials at a lower incubation pH could help understand whether the incorrect test conditions were responsible for the protein solubility not increasing. It should be noted that the Bradford assay may have underestimated the soluble protein concentration due to the use of BSA as a standard rather than the individual proteins being tested.

4.3.3 Digestion studies

4.3.3.1 SDS-PAGE

Results from the SDS-PAGE analysis are presented in Figure 4 below. Following digestion (lanes 2 and 4 for RFB, 6 and 8 for HQB, 9 for casein), a marked reduction or disappearance of protein bands was observed across all molecular weight ranges, demonstrating that digestion took place.

In the rapeseed-faba bean beverage samples after digestion (lanes 2 and 4) an intensification of bands below 10 kDa and above 250 kDa were observed. The presence of a band above 250 kDa in the digested (but not undigested) samples could be due the formation of glycated proteins during digestion, formed from the reaction of proteins and sugars in food. This may have been facilitated by the agave syrup in the beverage as it contains mainly fructose which is more reactive than glucose in promoting glycation (Yuan et al., 2025). Based on these results, agave syrup should be swapped for sweeteners without fructose to avoid reducing protein digestibility. The band below 10 kDa indicates the release of small peptides produced by the digestion process.

No visible difference in band pattern was observed between the phytase-treated and untreated samples for both beverages. As with the Bradford assay results, the enzymatic treatment did not appear to affect protein digestibility under the conditions tested. Future trials at lower incubation pH would be necessary to confirm if phytase can improve digestibility under optimal conditions.

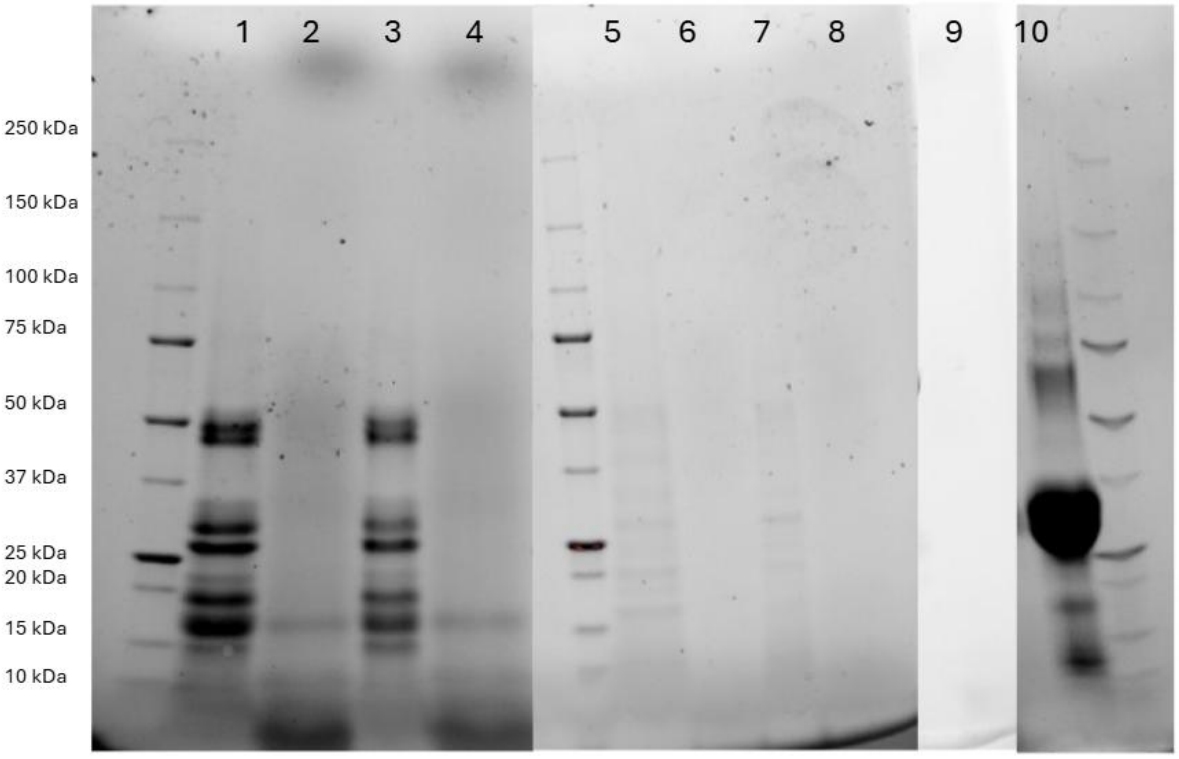


Figure 4. Results from the SDS-PAGE analysis performed on the samples: 1 (RFB, untreated and undigested); 2 (RFB, untreated and digested); 3 (RFB, phytase-treated and undigested); 4 (RFB, phytase treated and digested); 5 (HQB, untreated and undigested); 6 (HQB, untreated and digested); 7 (HQB, phytase-treated and undigested); 8 (HQB, phytase-treated and digested); 9 (casein, digested); 10 (casein, undigested). RFB, rapeseed-faba bean beverage; HQB, hemp-quinoa beverage.

4.3.3.2 Free amino acid content

Results from the OPA assay are presented in Table 18. The rapeseed-faba bean digested free amino acid concentrations were extrapolated beyond the linear range, and this is acknowledged as a limitation.

Table 18. Results obtained from the colourimetric assay (OPA) for both beverages

Sample	Undigested (mM)	Digested (mM)	% increase
HQB untreated	1.64 ± 0.021	4.00 ± 0.158	+144%
HQB phytase treated	1.62 ± 0.105	4.00 ± 0.126	+147%
RFB untreated	2.60 ± 0.074	7.40 ± 0.242	+185%
RFB phytase treated	2.60 ± 0.158	7.20 ± 0.158	+177%

Note: HQB, hemp-quinoa beverage; RFB, rapeseed-faba bean beverage.

