DEVELOPMENT OF PLANT-BASED STRAINED YOGURT

A Thesis

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by

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Abstract

Following the developments of the world's population, alternative sources of protein to the complement and / or replacement of animal proteins are subject to intense development efforts in research and industries. Climate change and a better understanding of the environmental impact of food production have created an urgent need to develop sustainable food systems. The demand for plant-based proteins is due to multiple factors, the vegetarian, vegan and flexitarian populations have promoted the use of plant-based proteins in the production of food products. In addition, there is a global challenge to address food security and to preserve land and water resources due to climate change, population growth and changing the diets. According to research, the world's population could grow to exceed 9 billion people by 2050, leading to a sharp increase in demand for food in general and for food protein and animal feed. During recent years, a sustainable lifestyle has gained a remarkable interest among consumers, closely related to both heath and ethical reasons. To achieve this goal, a major shift towards plant-based food stuff is necessary, in which plant-based proteins products represent a great segment. The aim of this review is to tackle the different extraction/purification techniques used for plant-based proteins, as well as the challenges associated with the processing of plant proteins and how these challenges could be overcome.

Chapter 1

i. Introduction

Food is an essential part for our survival on this planet, it's the fuel of the human body and a source of energy. Throughout the decades, humans were inventive with different patterns and habits of their diets. As meals are at once associated with human health, they have all started to pay expanded interest to the meals they eat. We live in a fast paced world, where we want everything now and fast, regardless of how it would affect us in the future. As consumers, we don't plan ahead how our future generations will suffer from our simpler choices. Consumers must make sustainable choices for the production and consumption of food because we have a responsibility to preserve our environment for generations to come. One way to make an eco-sustainable choice is to choose to produce or help to producing food in a way that will not only benefit us, but will have a huge positive impact on the environment.We must practice a sustainable mode of food production and consumption. What makes a diet sustainable? It is based on low-input agro-ecological staple food production including limited animal husbandry, short-distance production- consumption nets, minimal food processing and refining, important culinary skills, diet and nutrition education, and firm links to positive traits of ancestral local cultures as well as appropriate use of recent technology tools. Biodiversity improvement appears to be a key for sustainable food production and food consumption (Burlingame, 2012). A sustainable diet as defined by the Food and Agriculture organization (FAO), is a diet with low impact on the environment and is respectful and protective of biodiversity and ecosystems (Burlingame, 2012). According to EAT-Lancet Commissions' report, a sustainable diet was referred as a healthy and Planetary Health Diet, which consists of consuming mainly plant-based foods and small amounts of animal-based foods (Willett et al., 2019). It also aims to more than double the consumption of fruits, vegetables, legumes and nuts and more than half of the global consumption of red meat and added sugars by 2050. To achieve this goal, a major transformation of current westernized diets towards predominantly plant-based diets is necessary. Plant-based diets consist of a diverse family of eating patterns, defined in terms of low frequency of consumption of foods of animal origin (Satija & Hu, 2018). In recent years, European consumers have started to consider consuming plant-based diets and plant-based fermented foods such as tofu or other newly developed probiotic products, and so it is no longer a market of niche. Allergies, especially to nuts, gluten, milk protein and lactose, are on the increase and avoiding them is considered a healthy lifestyle (Rosario & Robin, 2018). A previous study has shown that plant-based dairy alternatives are attractive and tasty when fermented. Additionally, the same study found that fermenting plant-based dairy substitutes can potentially improve sensory attributes, nutritional quality, and texture. Plant-based yogurt substitutes are a good example of fermented dairy products that depend on different sources such as grains, legumes, nuts, seeds, or pseudo-grains (Mäkinen et al., 2016). Health, accessibility and the environment are the three key components of food consumption which must be balanced to provide a sustainable diet in accordance with health recommendations (Hwalla et al., 2021).

On the contrary, the Lebanese Mediterranean Diet is characterized by high consumption of animal-based foods and a decreased intake of plant-based foods such as fruits and vegetables (Hwalla et al., 2021). Lebanon continues to face damaging economic, health and socio-political challenges that are further worsen by the COVID19 pandemic. At the same time, the country is undergoing a significant nutritional transition that has contributed to the burden of malnutrition and noncommunicable diseases, all of which have serious repercussions on people's livelihoods, food security and health. Such circumstances have spurred public demand for advice on practical, healthy and sustainable food choices to alleviate the burden of this emerging unfortunate situation (Hwalla et al., 2021).

ii. Plant-based diet

1. Definition

A plant-based diet includes a variety of dietary patterns such as vegetarianism, veganism, lacto-vegetarianism, pescatarianism and other diets that fall under this category (Satija & Hu, 2018). It focuses on foods primarily from plants, and is also defined as the non-meat, fish, chicken, pork, eggs, dairy products or honey consumption (Tabanelli et al., 2018). Plant-based products are made from natural sources and ingredients, and are similar to the conventional animal-based foodstuff in terms of texture, sensory attributes and other criteria (Montemurro et al., 2021).

Some of these innovative food products are plant-based eggs, cultured meat, tofu, among others. (Rondoni et al., 2021; Tuomisto & Teixeira de Mattos, 2011; Zheng et al., 2020).

2. Advantages of plant-based diets

The increased demand for a plant-based diets is due to the nutritional, cultural, and ethical aspects, and most importantly its health benefits. (Tabanelli et al., 2018). For instance, the vegetarian trend has grown by 360% in ten years between 2007 and 2017 because people are more aware of the benefits that this diet offers which are lacking in other dietary patterns such as the animal-based diet (Aydar et al., 2020). It has been shown that a plant-based diet is highly beneficial and effective in treating cardio-metabolic diseases, decreasing blood lipids and blood pressure, controlling weight, treating and preventing diabetes type 2 (Kahleova et al., 2017). (Päivärinta et al., 2020). Plant-based diets in contrast to animal-based diets are more sustainable because they use fewer natural resources and are less harmful to the environment (Sabaté & Soret, 2014).

3. Plant-based products

In the 1980s, Dr. T. Colin Campbell introduced the term "plant-based diet" into the world of nutritional science to denote a low-fat, fiber-rich, plant-based diet, with a focus on health rather than ethics.

In recent years, more concerns about food allergies are increasing, more specifically milk proteins and lactose intolerance. Hence, non-dairy milk substitutes such as plant-based yogurt, cheese, kefir, butter, ice-cream, are essential to prevent these allergies and intolerances (Aydar et al., 2020). These products are fortified by plant proteins such as chickpeas, lentils, pea, fava bean (Sim et al., 2020) to give the final product its nutritional and functional values. Beside dairy alternatives, other plant-based products (Table.1) are available on the market. For example, cultured meat produced in *vitro* using tissue engineering techniques which is healthier and more efficient than animal meat (Tuomisto & Teixeira de Mattos, 2011).

iii. Plant-based yogurt-like products

The ability of milk to form a wide range of dairy products (hard/soft cheese, yogurt, fermented milk, etc.) is closely related to the various functional properties of its components (McClements et al., 2019).

1. Definition

A plant-based yogurt-like product represents a great segment among the dairy-free alternatives. It is similar to the conventional yogurt, but made from natural sources such as vegetables, legumes, cereals, grains, nuts, seeds, or even from plant-based milk (Grasso et al., 2020; Montemurro et al., 2021).

2. Conventional yogurt and strained yogurt (Labneh)

Yogurt is one of the most popular dairy products consumed worldwide due to its versatility in flavors and usage. It is a fermented milk produced using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus* (Savaiano & Hutkins, 2021). Yogurt is a good source of nutrients, proteins, vitamins, fibers and beneficial probiotics (Grasso et al., 2020; Sajdakowska et al., 2021; Zhi et al., 2018).

Strained yogurt or labneh is a semi-solid or concentrated yogurt with longer shelf-life (Mehanna et al., 2021). Traditionally, labneh is made by straining yogurt for several hours through cloth bags to remove the whey. The sensory, physicochemical and nutritional characteristics of labneh are affected by the different types of milk used in the production, the fat content (Table.3) and animal source. The type of milk significantly affects the apparent modulus, hardness, hardness and bonding strength, while the fat content significantly affects the hardness. It also significantly affects the sensory properties such as syneresis, firmness, goat smell and taste, flow rate, color, gloss, bitterness, density, melting speed and spreadability, while fat content only affects color, density and melting speed (Atamian et al., 2014).

3. Market and trends

The layout of novel PB and yogurt-like (YL) merchandise (PBYL) received excessive interest because of the brand-new possibilities supplied with the aid of using the global market. Furthermore, PBYL are taken into consideration as a financial opportunity to dairy merchandise in growing countries (Montemurro et al., 2021). Current market share forecasts show that plant-based milk substitutes will grow at a continuous annual growth rate of more than 8-12% within four years. Euromonitor data shows that the sales of plant-based milk substitutes have doubled in the six years from 2009 to 2015 (Puckorius, 2015).

4. Advantages and disadvantages of PBYL products

In the last decade, consumers tend more towards plant-based yogurts consumption than the ones produced of cow’s milk (Aydar et al., 2020). However, PBYL are different from the conventional dairy products in terms of nutritional composition. First of all, PBYL are usually lower in the protein content than milk, but rich in fiber and unsaturated fatty acids. Moreover, they do not contain any cholesterol (Aydar et al., 2020).

iv. Manufacturing process of PBYL products

1. Ingredients

Choosing the right ingredients is the first step in formulating a plant-based yogurt. Generally, grains, pseudo grains, and legumes are used in various combinations to achieve the best texture and nutritional quality of foods.

