Development of a novel ice cream with hemp milk based on chia seed mucilage as a stabiliser

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Weilun Zhang
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Abstract

The majority of commercial ice cream products are made from dairy, which can lead to health issues for consumers with lactose intolerance and casein allergies. Commercial stabilisers are used in ice cream formulas to provide maximum performance in meeting several requirements, including consumer demands, transport, and cost-effectiveness. As a result, there is a significant need for consumers to discover substitute products that offer comparable health benefits to dairy products and products with clean labels. If there is an ice cream that uses natural sources of stabilisers that can make the ice cream label look simpler and cleaner while having health benefits, it will probably be competitive in the marketplace.

Hemp seeds are rich in essential elements needed by the human body. Consequently, food manufacturers have taken an interest in hemp milk because of its exceptional nutritional content. Currently, the food industry has not made any progress in creating ice creams that contain hemp milk to improve their nutritional content. Hemp milk, on the other hand, has a specific flavour, and the direct use of hemp milk in ice cream production may have a negative impact on the product's flavour and aroma. The differences in the type and content of proteins and fats between hemp milk and cow milk may also have an unknown effect on the physical and chemical properties of the ice cream (e.g., texture, overrun, and ice crystal content). This study substituted five levels of hemp milk (0%, 25%, 50%, 75%, and 100%) for cow milk using chia seed mucilage (CSM) as stabiliser. The aim was to determine the impact of the added hemp milk on the ice cream's properties and its storage stability after 21 days to determine the most suitable hemp milk content. This study was conducted in four phases.

In the first phase, chia seeds were soaked, stirred, and centrifuged to separate CSM. The extracted CSM was dried and stored after measuring its water activity and moisture content. Then, hemp milk was extracted from hemp seeds using a high-shear blender, homogenised, and filtered. Its total solid content, dissolved sugar content, dietary fibre content, and nutritional properties were measured and stored under refrigeration.

In the second phase, five ice cream samples containing different amounts of hemp milk were prepared. The formulation containing 25% hemp milk was selected as the best-performing formulation based

on the summary of the opinions of the focus groups (n = 6), pH, overrun, colour, and melting properties.

In the third phase, the physicochemical and nutritional characteristics of the ice cream were analysed and discussed. In addition to this, a consumer sensory panel (n = 35) assessed the overall acceptability and Just-About-Right (JAR) of the best formulation ice cream sample. The average acceptability score of the ice cream containing 25% hemp milk was 6.75, and the JAR results showed that the JAR percentage was above 70% for all the attributes except for taste and aroma. The indicators of taste were found to fall within the noteworthy range by penalty analysis, but not in the high impact (>0.5) range. The results revealed that the ice cream formulation containing 25% hemp milk has ideal sensory and physicochemical properties.

In the fourth phase, the stability of the best formulation ice cream during storage (-19 °C) for a period of 21 days was evaluated by measuring the pH, colour, and hardness and by consumer sensory evaluation of the best formulation ice cream. Although the L* a* values in colour and hardness of the ice cream showed statistically significant changes ($p<0.05$) during storage, the b* values in the colour and pH of the ice cream remained stable ($p > 0.05$). Meanwhile, the consumer sensory evaluation results showed that the product was still considered acceptable after 21 days of storage.

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List of Abbreviations

a*	=	redness-greenness
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemist
APC	=	aerobic plate count
b*	=	yellowness-blueness
BGLB	=	Brilliant Green Lactose Bile
CBD	=	Cannabidiol
CFU	=	colony-forming units
EAAs	=	essential amino acids
EFAs	=	essential fatty acids
etc	=	et cetera
GLA	=	c-linolenic acid
HSO	=	hemp seed oil
HSPI	=	Hemp seed protein isolate
IDF	=	insoluble dietary fibre
L*	=	lightness
LA	=	ω-6 Linoleic Acid
LNA	=	ω-3 Alpha-linolenic Acid
LSES	=	Listeria Selective Enrichment Supplement
PDCAAS	=	The digestibility-corrected amino acid score
pH	=	potential hydrogen
SDA	=	stearidonic acid
SDF	=	soluble dietary fibre
TDF	=	total dietary fibre
THC	=	tetrahydrocannabinol
VRBA	=	Violet Red Bile Agar
w/w	=	weight per weight
WI	=	white index

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Chapter 1 Introduction

1.1 Background

Ice cream is a delightful frozen dairy product that is frequently consumed globally due to its pleasant flavour, smooth texture, and refreshing taste (Marshall et al., 2003). Historically, ice cream was prepared with milk, sugar, cream, and natural flavours. Nevertheless, to satisfy consumers' expectations, ice cream products have been enhanced with additional artificial additives to achieve the required texture, mouthfeel, taste, and colour characteristics. Furthermore, the additives also provide optimal melting properties throughout changes in temperature and storage stability (Clarke, 2004).

As consumer awareness grows, customers increasingly consider the processing methods, source, health implications, and components of food items. Consumer demands for 'clean label' products have pushed food producers to focus on the use of natural ingredients instead of artificial additives (such as preservatives, stabilisers, and emulsifiers) when creating new products (James, 2020). Although food manufacturers have prioritised this trend, the term 'clean label' currently has no official meaning. As a result, it is an unregulated and ambiguous descriptor. Both consumers and food producers have differing interpretations of "clean label", with each group having their subjective definition of the term (Galanakis, 2022b). Feizi et al. (2021) suggest that researchers developing clean label products should prioritise the usage of particular natural materials rather than electronic-numbered artificial additives.

Chia seed, formally known as *Salvia hispanica* L., is a member of the Labiatae family and is rich in mucilage. When water is absorbed, mucilage develops on the surface of the seed and transforms into a gelatinous body (Feizi et al., 2021). Chia seed mucilage (CSM) is a mucilage based on polysaccharides consisting largely of crude fibres and carbohydrates (Chavan et al., 2017). The mucilage from hydrated chia seeds can be used as an emulsifier and stabiliser in ice cream. This is because it can hold and absorb water, similar to traditional stabilisers used in food systems (Punia & Dhull, 2019). The water-holding capacity of CSM is similar to that of guar gum and about four times greater than that of arabic gum (Feizi et al., 2021). Chia seeds, also referred to as a superfood, are rich in protein (15–25%), fibre (18–30%), and fats (30–40%). These fats include polyunsaturated fatty acids, such as omega-3 (54-67%) and omega-6 (12%). Omega-3 fatty acids have significant health benefits due to their function as essential components of brain and heart muscle cells (Chavan et al., 2017).

With the gradual development of the consumer market and pluralistic dietary preferences, the ideal new product must not only offer health advantages but also satisfy the requirements of specific consumer groups, such as those who are lactose intolerant or follow a vegan lifestyle (Bekiroglu et al., 2022). Consequently, an increasing number of specialty and functional dietary ice creams are being introduced to address the market demand for options that are low in sugar, low in fat, and made with probiotics and plant-based milk (McClements & Grossmann, 2022). Altering cow's milk with plant-based milk such as soy, coconut, rice, oat, hemp, or almond in ice creams might result in a final product that shows similarity to cow milk ice creams (Leahu et al., 2022).

The hemp plant (*Cannabis sativa* L.) has been utilised for various purposes throughout history, including as a source of food, fibre, psychotropic/religious drugs, and medicine (Xu et al., 2021). However, confusion between the psychoactive compound tetrahydrocannabinol (THC) in cannabis (*Cannabis indica*) and industrial hemp (*Cannabis sativa*) has restricted the cultivation and production of hemp (BeŞİR et al., 2022). It is important to note that industrial hemp is a flexible plant that offers multiple purposes and contains minimal or no THC. In modern times, hemp is being acknowledged again as a valuable source of medicinal products, food product manufacturing, and fabrics (Borkowska & Bialkowska, 2019).

Hemp seeds are considered a superfood due to their high content of cannabidiol (CBD), a useful compound with nutritional and therapeutic effects that are beneficial for the human body (Chauhan, 2021). Hemp seeds are known for their significant nutritional value and hypoallergenic properties (Wang et al., 2023). Hemp seeds contain around 25–35% fat, 20–25% protein, 20–30% carbs, and 10-15% insoluble fibre. Additionally, they are abundant in vitamins and minerals. As a result, hemp seeds are regarded as a highly beneficial source of high-quality fat, protein, and dietary fibre (Pham, 2021). Hemp milk is characterised by its high nutritional value, low saturated fat content, and a significant proportion of polyunsaturated fatty acids (PUFA), such as omega-3 and omega-6. These qualities make hemp milk a highly promising and favourable choice for those seeking a plant-based milk alternative (Leahu et al., 2022).

1.2 Aim and Objectives

The main aim of this study was to develop a novel clean label ice cream containing hemp milk and CSM by replacing cow milk with different percentages of hemp milk in ice cream formulations while replacing conventional stabilisers with CSM.

The specific objectives are to:

1. Extract mucilage from chia seed and apply it to ice cream.
2. Extract hemp milk from hemp seeds and apply it to ice cream.
3. Select the optimal hemp milk ice cream formulation by analysing the physicochemical and focus group feedback of the ice cream samples.
4. Evaluate the performance of the best formulation ice cream by analysing the physicochemical and sensory characteristics of the ice cream.
5. Evaluate the storage stability of the best formulation ice cream sample by analysing the physicochemical and sensory properties.

Chapter 2 Literature Review

2.1 Introduction

Ice cream is a frozen mix of milk, sweeteners, stabilisers, emulsifiers, and flavourings, with the possibility of including other components such as egg products, colours, and starch hydrolysates. The ice cream mixes, which are not frozen, undergo pasteurisation and homogenization before being aged. The aged ice cream mixes are then rapidly frozen while stirring constantly to freeze and incorporate air, resulting in the intended smoothness and softness of the ice cream product. Ice cream is regarded as a nutritious food product as it contains valuable elements like protein, minerals (such as calcium, phosphorus, and iron), and vitamins, in addition to fat (Clarke, 2004). New Zealand is one of the top countries in the world, as it ranks for the amount of ice cream, frozen yoghurt, and frozen dessert items consumed per person each year. The average individual in New Zealand consumes 26.5 litres of these products yearly (Marshall et al., 2003).

The use of plant proteins obtained from agricultural and industrial by-products, while enhancing their functioning in various ways, has become an attractive topic in the field of food science in recent years. The growing interest in healthy eating and vegetarianism, driven by economic, ethnic, religious, and environmental factors, has led to an increased demand for plant-based proteins (Karabulut et al., 2022).

Hemp hearts or seeds are the reproductive units of the cannabis plant, which can be defined botanically as nuts but are often referred to as seeds or hearts. Anamika states that this superfood is consumed for its omega-3 and omega-6 fatty acids as well as its antioxidant properties (Chauhan, 2021). Hemp seeds are a highly nutritious food, particularly rich in unsaturated fatty acids and necessary amino acids. Food manufacturers have created several hemp products for retail, including nuts, oils, protein powders, energy bars, granola bars, nut butter, pasta, and ice creams, due to the acknowledged nutritional benefits of cannabis seeds (Leson, 2006).

The current research emphasis on hemp seeds is on hemp proteins, which serve as both nutritional supplements and functional components in processed foods to improve the qualitative characteristics of the products. Hemp proteins are hypoallergenic, which means they are less likely to cause allergic reactions compared to other plant proteins. This quality makes hemp seed protein products a viable substitute for regularly used proteins like casein, whey, wheat, and soy in some food products. As a result, the usage of hemp seed protein products as an alternative is increasing (Gram & Mortas, 2023). Hemp seed protein isolate (HSPI), derived from hemp seed meal, is a highly nutritious food ingredient that contains all the essential amino acids, similar to those found in egg white protein and soy protein. Although HSPI has a high nutritional value, it lacks usefulness in food formulations, which is why hemp protein is not commonly used as an ingredient in the food industry (Karabulut et al., 2022).What sets hemp seed apart from other plant seeds is the higher quality and proportion of proteins and fatty acids it contains. Hemp seed protein is composed of 65% high-quality edestin protein derived from hemp seeds, making it the most powerful protein among all plant sources. Furthermore, hemp milk tends to be an improved source of calcium, iron, vitamin A, magnesium, and zinc in comparison to soy milk, rice milk, almond milk, and cow's milk (Vahanvaty, 2009).