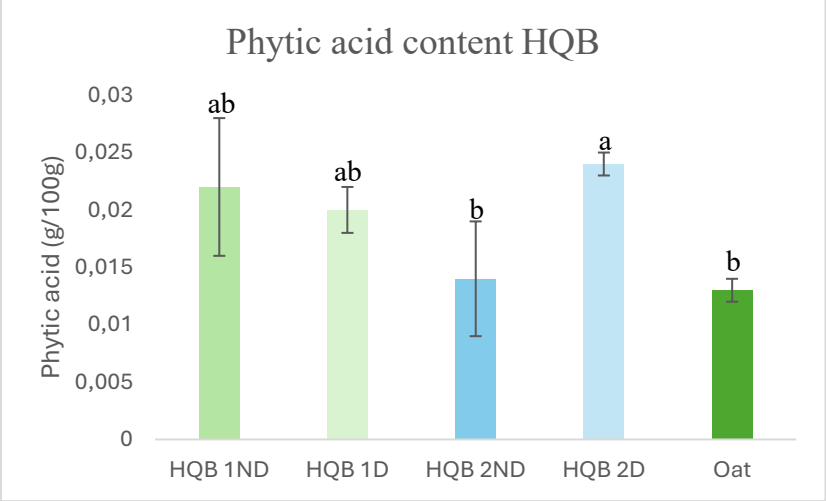
The increase in free amino acid concentration in all digested samples confirmed that the digestion was successful, consistent with SDS-PAGE results. The larger percentage increase for the rapeseed-faba bean drink compared to the raw ingredient one suggests that more hydrolysis occurred during digestion. This is consistent with the Bradford assay results, where RFB showed a higher soluble protein fraction, as soluble protein can be accessed more easily by digestive enzymes.

No statistically significant difference in free amino acid release was observed between phytase-treated and untreated digested samples in either beverage ($p = 0.16$ for HQB, $p = 0.07$ for RFB), suggesting that phytase treatment did not improve protein digestibility under the conditions tested. This, again, is consistent with the results from the Bradford assay, where the enzymatic treatment did not increase the soluble protein fraction, and from SDS-PAGE, where no difference was observable between digested treated and untreated samples. As before, possible explanations are incorrect enzyme incubation parameters or protein aggregation after their release from phytic acid complexes. These results indicate that under the applied conditions and with the utilised analyses, phytase did not improve protein digestibility.

A limitation of this analysis is that OPA reacts only with primary amines and does not detect proline, a secondary amine, therefore the amino acid content may be underestimated (Jasco, 2024).

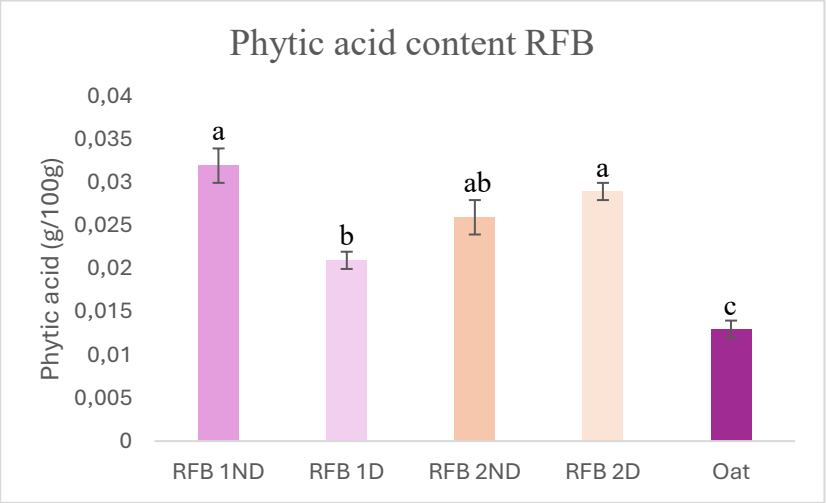
4.3.3.3 Phytic acid content

The results are presented in Figure 5 and Figure 6 below, where HQB refers to the hemp-quinoa beverage, while RFB refers to the rapeseed-faba beverage. The data used to get these graphs is presented in Table A 18 in the Appendix.



* 1: no phytase // 2: with phytase // ND: non-digested // D: digested

Figure 5. Phytic acid content in the hemp-quinoa beverage, along with the oat control sample (the columns with different superscript show that there is a significant difference ($p < 0.05$)



* 1: no phytase // 2: with phytase // ND: non-digested // D: digested

Figure 6. Phytic acid content in the rapeseed-faba beverage, along with the oat control sample (the columns with different superscript show that there is a significant difference ($p < 0.05$))

The results in relation to the hemp-quinoa beverage showed a significant reduction (36.4%) in the phytic acid content in the sample with phytase and before digestion (HQB 2ND) compared to the sample with no phytase (1ND); meanwhile, for the rapeseed-faba drink, the same sample (RFB 2ND) presented slightly lower phytic acid values (18.8% reduction) than the sample with no phytase before digestion (RFB 1ND). For both drinks, the sample with phytase and after digestion (2D) presented higher or similar values in phytic acid as the original drinks (1ND); this is acknowledged as a limitation in the results.

A study found on the treatment of a soy drink with phytase and that was also digested following the INFOGEST protocol, it was found that the use of phytases led to a complete reduction of the phytic acid in the soy drink (Lv et al., 2024), something that did not happened in both the digested HQB and the RFB, which presented an average of 0.024 g/100g and 0.029 g/100g, respectively. The reason could be that the pH used during the treatment was not the optimal suggested by the manufacturer, which could have hindered the activity of the enzyme.

Apart from the incubation conditions, like pH, another conclusion reached was how depending on the elements present in each of the matrices the interaction between phytic acid, minerals and proteins can lead to stronger or weaker bonds, and based on this, the phytase could have had a bigger or smaller impact on the phytic acid content of the drinks. This reasoning is linked to the results obtained from the samples with phytase and before digestion (2ND).

When comparing the results of this analysis with the previous ones, such as the Bradford and OPA assays, where the main conclusion reached was that the treatment with phytase was not linked with

a higher digestibility of the proteins, it is clear that more research would be needed to understand what exactly could be hindering the absorption and bioavailability rates of protein.

4.4 Limitations

Several limitations should be acknowledged. Stability assessments were based on visual observations. The sensory analysis was conducted with a focus group of untrained panellists. The Bradford assay may have underestimated the soluble protein concentration due to the use of BSA as a standard rather than the proteins tested. DIAAS was estimated using TID values of cooked, dehulled faba beans instead of faba bean isolate. PDCAAS was estimated for the quinoa-hemp beverage instead of DIAAS due to the absence of published ileal digestibility values for quinoa. The homogenisation was performed at bench-scale, with reduced results compared to industrial scale homogenisation. The simulated *in vitro* digestion was performed only once, and the actual activity of the enzymes was not measured. Also, amylase and gastric lipase were nor used, which could have affected the results from the simulated digestion as well. For the phytic acid content analysis, the samples with phytase after digestion presented higher phytic acid values than the original samples, and for this reason these samples were not considered reliable.