Starters are a key ingredient for the fermentation, which are exopolysaccharide-producing bacteria (EPS), and are selected depending on the main raw ingredient used for the production of a plant-based yogurt (Montemurro et al., 2021). For example, commercial thermophilic starters are used with brown rice, germinated brown rice or soaked rice (Cáceres et al., 2019). Other cultures such as *Limosilactobacillus reuteri and Streptococcus thermophilus* are used with almond (Bernat et al, 2015). The selected starter used in the production of a PBYL product should be able to quickly acidify, and prevent spoilage and microbial contamination. It should also fully improve the nutritional and sensory qualities, impart a pleasant aroma, and may improve the texture through exopolysaccharide (EPS) synthesis and survive at high cell density in cold storage conditions (Montemurro et al., 2021). Probiotics can be added to yogurt after or before fermentation, using starter cultures selected for their respective processing characteristics (Montemurro et al., 2021). For example, for the cereal/legumes-based yogurt, *Lacticaseibacillus rhamnosus* probiotic is used (Pontonio et al., 2020). In order to improve the nutritional value of a plant-based yogurt, pulse proteins can be used as alternative sources of protein, mainly derived from peas, lentils, chickpeas, beans and lupins (Vogelsang-O'Dwyer et al., 2021). Pulse proteins must have good functional properties, such as solubility and emulsification properties (Vogelsang-O'Dwyer et al., 2021). Pulse protein-based yogurts are commercially new and rare (Boeck et al., 2021).

2. Antinutritional factors in pulses

Pulse proteins are composed of high protein contents, carbohydrates (starch, non-starch and dietary fibers), minerals, vitamins and phytochemicals (i.e., phenolic acid, carotenoids) (Hall et al., 2017; Lam et al., 2018; Vatansever & Hall, 2019). Globulins, albumins, glutelins, prolamins are the major protein crop fractions in peas, fava beans and lentils (Vatansever & Hall, 2019).

Lentils has a downside of presence of antinutritional compounds (ANCs) and allergens which reduce their potential health benefits and bioavailability (Derbyshire, 2011). Removal of ANCs is necessary to improve the nutritional profile. Some of the ANCs found in lentils are lectins. Lectin is found in large quantities in lentils, but can be completely eliminated after a 72-hour fermentation treatment at to 42°C. However, lectins in lentils are not toxic (Campos-Vega et al., 2010). Besides lectin, other ANCs such as phytic acid, glucosinolate, phenolic compounds, and phytates need to be removed before incorporating them in the feed (Chéreau et al., 2016). On the other hand, fava beans, lupin and peas are a great source of ANCs as tannins, legumin, vicin and convicin. Fermentation has been shown to greatly reduce the ANCs total concentration (Boeck et al., 2021). Several processing methods were effective in reduction of ANCs in chickpeas, such as phytic acid, polyphenols, tannins, saponins, oxalates and trypsin inhibitor activity (TIU), by germination, boiling, pressure cooking and roasting (Rajni Mittal et al., 2012). Protein isolates are acceptable ingredients for dairy products because of their small particle size and dispersibility. It has been found that the pressure cooking method was the most efficient in removing the ANCs by up to 71.79% (Rajni Mittal et al., 2012)

v. Pulse protein production

Several technologies are used in the extraction of pulse proteins from a variety of sources (Table.5)

Fibers act as a bulking agent. Many researchers report that the rheological properties of yogurt vary depending on the type of fiber source (I. B. Hashim et al., 2009). Fibers play a major role in increasing water retention, stabilizing high-fat yogurt, improving viscosity and gelling properties. They can be used to strengthen yogurt to improve texture and reduce syneresis (Balthazar et al., 2016). For example, pea fibers work well with other ingredients and are a good filler. Due to their quality, they have the potential to be used in a variety of applications from the bakery to the dairy industry. High fiber content can increase the nutritional value of the final product, so pea fiber fortified yogurt supplements its health benefits (Dabija et al., 2018). Fortification and enrichment of yogurt by calcium, vitamin A, B1, B2, B12, D2 and E is also important and calcium concentration can be increased in the final product by the use of calcium trinitrate (Aydar et al., 2020).

vi. Manufacturing techniques of PBYL product

Different PBYL were produced using several technical options (Table.6), mainly based on the use of heat treatment, enzymes, and fermentation.

For instance, Montemurro et al, have developed a PBYL product by mixing the raw materials (cereals, legumes, vegetables, fruit purees or nuts & grains) with water, followed by pre-fermentation treatments (pasteurization and/or starch gelatinization and/or filtration and/or high-pressure homogenization). Isolated lactic acid bacteria strains from plants were added to start the fermentation, and/or probiotics. PBYL was supplemented with additives (flavor enhancers, fibers, sweeteners and sugars, structuring agents and probiotics). This technique used and mixed different plant-based ingredients and raw materials to modulate the nutritional composition (proteins, sugars, fats, fiber concentration) and create a PBYL formulation that meets the specific nutritional and sensory needs of the consumer.

Fermentation has been recognized as essential to achieving an adequate sensory profile, but it is also a powerful biotechnological tool to improve the nutritional and functional properties of ingredients of plant origin. Results revealed that grain-based yogurt also appears to have specific sensory properties, depending on the species under consideration. Unpleasant smells and tastes have been associated with the use of raw, unprocessed, or unrefined forms. derived from phenolic compounds found in the outer layers of whole grain products. Although oats are primarily used as the main ingredient in the commercial production of PBYL, they quickly develop a green and bitter taste if not properly post-harvest heat treatment aimed at inactivating lipolytic enzymes (Montemurro et al., 2021).

Other studies, focused on the fermentation of a plant-based milk in order to produce PBYL. Different types of milk were taken into consideration such as cashew, coconut, hazelnut, almond, rice milk and others (Aydar et al., 2020; Jacobowitz, 2019.). Plant-based milk was later fortified with pea and wheat protein isolates (Jacobowitz, 2019.). The participated panelists liked the control yogurt sample more than the fortified samples; however, the almon-based yogurt received more liking than the coconut-based one (Jacobowitz, 2019).

In the fava bean-based yogurt analogue, food preparation, amylase hydrolysis, oil addition, homogenization, and lactic acid bacteria fermentation were effective and beneficial for the manufacture of yogurt analogs from whole beans. The protein effectively stabilizes the emulsion and forms a typical gel network. The hydrolyzed starch compound improves the gel strength and viscosity of the yogurt analog (Jiang et al., 2020).

On the other hand, the homemade version of nut-based yogurt, the data obtained indicated the presence of spontaneous lactic acid fermentation which is responsible for the formation of taste and essential for improving the hygienic quality of the product. In the spontaneous fermentation, however, no pH value (4.4) could be reached that could inhibit the growth of *Listeria Monocytogenes* and the addition of NaCl at the end of the fermentation could not improve the selection and competitiveness of safe LAB. The use of local starter cultures separated from the natural substrate and inoculated in sufficient quantities can achieve a controlled fermentation process, so that the product has the characteristics required for industrial production, storage and distribution (for example, pH and <4.4) (Tabanelli et al., 2018).

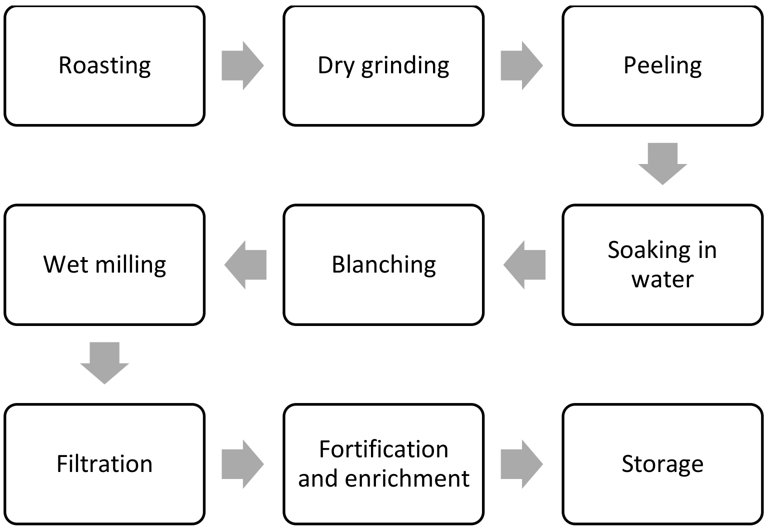

Figure 1. The following chart describes the manufacturing process of the milk substitute, starting from the raw material until the end product formation

Roasting is applied for nuts such as peanuts, almonds, hazelnuts, etc., it reduces acidity, total solids content, fat, protein, and prevents bitterness and chalky taste (Ahmadian-Kouchaksaraei et al., 2014). Dry grinding is dry milling of roasted grains (Aydar et al., 2020). Peeling consists of soaking the nuts or grains in water or acid or base for a period of time, depending on the raw material used, in order to remove the outer skin (Aydar et al., 2020). After this step, soaking in water is essential for the swelling and softening of the grains, thus reducing the blanching time

(Aydar et al., 2020). Blanching is applied for sesame, coconuts, peanuts, rice and quinoa. It has many benefits, such as reducing microbial load and inactivating enzymes (Aydar et al., 2020). In the wet milling process, water is added to the raw material and then grounded. The amount of water added, the milling temperature, the pH, the type of milling and the feed rate are the factors that affect the final product (Aydar et al., 2020). Filtration is applied to separate the cake and also the milk a part of the ground raw material, using different filtering materials such as cheesecloth (Ahmadian-Kouchaksaraei et al., 2014). Additional ingredients are added to the final product such as sweeteners, sugars, flavors, stabilizers, emulsifiers, and others (Aydar et al., 2020). Aseptic packaging and cold storage temperature are necessary for long shelf-life and high stability. The storage temperature must be +4°C (Aydar et al., 2020).

vii. Nutritional Properties

The nutritional properties of plant-based yogurt depend on the quality and type of raw materials used and the type of processing. Most commercial plant-based milks are fortified with calcium and vitamin D, and many manufacturers use cheap additives such as sweeteners, sugars and stabilizers to improve sensory and processing properties, which means lower nutritional value (Rincon et al., 2020). Chickpea and coconut-based milk display a good nutritional composition in comparison to cow's milk, highest protein content thus no required fortification. Whereas milk extracted from soy, almon and rice are poor in protein, calcium and iron (Rincon et al., 2020).