Today, there is a growing consumer preference for the processing methods, origins, health implications, and constituents of food items. Consumer demands for natural products are encouraging food and beverage businesses to create new products by removing artificial additives, such as emulsifiers, and introducing natural components. The food industry has lately changed to producing "clean label" products with reduced additives in response to consumer demand. Increasingly, the food company is recognising and utilising naturally produced plant gels (Galanakis, 2022b). Chia seeds are a rich source of gel. The surface of seed absorbs water, resulting in the formation of a gel-like substance called mucilage, which constitutes 6% of the seed's composition (Feizi et al., 2021). CSM is a mucilage mostly made up of polysaccharides, which largely consist of crude fibres and carbohydrates (Chavan et al., 2017). The gelatinous substance obtained from hydrated chia seeds can serve as an emulsifier and stabiliser in ice cream. This is because it has the ability to retain and absorb water, similar to conventional stabilisers commonly employed in food preservation (Feizi et al., 2021).

2.2 Clean label food

Nowadays, more and more consumers are becoming more interested in food processing, raw material origin, health, and composition. Among these factors, consumers are most concerned about food ingredients. At the same time, the ingredients of food are displayed through food labels, which can help people intuitively understand the channels of food (Galanakis, 2022b). According to Cao and Miao (2022) food labels are an important communication tool for food manufacturers and retailers because they inform consumers and help them make purchasing decisions. According to James (2020), health and sustainability are two factors that influence consumer purchasing preferences and prompt food and beverage manufacturers to develop new products that eliminate chemicals and artificial additives while adding natural ingredients. In support of this argument, Galanakis (2022b) emphasises that, in response to consumer demand, much of the food industry has recently begun to develop ‘clean label’ products that use fewer additives.

‘Clean label’ has become a popular concept in the food industry, but there is no standard and formal definition of this generic term. The term ‘clean label’ emerged in the 1980s, when consumers began to perceive electronic numbers listed on food labels as having negative health effects (James, 2020). Subsequently, the term clean label as an extension of ‘natural ingredients’ began to appear in consumers’ sights, and some influential authors began to advise consumers that they should not consume foods with more than five ingredients (Chen et al., 2022). Figure 2.1 shows the history of clean label development. First, the ‘free’ claim seems to be the basis of the "clean label" trend, especially ‘GMO (genetically modified organism) free’. The definition given by Galanakis (2022b) about clean label ingredients suggests that clean label ingredients should contain no ‘chemical’ additives in their ingredient list and be processed using conventional techniques with as few steps as possible. Cao and Miao (2022) presents a more detailed explanation of clean labelling, recommending that products be free of additives, preservatives, or 'organic', while it's best to keep ingredient lists shorter and minimally processed raw materials and avoid additives with chemical-sounding names. Furthermore, figure 2.2 shows the evolution of clean label trends from the past to the future.

Figure 2.1 The history of clean label development (Galanakis, 2022b).

Figure 2.2 The evolution of clean label trends from the past to the future (Galanakis, 2022b).

A few food ingredient manufacturers offer solutions to reduce additives, use natural ingredients, and address other formulation challenges posed by "clean label" concerns. Recently, a group of food ingredient companies conducted a study of more than 2,800 consumers in nine European countries on the importance of understanding shopper profiles, front-of-package claims, and ingredient labels in order to better demonstrate understanding and definition of ‘clean label’ from customers. (Chen et al., 2022). That study classified common food ingredients into three categories: acceptable, tolerated, and restricted to guide product formulation in the food industry (Table 2.1).

For this study, to develop a clean label ice cream, the restrictive ingredients mentioned in the above table should be avoided and replaced with acceptable and tolerated ingredients as much as possible. Avoid guar gum, carrageenan, and xanthan gum in the stabiliser portion of ice cream ingredients, and the sweetener part uses sucrose or glucose instead of fructose corn syrup. The fat portion uses animal cream instead of hydrogenated fats.

Table 2.1 Ingredient acceptability, tolerance, and restriction by European consumers (Galanakis, 2022b).

Accepted	**Tolerated**	**Restricted**
Natural flavours	Yeast extract	Guar gum
Natural colours	Ascorbic acid	Maltodextrin
Sugar, glucose	Corn syrup	Monosodium glutamate
Vegetal oils	Pectin	Xanthan gum
Potato starch	Whey protein	Hydrogenated fats
Starch	Lecithin	Carrageenan
Corn starch	Potato fibre	Mono/di-glyceride fatty acids
Gelatine	Artificial flavours	Caseinate
Soya protein	Artificial colours	MCC (microcrystalline cellulose gel)
Rice starch	Modified starch	CMC (sodium carboxymethyl cellulose)
Preservatives	Tapioca starch	HPMC (hydroxypropyl methylcellulose)

In addition to reducing the use of restricted ingredients in ingredient lists, food labels can also increase nutrition and health claims. Claims on food products can help consumers compare a product to similar products that do not have such nutrients and other substances added, giving the food with a claim a nutritional, physiological, or other health advantage. In this way, consumers can understand the total amount of each nutrient or other substance they are consuming. A claim is any information or representation, including any form of pictorial, graphic, or symbolic representation, that is not mandatory under community or national legislation, indicating or implying that a food has a specific characteristic (Galanakis, 2022b). In support of this argument, Cao and Miao (2022) states that food claims should also include printed content as well as food packaging for promotional purposes.

Nutrition claim means any claim that states, implies, or implies that a food has particularly beneficial nutritional properties due to: (1) the energy (caloric value) provided; (2) the content of the three basic nutrients; (3) sodium (and other mineral content); (4) other specific nutrients or substances included; (5) substances not included (Galanakis, 2022b). Finally, there are health claims and disease risk reduction claims, which means any health claim that states or implies that there is a relationship between a food category, a food, or one of its constituents and health or that one of its constituents significantly reduces a risk factor for the development of disease in humans (Chen et al., 2022).

As summarised in Galanakis (2022b)' research, most definitions agree that ‘clean label’ must include a short list of ingredients, along with words such as natural, organic, or additive-free. On the other hand, words that sound like chemicals or electronic numbers must be avoided. Due to the lack of legal definitions and specific regulations, the interpretation of ‘clean label’, especially terms such as ‘artificial’ and ‘natural’ ingredients, is subjective for both consumers and food manufacturers. Moreover, the definition is up to the parties involved (food and beverage manufacturers, ingredient suppliers, retailers, and consumers), each of whom has their own definition of a clean label (Cao & Miao, 2022). In addition, currently there is no special place for clean label food in supermarkets, and the words ‘clean label; are not seen on product labels, so consumers' acceptance of a product depends largely on consumers' perception of the product (Chen et al., 2022). Familiarity with food ingredient lists, raw materials, and processing processes. To summarise, in this research, if a new product is developed with a “clean label,” it must contain: (a) a natural, familiar, and simple ingredient list; (b) nutrient-dense ingredients; (c) sustainably produced, Animal welfare is considered without compromising the needs of future generations (Galanakis, 2022b).

2.3 Hemp

2.3.1 Hemp plant

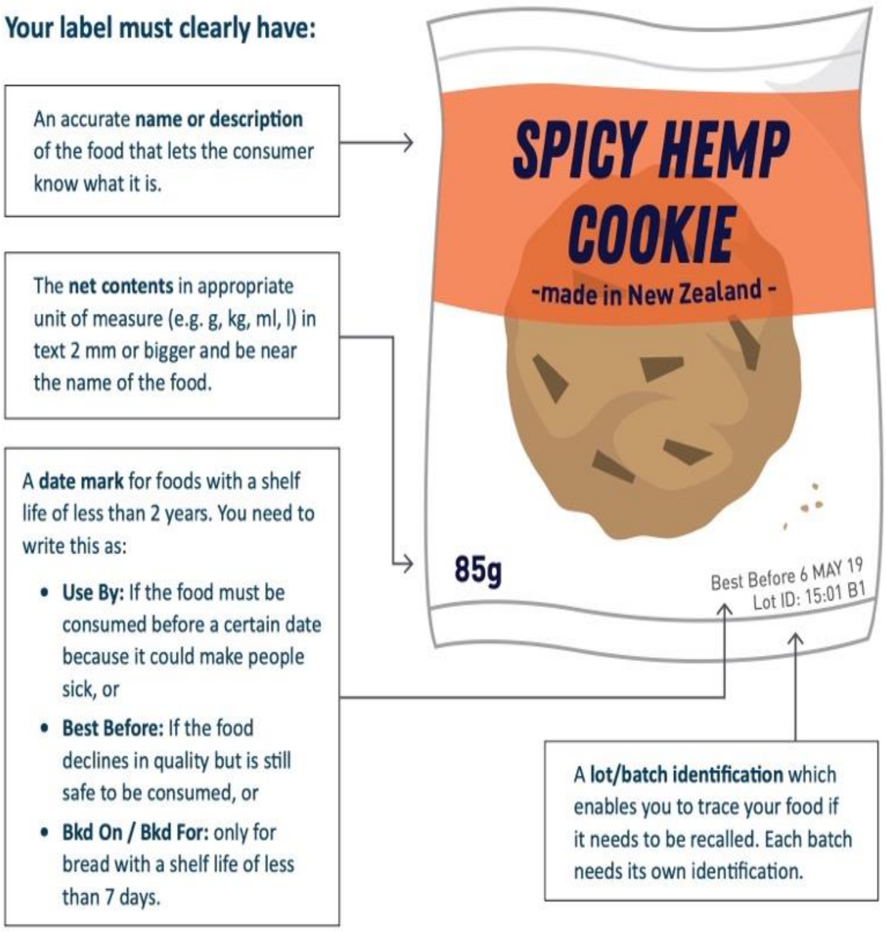
Hemp (Cannabis sativa L.) is an herbaceous annual belonging to the family Cannabinaceae. People often confuse the hemp plant with the cannabis plant, focusing solely on its addictive and psychoactive properties, ignoring its edible and medicinal value and its industrial applications. (Borkowska & Bialkowska, 2019). Chauhan (2021) conducted a questionnaire survey on 100 undergraduates, and 99% of the students believed that hemp was only psychoactive and used as a drug. Indeed, the edible industrial hemp and the addictive and psychoactive cannabis are distinct subspecies within the same family. However, the line between use, misuse, and abuse of substances with specific properties is blurry, and it is the responsibility of those in the food industry to draw a clear line between hemp's nutritional value, health benefits, and abuse (BeŞİR et al., 2022). Cannabis is subdivided into two main subspecies: Indica and Sativa (Chauhan, 2021). Borkowska and

Bialkowska (2019) added that in addition to two standard varieties of cannabis plants: Cannabis indica and Cannabis sativa, there is a third standard variety, Cannabis ruderalis, and some hybrid plants that mix these three subspecies. But these subspecies of the cannabis plant all contain varying levels of tetrahydrocannabinol (THC).