5. Conclusion

The aim of this thesis was to address a gap in the plant-based beverage market, which is the lack of drinks with complete and digestible proteins that could represent nutritionally competitive alternatives to animal-based milk.

This work demonstrated that it is possible to formulate plant-based beverages with almost complete amino acid profiles and high protein quality using complementary protein sources, both from whole ingredients and protein isolates. Although the formulated drinks did not achieve a fully complete amino acid profile, the gaps were so minor (1-4%) that simply adjusting the ingredient ratio or formulation would give protein completeness. The rapeseed-faba bean beverage is a promising alternative in terms of protein content and quality to cow's milk, while the quinoa-hemp beverage is more favourable compared to most plant-based beverages in terms of protein quality and amino acid profile. While the phytase treatment did not produce a measurable improvement in protein digestibility under the tested conditions, it was able to reduce phytate content in the samples before digestion, even though moderately, highlighting the importance of continuing the research on methods to improve protein digestibility of plant-based products, as they are becoming increasingly relevant in today's food industry. These findings contribute knowledge on the development of plant-based beverages with almost complete amino acid profiles and emphasize the importance of continuous innovation in this expanding product category.

For future work, there are several directions to be explored based on the findings of the thesis. To improve protein digestibility, future trials could test different incubation parameters for phytase or other food-grade enzymes on the beverages, and the results compared with the thesis results. To achieve protein completeness for both beverages, an analysis of the IAA content should be performed on the ingredients used beforehand to calculate a targeted ratio and ensure a complete amino acid profile. To improve consumer acceptability of the rapeseed-faba bean beverage, high-pressure homogenisation could solve the grittiness issue, while increasing the concentration of gellan gum and cocoa powder could further improve viscosity and taste.

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Appendix

Table A 1. IAA content (mg/g protein) for rapeseed isolate, potato isolate, faba bean isolate, and hemp concentrate compared to FAO adult requirement patterns (mg/g reference protein)

IAA	Adult requirements[a] (mg/g reference protein)	Rapeseed[b] (mg/g protein)	Potato[c] (mg/g protein)	Faba bean[d] (mg/g protein)	Hemp[e] (mg/g protein)
Histidine	15	32	18	30	39
Isoleucine	30	37	60	54	39
Leucine	59	74	99	99	63
Lysine	45	64	76	76	42
SAA	22	54	34	18	30
AAA	38	62	125	99	80
Threonine	23	38	47	41	35
Tryptophan	6.0	14	15	12	6.0
Valine	39	51	86	41	51
Tot. IAA	277	426	560	470	385

Note. Data are from FAO (2013)[a], DSM-Firmenich (2026)[b], SaporePuro (n.d.)[c], Green Goddess (n.d.)[d], and Rawfoodshop (n.d.)[e]. SAA, sulphur amino acids; AAA, aromatic amino acids.

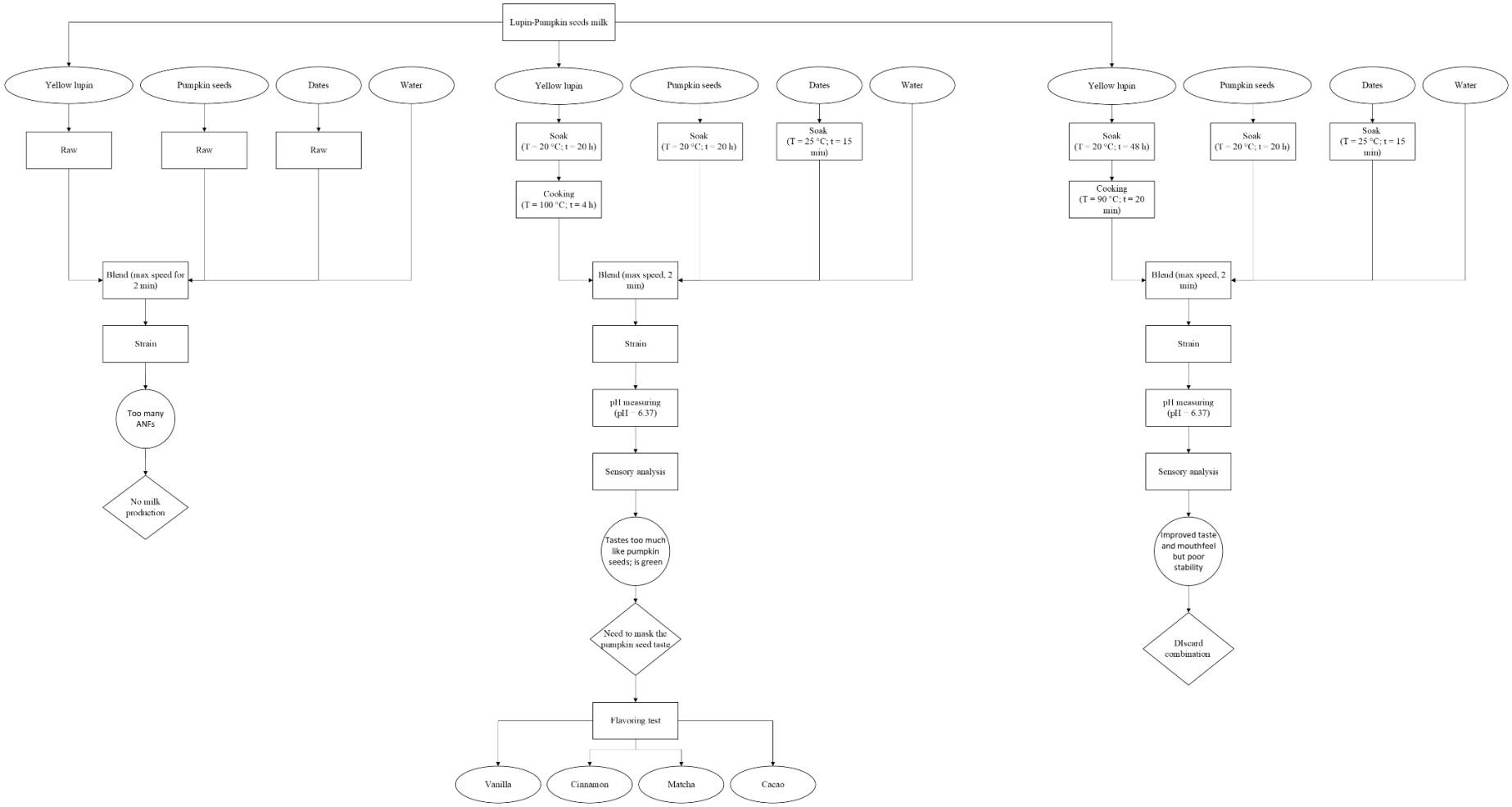

Figure A 1. Flow chart of the different steps followed during the formulation of the yellow lupin-pumpkin seeds milk