Another study assessed, that the fermentation of a blend of cereals and legumes by a selected lactic acid bacteria strain (LAB) (Lactoplantibacillus plantarum and Levilactobacillus brevis) in comparison to an unfermented control sample, induced a significantly higher concentration of free amino acids and a lower content of anti-nutritional factors (i.e., phytic acid) (Pontonio et al., 2020). Physicochemical analysis is essential to evaluate the storage, quality and performance properties of a plant-based yogurt. Plant-based yogurts have creamy-like appearance, long stability thus long shelf-life and low-viscous texture (McClements, 2020). Determining the pH, acidity, particle aggregation, viscosity, color index, and chemical degradation are crucial. Acidity and viscosity vary with storage temperatures and time, thus the higher is the temperature and storage time, the more the acidity is and less storage shelf-life of the product. Zhi and her colleagues, observed increased acidity with increased temperature and a drop in viscosity (Zhi et al., 2018). Comparing the total titratable acidity between dairy and plant-based yogurt, showed higher values for the dairy samples than the plant-based ones (1.38 mL NaOH/g and 0.12–0.78 mL NaOH/g respectively) (Grasso et al., 2020). These changes lead to samples deterioration. The brightness of yogurt depends on the particle size of fat globules and protein and type of raw material, which affects their light reflection and light scattering capabilities. According to a study, cashew had L-value of 84.67 indicating an off white color, whereas the dairy sample had a higher L-value of 94.47 (Mattison et al., 2020).2

viii. Sensory Attributes

Sensory evaluation can guide and promote the development of plant milk substitutes. Consumers' overall perception and acceptance of products are based on the integration of multiple sensory attributes which are appearance, odor, texture, flavor and color. However, when evaluating the sensory properties of plant-based milk, some additional factors need to be considered because it has some unique properties, including "beans" and "green" flavors, plant-based aromas, and specific color properties (for example, Beige or yellow) (Grossmann et al., 2021). The assessment of fermented yogurt with specific LAB strains, showed that the novel yogurt was characterized by acidic and creamy odor and taste (Pontonio et al., 2020). Fermented crude brown rice showed bitter and sour flavor, and creamy texture (Cáceres et al., 2019).

Table 1. Examples of plant-based products

Animal-based product	**Plant-based product**	**Reference**
Bovine milk	Almond, cashew, oat, coconut, soy, rice, etc.	(McClements, 2021; McClements et al., 2019)

Yogurt	Cashew/peanut/chickpea/rice/lupin/coconut-based yogurt	(Cáceres et al., 2019; Grasso et al., 2020; Hickisch et al., 2016; Mattison et al., 2020; McClements, 2021)
Meat	Cultured meat	(Tuomisto & Teixeira de Mattos, 2011)
Eggs	Plant-based eggs	(McClements, 2021; Rondoni et al., 2021)
Cheese	Plant-based cheese & tofu	(McClements, 2021; Zheng et al., 2020)

Table 2. Describes the analysis of labneh (Atamian et al., 2014)

Attribute	**Description**
Color	From chalky white to light ivory, yellow shades on the surface
Flavor	Salty, sour/fermented, milky, bitter
Texture	Lumpy, sticky, residual film
Odor	Sour, milky, goat

Appearance	Shiny

Table 3. The effect of milk fat content on the chemical composition of labneh (Atamian et al., 2014)

Attribute	**Full fat**	**Reduced fat**	**Low fat**
Moisture (%)	73.88	76.86	80.01
Fat (%)	9.18	4.79	0.35
Protein (%)	8.14	9.30	10.65
Ash	1.56	1.58	1.66
Sodium (mg/ 100 g)	158.92	183.40	204.00
Calcium	0.30	0.30	0.32
Magnesium (mg/ 100 g)	4.05	4.57	4.78
pH	3.76	3.73	3.74
Acidity (%)	1.12	1.19	1.22

Table 4. Summarizes the most commonly used protein pulses, ANCs and their reduction processes

Protein pulses	ANCs	Process	Reference
Lentils	lectin - phytic acid, glucosinolate, phenolic compounds & phytates	72 h fermentation treatment at to 42°C	(Campos-Vega et al., 2010; Chéreau et al., 2016)
Fava beans, peas and lupin	Tannins, legumins, vicin and convicin	Fermentation	(Boeck et al., 2021)
Chickpeas	phytic acid, polyphenols, tannins, saponins, oxalates and trypsin inhibitor activity (TIU)	Germination (soaked in water for 2 days at room temperature 22 °C and dried in hot air drier at 30-35 °C), boiling (in ratio 1:7 w/v for 10 min), pressure cooking (in a pressure cooker at 15 psi using water ratio 1:2 for 15 minutes followed by drying) and roasting (for 15 min at 120 °C)	(Rajni Mittal et al., 2012)

Table 5. The different existing techniques used in the extraction of pulse proteins

Manufacturing process	Reference
Milling – Protein extraction (alkaline/neutral/acid) – centrifugation/filtration – isoelectric precipitation – washing/centrifugation – resuspension – drying – protein isolate	(Vogelsang-O'Dwyer et al., 2021)
Chickpea germination (at 22°C for 2 days, then hot air dried at 30°C-35°C) – boiling in water – pressure cooking – roasting – ground	(Rajni Mittal et al., 2012)
Deffated legumes + water – centrifugation – precipitation (protein isolate) – neutralization – freeze drying - storage	(Garba & Kaur, 2014)

Table 6. Manufacturing techniques of PBYL product

Ingredients	Manufacturing technique	Main findings	Reference
Cereal flour/flakes, legumes, vegetables,	Mixing with water – pre-fermentation treatments (pasteurization and/or starch	Formulation meeting the specific nutritional and sensory needs	(Montemurro et al., 2021)

fruits purees, nuts or grains	gelatinization and/or filtration) – starter's inoculum and/or probiotics – supplementation (addition of flavors, emulsifiers, fibers, sweeteners, probiotics,etc)		
Almond milk and coconut milk	Heating the almond milk – lactic acid bacteria addition – incubated for 10 hours until the pH is between 4.41 and 6.00 – almond and coconut yogurt are supplemented with 5g of pea protein and wheat gluten protein each.	Almon-based yogurt received more liking than the coconut-based one	(Jacobowitz, n.d.)
Cashew nuts (Homemade) (Pilot-scale)	Cashew nuts (5kg) soaked for 8 hours at room temperature with water – drained and rinsed – 2 kg of soaked cashew nuts were ground in a domestic mixer with fresh water (40%) – the cream was fermented at room temperature for 48 h with autochthonous LAB starters – salt, olive oil and lemon juice were added at the end – cold storage	Presence of spontaneous lactic fermentation	(Tabanelli et al., 2018)
Rice, lentil, chickpea	Mixing the raw materials (2:1:1) with water – thermal	To be similar to conventional yogurt for	(Pontonio et al., 2020)

	treatment at 80°C for 15 min – cooling at 4°C then heating at 30°C – inoculum of L. plantarum DSM33326 and Le. brevis DSM33325 – fermentation at 30°C for 16 h – cooling at 4°C – homogenization – additional ingredients - packaging	the high density of viable LAB and the creamy structure (or texture)	
Fava bean	Thermal pre-treatment – dehulling/milling – mixing with water – centrifugation – heating at 95 °C – water extract adding oil and homogenization – heating at 95 °C and cooling – starch free emulsion - LAB fermentation at 37 °C	Starch hydrolysis improves gel strength and viscosity while avoiding a fractionation step	(Jiang et al., 2020)
Lupin	Lupin seeds are boiled with 0.5% NaHCO3 at 93°C for 6h then cooled at room temperature – after manual dehulling, the soaked lupin is ground until smooth and sieved – water is added to a ratio 1:9 w/v – 0.1M NaOH is added until pH 6.5-7 – the milk is heated at 93°C for 20 min then cooled at 4°C For the yogurt preparation:		(Jiménez-Martínez et al., 2003)

	Lactose and sucrose milk fortification – pasteurized at 90°C for 15 min – cooled at 45 °C and inoculated with 3%(v/v) of the starter for 8h	

Table 7. Represents the production techniques of plant-based milk

Ingredients	**Manufacturing process**	**Reference**
Shelled raw material (walnut, sesame, cashew, lupin, peas, chickpeas, almond, lentils and beans)	Soak in hot water until soft and swollen Dehulling (to remove the outer skin) using a huller machine Cashew will be soaked in deionized water with NaHCO3 to remove off flavors. The rest after dehulling undergo peeling with diluted acid or base at high temperatures, then soaking in deionized water. Draining. Cashew undergo wet milling directly after draining. Cooking or blanching.	(Aydar et al., 2020)