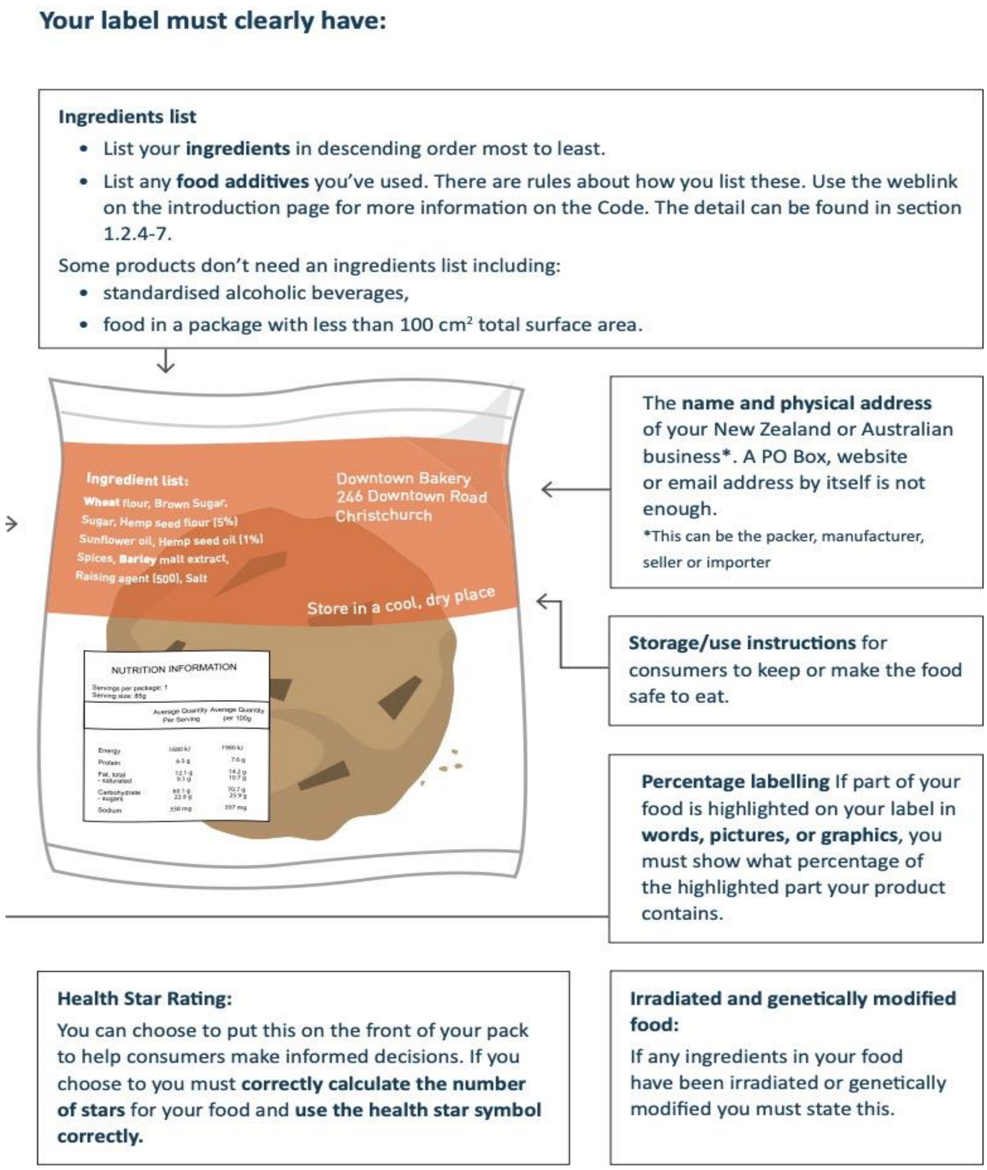
THC is a strictly regulated organic compound that primarily affects the human central nervous system. Historically, people have used marijuana as an anaesthetic, but the narcotic ingredient, THC, is no longer in use (Vahanvaty, 2009). On the other hand, Chauhan (2021) criticised indica variety contains higher levels of THC, while the Sativa variety contains more cannabidiol (CBD), which has nutritional benefits and medicinal properties and is an ingredient that is beneficial to the human body. In fact, all the subspecies contain varying levels of THC and CBD, and the concentration of THC they contain depends on the variety and processing of the hemp plant. Therefore, in order to standardise the management and define the use of hemp, many countries have issued relevant regulations based on the content of THC. For example, in most European countries, the actual legal limit for the cultivation of hemp seeds is no more than 0.2% of the total dry weight of the plant for the psychoactive ingredient THC (Trovato et al., 2023). Moreover, the FDA stipulates that the THC content in industrial hemp cannot exceed 0.3%. Under federal law, any cannabis plant that contains more than 0.3 percent THC would be considered non-hemp cannabis or marijuana (Leonard et al., 2020).

Although New Zealand requires more strict levels of THC in hemp plants than other regions or countries, Ministry of Primary Industries (2019) suggested that the plant's THC level should generally be below 0.35% and not above 0.5%. However, New Zealand has many other stricter regulations for hemp as a food. For example, the only part of the low-THC hemp plant that is allowed to be sold as food or used as a food ingredient is the seed, which means that the leaves, flowers, buds, stems, or any other part of the low-THC hemp plant are not allowed to be made into food. Despite the health benefits of CBD for humans, Ministry of Primary Industries (2019) prohibits the sale or use of medicinal cannabis (THC content higher than 0.5%) and CBD products (such as CBD oil) as food or food ingredients. Ministry of Primary Industries (2019) also suggested the hulling (removal of the outer shell) of retail hemp seeds and their addition to food and prohibits their cultivation. Furthermore, food cannot contain the whole seed, but it can serve as an ingredient by grinding it into a powder or extracting it for oil. Significantly, CBD cannot be extracted and added to food as an additive, so the CBD in all hemp can only be found naturally in or on the seeds. And its CBD content shall not exceed 75 mg/kg (Ministry of Primary Industries,2018).

In addition, Ministry of Primary Industries (2018) also has regulations on the labelling of foods containing hemp, and the label prohibits the inclusion of cannabis, marijuana, or words with the same meaning and cannot contain images of any part of the cannabis plant (except seeds). It is also prohibited to suggest or imply that the product may alter mood, behaviour, or induce hallucinations (e.g., psychoactive effects). One point that is more easily overlooked is that any nutritional statement or health claim about CBD, or even the name, concentration, and content of CBD, cannot be reflected on the label (Ministry of Primary Industries,2018). More specific labelling guidelines are shown in Figure 2.3.



(a)



(b)

Figure 2.3 Guideline of Food Containing Hemp Seeds, front (a) and back (b) (Ministry of Primary Industries,2018).

2.3.2 Basic nutrients of hemp seeds

Nutrients can be defined as the chemical constituents of food that must be supplied to the body in proper amounts (Leonard et al., 2020). They perform specific functions in our bodies and are required in varying amounts. Our bodies require large amounts of nutrients such as carbohydrates, protein, fat, and water, known as macronutrients. Vitamins and minerals are micronutrients that our bodies need in small amounts. Getting the right amount of nutrients helps maintain proper nutritional status (Chauhan, 2021).

Hemp seed can be used as a superfood due to the above-mentioned CBD, which is beneficial to the human body. It is also rich in protein, fat, dietary fibre, and various vitamins and minerals. Research by Pham (2021) supports that hemp seeds contain approximately 35% (w/w, wet basis) fat, 25% (w/w, wet basis) protein, a considerable amount of dietary fibre (especially non-digestible), vitamins, and minerals (Callaway, 2004). The basic nutrient content of hemp seed is shown in Table 2.2. After hulling, the percentages of fat and protein increase to about 47% and 36%, respectively, so hemp seeds are considered to be an excellent source of edible oil, protein, and dietary fibre (Pham, 2021).

Table 2.2 The basic nutrient content of hemp seed (Callaway, 2004).

Constituent (%)	**Whole hemp seed**
Oil	35.5
Protein	24.8
Carbohydrate	27.6
Moisture	6.5
Ash	5.6
Total dietary fibre	27.6
Digestible fibre	5.4
Non-digestible fibre	22.2
Energy (kJ/100 g)	2200

2.3.3 Application history of hemp seeds

Hemp has been cultivated in temperate Eurasia for thousands of years but was first introduced to North America in 1606. However, the global and decades-long prohibition on hemp cultivation led to the long-term non-cultivation of this famous plant, resulting in the loss of this highly valuable product and the removal of nutritious hemp seeds and oils from the human diet. Moreover, before the outbreak of World War II, hemp oil was the most common and most frequently consumed vegetable oil (Xu et al., 2021).

In 1996, the European Union began to support the return of hemp to its market (Borkowska & Bialkowska, 2019). Canadian farmers planted approximately 8,000 hectares of cannabis in 2005, almost exclusively for the production of food and body care product seeds. Canadian cannabis acreage has continued to increase, from 1,300 hectares in 2001 to double since 2004. The rapid growth of hemp acreage is entirely driven by strong demand for hemp food (Leson, 2006). For the first time since 2015, its cultivation as a dual-purpose crop for seeds and fibres exceeds 20,000 hectares (Xu et al., 2021). Canadian farmers, processors, and hemp food manufacturers have learned how to produce high-quality, safe raw materials from hemp seeds, including hulled seeds (nuts), cold-pressed oils, and, increasingly, protein-rich seed cakes or flour. Meanwhile, food manufacturers have developed a wide range of retail products, including nuts, oils, protein powders, energy bars, granola, hemp nut butter, pasta, and ice cream, among others (Leonard et al., 2020). Manufacturers have also been very successful in launching their products in retail stores(Leonard et al., 2020). Over the past decade, hemp has re-emerged as a valuable food and fibre industrial crop in Canada and European countries. Hemp seeds can be legally consumed as food in the United States and Canada, so hemp seeds and hemp seed edibles have become available to the public in these countries. But in the United States, the cultivation of any variety of marijuana is illegal (Callaway, 2004). Global demand for industrial hemp was approximately $4 billion in 2018, and according to trend estimates, this demand will increase to $11 billion by 2025 (Leson, 2006).

While the potential of hemp seeds as a human food has not yet reached the mass consumer market in the West, its nutritional properties have long been recognised and valued by people in Asia, India, Russia, and Eastern Europe. In China, street vendors still sell roasted hemp seeds as a snack. In Russia, hemp oil has been pressed from hemp seeds and used as an alternative to more expensive and less healthy sources of dietary fat, such as butter and hydrogenated margarine. The roasted form of hemp seeds is also very popular in Turkey. In Germany, ‘cannabis soup’ is becoming more and more popular. Cannabis butter is consumed in the Baltic States and Russia, while in Iran, cannabis is known as the ‘king of seed’. Some traditional hemp seed foods can still be found in the Baltic countries, especially Latvia and other Eastern European countries(Callaway, 2004). Hemp has many advantages in addition to being used in the food industry, such as pharmaceuticals, cosmetics, textiles, cellulose and paper, construction materials, etc (Borkowska & Bialkowska, 2019).

2.3.4 Hemp seeds oil

Oil extracted from hemp seeds is used as human food and nutritional supplements. However, it occupies only a small portion of the market for edible oils, with approximately 90% of global edible oil production coming from palm, soybean, canola, and sunflower(Xu et al., 2021). This is because the oxidative instability and high heat sensitivity of hemp seed oil (HSO) limit its application range.

The basic properties of HSO are shown in Table 2.3. Compared with other vegetable oils, hempseed oil has a lower iodine value, reflecting its high degree of unsaturation (Pham, 2021). At the same time, the high content of polyunsaturated fatty acids also means that HSO's oxidative instability is high, which is one of the most critical factors affecting oil shelf life. The low saturation of fatty acids in HSO means that its crystallisation and melting transition points are lower than those of rapeseed oil and linseed oil, indicating that it has higher heat sensitivity. Hemp seed oil begins to oxidise in the temperature range of 130 to 140 °C and then reaches its peak oxidation temperature at 150 to 165 °C. (Leonard et al., 2020). Due to the low smoke point of HSO at 165°C, it limits its role in frying or cooking oil because most cooking oils should have high smoke point characteristics, such as a smoke point higher than 200°C.

Table 2.3 The basic properties of HSO (Xu et al., 2021).

Parameters	**Value**
L*	18.13 ± 0.11
a*	2.40 ± 0.36
b*	30.87 ± 0.14
Moisture content (%)	0.72 ± 0.10
Unsaponifiable matter (%)	0.26 to 1.92
Density (24 ◦C, mg/mL)	0.92
Refractive index (40 ◦C)	1.48
Iodine value (g of I/100 g of oil)	153.60 to 169.12
Peroxide value (mg peroxide/kg)	1.94 ± 0.15
p-Anisidine values (unit)	0.62 ± 0.11
Specific extinction 232 nm (unit)	1.53 to 4.18
Specific extinction 270 nm (unit)	0.02 to 1.43
Acid value (mg KOH/g of sample)	1.76 ± 0.05
Saponification value (mg KOH/g of sample	184.00 to 192.96

Unrefined HSO is liquid, contained in oil body spherical organelles in hemp seeds, and is dark green in colour due to the presence of a large amount of chlorophyll (Pham, 2021). Compared to linseed and rapeseed oils, hemp oil has significantly lower L* (lightness) and a* (red) values(Leonard et al., 2020). Tura et al. (2023) stated that, compared to other vegetable oils, hemp seed oil extracted by the cold pressing process has a large amount of chlorophyll present. This allows the oxidation process of the hemp seeds to be catalysed, as high levels of chlorophyll increase the oxidation sensitivity of the oil, leading to rancidity and a decrease in the quality of the hemp seed oil. This is because, as a phytochrome, chlorophyll is susceptible to photooxidation and acts as a pro-oxidant in oils and oily products.