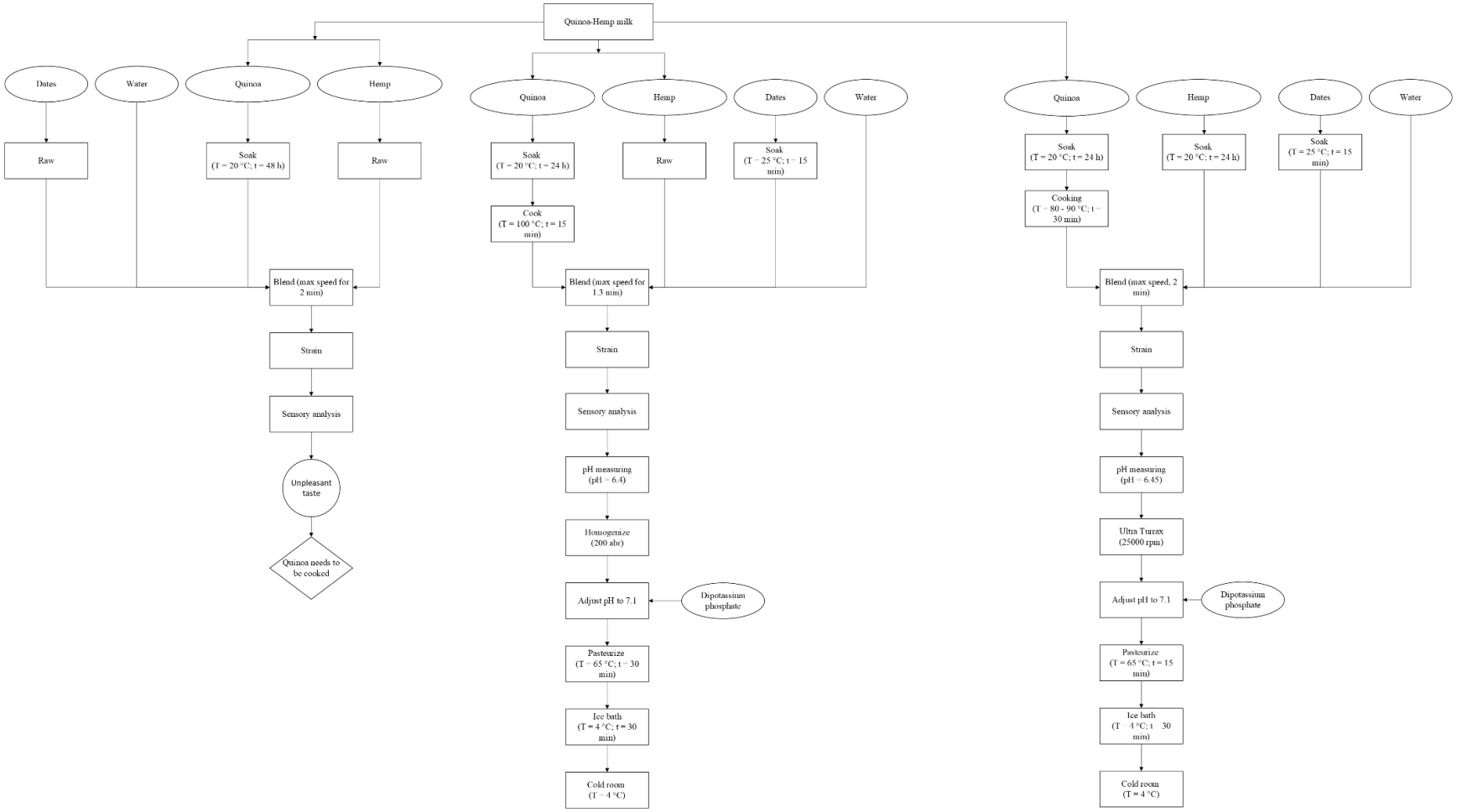

Figure A 2. Flow chart of the different steps followed during the formulation of the quinoa-hemp milk

Table A 2. Sensory properties of the different blends accomplished during the formulation of the drink made from raw ingredients

Combination	Appearance	Taste	Mouthfeel	Aroma	pH
*LPB 1	Green with yellow-grey undertones	Pumpkin seeds	Light Sandy Thick	Pumpkin seeds	6.6
LPB 2	Pale pastel green	Pumpkin seeds (< trial 1)	Sandy Lighter that trial 1 (liquid)	Pumpkin seeds (< trial 1)	6.45
LPB 3	Green with yellow undertones	Pumpkin seeds A bit beanie	Sandy Light Slightly creamy	Pumpkin seeds	6.4
*HQB 1	White with yellow undertones	Grassy Unpleasant Bitter Astringent	Thick Sandy Unpleasant	Grassy Starchy	6.3
HQB 2	White with black spots	Neutral Can taste the hemp Slightly earthy Slightly sweet	Creamy A bit too thick Slightly sandy	Neutral to earthy	6.4
HQB 3	White with small dark spots	Neutral Earthy Hemp aftertaste	Creamy Has good body	Earthy to neutral	6.45

*LPB: Lupin-Pumpkin seeds beverage // HQB: hemp-quinoa beverage

Table A 3. AAS for each IAA for four rapeseed-faba bean blends calculated based on adult requirement patterns (FAO, 2013)

		Rapeseed / faba bean blend			
AA	**Adult requirements (mg/g reference protein)**	**AAS ratio A (50/50) (%)**	**AAS ratio B (60/40) (%)**	**AAS ratio C (70/30) (%)**	**AAS ratio D (56/44) (%)**
Histidine	15	207	208	209	207
Isoleucine	30	152	146	141	149
Leucine	59	147	143	138	144
Lysine	45	156	153	150	154
SAA	22	163	179	195	173
AAA	38	212	202	192	206
Threonine	23	172	171	170	171
Tryptophan	6.0	220	224	228	223
Valine*	39	117	120	122	119
AAS (%)		**117**	**120**	**122**	**119**