	Wet milling (grinding the raw material with water according to specific milling conditions) Filtration (cheese-cloth or filtering paper). Sterilization (pasteurization or sterilization) Homogenization Cold storage.	
Unshelled raw material (rice)	drying soaking in hot water draining blanching. Wet milling (grinding the raw material with water according to specific milling conditions) Filtration (cheese-cloth or filtering paper). Sterilization (pasteurization or sterilization) Homogenization Cold storage	(Aydar et al., 2020)
Raw chickpea	Soak for 12 h in water – cook under pressure (2atm) at 120 °C for 20 min – cooked chickpea + 20L water in thermomixer –	(Rincon et al., 2020)

	filtration with cloth – chickpea extract	
White beans (*P. vulgaris*)	Germination at 25 °C for 3 days – mix with drinking water (1:9) – sterilization at 121 °C for 15 min – cooling at 5 °C	(Ziarno et al., 2020)
Lupin seeds	Grinding 100g of lupin – gentle mixing with water – addition of 1M NaOH – stirring for 90 min at room temperature – filtration – 1M of HCL to the filtrate – centrifugation for 15 min at 8000G – resuspension in 1L water – addition of 100 mg coffee mate and stirred at 85 °C – filtration and cooling at 5 °C. (Coffee-mate is a source of fat/sugar/emulsifier/food color/minerals)	(Snowden et al., 2007)

Table 8. The nutritional profile of cow's milk and commercial plant-based milk alternatives (Erdal, 2021)

Milk type	**Fat (g/100g)**	**Protein (g/100g)**	**Carbohydrates (g/100g)**	**Calcium (mg)**	**Reference product**
Whole cow's milk	3.5	3.0	4.5	120	Sütaş
Almond milk	1.9	0.7	2.0	120	Fomilk
Cashew milk	1.1	0.5	2.6	120	Alpro
Rice milk	1.0	0.1	9.5	120	Alpro
Soybean milk	1.8	3.0	2.5	120	Alpro
Walnut milk	4.6	1.3	0.4	10	Elmhurst
Fava bean milk	4.36	90.1	0.34	-	Ensure

Table 9. Represents the different sensory attributes of plant-based yogurt-like products based on the used raw materials

Attribute	Description	Reference
Color	Light brownish, off-white	(McClements et al., 2019)
Odor	Nutty, beany, cooked grain, starchy, caramel, leguminous (Soy, almond, coconut)	(Grossmann et al., 2021; Vaikma et al., 2021)
Flavor	Beany, bitter, astringent, grassy, rancid, metallic, earthy, watery, umami (Soy, almon, cashew, rice, oat)	(Grossmann et al., 2021; Vaikma et al., 2021)
Texture	Mouthcoating, thick, chalky, oily, lumpy (Soy, cashew, almond)	(Grossmann et al., 2021; Vaikma et al., 2021)
Appearance	Greenness, heterogeneity, darkness, redness	(Vaikma et al., 2021)

Chapter 2

I. Introduction

During recent years, sustainable dietary patterns as a part of a healthy and eco-conscious lifestyle have gained a remarkable interest among consumers closely related to both health and ethical reasons. For instance, the popularity of the vegetarian diet has grown by 360% in ten years between 2007 and 2017 due to people awareness of the health and ecological benefits this diet offers over others particularly the animal-based diet (Aydar et al., 2020). These benefits offer a contribution to solve worldwide challenges in addressing food security, preserving land and water resources , and mitigating the negative consequences of climate change (Ismail et al., 2020). Dairy products are vital for the humans health referred as whole foods since they provide a great source of minerals, proteins and vitamins (Makarem et al., 2019). Unfortunately, milk products are an excellent medium for molds and toxins growth due to the high nutritional profile and unfavorable storage conditions (Makarem et al., 2019). Aflatoxins (AFs) are carcinogens (Iqbal et al., 2011). Another global concern that has been raised recently is finding solutions for the health problems caused by food allergies more specifically milk proteins and lactose intolerance (Aydar et al., 2020). Intense efforts have been focused on the reduction of this latter problem, by the development of plant-based dairy alternatives that are gaining popularity due to their versatility (Chalupa-Krebzdak et al., 2018; Sethi et al., 2016). For instance, the popularity of plant-based yogurt has grown exponentially, with the UK taking the global lead for the number of vegan products launched in 2018. Similarly, in Europe it held more than 50% of the market revenue share in 2019 and is expected to grow at a CAGR (Compound annual growth rate) of 3.2% during the forecast period 2021-2026. Even though in some countries such as the US and Middle East it is still in its infancy stage, experts see very high potential in this market expand. In Lebanon, a Middle Eastern country, the consumers are more familiar with the dairy strained yogurt, but the plant-based local production is still a niche concept and such products are exclusively imported and thus, expensive on the market shelves. Plant-based beverages are complex media containing fat droplets, protein particles or plant cell fragments, dispersed in an aqueous medium. The physical and chemical properties of these particles determine the functional, physical, sensory and nutritional properties of the end product as well as the bioavailability of each component

(Aydar et al., 2020; McClements, 2020; Ranadheera et al., 2017; Sethi et al., 2016). The main challenges for plant-based yogurt manufacturers are in appearance and textural properties, as these products generally exhibit textural issues caused by phase separation. Upon acidification of such plant-based systems, protein destabilization leads to the formation of a discontinuous weak gel, resulting in syneresis (Grasso et al., 2020). For this reason, hydrocolloids (thickeners, emulsifiers, gel-forming agents) are often used in the formulation of plant-based yogurts to stabilize suspended particles, help structure and help mimic the properties of a milk-based yogurt (Guo & Yang, 2015). The majority of plant-based yogurt products are cereals and nuts-based. Legumes are a staple food worldwide and the most consumed varieties in Lebanon are chickpeas, beans, lentils and lupins (Bouchenak & Lamri-Senhadji, 2013; Campos-Vega et al., 2010). Such legumes are typically low in fat and cholesterol-free, with a favorable fatty acid profile dominated by unsaturated fatty acids. They are also a good source of iron, calcium, zinc, selenium, magnesium, phosphorus, copper, potassium and B vitamins (Grasso et al., 2020). Legumes fermentation can improve the proteins digestibility and upon the release of free amino acids and bioactive compounds, ANFs (Antinutritional factors) will be greatly reduced (Arbab Sakandar et al., 2021; Pontonio & Rizzello, 2021; Starzyńska–Janiszewska & Stodolak, 2011).

Besides the health values and convenience, price of such legumes is still the lowest among other types of legumes in the Lebanese market. To the best of our knowledge, no previous studies have been performed in Lebanon about nationally produced plant-based strained yogurt from available crops such as white beans and chickpeas. The aim of this research work is to produce a plant-based strained yogurt from available raw materials in the Lebanese market such as chickpeas, white beans and lupin, and to determine and assess the physicochemical, rheological, sensory attributes and nutritional profile of the end product. The second objective is to study and extend its shelf-life by freeze-drying. Finally, it aims to investigate how the selected panels perceive the reconstituted freeze-dried end product in comparison to the commercial Lebanese dairy-based strained yogurt known as labneh according to a sensory evaluation. The importance of this project is to launch locally produced dairy-alternatives for celiac patients, gluten-free, vegans and vegetarians.

II. Materials & methods

1. Materials

The dried chickpeas (Cicer arietinum L.), Lupin (Lupinus campestris) and white beans (*Cannellini*) were purchased from Aoun Food Co, Lebanon. Each bag of the dried pulses weighed 900g, with the nutrition facts (Table 1) respectively.

Table 10. Nutrition facts of the dried chickpeas and white beans (Cichońska & Ziarno, 2021).

Nutrition facts Per 100g	Chickpeas	White beans	Lupins
Energy (kcal/KJ)	380	333	371
Total Fat (g)	6	0.85	9.74
Total carbohydrates (g)	60	60.27	40.38
Proteins (g)	20	23.36	36.17
Vitamin C (mg)	4.8	0	4.8
Calcium (mg)	120	240	176

The freeze-dried strains of *Lactobacillus delbrueckii subsp. bulgaricus Streptococcus thermophilus* were provided by CHR Hansen, a bioscience company located in Hørsholm, Denmark, and stored at < -18 °C / < 0 °F. Glucose was inoculated with the starter culture as a source of nutrients.

2. Milk preparation

The plant-based milk preparation was carried out in the Chemistry Laboratory at the Notre Dame University – Louaize (NDU). The following steps were used in common for the different trials: the dried pulses were weighed and soaked in tap water at room temperature for 24 hours at a ratio of (1:2). The soaked beans were then strained and rinsed with clean water in order to discard any antinutritional factor. Next, the beans were mixed in a magic bullet blender (https://www.nutribullet.com/shop/blenders/magic-bullet/) with filtered water at a ratio of (1:1) to obtain the milk at room temperature.

The obtained milk was filtered with a cheese cloth to separate the milk from the pulp and cake-press and divided into different batches to be heat treated under different temperatures at 72°C for 15 seconds and 90°C for 10 seconds with constant stirring. After each pasteurization at the 2 different temperatures, the milk was then cooled down until a temperature of 43°C.

3. Milk fermentation

For each batch volume, different amount of the culture was inoculated; for example, 0.015g for 250ml, 0.02g for 300ml, 0.04g for 600ml and 0.05g for 900ml. Also, *1.5% (v/v)* of glucose was added in parallel as a source of nutrients for the culture fermentation. When each milk batch was cooled until the temperature reached to 43°C, the culture was activated with small amount of the milk and then added to the whole batch. The incubation was carried out in an incubator at 43°C for 24 hours. The following flow-chart describes the whole yogurt procedure.