Peroxide values indicate the degree of primary oxidation products, while p-Anisidine values indicate the amount of secondary oxidation products. The amounts of free fatty acids, peroxides, and dienes that can be seen at 232 nm are shown by their specific extinction values. Whereas the specific extinction value at 270 nm indicates the amount of secondary oxidation products (aldehydes and ketones). Based on these measurements, Leonard et al. (2020) concluded that the oxidative stability of hemp seed oil was within acceptable quality for commercial oils.

Researchers agree that hemp seeds are an excellent source of nutritional value and offer incredible health benefits. For example, hemp seed oil has over 80% polyunsaturated fatty acids and is high in w-6 and w-3 essential fatty acids (EFA), with concentrations of about 50% and 15–20%, respectively (Tura et al., 2023). The polyunsaturated fatty acids contained in the hemp seeds are shown in Table 2.4. EFAs metabolise and produce a large amount of ω-6 Linoleic Acid (LA), ω-3 Alpha-linolenic Acid (LNA), c-linolenic acid (GLA), and stearidonic acid (SDA) in the human body, it is worth mentioning that these essential fatty acids such as LA and LNA cannot be synthesised and produced by the human body and must be ingested from the daily diet (Xu et al., 2021). What's more, the polyunsaturated fatty acids in hemp oil, such as gamma linolenic acid (GLA; omega-6) and stearidonic acid (omega-3), are comparable to those found in fish, but unlike fish, hemp does not contain heavy metals such as mercury (Vahanvaty, 2009).

Table 2.4 The polyunsaturated fatty acids contained in the hemp seeds (Callaway, 2004; Leonard et al., 2020; Tura et al., 2023).

Type of fatty aid		**percentage**
C14:0	Myristic Acid	0.03
C15:0	Pentadecanoic Acid	0.01
C16:0	Palmitic Acid	5.37
C16:1(ω-7)	Palmitoleic Acid	0.11
C17:0	Heptadecanoic acid	0.05
C18:0	Stearic Acid	1.56
C18:1(ω-9)	Oleic Acid	11.51
C18:2(ω-6)	Linoleic Acid	59.16
C18:3(ω-3)	Alpha-linolenic Acid	17.96
C18:3(ω-6)	Gamma-linolenic Acid	3.48
C18:4(ω-3)	Stearidonic Acid	1.36
C20:0	Arachidic Acid	0.18
C20:1	Eicosenoic Acid	0.80
C20:2	eicosadienoic acid	0.04
C22:0	behenic acid	0.27
C22:1	erucic acid	0.01
C24:0	lignoceric acid	0.12
ω6/ω3 ratio		3.29
Essential fatty acid (EFA)		70.37
Saturated fatty acid (SFA)		11.90
Unsaturated fatty acid (UFA)		88.10
Monounsaturated fatty acid (MUFA)		11.71
Polyunsaturated fatty acid (PUFA)		80.60
Polyunsaturated/saturated ratio		10.42

Moreover, factors such as ageing, diet, and pathology may negatively affect the body's GLA metabolism process, and dietary GLA supplementation can have a positive impact on rheumatoid arthritis and cardiovascular, psychiatric, and immune system diseases. And in addition to its nutritional value and health benefits to the human body, hemp seed oil is also good for some metabolic processes in the human body, such as lipid metabolism and immune regulation (Xu et al., 2021). Consumption of hemp seed oil has been reported to cause significant changes in plasma fatty acid profile and improve clinical symptoms of atopic dermatitis due to a balanced and adequate supply of polyunsaturated fatty acids (PUFAs) (Callaway, 2004).

The ratio of omega-6 and omega-3 fatty acids in the human diet has a very important impact on human health. The most important thing is that the fatty acid ratio balance must be suitable for the human body, so that the 3:1 ratio of omega-6 to omega-3 fatty acids in HSO promotes cardiovascular health (Chauhan, 2021). Research by Vahanvaty (2009) also supports that the ratio of omega-6 and omega-3 fatty acids in hemp oil is close to the ideal ratio required by the human body, and this may be important to the essentialist. Almost all studies only mention that the ratio of fatty acids in hemp seed oil is almost perfect, but no explanation is given. The research by Callaway (2004) explains that this is because linoleic acid (C18:2 omega-6) and alpha-linolenic acid (18:3 omega-3) are metabolized into gamma-linolenic acid after ingestion (18:3 omega-6, ‘GLA’) and stearidonic acid (18:4 omega-3, ‘SDA’), both utilize the same enzyme in this metabolic process: delta-6 desaturase, which means that there is metabolic competition between these two essential fatty acids, so that an excess of alpha-linolenic acid (omega-3) in the diet can lead to a deficiency of omega-6 metabolites, which upsets the metabolic balance.

However, both GLA and SDA exist in HSO, and the ratio of ω-6/ω3 in most commercial HSO is close to 2.5:1. This allows the enzymatic step using delta-6-desaturase to be effectively bypassed, maximising the availability of fatty acids in the body. Trovato et al. (2023) proposed that the ratio of (ω-6) and (ω-3) in HSO is 4:1, which is different from the 3:1 mentioned in the above literature. Combined with the research by Tura et al. (2023), we can know that not all hemp oils have the same content, and the fat composition of hemp oil produced by different varieties or subspecies of hemp seeds is also quite different.

In addition, the processing technology of hemp oil also affects the content of unsaturated fatty acids. High temperatures and oxygen will oxidise and change the unsaturated fatty acids in HSO. Although hot pressing oils can provide some extra special flavour, they also lead to the loss of some nutrients and the formation of some toxic compounds, such as benzopyrene and 3-chloro-1,2-propanediol ester, during the roasting of seeds. Using cold pressing processes, solvent extraction, nitrogen protection during processing, and even carbon dioxide supercritical extraction can greatly increase the yield of hemp seed oil (Trovato et al., 2023).

The above polyunsaturated fatty acids and other components are beneficial to the cardiovascular system and help maintain low blood cholesterol and triglyceride levels (Trovato et al., 2023). In addition, some unsaponifiable lipid fractions, such as sterols, phytols, and tocopherols, also have health benefits for the human body. Their mechanism of action is to inhibit the absorption of cholesterol from dietary fat to reduce the risk of cardiovascular disease, as well as some antiviral, fungal, and anti-inflammatory effects. Table 2.5 shows the fraction of unsaponifiable lipids in HSO (Leonard et al., 2020).

Table 2.5 the quantity of unsaponifiable lipids in HSO (Leonard et al., 2020).

Constituent	**Quantity (mg/kg of oil)**
β - Sitosterol	1905.07
Campesterol	505.69
Δ5 - avenasterol	142.80
Total Sterol	2793.73
Phytol	167.59
Geranylgeraniol	26.06
Total policosanols (linear aliphatic alcohol)	226.94
α - Tocopherol	30.22
γ - Tocopherol	733.38
Total tocopherol	800.28
Total linear hydrocarbons	451.77
Total waxes	43.35

Dietary antioxidants other than tocopherols, such as sterols (up to 15%) and other phenolic compounds, which are present in higher levels in hemp seeds, are cardioprotective and anti-inflammatory (Xu et al., 2021). According to Leonard et al. (2020), the sterol content in hemp seed oil is twice that of olive oil, and supplementing phytosterols can effectively enhance blood cholesterol regulation. The total content of other antioxidants in this part is about 2%, including the most interesting bioactive compounds, b-sitosterol (1905 ppm), campesterol (506 ppm), phytol (168 ppm), and cycloartemisinin (91 ppm). Clinical trials have shown that treatment with β-sitosterol can significantly improve the symptoms of benign prostatic hyperplasia(Leonard et al., 2020). Phytol, a component of chlorophyll, has been demonstrated in several in vivo and in vitro studies for its antioxidant, analgesic, and antiparasitic properties. Therefore, these ingredients have the potential to be used in the development of novel foods and even non-food applications (Xu et al., 2021).

Callaway (2004) also reported that supplementation with phytosterols had a significant effect on regulating blood cholesterol. In addition, tocopherol is the primary ingredient in foods that promote vitamin E activity. Their ability to react with and inactivate free radicals makes them very effective antioxidants, preventing the oxidation of unsaturated fatty acids. Tura et al. (2023) summarises the positive effect of tocopherol on atherosclerosis, Alzheimer's disease, cataracts, and cancer. The total tocopherol content of hemp seeds is approximately 80 to 90 mg per 100 g of oil, which is higher than most vegetable oils such as sunflower, corn, sesame, and flaxseed oils. Along with sterols and tocopherols, the unsaponifiable part of HSO has linear fatty alcohols. Phytol, which is a part of chlorophyll in hemp seeds, is the most important of these. Several in vivo and in vitro studies have demonstrated the antioxidant, analgesic, and antiparasitic properties of alcohol (Leonard et al., 2020).

2.3.5 Hemp protein

Hemp protein is an emerging protein source that has been underutilized for a long time, accounting for 25%~30% of the composition of hemp seed. Hempseed protein is rich in essential amino acids (EAA) and easily digestible, comparable in nutritional value to soy protein and even many other proteins, such as egg whites (Tang et al., 2006).

In the study by Xu et al. (2021), the chemical composition of hemp seeds of 13 varieties was analysed, and the results showed that hemp seeds are a valuable source of protein. In detail, the crude protein content in the hemp seeds of the 13 varieties tested ranged from 26.48% to 32.03%, with an average value of 28.48%. Moreover, the shelling of hemp seeds had no significant effect on the protein composition, but the protein extraction rate was greatly improved, indicating that most of the proteins in hemp seed were present in the cotyledons. A total of 181 proteins were identified in hemp seeds, most of which were two proteins, globulin and albumin (Leonard et al., 2020). These two important fractions have distinct amino acid profiles and functional properties (Pham, 2021).

2.3.5.1 Albumin

Albumin (a globular protein) and edestin (a legumin), the two main proteins in hemp seeds, are rich in amino acids that are essential for human health (Callaway, 2004). Hemp seed protein consists of 65% premium edestin protein, the most potent protein of all plant sources, with the remaining 35% provided by albumin and essential amino acids (Vahanvaty, 2009). So, hemp protein is characterised by a particularly high content of arginine and glutamic acid. These two cannabinoid proteins were found to have positive effects on regulating organ function and human metabolism (Leonard et al., 2020).

2.3.5.2 Edestin

Edestin accounts for about 85% of the total cannabinoid proteins. This fraction is soluble in saline solution but extremely poorly soluble in water, with the lowest solubility at pH 5 (Tang et al., 2006). Edestin is mainly composed of 11S and 7S protein types, and 11S protein also has high nutritional value due to its high content of sulphur-containing amino acids, arginine, and essential amino acids (Wang et al., 2023).

According to the sedimentation coefficient analysis, the edestin fraction is composed of 93% legumin-type edestin (also known as macin) and 7% vicilin-type edestin. These two components of edestin can be precipitated by adjusting the pH to pH 6.4 and pH 4.6, respectively. Two of the cysteine residues form intrachain disulfide bonds, and the remaining cysteine residues keep the thiol groups free. Dissociation or recombination of the disulfide bonds is thought to be responsible for the poor solubility of the cannabinoid protein (Leonard et al., 2020).

The albumin fraction makes up about 15% of the hemp protein (Tang et al., 2006). Albumin is water-soluble. Pham (2021) reported that the protein fraction consists of seven polypeptide molecules with a molecular weight ranging from 6 to 35 kDa. Of these, most are proteins with a molecular weight below 18 kDa. However, this protein fraction is rich in sulphur-containing amino acids, accounting for approximately 18% of albumin's total amino acids. In support of evidence, Xu et al. (2021) argued that albumin (2S) mainly consists of two polypeptide chains with 27 and 61 amino acid residues, respectively, including 18% wt sulphur-containing amino acids (cysteine and methionine). Sulphur-rich proteins can be used as rich thiol resources to formulate highly nutritious foods, and they can also be used as nutritional tools to improve the body's antioxidant capacity (Wang et al., 2023).