Table A 4. DIAAS for each IAA for four rapeseed-faba bean blends calculated based on adult requirement patterns (FAO, 2013)

		Rapeseed / faba bean blend			
AA	**Adult requirements (mg/g reference protein)**	**DIAAS ratio A (50/50) (%)**	**DIAAS ratio B (60/40) (%)**	**DIAAS ratio C (70/30) (%)**	**DIAAS ratio D (56/44) (%)**
Histidine	15	171	171	171	171
Isoleucine	30	121	114	107	117
Leucine	59	118	112	107	115
Lysine	45	135	131	127	132
SAA	22	142	155	167	150

AAA	38	167	155	144	160
Threonine	23	139	135	131	136
Tryptophan	6.0	183	183	184	183
Valine*	39	94	94	94	94
DIAAS (%)		**94**	**94**	**94**	**94**

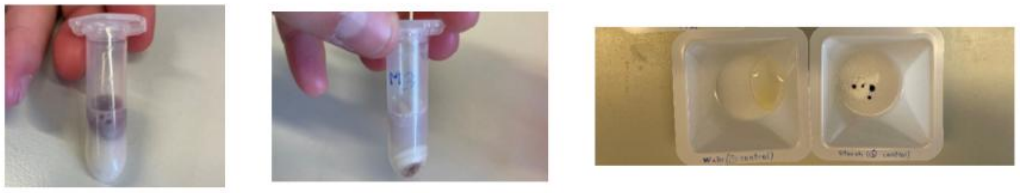

Figure A 3. Result obtained from the iodine test performed on: a) The drink itself (no treatment). b) Supernatant of the drink after it was centrifuged. c) Negative (yellow) and positive (black) control samples

Table A 5. Data used for estimating nutritional composition of the rapeseed-faba bean beverage

Nutrient (unit)	**RPI** [a]	**FPI** [b]	**Agave syrup** [c]	**Rapeseed oil**[d]	**Inulin** [e]	**Cocoa powder**[f]	**Vanilla extract** [g]	**Lecithin** [h]	**Gellan gum**[i]	**Salt** [j]
Weight standard (g)	100	100	100	100	100	100	100	100	100	100
Energy (kcal)	409	427	300	828	200	394	0	800	357	0
Fat, total (g)	0.2	7.7	0.1	92	0	22	0	50	1.04	0
Saturated fat (g)	0.1	1.4	0.1	7	0	13	0	10	0.21	0
Carbohydrates (g)	0.5	0	75	0	7	11	0	10	73.4	0
Sugar (g)	0.2	0	75	0	7	0	0	0	0	0

Protein (g)	97.1	89	0.1	0	0	24	0	0	12.2	0
Fiber (g)	0.5	0	0	0	90	0	0	0	0.7	0
Salt (g)	0.26	2.3	0.1	0	0.1	0.02	0	0	6.23	100
% in drink	2.13	1.67	2.5	2.5	2.5	1	0.8	0.25	0.05	0.05

Note: RPI, rapeseed protein isolate; FPI, faba bean protein isolate. Data are from DSM-Firmenich (2026)[a], Green Goddess (n.d.)[b], ICA (n.d.-a)[c], Willys (n.d.)[d], Private Label Food Manufacturing (n.d.)[e], ICA (n.d.-c)[f], The Spice & Tea Exchange (n.d.)[g], PhytoTherapy (n.d.)[h], OPAL Biotech. (n.d.)[i], and ICA (n.d.-b)[j].

Table A 6. Amino acid profile (hemp-quinoa beverage)

IAA	Adult requirements (mg/g reference protein) [a]	Children requirements children (mg/g protein) [a]	White quinoa (mg/g protein) [b]	Hemp (mg/g protein) [c]	Final recipe (mg/g protein)
Histidine	15	18	29	24	8.45
Isoleucine	30	31	36	35,3	10.68
Leucine	59	63	59	55,6	17.43
Lysine	45	52	54	32	15.28
Methionine + cysteine	22	26	36	35	10.67
Methionine	16		22	21	6.51
Cysteine	6		14	14	4.17
Phenylalanine + tyrosine	38	46	61	62,1	18.19
Threonine	23	27	30	30,5	8.94
Tryptophan	6	7,4	12	18,6	3.81
Valine	39	42	42	41,2	12.47
Tot. indispensable AA	277	312,4	359	334,3	**105.92**

* Data obtained from: (FAO/WHO, 2013)[a], Nowak et al. (2016)[b], Teleszko et al. (2022)[c]

Table A 7. Amino acid score for the hemp-quinoa beverage

Ingredient	Histidine	Isoleucine	Leucine	Lysine	Met + Cys	Phenylalanine + tyrosine	Threonine	Tryptophan	Valine
Quinoa[b]	29	36	59	54	36	61	30	12	42
Hemp[c]	24	35.3	55.6	32	35	62.1	30.5	18.6	41.2

Target (children)	18	31	63	52	26	46	27	7.4	42
Target (adult)	15	30	59	45	22	38	23	6	39
Total	26.7	35.7	57.4	43.7	35.5	61.5	30.2	15.1	41.6
AAS (children)	148.1	115.1	91.1	84.1	136.7	133.7	111.9	203.8	99.1
AAS (adult)	177.8	118.9	**97.3**	**97.2**	161.5	161.9	131.4	251.3	106.7

Table A 8. PDCAAS calculation for the hemp-quinoa beverage

Ingredient	Percentage in formulation	Protein content (g/100g ingre.)	Protein digestibility	Recipe protein digestibility	AAS	PDCAAS
Quinoa	26.17%	15	92%	90.22%	97%	0.87
Hemp	3.58%	32	84.1%			

Table A 9. Data used for estimating DIAAS values

IAA	Adult requirements[a] (mg/g protein)	Rapeseed IAA[b] (mg/g protein)	Rapeseed SID[c]	Faba bean IAA[d] (mg/g protein)	Faba bean TID[e]
Histidine	15	32	0.806	30	0.851
Isoleucine	30	37	0.708	54	0.884
Leucine	59	74	0.730	99	0.876
Lysine	45	64	0.816	76	0.916
SAA	22	54	0.824	18	0.924
AAA	38	62	0.688	99	0.884

Threonine	23	38	0.719	41	0.892
Tryptophan	6	14	0.763	12	0.898
Valine	39	51	0.715	41	0.884

Note: data are from FAO (2013) [a], DSM-Firmenich (2026) [b], Bailey and Stein (n.d.)[c], Green Goddess (n.d.)[d], Itkonen et al., (2024)[e].