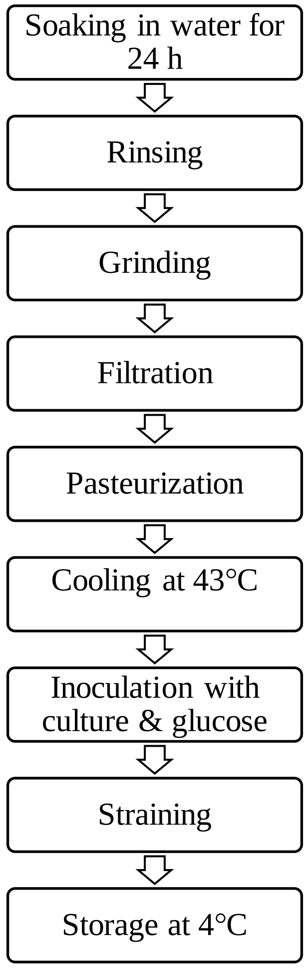

Figure 2. Flow chart of the plant-based yogurt procedure

The yogurt is then strained using a cheese cloth for 12 hours to obtain the strained yogurt.

4. Preliminary stages

As mentioned above, different trials were carried out and divided into different batches. pH was monitored before heat treatment and after fermentation at 72°C and 90°C respectively. The 72°C temperature was carried out for pasteurization purposes in order to inactivate pathogens and microorganisms. The majority of non-spore forming bacteria are inactivated at 90°C, and can cause spoilage of milk so a temperature of 90°C will be enough for their inactivation (Deeth, 2021).

Table 11. Set of trials at 90°C and 72°C

Trial number	Chickpeas %	White beans %	Lupin beans %	Pasteurization temperature	
1	100%	-	-	72°C	90°C
2	-	100%	-	72°C	90°C
3	-	-	100%	72°C	90°C
4	50%	50%		72°C	
5	40%	60%		72°C	
6	60%	40%		72°C	
7	30%	70%		72°C	
8	70%	30%		72°C	

Other series of trials was carried out but with heat treatment at 72°C only and at different chickpeas and white beans ratios to obtain the final product. The trials were reconducted and divided into triplicates.

5. Characterization of physicochemical properties

a. pH and Total Titratable Acidity (TTA)

The pH was directly measured using a digital pH-meter (METTLER TOLEDO, Seven Compact) before and after fermentation. For the total titratable acidity analysis, 15 g of the yogurt sample was collected in a 250 mL conical flask with 4 drops of phenolphthalein indicator, then titrated with 0.1M NaOH with continuous mixing until the appearance of persistent pink color. The titration is repeated twice. Titratable acidity is an approximation of the total acidity in a substance. It determines how much of a base (NaOH) is required to neutralize an acid (Belitz et al., 2004; Nielsen, 2010).

Calculation of Titratable acidity:

% acid = $\frac{N \times V \times Eq\ wt}{W \times 1000} \times\ 100$

Where:

N = normality titrant, usually NaOH (mEq/ml)

V = volume of titrant (ml)

Eq.wt. = equivalent weight of predominant acid (mg/mEq)

W = mass of sample (g)

1000 = factor relating mg to g (mg/g)

(1/10 = 100/1000)

b. Moisture

About 20 g of purified sand and a short glass-stirring rod were placed in a numbered dish made from aluminum. Dried in an oven at 100 - 102°C for 30 min then cooled in a desiccator at room temperature and weighed. Approximately 5g of the yogurt sample was weighed in the clear space of the dish and stirred. Placed on a warm hotplate for 10 min, stirring at intervals in order to obtain a well-ventilated mass. Dish and rod were transferred to the oven and dried at 100ºC overnight. Cooled in a desiccator (do not seal completely) and reweighed. Repeated in triplicate for accuracy (Nielsen, 2010).

Moisture and total solids contents of foods can be calculated as follows using oven drying procedures (Maynard a. Joslyn, 1970):

% Moisture (wt/wt) = $\frac{wt\ of\ wet\ sample - wt\ of\ dry\ sample}{wt\ of\ wet\ sample} \times 100$

c. Protein

Total amount of protein was determined using Kjeldahl (Gerhardt) method. The procedure is carried out in triplicate. The Kjeldahl method can be divided into 3 steps: digestion, neutralization, and titration (Nielsen, 2010).

About 1.5 to 2 g sample was weighed on an ashless filter paper. A piece of blank paper rolled up and inserted into the neck of the Kjeldahl flask. This will keep the neck of the flask clean while you add the sample. The sample is placed inside a labeled digestion flask. To the flask 7 g of potassium sulfate (K_2SO_4) was added, and 0.1 g of copper sulfate (CuSO4). Finally, using a dispenser, 20 mL of concentrated sulfuric acid (H_2SO_4; 98%) added into the flask. The digestion (Kjeldahl) flask was placed on the flask heater in the Gerhardt apparatus.
After 15 mins cooling off the Hence, different ratios of chickpeas and white beans extracts were combined to find the ultimate product, according to the nutritional value and acceptability.
flasks with ice and addition of cold water into each flask, one boiling chip was added with 50 mL of 2% Boric acid (H_3BO_3) and then 70mL of 32% caustic soda (NaOH) and 4 drops of the indicator mixture (Methylene blue and red Methyl).
The last step is the titration of ammonia collected with 0.1 M HCl solution until a pink color persisted.

The percentage of proteins can be afterward calculated simply using the following equation:

$$\%N = N\ HCl \times \frac{\text{Corrected acid volume}}{\text{g of sample}} \times \frac{14\ g\ N}{mol} \times 100$$

% protein = % nitrogen / 0.16

d. Fat

Soxhlet (SER 148 VELP Scientifica) was used.
5 g of the sample was weighed into a Soxhlet thimble then weighed again with a cotton wool on top. The Soxhlet extraction beaker was weighed and 70 mL of extraction solvent: petroleum ether (40-60°C) was added. The thimble and the extraction beaker were placed in their position in the Soxhlet extraction.

After extraction and recovery, in an oven at 103°C for 30 minutes both: (1) the extraction beaker with extract and boiling stones, and the thimble placed in a pre-weighed beaker.
After drying that removed the last trace of solvent, (1) the extraction flask containing the extracted oil, and (2) dry thimble containing the nonfat residue were weighed.
(Nielsen, 2010).

Calculations:
Weight of fat in sample = (beaker + fat) - beaker (Akoh & Min, 2008)
% Fat on dry weight basis = g of fat in sample/g of dried sample $\times$ 100 (Belitz et al., 2004)

e. Ash content:

AOAC International has several dry-ashing procedures (e.g., AOAC Methods 900.02 A or B, 920.117, 923.03) for certain individual foodstuffs. The general procedure includes the following steps:
A 10 g of the yogurt sample was weighed into a tared crucible. Pre-dry if the sample is very moist. Crucibles were placed in a cool muffle furnace. The procedure was ignited 12–18 h (or overnight) at about 550◦C. Muffle furnace was turned off and waited to open it until the temperature has dropped to at least 250◦C, preferably lower. Using safety tongs, crucibles were quickly transferred to a desiccator with a porcelain plate and desiccant. Crucibles were covered, desiccator was closed , and crucibles were allowed to cool prior to weighing (Nielsen, 2010)

6. Sensory evaluation

Hedonic tests were performed in order to evaluate the sensory attributes of the trials of set 3 and the final product. The sensory evaluation was held during class with 12 untrained students. The questionnaire was based on a scale of five points, ranging from “1 = Like a lot” to “5 = Dislike a lot”, also, each attribute (appearance, flavor, odor, color and texture) was evaluated on a 5-point Likert-scale (1= Unappealing; 2= Needs improvement; 3=Ok; 4= Appealing; 5= Very appealing). Four samples were served at refrigerator temperature 4°C in a randomized order. Participants received 4 cups, each labeled in the following way; 1, 2, 3 & 4.

7. Statistical Data Analysis

The results were analyzed by using IBM® SPSS® (Statistical Package for Social Sciences) version 26. One way-ANOVA followed by Tukey's multiple comparison test was employed to test the effect of the different ratios on the different organoleptic properties of the strained yogurt and the significance level was $P \leq 0.05$. The Homogeneity of variance test was carried out to compare variance values. To find the most chosen sample, a frequencies test was carried out.

III. Results and discussion

1. Preliminary results

The preliminary studies were conducted to reduce the number of trials by assessing the physical characteristics and sensory attributes.

Table 2 shows the different trials conducted at two different temperatures (72°C and 90°C).

For trial 1 (100% chickpeas), after 4 hours of fermentation, both batches with the different heat treatment temperatures gave a firm, gel-like yogurt (Fig 1). Coagulation was observed at a higher ratio and firmer at 90°C than the one at 72°C, hence why after straining the latter gave a crumbly and dry strained yogurt (Fig 2), whereas the one at 72°C the strained yogurt obtained was smooth and creamy in texture. Both samples had a strong acidic odor and a flavorless taste.

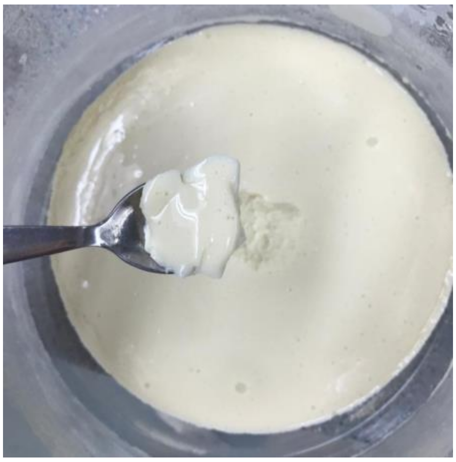

Figure 3. 100% chickpea yogurt before straining

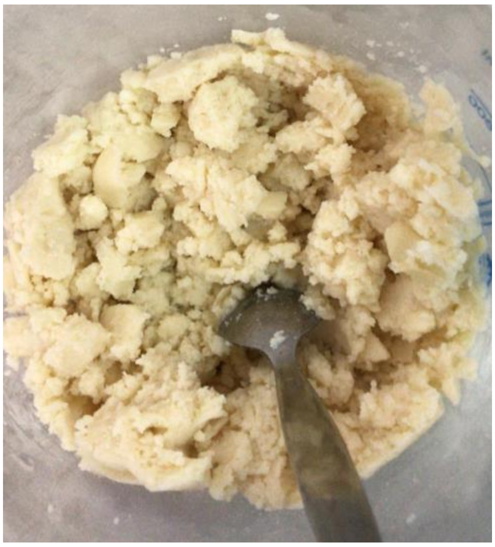

Figure 4. 100% chickpea yogurt after straining

Results revealed that grain-based yogurt also appears to have specific sensory properties, depending on the species under consideration. Hence why, the incubation time was increased to 24 hours for the rest of the remaining trials at 43°C. Fermentation has been recognized as essential to achieving an adequate sensory profile, but it is also a powerful biotechnological tool to improve the nutritional and functional properties of ingredients of plant origin (Boeck et al., 2021). For trial 2 (100% white beans), the batch at 72°C gave a weak coagulation or gel-like structure with high syneresis, the batch at 90°C gave a firmer gel-like yogurt.