The contents of aromatic amino acids (Tyr, Phe, Trp) and hydrophobic amino acids (Ala, Cys, Val, Met, Ile, Leu) in albumin were lower than those in edestin. Therefore, albumin has a more flexible structure and higher water solubility and does not change drastically with pH (Tang et al., 2006). The solubility of albumin is low at pH 3 but increases with increasing pH, which may be due to the fact that albumin has a higher foaming capacity but a lower foaming stability than edestin (Pham, 2021). Albumin has fewer disulphide bonds compared to the edestin molecule, therefore albumin is more compact and flexible, which has been supported by Xu et al. (2021).

2.3.5.3 Hemp seed protein isolate

hemp protein concentrates contain at least 65 % protein. Hemp seed protein isolate (HSPI) is typically produced by removing the non-protein components from defatted cannabis seed cake. The main way of removing the non-protein components is by hydrolysing the non-protein components of the cannabis seed cake using a mixture of enzymes including cellulases, hemicellulases, xylanases, and phytases (Tang et al., 2006). Wang et al. (2023) combines enzymatic hydrolysis and membrane filtration to convert the low-protein hempseed kernel meal into cannabinoid protein concentrates, which have an elevated protein content of about 70% by weight.

HSPI has at least 90% protein content (Tang et al., 2006). The separation method usually uses isoelectric precipitation, and the separation process is usually applied to defatted hemp seed or hemp seed powder. The protein is dissolved in the environment at pH 8–10. The solubilized protein is then subjected to isoelectric precipitation at pH 4.5–5.0, although this method yields high protein purity. The extraction rate is low, though. This could be because using alkaline conditions for extraction could hurt the final extract, causing problems with the protein, making the protein less digestible, losing some amino acids, and making it hard to control the final product properties and process conditions (Pham, 2021).

HSPI contains high levels of essential amino acids, sufficient to meet the FAO/WHO requirements for children aged 2 to 5 years (Tang et al., 2006). HSPI contains highly digestible amino acids compared to other protein sources such as rapeseed meal and borage meal, making it an excellent natural source (Xu et al., 2021). HPI shows high digestibility, while the in vivo and in vitro digestibility of hemp seed protein and soy protein are shown in Table 2.6. Wang et al. (2023) performed an in vitro digestion experiment with soybean protein isolate (SPI) and HPI. It can be clearly seen from the reducing SDS-PAGE profiles: after digestion with pepsin and trypsin, the in vitro digestibility of hemp protein isolate was significantly higher than that of soybean protein isolate (Figure 2.4). But, depending on the source and variety of hemp, the digestibility of dehulled hemp seeds can range from 90.8% to 97.5%, while casein has a digestibility value of 97.6%.

Table 2.6 The in vivo and in vitro digestibility of hemp seed protein and soy protein (Wang et al., 2023).

plant material		***in vivo***	***in vitro***
hemp	whole seed	85	-
	hulled seed	95	-
	meal	87	85
	protein isolate	99	88
soy	bean	93	-
	flour	86	-
	meal	80	-
	protein isolate	95	80

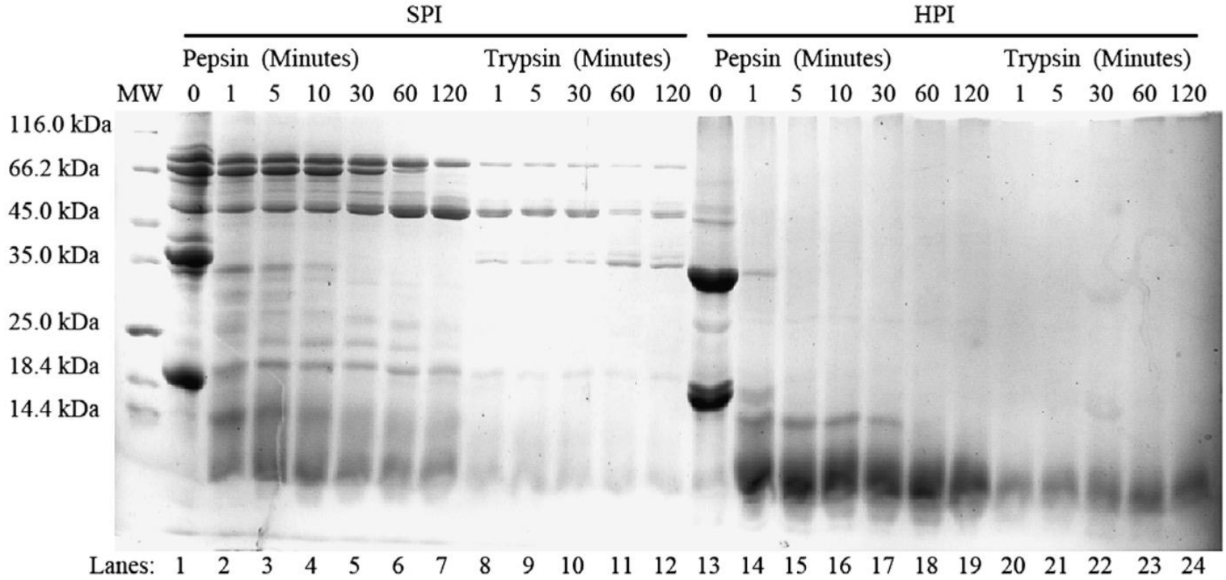


Figure 2.4 Reducing SDS-PAGE profiles for soybean protein isolate (SPI) and HPI digested with sequential pepsin and trypsin (Wang et al., 2023).

Although shelled hemp seeds have a lower digestibility than soybeans, shelled hemp seeds, hemp seed meal, and hemp protein isolate have a higher digestibility than soybeans and soybean products. So, removing the husk helps to improve the digestibility of hemp protein and its bioavailability because the presence of fibre limits the digestion of the protein (Paul et al., 2020). Second, because the hemp fibre shell part contains some antinutrients. Research by Martin et al. (2023) also verified that the digestibility score of protein in shelled hemp seeds (61 points) is higher than that of whole hemp seeds (51 points) and protein in hemp seed powder (48 points). This suggests that high concentrations of antinutritional factors, such as phytic acid and fibre, may hinder protein absorption.

The digestibility-corrected amino acid score (PDCAAS) for hemp protein ranges from 0.48 to 0.61(Wang et al., 2023). Lower lysine and tryptophan contents give hemp protein a lower PDCAAS than soy protein isolate (0.92). Regardless, PDCAAS based fecal nitrogen digestibility may not reflect the digestibility of all amino acids. At the same time, another limitation of the PDCAAS method to measure whether the protein is high-quality is that this method mainly focuses on whether the amino acids contained in the measured protein meet the daily amino acid intake requirements of humans and also uses the protein requirements of children with a proportionally greater lysine requirement as a reference, so it is very likely that this approach ignores other potential properties and values of hemp isolate. For example, hemp protein isolate contains a higher proportion of arginine compared to other edible foods, which may position hemp protein as a good source of digestible arginine (Pham, 2021).

2.3.5.4 Amino acids in hemp seed protein

Hemp seed protein is rich in essential amino acids (EAAs) and contains all nine EAAs required by humans. The amino acid composition of hemp seed protein is shown in Table 2.7. Its amino acid profile is characterised by a very high content of arginine and glutamic acid, with a moderate amount of sulphur-containing amino acids (Karabulut et al., 2022). The amino acid composition of hemp protein is similar to that of egg white and soy, with high concentrations of arginine, glycine, and histidine(Callaway, 2004).

Table 2.7 The amino acid composition of hemp seed protein (Callaway, 2004).

Amino acid	**Soybean (g/100g)**	**hemp seeds (g/100g)**
Alanine	1.39	1.28
Arginine	2.14	3.10
Aspartic acid	3.62	2.78
Cystine	0.54	0.41
Glutamic acid	5.89	4.57
Glycine	1.29	1.14
Histidine*	0.76	0.71
Isoleucine*	1.62	0.98
Leucine*	2.58	1.72
Lysine*	1.73	1.03
Methionine*	0.53	0.58
Phenylalanine*	1.78	1.17
Proline	1.65	1.15
Serine	1.54	1.27
Threonine*	1.35	0.88
Tryptophan*	0.41	0.20
Tyrosine	1.14	0.86
Valine	1.60	1.28
Typical protein content (%)	32%	25%

Essential amino acids are indicated by an asterisk (*)

Hemp protein is considered a good source of sulphur-containing amino acids (i.e., methionine and cysteine), which are not usually present in other plant proteins (Pham, 2021). Hemp protein isolate was compared to soy protein isolate and found to have higher levels of sulphur-containing amino acids, a higher ratio of essential amino acids to total amino acids, and higher enzymatic digestibility (Leonard et al., 2020).

In addition, the high amount of arginine (a metabolic precursor of nitric oxide) in hemp seed protein is a chemical necessary for the normal regulation of blood pressure. Moreover, arginine has been recognised by several clinical studies for its important role in foetal growth and reducing insulin resistance (Xu et al., 2021). In addition, Leonard et al. (2020) also emphasised the important role of glutamate as a neurotransmitter in the brain.

Among them, lysine and tryptophan are the main limiting amino acids in hemp seed protein(Xu et al., 2021). Raw hemp seeds are low in lysine, one of the essential amino acids that the body cannot synthesize. The amino acid scores for lysine, leucine, and tryptophan in hemp protein range between 0.5 and 1.0 and indicate that hemp seeds alone are not sufficient to meet the minimum daily intake recommended by the FAO/WHO for this amino acid (Martin et al., 2023). In addition, food processing conditions may further exacerbate this loss because the ε-amino group of lysine is susceptible to the Maillard reaction. Therefore, food manufacturers may need to further supplement hemp seed-containing products with lysine to achieve the good amino acid profile required for human intake (Leonard et al., 2020).

Compared with soy protein isolate, hemp protein is considered a good nutritional source of plant protein for infants because, except for lysine, the content of other essential amino acids is similar to or higher than that of soy protein isolate. The EAAs of hemp protein meet the FAO/WHO amino acid requirements for infants aged 2 to 5 years (Tang et al., 2006). In detail, compared with soy protein isolate, hemp protein isolate has a higher content of arginine, methionine, and cysteine but is lower in aspartic acid, glutamic acid, and lysine. Other amino acids are found in similar amounts in hemp and soy protein isolates (Pham, 2021).

On the downside, hemp seeds contain some anti-nutritional factors, most notably phytic acid and trypsin inhibitors (Leonard et al., 2020). Compared to soy protein, hemp protein has very low levels of trypsin inhibitors, which are considered antinutrients and can be inactivated by heat treatment. This results in lower levels of this antinutrient, making the hemp protein more digestible (Pham, 2021). In addition, several antioxidant bioactive peptides have been isolated from hemp seed protein, but fat would limit the extraction of one of the components of hemp protein from plant material by forming cross-links with the protein. Therefore, the input material for the protein extraction process is usually defatted (Wang et al., 2023).

When protein is used in the food industry, its lower limit often determines its application, not how high its upper limit is. Because the protein's structure determines its function, the structural conformation of the hemp protein may change when exposed to high temperatures, causing negative effects, as shown in differential scanning calorimetry analysis(Leonard et al., 2020). In detail, HSPI start denaturation at 86 °C and reach the denaturation peak temperature at 95 °C. These transition points were attributed to the strength of disulfide bonds rather than hydrophobic interactions (Tang et al., 2006). Proteins in legumes such as lupines and chickpeas are also denatured at the same temperature, with a denaturation temperature range of 85 to 95 °C common among proteins derived from beans and legumes (Leonard et al., 2020).

The function of hemp seed protein is generally pH dependent. Its solubility, emulsion stability/activity, and foaming ability/stability are minimal in the pH range of 4.0 to 6.0, where it has an isoelectric point, and sharply increase above pH 9.0. Similar trends were observed for other functional properties, including emulsion and foaming ability/stability. This phenomenon can be explained by the aggregation of ededin at a pH below neutral (Leonard et al., 2020). Furthermore, Xu et al. (2021) attributed the low emulsifying activity and stability of rapeseed and linseed isolates to their poor protein solubility.