Table A 10. Calculation of dry matter content (hemp-quinoa beverage)

Sample	Sample weighed (g)	Dish weight (g)	Sample weight after 24 h (g)	Dry matter (%)	Average dry matter (%)
Sample 1	3.03	2.06	2.27	6.93%	
Sample 2	3.10	2.47	2.68	6.77%	**6.9%**
Sample 3	3.00	2.47	2.68	7%	

Table A 11. Calculation of dry matter content (rapeseed-faba bean beverage)

Sample	Sample weight (g)	Disk weight (g)	Sample weight after 24 h (g)	Dry matter (%)	Average dry matter (%)
Sample 1	3.92	2.46	2.96	12.7%	
Sample 2	2.99	2.45	2.83	12.6%	**12.4%**
Sample 3	3.93	2.46	2.93	11.9%	

Table A 12. Raw data of the total protein content of both beverages

Sample	Total nitrogen	Dry protein (%)	Original protein (%)
HQB 1	2.59	16.20	1.12
HQB 2	2.54	15.85	1.09

HQB 3	5.65	16.59	1.14
RFB 1	4.32	27.02	3.35
RFB 2	4.33	27.08	3.36
RFB 3	4.34	27.13	3.36

Table A 13. Total amino acid analysis from Eurofins for the hemp-quinoa beverage

Amino acids	**AA content (g/100g)**	**Uncertainty (%)**
Tryptophan (total)	0.211	± 10
Alanine	0.726	± 14
Arginine	1.83	± 14
Aspartic acid	1.60	± 14
Glutamic acid	2.67	± 14
Glycine	0.745	± 14
Histidine	0.448	± 14
Hydroxyproline	< 0.2 (LOQ)	
Isoleucine	0.619	± 14
Leucine	1.06	± 14
Lysine	0.702	± 14
Ornithine	< 0.05 (LOQ)	
Phenylalanine	0.714	± 14
Proline	0.620	± 14
Serine	0.750	± 14
Threonine	0.550	± 14
Tyrosine	0.520	± 14

Valine	0.800	± 14
Cysteine + Cystine	0.245	± 14
Methionine	0.372	± 14

Table A 14. Total amino acid analysis for the rapeseed-faba bean beverage from Eurofins

Amino acids	AA content (g/100g)	Uncertainty (%)
Tryptophan (total)	0.265	± 10
Alanine	0.883	± 14
Arginine	1.54	± 14
Aspartic acid	1.76	± 14
Glutamic acid	4.47	± 14
Glycine	0.941	± 14
Histidine	0.582	± 14
Hydroxyproline	< 0.2 (LOQ)	
Isoleucine	0.841	± 14
Leucine	1.58	± 14
Lysine	1.26	± 14

Ornithine	< 0.05 (LOQ)	
Phenylalanine	0.866	± 14
Proline	1.22	± 14
Serine	0.940	± 14
Threonine	0.765	± 14
Tyrosine	0.534	± 14
Valine	1.03	± 14
Cysteine + Cystine	0.453	± 14
Methionine	0.317	± 14

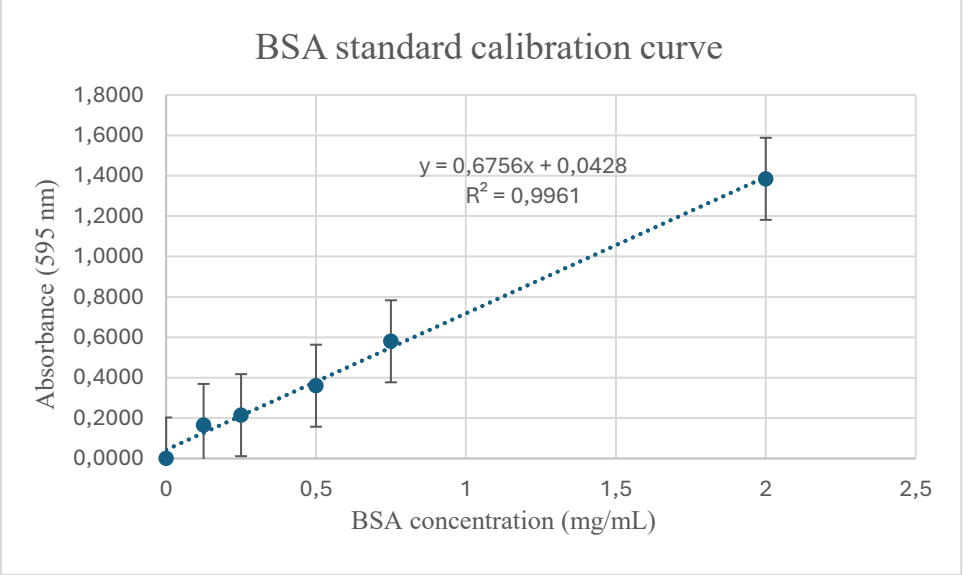

Figure A 4. BSA standard calibration curve for Bradford assay

Table A 15. Bradford assay raw data

Sample	Absorbance (595 nm)	Average absorbance (595 nm)	Absorbance-blank (595 nm)	Concentration (mg/mL)	Concentration not diluted (mg/mL)
Blank’	0.439	0.446 ± 0.007	-	0.0	-
Blank‘’	0.452				
Blank‘’’	0.446				
STD 1’	1.820	1.831 ± 0.010	1.385	2.0	-
STD 1‘’	1.838				
STD 1‘’’	1.834				
STD 2’	1.030	1.026 ± 0.006	0.580	0.75	-
STD 2‘’	1.032				
STD 2‘’’	1.016				
STD 3’	0.793	0.806 ± 0.011	0.360	0.5	-
STD 3‘’	0.813				
STD 3‘’’	0.812				
STD 4’	0.653	0.660 ± 0.007	0.214	0.25	-
STD 4‘’	0.661				
STD 4‘’’	0.667				
STD 5’	0.606	0.612 ± 0.005	0.166	0.125	-
STD 5‘’	0.614				
STD 5‘’’	0.616				
PPI pH 3.1’	1.659	1.672 ± 0.012	1.273	1.82 ± 0.018	-
PPI pH 3.1‘’	1.681				