Figure 5. 100% white beans after straining

The same results were obtained after straining with this trial, crumbly and dry strained yogurt at 90°C and creamier strained yogurt at 72°C (Fig 3). Based on these observations, heating the milk at 90°C gave a firmer gel-like yogurt with a dry and crumbly strained yogurt whereas the ones at 72°C gave more pleasant results according to texture, appearance and flavor. A third trial was conducted with 100% lupin, in which the results were negative, no coagulation was observed it was a failed trial.

Subjecting plan-based ingredients to heat in the presence of water, induce starch gelatinization and a consistent gel-like texture will be formed. The heat-induced gelatinization process strictly depends on the used raw materials and their composition, and it causes an irreversible swelling of the starch thus, a significant increase in viscosity will occur (Montemurro et al., 2021). Even though all the 100% chickpeas, 100% white beans and 100% lupins, were subjected to the same heat and time conditions, the lupin milk remained liquid, this might due to the type of culture used and fermentation temperature. Results of a study conducted on the production of lupin-based yogurt, showed that the yogurt had a similar viscosity and texture as the dairy-based one (Fernandez-Avila & Trujillo, 2016; Jiménez-Martínez et al., 2003). The difference between the results of this study and the current one, is that (Fernandez-Avila & Trujillo, 2016; Jiménez-Martínez et al., 2003), used only *Lactobacillus delbrueckii ssp.bulgaricus* and fermentation was carried out for 72 h at 25°C. Whereas this present study, fermentation was carried out for 24 h at 43°C in the presence of 2 strains *Lactobacillus delbrueckii ssp.bulgaricus* and *Streptococcus thermophilus*. In this study, more intense heat treatment such as UHT is needed for the lupin-based yogurt (Harper et al., 2022) in order to improve the coagulation rate and rheological properties of the fermented product.

As observed in Table 1, comparing the carbohydrates content of the legumes, chickpeas and white beans have high amounts compared to lupin. Hence why, lupin was a failed trial coagulation due to the low amount of carbohydrates necessary for the fermentation. Pasteurization usually takes place at a temperature below 100 °C, which destroys pathogenic microorganisms. Hence why in the present study, temperature at 90°C have a thick custard-like beverage due to the aggregation and denaturation of proteins at a temperature close to 100°C which affected the high firmness of the end product, whereas at 72°C, pasteurization did not affect the aggregation of oil-coated droplets nor the thickness of the plant-based milk. Pasteurization is a key step that enhances protein-protein interactions that can result in aggregation and sedimentation or gelling (Mäkinen et al., 2016).

Therefore, the next set of trials were pasteurized at 72°C and fermentation was carried out for 24 hours at 43°C. One of the limiting factors in using chickpea milk is its distinctive bean flavor and yellow appearance. To mitigate these factors, mixing white bean-based milk is a potential alternative. Comparing carbohydrates content of both legumes, chickpeas have lower fermentability than white beans due to their nutritional composition, hence why chickpeas were preferrable at lower ratios than white beans. Also, white beans are higher in protein, for that reason, higher chickpeas ratios are associated with lower gel-like structure formation and viscosity. The low protein content and coagulation properties of proteins in plant-based yogurt are related to the firmness of the product (Harper et al., 2022). White beans improved the sensory attributes of the product whereas chickpeas improved the nutritional value. The 100% white beans, gave a low degree of coagulation but straining it gave a gel-like, creamy yogurt with a strong acidic flavor and astringent odor. Since chickpeas improve the nutritional values of the product, 50% white beans and 50% chickpeas was conducted. The results gave a hummus-like texture with a yellowish color after straining. The higher chickpeas ratios (70% and 60%), gave a gel-like yogurt, but after straining, the strained yogurts were grainy, yellowish, with a foul and pungent odor. Based-off these observations, the chickpeas were then used at lower ratios in comparison to the white beans. For 70% white beans, 30% chickpeas were used and for 60% white beans a ratio of 40% chickpeas. The 70% and 60% white beans gave a semi-liquid structure, but the lower white beans content gave a firmer gel-like network. According to appearance, the 60% white beans trial gave an off-white creamy appearance whereas the 60% chickpeas gave a yellowish grainy appearance, in which this factor facilitated the elimination of the 60% chickpeas (Fig 4).

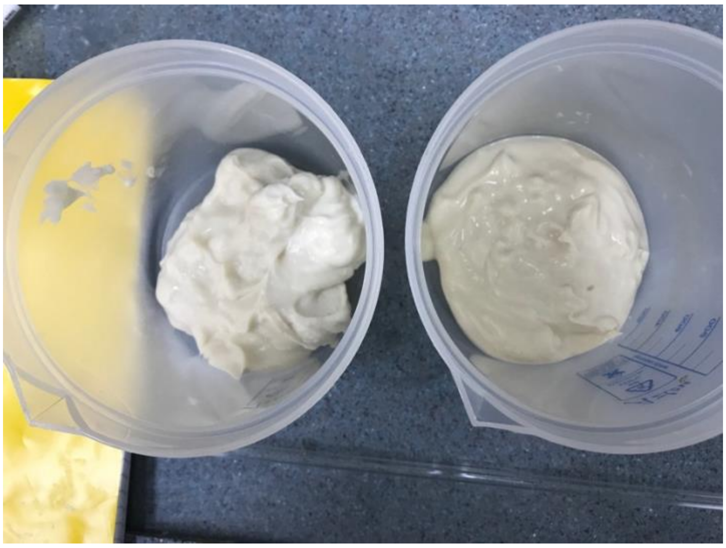

Figure 6. On the left: 60% white beans; On the right: 60% chickpeas

Comparing the 70% white beans and 70% chickpeas, 70% white beans had an acidic taste with a creamy texture, whereas the 70% chickpeas had a bitter aftertaste with a strong chickpeas flavor and a grainy mouthfeel (Fig 5). Also, this factor was determinant in eliminating the 70% chickpeas.

Figure 7. 70% chickpeas strained yogurt

In conventional yogurt, through acidification, fermentation directly affects the stability of the casein micelles by reducing their charge, breaking some of the insoluble calcium phosphate crosslinks, and modifying the internal binding between proteins. Reaching a pH value below the isoelectric point of the caseins causes them to gel (Montemurro et al., 2021). On the contrary in plant-based yogurt, the formation of this protein network imposes one of the main issues. In this case, fermentation lead to the destabilization of the protein structure thus leasing to a weak structure and syneresis during storage (Montemurro et al., 2021). According to these results, trials were narrowed down to the 4 remaining batches: 100% white beans, 50% white beans, 60% white beans and 70% white beans. This next set of trials were conducted in triplicates, pasteurized at 72°C, fermented at 43°C for 24 hours.

100% white beans showed a low degree of coagulation, after straining taste was bland with a bean aftertaste. White beans are well-known for containing high amounts of carbohydrates (60.27g per 100g), soluble dietary fibers are a component that is less available for microbial degradation and protein digestibility (Hughes et al., 1996; Thompson et al., 2020). For Trial 2 (50% chickpeas & 50% white beans), the first batch was discarded due to contamination with the appearance of pink sports on the surface and foul odor. The second and third batches were very acidic with bitter aftertaste. The bitter off-flavor in plant-based yogurts is due to the presence of various components such as phenols, glycosylates and flavonoids (McClements et al., 2019).

A better coagulation was observed with 60% white beans and the 70% white beans. Trials with higher white beans % than chickpeas, were more appealing in appearance and color. Hence why, the first trial (100% white beans) was discarded, the others were conducted for another try with a new trial added (80% white beans and 20% chickpeas) under the same conditions, where this final set of trials was evaluated by a sensory session. The 80% white beans gave a low grade of coagulation (Fig 6), but after straining it gave a creamy, smooth and chalky white strained yogurt with a mild acidic odor. Yogurt is a non-Newtonian fluid, in which exhibits various non-Newtonian effects such as shear thinning, yield stress, viscoelasticity, storage modulus G' and loss modulus G" (Prajapati et al., 2016), in which yield stress is the minimum stress at which yogurt undergoes deformation without an increase in the external force, storage modulus is the elasticity or the solid-like state and loss modulus is the liquid character of the gel network (Ozcan, 2013). Rheological properties of yogurt are influenced by the total solids content, the physical state of proteins and fat droplets, the amount and type of the culture strain, incubation time, fermentation temperature and heat treatments (Prajapati et al., 2016). According to research, the most viscous yogurts are those with a long fermentation time and viscous strains (Beal et al., 1999). Another study found that G' and G" for solid yogurts and stirred yogurts, increased with time and temperature as well as the protein concentration. They concluded that G' values resulted from strong protein bonds and G" values are due to weak gel-like network (Tunick, 2000). Based on these results, we can assume that weak gel network in all the trials is associated with low G" values and for the lupin trial, protein content of the milk, type and amount of the strain and the incubation type have affected the rheological properties causing a failure in coagulation and the gel network formation. A study investigated the rheological properties of strained yogurt (labneh), and found when G' > G" the gel network had a weak viscoelasticity and hence both moduli increased with protein content of the milk (Ozer et al., 1997).