Compared to most other plant proteins, hemp protein's hypoallergenic properties also make it a good alternative to other proteins in some foods. The use of hemp protein products as an alternative to commonly used casein, whey, wheat, and soy proteins is increasing. For example, some studies have shown that hemp protein products can be used as value-added ingredients in bread production, increasing protein and macro- and micro-element content and reducing baking losses and baking time (Chauhan, 2021; Xu et al., 2021).

In general, hemp seed protein has poorer functional properties than other plant proteins. Tang et al. (2006) reported that hemp seed protein was less soluble, less able to emulsify, less stable in emulsions, and less able to bind water than soy protein isolate. The fact that hemp seed protein has more free sulfhydryl groups shows that there are covalent disulfide bonds there. Hence, a tendency to aggregate. Differences in functional properties may also be due to the different protein compositions and aggregation properties of the glycinin and ededin fractions between soy protein and hemp seed protein (Leonard et al., 2020).

2.3.6 Trace elements and minerals in hemp seeds

Hemp seeds are rich in vitamin E, minerals, antioxidants, and fibres. The contents of various trace elements and minerals in hemp seed are shown in Table 2.8. Hemp milk appears to be a better source of calcium, iron, vitamin A, magnesium, and zinc than soy milk, rice milk, almond milk, and cow's milk (Callaway, 2004). The mineral content of hemp seeds is shown in Figure 2.5. Although not graphically represented, there was no significant difference in B vitamin content between various plant milks, but mineral content varied significantly between various plant milks and milk (Vahanvaty, 2009).

Table 2.8 The contents of various trace elements and minerals in hemp seed (Callaway, 2004).

Ingredient	**Content (mg/100g)**
Vitamin E	90.0
Thiamine (B1)	0.4
Riboflavin (B2)	0.1
Phosphorous (P)	1160.0
Potassium (K)	859.0
Magnesium (Mg)	483.0
Calcium (Ca)	145.0
Iron (Fe)	14.0
Sodium (Na)	12.0
Manganese (Mn)	7.0
Zinc (Zn)	7.0
Copper (Cu)	2.0

Figure 2.5 Summary of the mineral content of several common plant milks (Vahanvaty, 2009).

Vitamin E is a well-known bioactive compound present in hemp seed oil, along with other minor bioactive compounds, including polyphenols, carotenoids, phytosterols, other vitamins, and dietary minerals. These compounds contribute to the nutritional value of the oil (Callaway, 2004). Vitamin E comes in eight forms, including the four tocopherols (a-, b-, c-, and d-) and four tocotrienols (a-, b-, c-, and d-), of which type a has the highest biological activity and type c has the highest antioxidant activity. In general, vegetable oils are much more present in tocopherols than tocotrienols; therefore, past studies have focused on tocopherols. Recent studies have shown that tocotriol has better properties than tocopherol in anti-oxidation, anti-cancer treatment, and cholesterol reduction, so it has received more and more attention (Leonard et al., 2020; Tura et al., 2023). Hemp seed oil ranges from 800 to 1500 ppm, which is higher than the 600–1150 ppm found in common vegetable oils such as soybean oil, sunflower oil, and rapeseed oil (Callaway et al., 2005). The content of tocopherols may vary depending on the varieties, agronomic conditions, and extraction methods. Depending on storage conditions, the major isomer is c-tocopherol, which accounts for 85–91% of the total tocopherols in cannabis oil (Xu et al., 2021).

Trace components such as tocopherols, polyphenols, and phytosterols have strong antioxidant properties, can protect fat components from oxidation, and can also provide health benefits to humans (Xu et al., 2021). Because of their ability to react with and inactivate free radicals, they are very effective antioxidants, preventing the oxidation of unsaturated fatty acids. The addition of α-tocopherol to cooked turkey significantly improved oxidative stability. Meanwhile, fortification of γ-tocopherol in energy bars fortified with fish oil reduced lipid oxidation, especially at high fish oil concentrations (>440 μg/g) (Leonard et al., 2020).

Phenolic compounds comprise more than 10,000 molecules with various therapeutic properties that provide antioxidant and anti-inflammatory effects. In particular, their presence has been associated with a reduced risk of stroke, myocardial infarction, and diabetes, as well as improved insulin resistance and systemic inflammatory conditions (Trovato et al., 2023). These mainly include hydroxycinnamic acids, lignanamides, and cannabinoids. Hydroxycinnamic acids are the result of the phenylpropanoid pathway with tyrosine and phenylalanine as precursors, whereas lignanamides are derived from hydroxycinnamic acid amide monomers derived from the oxidative coupling between them. Fourteen lignanamide compounds were identified from hemp seeds, which have good antioxidant activity and acetylcholinesterase inhibitory activity, which may provide good options for the treatment of multi-target Alzheimer's disease (Xu et al., 2021).

As for cannabinoids, they can also be classified as terpenephenolic compounds, biosynthesized from phenolic acids, olive fruit acid, and monoterpene geranyl pyrophosphate. To date, cannabinoids have shown potential benefits such as antiepileptic, analgesic, neuroprotective, and anticonvulsant effects (Trovato et al., 2023). These components are frequently produced as part of plant defense mechanisms against pathogens or UV radiation.

In addition to this, the presence of bioactive compounds such as carotenoids and polyphenols has led to the use of hemp seeds as a beneficial food, and other trace amounts such as terpenes may also contribute to the overall beneficial effect (Martin et al., 2023).

2.4 Chia seeds

2.4.1 Nutrition of chia seeds

Chia is a plant native to southern Mexico and Guatemala with slightly walnut-flavoured seeds and a crunchy structure. While chia seeds may seem to be a new nutrient, they are actually an ancient food source (de Falco et al., 2017). In many studies, chia seeds have been described as a good source of fat, protein, dietary fibre, minerals, and polyphenolic compounds (Chavan et al., 2017). In addition, chia seeds are free of toxic compounds and gluten, making them a safe ingredient for a gluten-free diet (Campos et al., 2016).

In a study by Velazquez-Gutierrez et al. (2015), the main food components of chia seeds were identified as dietary fibre (33.4%), fat (32.2%), and protein (18.2%) . Whereas in the study by (Chavan et al., 2017), each ounce of chia seeds contained 9% of the daily intake of protein, 13% of oil, and 42% of dietary fibre, the nutrient content, especially protein, fat, and fatty acid content, varied according to the ecosystems (e.g., temperature, light, soil structure, and variety) (Dinçoğlu & Yeşildemir, 2019) and genetic modifications (de Falco et al., 2017). Due to their chemical composition, chia seeds are beneficial to human health. They are rich in dietary fibre and polyunsaturated fatty acids, especially a-linolenic acid. In addition, chia seed contain a large number of polyphenols, including caffeic acid, chlorogenic acid, populin, quercetin, and kaempferol, which have strong antioxidant activity (Punia & Dhull, 2019). Due to their use in the food industry and their health effects, chia seeds are considered a functional food. However, the results of studies demonstrating the effects of chia seeds on diseases have been controversial, and many of the relevant studies were animal experiments (Dinçoğlu & Yeşildemir, 2019).

2.4.2 Characteristics of CSM

Chia seeds and chia oils are becoming increasingly popular in many countries, especially in Europe, due to their nutritional and health benefits. Nowadays, chia seeds are used in a variety of industrial applications, such as functional foods, animal feed, and cosmetics (Arnak & Tarakçi, 2021). Dietary fibre is one of the important components of chia seeds, and it has been found that its insoluble and soluble fractions can be used as foam stabilisers, suspending agents, and emulsifiers in foods and pharmaceuticals due to their physical properties, including water holding capacity and viscosity (de Falco et al., 2017). Chia seeds are a good source of total dietary fibre (TDF), which consists of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). SDF is a class of compounds that includes oligosaccharides and polysaccharides such as cellulose and hemicellulose, which may be associated with other constituents such as lignin, pectin, gums, and mucilage (de Falco et al., 2017). TDF can absorb water and swell up. This is because the carbohydrates contain free polar groups that interact with the hydrophilic chains in the matrix to create a gel (Moreira et al., 2010). CSM is a high-molecular-weight (0.8–2 × 106 Da) polysaccharide that was described by the Food and Agriculture Organisation of the United Nations (FAO) in 1996 as a potential source of polysaccharide gels because of its specific mucilaginous properties at low concentrations in aqueous solutions (Muñoz et al., 2012). Part of the fibre extrudes from the surface of the fruit as a transparent mucilaginous capsule after hydration and adheres firmly to the fruit (Kulczynski et al., 2019). de Falco et al. (2017) used scanning electron microscopy (SEM) to describe this process in chia seeds after being subjected to moisture for 5, 10, 30, and 60 minutes (Figure 2.6).

Figure 2.6 The chia seeds were observed by scanning electron microscopy (SEM) after 5, 10, 30 and 60 minutes of immersion (Salgado-Cruz et al., 2013).

By treating chia seeds with water, gums can be extracted from their dietary fibres and used as additives to control viscosity, stability, texture, and consistency in food systems (Bruna de Falco). Mucilaginous gels obtained from hydrated chia seeds can be used to stabilise food systems in a way that is comparable to traditional stabilisers due to their water-holding and absorptive capacity (Salgado-Cruz et al., 2013). The addition of BSG reduces melting rate, roughness, and coldness, resulting in creamy ice cream. In addition to this, the addition of BSG as a stabiliser (0.1% and 0.2%) in ice cream reduced the recrystallization rate (Feizi et al., 2021). This mucilage also reduced the melting rate and increased the fat particle size distribution, suggesting that CSMs may have a different structure by reducing the interfacial tension (Velazquez-Gutierrez et al., 2015). Generally, hydrophilic colloids have been shown to be a good choice for stabilising food systems due to their ability to hold water and absorb water. Hydrophilic colloids are widely used in different areas of the food industry due to their water-retention capacity(Velazquez-Gutierrez et al., 2015). They also have significant thickening and gelling properties, hysteresis control, emulsion stabilisation, etc.(Muñoz et al., 2012).

2.5 Ice cream

2.5.1 Composition of ice cream

According to Marshall et al. (2003), when commercial ice cream was first introduced to the consumer market, its base ingredients consisted of cream, liquid milk, sugar, eggs, and gelatin. As the ice cream trend evolved, condensed milk, non-fat milk powder (NDM), and butter also became popular ice cream ingredients. Since then, technological advancements and changes in marketing and economic conditions have encouraged the development of ingredients from multiple sources.

Dairy ingredients provide milkfat and non-milkfat solids (NFMS), which play an important role in ice cream (Feizi et al., 2021). Depending on the ice cream formulation, there are some dairy products that provide fat, such as butter. Some dairy products, such as skimmed milk powder and skimmed milk, only provide NFMS. Some dairy products, such as whole milk and cream, provide both fat and NFMS. Some dairy products provide only some of the NFMS. For example, whey powders provide only whey proteins, whereas casein powders provide only casein proteins (Clarke, 2004). Non-dairy products include sweeteners, stabilisers, emulsifiers, egg products, fruit, nuts, flavours, specialty products, and water. The components in the ingredients give the mixture a variety of functional properties (Campos et al., 2016).

Clarke (2004) classified the ice cream components into three types according to their quantity:

- Primary ingredients (present in ice cream in large quantities, amounting to more than 1% of the total weight), such as milk proteins, sugars, fats, and water.
- Minor ingredients (present in ice cream in small quantities, amounting to more than 1% of the total weight), such as emulsifiers, stabilisers, colours, and flavours.

Combination ingredients, which are not essential to the production of ice cream but are added to achieve a certain flavour or theme, such as chocolate, biscuits, cereals, sliced fruit, and nuts.