PPI pH 3.1‘’’	1.677				
PPI pH 6.5-6.9’	1.093	1.108 ± 0.013	0.709	0.99 ± 0.019	-
PPI pH 6.5-6.9‘’	1.112				
PPI pH 6.5-6.9‘’’	1.118				
RPI pH 7.5’	1.488	1.491 ± 0.014	1.092	1.55 ± 0.021	-
RPI pH 7.5‘’	1.479				
RPI pH 7.5‘’’	1.505				
RPI pH 6.5-6.9’	1.591	1.594 ± 0.006	1.195	1.70 ± 0.009	-
RPI pH 6.5-6.9‘’	1.585				
RPI pH 6.5-6.9‘’’	1.607				
FPI pH 6.5’	0.832	0.839 ± 0.009	0.440	0.59 ± 0.013	-
FPI pH 6.5‘’	0.845				
FPI pH 6.5‘’’	0.841				
HPC pH 7’	0.511	0.517 ± 0.006	0.118	0.11 ± 0.009	-
HPC pH 7‘’	0.519				
HPC pH 7‘’’	0.522				
HPC pH 6.5-6.9’	0.521	0.526 ± 0.006	0.127	0.13 ± 0.009	-
HPC pH 6.5-6.9‘’	0.533				
HPC pH 6.5-6.9‘’’	0.525				
HQB’	0.522	0.537 ± 0.013	0.091	0.071 ± 0.019	0.710 ± 0.19

HQB‘’	0.545				
HQB‘’’	0.543				
HQB P’	0.518	0.526 ± 0.009	0.081	0.056 ± 0.013	0.560 ± 0.13
HQB P‘’	0.525				
HQB P‘’’	0.536				
RFB’	0.864	0.874 ± 0.012	0.428	0.571 ± 0.018	5.71 ± 0.18
RFB‘’	0.871				
RFB‘’’	0.888				
RFB P’	0.815	0.836 ± 0.044	0.390	0.514 ± 0.065	5.14 ± 0.65
RFB P‘’	0.886				
RFB P‘’’	0.806				

Note: PPI, potato protein isolate; RPI, rapeseed protein isolate; FPI, faba bean protein isolate; HPC, hemp protein concentrate; HQB, hemp-quinoa beverage (untreated with phytase); HQB P, hemp-quinoa beverage treated with phytase; RFB, rapeseed-faba bean beverage (untreated with phytase); RFB P, rapeseed-faba bean beverage treated with phytase.

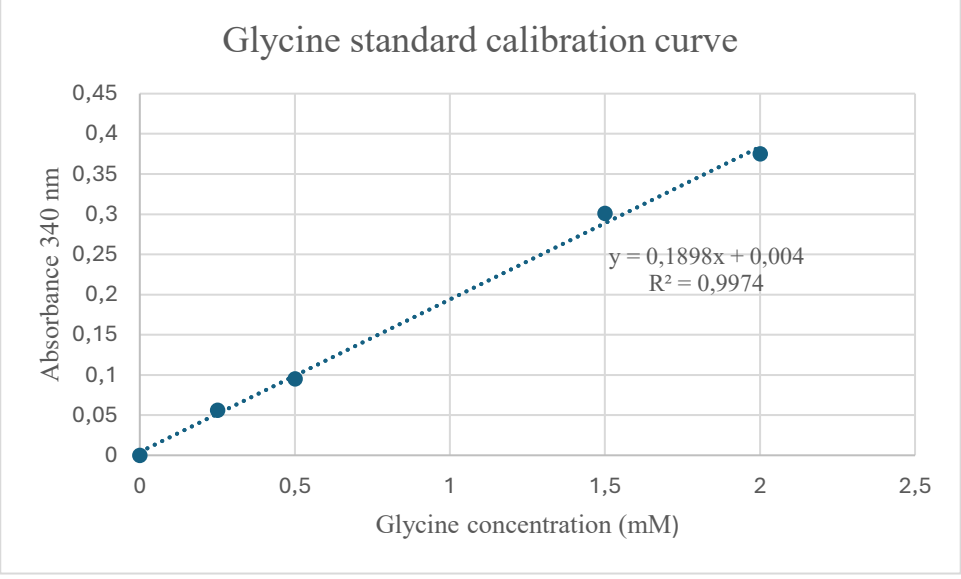

Figure A 5. Glycine standard calibration curve for the free amino acid colorimetric assay (OPA)

Table A 16. Raw data used to calculate free amino acid content in the OPA colourimetric analysis

Sample	Abs. 340 nm Replicate 1	Abs. 340 nm Replicate 2	Abs. 340 nm Replicate 3	Abs. Avg. 340 nm	Free AA in samples (mM)	Free AA in beverage (mM)
STD 1	-	-	-	0.056	0.25	-
STD 2	-	-	-	0.095	0.5	-
STD 3	-	-	-	0.301	1.5	-
STD 4	-	-	-	0.375	2.0	-
HQB 1 ND	0.162	0.160	0.158	0.160±0.002	0.82±0.011	1.64±0.021
HQB 1 D	0.401	0.373	0.379	0.384±0.015	2.0±0.079	4.0±0.158
HQB 2 ND	0.169	0.152	0.153	0.158±0.010	0.81±0.053	1.62±0.105
HQB 2 D	0.394	0.371	0.377	0.381±0.012	2.0±0.063	4.0±0.126
RFB 1 ND	0.261	0.231	0.241	0.244±0.015	1.3±0.079	2.6±0.158
RFB 1 D	0.724	0.681	0.714	0.706±0.023	3.7±0.121	7.4±0.242
RFB 2 ND	0.259	0.246	0.249	0.251±0.007	1.3±0.037	2.6±0.074
RFB 2 D	0.703	0.674	0.696	0.691±0.015	3.6±0.079	7.2±0.158

Note: The blank was already subtracted from the shown absorbance values. For the standards, only the average absorbance was recorded, therefore the standard deviation could not be calculated, and this is recognised as a limitation of the analysis. The RFB digested concentrations were extrapolated beyond the linear range, and this is another acknowledged limitation. For the samples: HQB, hemp-quinoa beverage; RFB, rapeseed-faba bean beverage; 1, not treated with phytase; 2, phytase-treated; ND, not digested; D, digested.