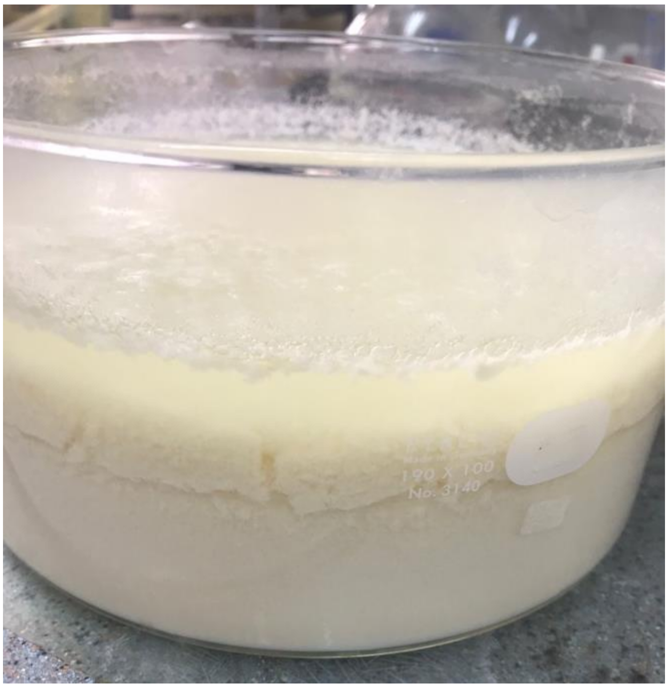

Figure 8. Low grade coagulation of 80% white beans

2. Post-fermentation

Fermentation parameters, including choice of substrate, microbiological culture, time and temperature, must be carefully controlled to obtain a product that is safe for consumption and has satisfactory stability and sensory standards (Penha et al., 2021). Starters selected for yogurt production ought to give a quick acidification that may inhibit contamination from spoilage microorganisms (Sivamaruthi et al., 2018). In addition, some LAB generate exopolysaccharides during the fermentation and have been identified as key components in developing the desired flavor and texture in fermented dairy products (Korcz & Varga, 2021). Also exopolysaccharides are able to improve the viscosity and decrease the syneresis of the product during storage (Harper et al., 2022). Plant-based beverages tend to be much lower in fat than dairy-based milk, so the selection of LAB strains that produce exopolysaccharides is important to produce plant-based yogurt with the desirable texture profile (Chalupa-Krebzdak et al., 2018).

During fermentation, the chickpeas and white beans milk's carbohydrates are a support for microbial growth and protein digestion, followed by a decrease of pH due to organic acid production (Thompson et al., 2020). The results of a study conducted by Chen et al, on yogurt fortification with chickpea flour; the addition of chickpea flour greatly improved the acidification process for a pH reaching between 5.5-4.5 (Chen et al., 2018). Furthermore, gel development is pH dependent, with the isoelectric point for the majority of proteins is around a pH 4.6 (Wang et al., 2018). The following graphs show the pH variation before and after fermentation at the different pasteurization temperature for all the conducted trials.

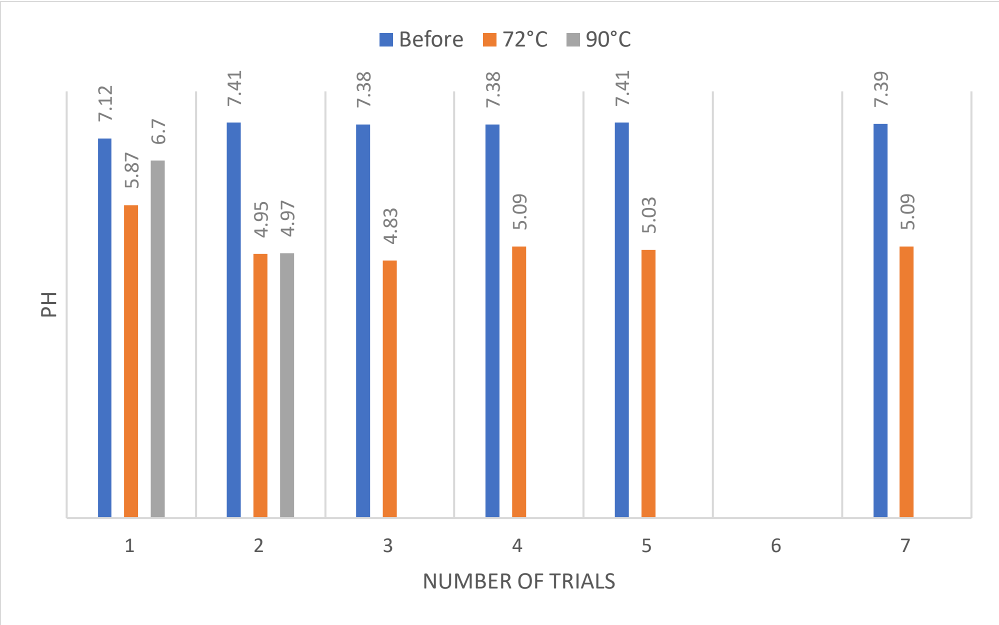

Figure 9. Set 1 trials pH variations of pasteurized milk at 72°C & 90°C before and after fermentation

For example, in Fig 8, For 100% white beans, the pH before fermentation was 7.47, which decreased after fermentation to 4.96, 4.99 and 5.06 to its different batches respectively. For 50% chickpeas & 50% white beans, the pH before fermentation was 7.48 and decreased after fermentation to 4.75 and 4.85 to its different batches respectively.

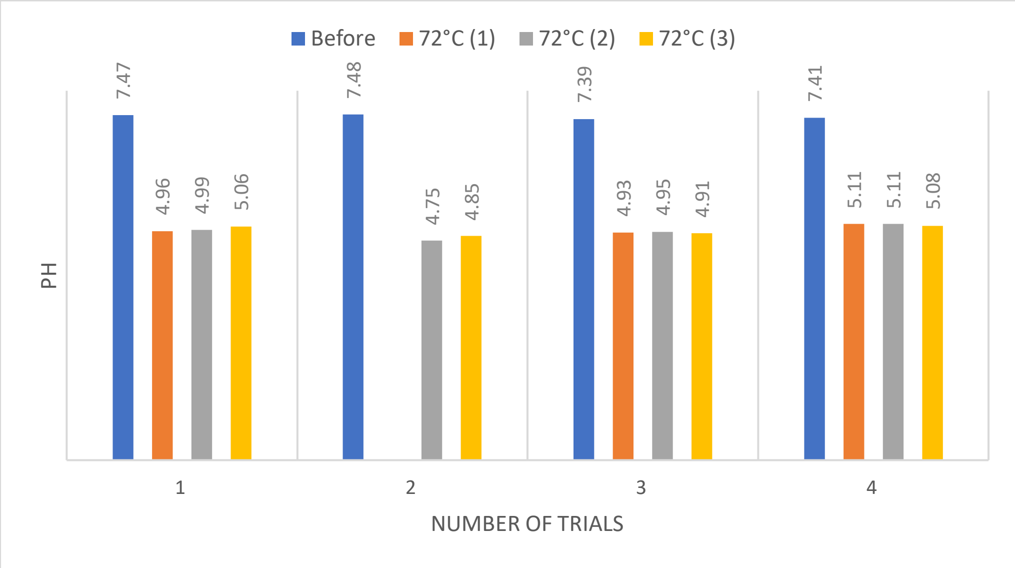

Figure 10. Set 2 triplicate trials pH variations of pasteurized milk at 72°C before and after fermentation

3. Chemical analysis

Plant-based yogurts are intended to be consumed after a short period of storage time under refrigerated conditions, similar to the conventional ones (Montemurro et al., 2021). The importance of determining the pH and total titratable acidity is emphasized as they are important parameters associated with the growth of microorganisms in food. More specifically, pH affects the appearance and distribution of microorganisms, and less acidic foods are more prone to spoilage due to microbial proliferation as well as growth of mold and yeast (Coda et al., 2011; Donkor et al., 2005; Pontonio & Rizzello, 2021). Besides, the protein extraction is enhanced in acidic medium, that is, at a low pH (Pineli et al., 2015). Total Titratable Acidity reflects the amount of acids present in food. Dairy-based strained yogurt has a minimum Total Titratable Acidity value of 0.6% (Food and Agriculture Organization of the United Nations, 2007). The value of the final sample was 1.09%, in which is acceptable within the range of cow's milk strained yogurt (Food and Agriculture Organization of the United Nations, 2007). Table 5 shows the results of the chemical analysis of the final sample (80% white beans & 20% chickpeas).