Because the ice cream making process involves whipping, most ice creams also contain a significant percentage of air, although they are not usually considered official constituents. The main ingredients of ice cream can be obtained from a variety of raw materials: for example, milk proteins, fats, and water can be supplied together in the form of milk or cream, or alternatively, they can be derived from different pure raw materials, i.e., skimmed milk powder, pure cream, and purified water (Marshall et al., 2003). The different sources do not have a significant effect on the final presentation of the ice cream, and the choice of each raw material depends largely on the manufacturers who are prepared to produce ice cream on a large scale, as the cost and availability of the different raw materials may have a large impact on the costs and profitability. Furthermore, the cost of transportation should be considered. For example, the cost of transporting powdered milk purchased from another region differs considerably from the cost of transporting fresh milk (Clarke, 2004). The typical ice cream ingredients are shown in Table 2.9.

Table 2.9 Component of typical ice cream (Homayouni et al., 2018).

Component	*Range (%)*
Milkfat	10–16
Milk solids-not-fat	9–12
Sucrose	9–12
Corn syrup solids	4–6
Stabilizers/Emulsifiers	0–0.5
Total solids	36–45
Water	55–64

Total solids are the sum of all ingredients, except water. In general, high total solids formulations produce high-quality ice cream, but too high a solids content may also negatively affect the texture and shape of the ice cream, so configuring and balancing the ice cream mix ensures each ingredient's proper functionality (Clarke, 2004). In addition to this, ingredients of superior quality, as well as intelligent processing, freezing, and hardening of the product, are essential to producing the highest quality ice cream. The view of Marshall et al. (2003) suggests that the selection of superior ingredients is undoubtedly the most important factor in the successful production of frozen desserts because only by using ingredients that have been carefully produced and processed can clean, fresh, and creamy flavours in ice cream be repeatedly ensured. The more times an ingredient has been handled or processed and the longer it has been stored, the less desirable its flavour and functionality in ice cream will be. However, Clarke (2004) argued that the formulation of ice cream can have a significant effect on the defining traits of ice cream.

Ice cream is a good source of essential amino acids from milk proteins, vitamins, and minerals (Clarke, 2004). Ice cream contains a high concentration of NMS and a high percentage of milk protein (34–36%). Proteins, as components of the cytoplasm of every living cell, play a very critical role in an animal's entire life cycle, and the milk proteins contained in ice creams have an excellent biological value because they contain all the essential amino acids required by the human body (Liu et al., 2023). It is well known that milk proteins not only have a complete amino acid profile and are an important source of tryptophan and lysine, but also that ingested milk proteins have a higher level of anabolism, which is generally 5–6% more complete than other proteins (Marshall et al., 2003).

In addition, its fat and sugar content make it a high-energy-density food. The primary focus has been on the nutritional and functional properties of fat in ice cream. It provides energy, essential fatty acids, fat-soluble vitamins, saturated and unsaturated fatty acids, sterols, and cholesterol (Narala et al., 2022). In addition to this, milk fat provides a unique flavour, carries some fat-soluble flavouring substances, lubricates the mouth, and influences the structure of frozen desserts, thus affecting their texture (Kurt & Atalar, 2018). Sugar, on the other hand, acts mainly to enhance the sweetness of ice cream to provide taste pleasure to the consumer, and secondly, sweeteners also play a role in the structure of ice cream, thus affecting properties such as mouthfeel and texture of the ice cream (Marshall et al., 2003).

Consumers are mainly concerned with the protein and fat in ice cream, but the mineral and vitamin content are easily overlooked (Clarke, 2004). Ice cream, like other dairy products, is one of the richest sources of calcium, and the amount of calcium and phosphorus contained in ice cream is almost entirely derived from skimmed milk solids, making it proportional to the amount of NMS. Like milk, ice cream is an important source of multivitamins, the content of which depends mainly on the content of milk solids and the weight of the portion. Fat-soluble vitamins A, D, E, and K are primarily found in fat and are generally not present in unfortified non-fat products (Marshall et al., 2003). The amount of water-soluble vitamins in ice cream is proportional to the concentration of NMS. Skim ice cream is expected to have the highest concentration, while high-fat ice cream has the lowest. Clarke (2004) agreed that milk fat is a good source of vitamin A, so manufacturers need to add vitamin A to the production of low-fat and skimmed ice creams, and if fruits and nuts are added to the ice cream, then they provide some of these vitamins as well. Ice cream is considered a good source of riboflavin, as well as providing large amounts of thiamine, pyridoxine, and pantothenic acid.

The high palatability of ice cream is one of the major factors that make it one of the most popular foods to provide high food value in such a tempting and non-repulsive way that it is even loved and spread by consumers in general. Most of the flavours of ice cream don't need to be chewed, and their smooth, velvety texture soothes the taste buds (Feizi et al., 2021). In summer, the cool temperature of the ice cream allows the consumer to get a sense of comfort. Another factor is that ice cream is generally more digestible, except for those who are lactose intolerant. So now more and more dairy-free ice creams are being developed to expand the audience of ice cream to lactose-intolerant and dairy allergy groups, and even vegan ice creams are being offered to vegetarians (Marshall et al., 2003).

As consumers become more aware of their food requirements, many ice creams have been developed to meet the needs of the consumer market, with a variety of special labels or for specific groups. For example, in 'natural' ice cream, artificial emulsifiers, colours, and flavours are avoided. There are low-fat or low-sugar ice creams and cholesterol-free ice creams for overweight people or people with chronic diseases. Newly developed ice creams rich in vitamins, calcium, or fibre, as well as ice creams containing polyunsaturated fats and prebiotic or probiotic ice creams, promote the growth of 'good' bacteria in the intestinal tract (Clarke, 2004).

2.5.2 Non-fat milk solid

Milk is made up of about 87% water, fat (4%), protein (3.5%), lactose (4.8%), and small amounts of inorganic salts, especially calcium and phosphate (0.29%), but this exact percentage depends on the breed of cow, in addition to the latitude, longitude, and season of the cow's habitat and the cow's diet, all of which can affect the percentage of each component in milk (Marshall et al., 2003). All of the components of milk, other than fat and water, are collectively referred to as non-fat milk solids (NFMS) because they are provided together in whole milk or skimmed milk powder (Clarke, 2004).

Milk proteins serve two important functions in ice cream. Firstly, they stabilise water-continuous emulsions and foams because they are surface-active. So, this has an important influence on the formation and stability of bubbles during ice cream aerating and freezing. Secondly, they contribute to the formation of recognisable characteristic flavours unique to dairy products (Clarke, 2004).

Milk contains two main types of proteins: casein (80%) and whey (20%). The difference between casein and whey proteins is mainly due to their solubility, with casein being insoluble at pH 4.6 (20 °C) and whey proteins being soluble. There are four main caseins: α_{s1}, α_{s2}, β and κ caseins. Most of the casein in milk is in the form of colloidal particles, called casein micelles, with a particle size of about 100 nm. Casein micelles scatter natural light, which is why skimmed milk is opaque even after the milk has separated the fat. Their natural function is to carry insoluble calcium phosphate, which is necessary for the growth of mammal pups (Clarke, 2004). Caseins are relatively small proteins. They are highly surface active because one end of the molecule is composed primarily of hydrophilic amino acids (e.g., serine and glutamic acid) and the other end is composed primarily of hydrophobic amino acids (e.g., leucine, valine, and phenylalanine). Casein is thermally stable but may be denatured by excessive temperatures, leading to aggregation and precipitation (Bahramparvar & Mazaheri Tehrani, 2011). There are also four types of whey proteins: lactoglobulin, lactalbumin, albumen, and immunoglobulins. These are globular proteins that are also surface-active. They are more sensitive to heat than casein and lose surface activity upon thermal denaturation (Clarke, 2004).

Milk also contains a third type of protein: various enzymes. These enzymes do not provide nutritional benefits but simply serve as proteins that can catalyse specific chemical reactions (Clarke, 2004).

2.5.3 Fat

Fat is often considered the main ingredient in ice cream production, determining the overall physical and organoleptic properties of the ice cream, and performing a variety of functional roles (Liu et al., 2023). Milk fats naturally provide desirable flavours, act as flavour carriers, and provide a smooth texture to the product. Milk fat also has nutritional value, providing some fatty acids and energy, as well as acting as a carrier for some fat-soluble vitamins. Triacylglycerols make up most of milk fat, but there are also some free fatty acids, phospholipids, and small amounts of mono- and diglycerides (Marshall et al., 2003).

In addition to this, the most important contribution of milk fats to the properties of ice cream is based on their interactions during processing (especially in combination with emulsifiers), such as the coalescence of fat globules and the adsorption of air bubbles. This has a significant impact on the structure, formation, appearance, texture, and melting behaviour of ice cream (Akalın et al., 2007). Due to their low thermal conductivity, milk fats can influence the growth of ice crystals and optimise the melting behaviour of ice cream through their higher melting temperatures. However, since fats are mixtures of triglycerides, they do not have a single melting point but will actually melt over a range of temperatures. The fatty acid content and melting point of milk fats are shown in Table 2.10. Fatty acid chain length and degree of unsaturation affect the melting curve: short-chain and highly unsaturated fats melt at low temperatures, whereas long-chain and highly saturated fatty acids melt at higher temperatures (Xiao, 2020).

Table 2.10 The fatty acid content and melting point of milk fats (Marshall et al., 2003).

Fatty Acid	% of Total Fatty Acid Content	Melting Point °C
butyric	3.0 – 4.5	-7.9
caproic	1.3 – 2.2	-1.5
caprylic	0.8 – 2.5	16.5
capric	1.8 – 3.8	31.4
lauric	2.0 – 5.0	43.6
myristic	7.0 – 11.0	53.8
palmitic	25.0 – 29.0	62.6
stearic	3.0 – 7.0	69.3
oleic	30.0 – 40.0	14.0
linoleic	2.0 – 3.0	-5.0
linolenic	< 1.0	-5.0
arachidonic	< 1.0	-49.5

Good-quality ice cream can only be made with fats that have the right melting characteristics. Fats that melt at high temperatures give ice cream a waxy texture. However, due to the structural properties of unsaturated fats, it is difficult to produce stable foam from fats melted at low temperatures (Xiao, 2020). Creamy fats have the correct melting curve to give ice cream good resistance to melting and also provide a smooth, creamy texture and dairy flavour (Feizi et al., 2021).

2.5.4 Sweetener

The main purpose of sweeteners is to enhance the sensory experience of a product by sweetening it with a pleasant, creamy flavour. Insufficient sweetness produces a bland taste; too much sweetness can mask the desired flavour and be unpleasant. Total sweetness in terms of sucrose can range from 12 to 20 percent, with 14 to 16 percent usually optimal (Marshall et al., 2003). Sweeteners increase the viscosity and total solids (TS) of the mix, which gives them two main functions in ice cream, the first being to sweeten it and to control the amount of ice in the ice cream system and therefore the softness of the ice cream (the higher the ice content, the harder the ice cream). By varying the amount and type of sugar, ice cream manufacturers can obtain a softer ice cream with an ice content of less than 45%. Secondly, sugar can affect the texture of the ice cream in another way, as the amount of sweetener has a direct effect on the viscosity of the matrix (Clarke, 2004). As the molecular weight of the sugar increases, the viscosity of the matrix also increases. Increasing the viscosity of the ice cream will result in a higher overrun and softer ice cream. At the same time, too high a viscosity would have a detrimental effect on the ice cream. For example, a high-viscosity matrix tends to produce an ice cream texture that is stickier and harder to scoop (Marshall et al., 2003).

Sucrose is the most commonly used sugar in ice cream. It is a disaccharide, consisting of dextrose molecules linked to fructose molecules. It is derived from sugar cane grown in tropical climates or sugar beets grown in temperate climates. When refined, both sources yield the same pure crystalline material(Marshall et al., 2003). Sucrose can be hydrolysed by heating with acid or using invertase enzymes to obtain a mixture of glucose and fructose in equal parts, called invertase, which is occasionally used as an ice cream ingredient. In recent years, mixtures of sucrose and corn syrup have been increasingly used in ice cream formulation, usually in proportions of 9–12% and 4–6%, respectively (Xiao, 2020).