Table A 17. Data used to calculate the standard curve for the phytic acid content analysis

STD sample	A655	Average ± std deviation (A655)	Phosphorus (µg)	ΔA phosphorus	M (µg/ΔA phosphorus)	Mean M (µg/ΔA phosphorus)
STD 0	0.076					
STD 0'	0.076	0.076 ± 0	0	0	0	
STD 0''	0.076					
STD 1	0.175					
STD 1'	0.177	0.176 ± 0.001	0.5	0.1	5	
STD 1''	0.176					5.139
STD 2	0.572					
STD 2'	0.566	0.569 ± 0.003	2.5	0.493	5.071	
STD 2''	0.568					
STD 3	1.047	1.045 ± 0.003	5	0.969	5.160	

STD 3'	1.042				
STD 3''	1.046				
STD 4	1.182				
STD 4'	1.482	1.484 ± 0.003	7.5	1.408	5.327
STD 4''	1.488				

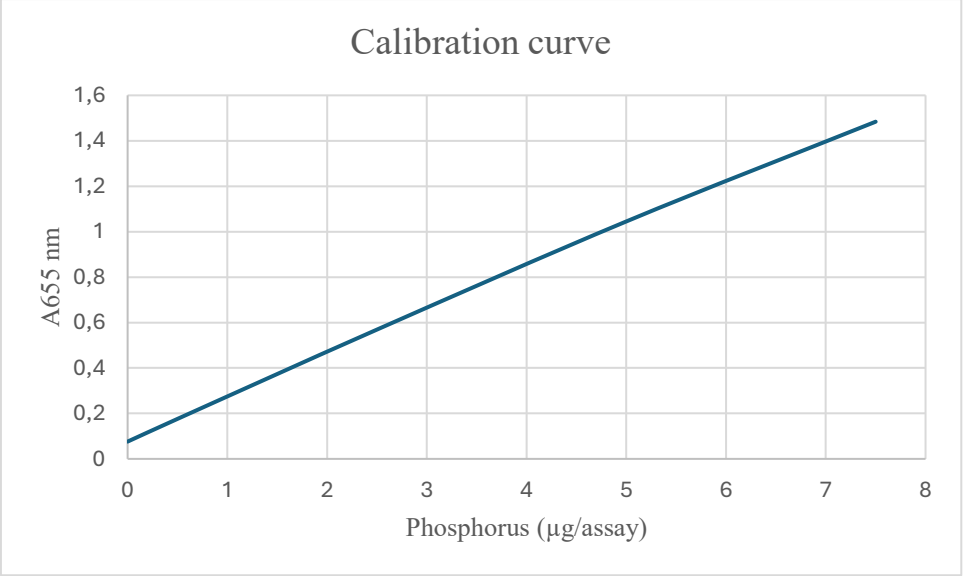

Figure A 6. Standard curve - phytic acid analysis

Table A 18. Phytic acid analysis raw data

Sample	A655	Sample (ΔA)	ΔA	Phosphorus (g/100g)	Phytic acid (g/100g)

HQB 1NDF	0.096	***HQB 1ND***	0.014	0.008	0.028
HQB 1NDF ‘	0.094	***HQB 1ND ‘***	0.010	0.006	0.020
HQB 1NDF ‘’	0.095	***HQB 1ND ‘’***	0.008	0.005	0.016
HQB 1NDT	0.110				
HQB 1NDT ‘	0.104				
HQB 1NDT ‘’	0.103				
HQB 2NDF	0.135	***HQB 2ND***	0.005	0.003	0.010
HQB 2NDF ‘	0.136	***HQB 2ND ‘***	0.006	0.003	0.012
HQB 2NDF ‘’	0.133	***HQB 2ND ‘’***	0.010	0.006	0.020
HQB 2NDT	0.140				
HQB 2NDT ‘	0.142				
HQB 2NDT ‘’	0.143				
HQB 1DF	0.080	***HQB 1D***	0.011	0.006	0.022
HQB 1DF ‘	0.080	***HQB 1D ‘***	0.009	0.005	0.018
HQB 1DF ‘’	0.079	***HQB 1D ‘’***	0.010	0.006	0.020
HQB 1DT	0.091				
HQB 1DT ‘	0.089				
HQB 1DT ‘’	0.089				
HQB 2DF	0.087	***HQB 2D***	0.011	0.006	0.022
HQB 2DF ‘	0.086	***HQB 2D ‘***	0.012	0.007	0.024
HQB 2DF ‘’	0.085	***HQB 2D ‘’***	0.012	0.007	0.024
HQB 2DT	0.098				
HQB 2DT ‘	0.098				
HQB 2DT ‘’	0.097				

RFB 1NDF	0.080	***RFB 1ND***	0.017	0.010	0.034
RFB 1NDF ‘	0.080	***RFB 1ND ‘***	0.015	0.009	0.030
RFB 1NDF ‘’	0.080	***RFB 1ND ‘’***	0.015	0.009	0.030
RFB 1NDT	0.097				
RFB 1NDT ‘	0.095				
RFB 1NDT ‘’	0.095				
RFB 2NDF	0.115	***RFB 2ND***	0.012	0.007	0.024
RFB 2NDF ‘	0.113	***RFB 2ND ‘***	0.014	0.008	0.028
RFB 2NDF ‘’	0.113	***RFB 2ND ‘’***	0.013	0.007	0.026
RFB 2NDT	0.127				
RFB 2NDT ‘	0.127				
RFB 2NDT ‘’	0.126				
RFB 1DF	0.081	***RFB 1D***	0.011	0.006	0.022
RFB 1DF ‘	0.080	***RFB 1D ‘***	0.010	0.006	0.020
RFB 1DF ‘’	0.080	***RFB 1D ‘’***	0.010	0.006	0.020
RFB 1DT	0.092				
RFB 1DT ‘	0.090				
RFB 1DT ‘’	0.090				
RFB 2DF	0.083	***RFB 2D***	0.015	0.009	0.030
RFB 2DF ‘	0.082	***RFB 2D ‘***	0.014	0.008	0.028
RFB 2DF ‘’	0.083	***RFB 2D ‘’***	0.014	0.008	0.028
RFB 2DT	0.098				
RFB 2DT ‘	0.096				
RFB 2DT ‘’	0.097				

Oat F	0.089	***Oat***	0.006	0.003	0.012
Oat F ‘	0.088	***Oat ‘***	0.007	0.004	0.014
Oat F ‘’	0.088	***Oat ‘’***	0.006	0.003	0.012
Oat T	0.095				
Oat T ‘	0.095				
Oat T ‘’	0.094				

* 1: no phytase // 2: with phytase // ND: non-digested // D: digested // F: free phosphorus // T: total phosphorus // ‘: replicate 1 // ‘’: replicate 2