Table 12. Total Titratable Acidity, Moisture, Ash, Protein and Fat of the 80% white beans and 20% chickpeas sample

Parameter tested for the final sample	Total Titratable Acidity (TTA)	Moisture	Ash	Protein	Fat
Average	1.09%	11.03	8.5%	7.59%	<1

Table 6 represents a comparison between labneh (dairy strained yogurt) and the plant-based sample. The composition and nutritional values of plant-based milk beverages depend on the type of used raw material and the production process used (Aydar et al., 2020). In most cases, however, they are high in carbohydrates and low in protein, containing up to 30 times less protein than dairy-based milk (Aydar et al., 2020; Fructuoso et al., 2021). Despite this, plant-based protein is inferior due to the presence of limiting amino acids (lysine in grains and methionine in legumes) and poor digestibility. In most cases, plant-based milks are low in fat unless enriched with plant oils (Alcorta et al., 2021; Mäkinen et al., 2016b). Upon fermentation, LAB's enzymatic activities result in proteolysis, leading to an increase of protein digestibility and the concentration of bioactive peptides (Abd El-Fattah et al., 2018; Hewitt & Bancroft, 1985). In legumes-based fermented beverages, the protein fraction is around 3.0–4.0% similarly to cow's milk (i.e., 3.3-3.5%) whereas cereal-based ones have a lower fraction of 0.1-1.0% (Lopes et al., 2020; Qamar et al., 2020). As reported by a study, when quinoa, lupin, soy, lentils and chickpeas are the main ingredient of the yogurt production, the protein content ranges between 3 to 5% (Hickisch et al., 2016; Lorusso et al., 2018; Pontonio & Rizzello, 2021).

Based on the results of our study, the protein content has a value of 7.49% which is above the average limit of the plant-based yogurt alternatives. This indicates that the combination of chickpeas and white beans offer the ultimate source of protein no additional fortification is needed. But in comparison to dairy-based strained yogurt, it was lower than the full fat of

8.14% but not significantly lower. The protein content of our product in comparison with the low fat one of 10.65% was significantly lower, and according to research, low fat strained yogurts are enriched with whey protein isolates (WPI) to improve their quality and nutritional values (M. A. Hashim et al., 2021). Fat content also depends on the used raw material for example; in coconut-based yogurt the fat is 9.8%, in almond and cashew-based yogurts is 5.4% and 4.9% respectively. According to the nutritional profile of the chickpeas and white beans legumes, the fat content per 100g is 6 and 0.85 respectively. Hence why, the strained yogurt was very low in fat (<1). Approximate acidity % values among all samples, indicate that the bacterial fermentation was achieved similarly in the dairy and the plant-based proteins. Comparing the ash content, the dairy alternative had a higher amount of 4.63% than the dairy samples, showing a significant amount is present in the strained yogurt and no fortification of minerals is required.

Table 13. Difference of nutritional values between dairy strained yogurt and plant-based strained yogurt

Attribute	**Full fat**	**Reduced fat**	**Low fat**	**White beans & chickpea-based strained yogurt**
Moisture (%)	73.88	76.86	80.01	85.38
Fat (%)	9.18	4.79	0.35	< 1
Protein (%)	8.14	9.30	10.65	7.49
Ash (%)	1.56	1.58	1.66	4.63
Acidity (%)	1.12	1.19	1.22	1.09

4. Sensory analysis

Sensory perception, specifically "taste," is the most important dimension of quality evaluation in a consumer's purchasing criteria (Shori et al., 2021). In the present study, the different legumes ratios improved the strained yogurt final organoleptic properties such as appearance. Table 5 represents the mean score obtained in the sensory evaluation of the 4 strained yogurt samples at different ratios. The lowest score value represents the highest acceptance rate or the most preferable sample since the 5-point hedonic scale ranges from 1 for "Like a lot" and 5 for "Dislike a lot". The appearance mean score value of sample 4 (80% white beans), is 1.18 lower than the score values of samples 2.83, 2.45 & 1.50 respectively. The same is for the taste and texture attributes where they had the lowest score of 2.18 and 1.42 respectively. This indicates that sample 4 was the preferrable sample among the 4 samples in accordance to appearance, taste and texture. For the odor attribute, sample 3 (70% WB) has a score value of 3.42 lower than the score values among the remaining samples. Sample 3 was preferrable among the 4 samples in accordance to odor. For color, sample 2 (60% WB) has the lowest score of 2.67 in comparison to the other 3 samples, which means sample 2 was preferred in accordance to color.

Table 14. Hedonic scale results for the sensory evaluation of the strained yogurt samples

Parameter	50% WB; 50% C Sample 1	60% WB; 40% C Sample 2	70% WB; 30% C Sample 3	80% WB; 20% C Sample 4
Appearance	2.83^{b}	$2.45^{a,b}$	$1.50^{a,b}$	1.18^{a}
Odor	3.67^{a}	3.55^{a}	3.42^{a}	3.45^{a}
Taste	3.17^{a}	2.40^{a}	2.92^{a}	2.18^{a}
Color	3.00^{a}	2.67^{a}	2.91^{a}	3.55^{a}

Texture	1.75[a]	1.90[a]	2.17[a]	1.42[a]

WB: White beans; C: Chickpeas SD: Standard Deviation
Values followed by different superscript letters (a–b) in the same column are significantly different ($p < 0.05$).

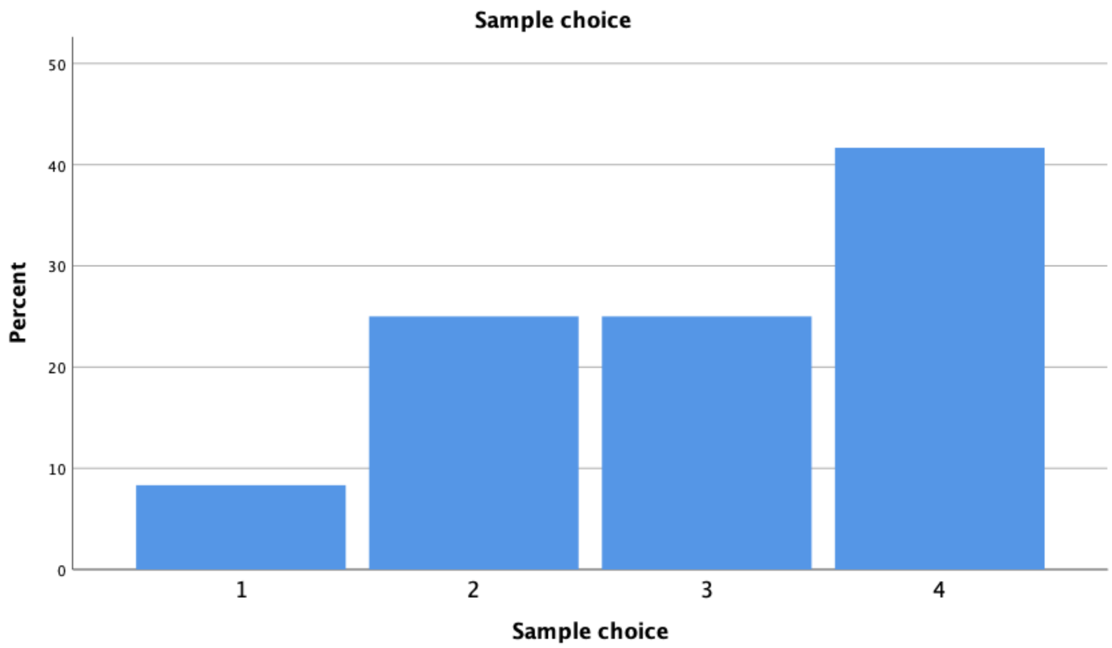

Figure 11. Bar chart showing the percent of panelists vs. the sample choice

Based on Fig 2, sample 4 (80% WB) is the chosen sample or the sample that had the highest percentage of choice of 42% among the 4 samples.

IV. End product applications and cost

Labneh or strained yogurt, is widely consumed with salty crackers, bread and with flavorings such as olive oil, spices, thyme, etc. One of the objectives of this study is producing a product where all people, at all times, have physical, social and economic access to sufficient, safe and nutritious product. Freeze-drying is an application to extend the shelf-life of the final product. Another objective is producing a product at a lower cost. For 500g batch, Khoury Labneh costs between 73 000 lbp and 79 000 lbp, Taanayel Labneh costs 95 000 lbp for 500g. The total cost of the final sample is 65 000 lbp which is lower than all the dairy-based ones. Vicky's plant-based labneh costs 112 00 lbp for 250g which was way higher than the final sample.

V. Conclusion

A strained yogurt with adequate physical, chemical and sensory characteristics was achieved from the white beans and chickpeas combination. While not widely used in plant-based yogurt alternatives, white beans & chickpeas possess many properties that make them an excellent alternative to soy-based and almond-based alternatives available in the market. People tend to consume dairy products for their calcium and protein content. The product displayed a good nutritional profile (such as protein, fat and carbohydrates) when compared to the dairy based without any additional fortification. Post-acidification is an undesirable process in yogurt because it shortens the shelf-life due to high acids content and syneresis. For that reason, pH monitoring and acidity is essential during storage days to assess the shelf-life of the end product on the market shelves. Also, our sensory results showed that white beans and chickpeas gave a weak gel-like structure after fermentation, and gelling ability of proteins is influenced by the minerals content. From this perspective, we assume that the content could be low or inadequate for the formation of a rigid and stable gel thus, further minerals testing is needed. In addition, rheological characteristics such as apparent viscosity, consistency and elasticity need to be assessed. The overall product mimicked the dairy-based strained yogurt in color, taste and odor. The results of the sensory analysis show that the product is a promising replacer for the conventional labneh or strained yogurt, even though the sample of participants is not representative for the Lebanese population, the sensory session should be carried out for a more representative and bigger sample. It is possible to use flavoring agents such as oregano, thyme. The lupin legume is an underutilized legume, a further number of trials should be conducted with the needed alterations. The sustainability and eco-friendly principles of the plant-based diet were achieved; thus, all waste was organic. According to literature, aflatoxins were detected at high concentration in chickpeas and chickpeas-based products (Ramirez et al., 2018). Such toxins can be eliminated by the processing of chickpeas, so testing the levels of these toxins will set the record about the safety consumption of such products in comparison to dairy-based yogurts.

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