Finally, it is worth noting that in recent years, low-sugar products have begun to capture a larger share of the ice cream market. For low-sugar products, sugar alcohols are frequently chosen. These non-nutritive sweeteners provide low calories and are popular with diabetics and dieters (Xiao, 2020). Sugar alcohols are formed when glucose reacts with hydrogen in the presence of a catalyst, such as sorbitol from glucose, lactitol from lactose, and mannitol from mannose. They are slightly less sweet than sucrose. They are not fully digestible by the human digestive system, so their calorific value is about half that of sugar (Clarke, 2004). However, while sugar alcohols themselves can provide a good freezing point reduction and an effective sweet flavour, they are associated with a number of digestive problems, and the potential hazards of these sugar substitutes for humans have been the subject of much controversy in recent years, so the amount of these materials used in formulations has been somewhat limited (Marshall et al., 2003).

2.5.5 Stabiliser

Stabilisers are a group of water-soluble or water-dispersible biopolymers used in small quantities (usually less than 0.5% added) in ice creams, fruit juice sorbets, water ice, and other food products(Marshall et al., 2003). Hydrocolloids are a type of large, high-molecular-weight molecules that have a lot of hydroxyl groups in their structure (Xiao, 2020). This makes them hydrophilic, which means they like water and can stick together well in it. Most stabilisers are polysaccharides of plant origin, such as alginate and carrageenan gums (from seaweed), acacia bean and guar gums (from tree seeds), pectin (from fruits), and sodium carboxymethyl cellulose (from cotton). Xanthan gum (a bacterial polysaccharide) and gelatin (a polypeptide of animal origin) are also sometimes used. These biopolymers are polydisperse and multimolecular because their structure varies with source and environmental conditions. From a nutritional point of view, stabilisers are a source of soluble fibre. Although they come from natural sources, they are considered food additives under European law and therefore have an associated E-number (Feizi et al., 2021; Marshall et al., 2003).

Stabilisers are straight or branched polymers containing hydroxyl groups that form hydrogen bonds with water molecules. Typically, they contain 103 monomer units and have a molecular weight of 105–106. Because they are so large, stabilisers do not dissolve in water as easily as small molecules(Marshall et al., 2003). Some stabilisers require high temperatures or shear forces to fully hydrate. When dissolved, they produce highly viscous solutions at low concentrations. Some stabilisers in solution can form gels when heated and/or cooled or when cations are added. Other solutions have complex solution properties such as shear-thinning behaviour or exceptionally high viscosity (Kasapoglu et al., 2023).

Stabilisers have a variety of beneficial effects on ice cream manufacture, storage, and consumption. Some of these are non-specific effects. For example, they are achieved by increasing the viscosity of the matrix phase, independent of the type of stabiliser used. The stabiliser may:

- Produce a smooth texture when consumed.
- Increase mixing viscosity
- Enhance the resistance of ice cream to melting during temperature fluctuations (such as heat shock) and reduce the rate of melting.
- Prevent ice cream from shrinking in volume and slow moisture migration during storage.
- Cover for the sensation of ice crystals in the mouth when eating.
- Facilitates air incorporation and helps to produce a stable foam.
- Stabilises the mixture to prevent layering by whey shedding.
- Help to suspend flavour and colour particles (Clarke, 2004).

Some stabilisers also have specific effects due to their special properties. For example, some stabilisers can retard the growth of ice crystals during storage. Finally, the interaction of two stabilisers results in synergistic effects, such as a more significant viscosity rise compared to each component separately or the formation of a gel when the individual components cannot gel on their own (Marshall et al., 2003). Many of these functions are attributed to the improved viscosity of the unfrozen phase in ice cream. The stabiliser must also have a pure neutral flavour without off-flavours that would combine with the flavour of the ice cream and bring about a melting that contributes to an acceptable melting of the ice cream and provides a desirable mouthfeel at the time of consumption. Limitations to the use of stabilisers include high mix viscosity, creating a heavy, cloying texture, and undesirable melting characteristics. Although stabilisers increase mix viscosity, they have little effect on freezing point (Clarke, 2004). However, Marshall et al. (2003) reported that stabilisers in ice cream limit the rate of ice crystal growth during recrystallization. The most important factor to consider during ice cream storage is ice recrystallization, which is caused by temperature fluctuations during the processing, storage, delivery, or consumption stages. As the temperature changes, some ice crystals may melt and deposit on the existing ice crystals around them. The most important function of stabilisers in ice cream is to provide its smooth properties by controlling the recrystallization process of the ice (Xiao, 2020). The mechanism by which stabilisers limit the recrystallisation of ice is still unclear. One possible suggestion is that as the viscosity of the ice cream mixture and the unfrozen phase increase, the fully swollen stabiliser molecules will adsorb a large number of water molecules, transforming the water into a bound state. These bound water molecules will be difficult to diffuse and participate in the recrystallisation process of the ice (Feizi et al., 2021).

The most common stabilisers are polysaccharide materials such as alginate, carrageenan, guar gum, acacia bean gum, and xanthan gum. Of these, acacia bean gum and guar gum are almost universally used in the ice cream industry, and combinations of these two materials are generally considered to be the most effective in retarding ice recrystallisation. However, they have certain drawbacks, primarily due to their insolubility with the proteins in the mix. Due to solubility competition between the stabilisers and the proteins, some proteins, especially casein micelles, undergo phase separation, which results in an unstable mix during storage and the release of clear serum during melting, thus producing negative whey shedding(Xiao, 2020). Secondly, the safety of these stabilisers has not been tested in humans, so there are food safety risks (Marshall et al., 2003). In addition to this, the concept of clean labelling is being promoted by more and more consumers as they become more aware. Additives with E numbers as well as chemical and industrial names should be avoided (Feizi et al., 2021).

2.5.6 Emulsifiers

An emulsifier is a substance that enables two liquids that do not mix naturally (usually oil and water) to form a stable suspension (Marshall et al., 2003). Generally, two immiscible phases exist in two separate layers, and the main function of an emulsifier is to disperse the two immiscible phases evenly. In nature, the two simplest forms of emulsions are oil-in-water (O/W) emulsions and water-in-oil (W/O) emulsions (Xiao, 2020). Emulsifiers are amphiphilic molecules with both hydrophobic and hydrophilic portions. Dairy systems contain a variety of natural emulsifiers, such as casein micelles, whey proteins, and phospholipids, which are involved in the stabilisation of both fat globules and air bubbles (Wang et al., 2023). As a result, traditional emulsifiers are less necessary to emulsify the fat in ice cream. However, it is well known that fats in ice cream emulsions are partially destabilised during freezing by a mechanism known as partial coalescence, which improves the quality of the ice cream (Xiao, 2020). The mechanism of action of emulsifiers to promote fat destabilisation can be summarised as follows: Emulsifiers reduce the interfacial tension of fat/water in the mix, which removes the proteins from the surface of the fat globules. At the same time, it reduces the stabilisation of the globules, causing them to be destabilised during the whipping and partially coalesce during the whipping and freezing processes. This causes the frozen product to form fat structures, which have a significant impact on texture and thawing characteristics (Marshall et al., 2003).

Emulsifiers have been used to produce ice cream mixes for many years. Emulsifiers are usually mixed with stabilisers, but emulsifiers and stabilisers have separate functions in the structure and composition of ice cream. They are used to:

- Promote fat nucleation during the ageing process, thus saving time in the ageing of the ice cream.
- Improve the whipping capacity of the mix due to their action at the air interface, thus reducing the size of the air bubbles mixed into the ice cream matrix and providing an even distribution of air in the ice cream.
- Enhance fat instability and promote partial coalescence of fats to increase the hardness of the ice cream to meet specific production requirements such as moulding, fancy extrusion, and sandwich manufacture.
- Increase resistance to shrinkage and thermal shock during storage due to the combined effect of the above two factors.
- Increase resistance to roughness and ice crystal texture due to partial coalescence of fats, more air bubbles, and thinner lamellar structures between neighbouring bubbles reduce the size and retard the growth of air bubbles, resulting in a smooth texture of the finished product (Clarke, 2004).

Modifying the microstructure of ice cream through the addition of emulsifiers is one of the main structural ways to change the texture of ice cream. To achieve the manufacturer's desired range of partial coalescence levels, commercial emulsifiers must be used for conditioning. Partial coalescence of fat globules is usually caused by shear forces, and the primary sign of this phenomenon is the rupture of the fat globule membrane (Marshall et al., 2003). The membrane formed by the adsorption of proteins during homogenization gives the fat globules a high degree of stability during freezing, to the extent that partial coalescence is very likely to occur (Clarke, 2004). The addition of synthetic emulsifiers (e.g., monoglycerides) replaces the protein layer on the surface of the fat globules, making them fragile and susceptible to rupture and coalescence by collision with other droplets in the ice cream freezer's high shear conditions (Marshall et al., 2003). Partial coalescence optimises certain properties of ice cream, such as whipping quality, melting characteristics, dryness of texture, smoothness, and hardness (Xiao, 2020).

The types of emulsifiers typically used to displace proteins in commercial production are small molecule emulsifiers derived from lipids. The most commonly used emulsifier in ice cream production is mono/diglycerides (E471). Lipids are triglycerides (esters formed by glycerol with three fatty acid molecules), monoglycerides are esters formed by glycerol with one fatty acid molecule, and diglycerides are esters formed by glycerol with two fatty acid molecules. They are all surface-active because the glycerol end of the molecule is hydrophilic and the fatty acid end is hydrophobic (Xiao, 2020).

Mono/diglycerides are made by partially hydrolysing vegetable fats (soybean oil and palm oil). Animal fats based on emulsifiers are not commonly used because they are not suitable for vegetarian and some religious diets (Marshall et al., 2003). They usually contain 40–60% monoglycerides, as well as diglycerides and a small amount of triglycerides. For this reason, such products are often named MDG, a mixture of monoglycerides and diglycerides. In aqueous systems, they are difficult to disperse because they become very viscous and form gels. However, this may be helpful in some applications (Clarke, 2004). For example, gelling properties have been used to make very low-fat ice cream. Food emulsifiers may be in liquid, powder, or tablet form. The different physical states are largely determined by the properties of the attached fatty acids. Attached long-chain fatty acids and saturated fatty acids produce solid emulsifiers. They can be sprayed as powder and mixed with other ingredients to make ice cream powder (Marshall et al., 2003).

The traditional ice cream emulsifier in older formulation is egg yolk. This is because egg yolks also contain a number of ingredients with emulsifying properties, notably lecithin, which is commonly used in all-natural, premium, or homemade ice cream. However, like gelatine as a stabiliser, the use of eggs or egg yolks in modern formulations has been replaced by specific ingredients that provide greater functionality at a lower cost. The approximate composition of egg yolk by weight is 50% water, 16% protein, 9% lecithin, 23% other fats, 0.3% carbohydrates, and 1.7% minerals (Clarke, 2004). Egg yolks used in the production of ice cream are subjected to a pasteurisation process to eliminate the possible introduction of pathogenic bacteria. The amount of egg yolk solids is usually about 0.5–3 percent. High concentrations are used only in ultra-premium products to give ice cream an eggy flavour, which is considered beneficial for marketing purposes (Xiao, 2020).

2.6 Conclusion

The objective of this review is to provide a comprehensive understanding of the fundamental information regarding ice cream, hemp milk, and chia seed. This includes their chemical composition, structural and functional characteristics, health advantages, and prospective applications. Hemp milk exhibits similar characteristics as cow milk, including physicochemical features and nutrient composition. As a result, it might be used as a replacement for NFMS in ice cream. Hence, to develop an ice cream with hemp milk, it is essential to replace cow milk with hemp milk in the ice cream formulation. Additionally, it is important to identify the optimal amount of replacement that will result in the highest consumer acceptance score while also ensuring the stability of the physicochemical and sensory characteristics of the ice cream during its storage period. The inclusion of hemp milk and CSM in the product provides multiple bioactive components that offer health benefits, so this novel product has multiple possible applications. Additionally, these ingredients enable the product to be classified as a clean-label food. Nevertheless, the challenging parts of developing this ice cream formulation containing hemp milk consist of two main areas: i) determining the appropriate amount of hemp milk to replace cow milk and figuring out the resulting changes in physiochemical characteristics and sensory attributes; and ii) evaluating consumer feedback and acceptance for this novel product.

Based on the information collected through the literature review, we have determined that hemp milk could be successfully replaced by the NFMS component in normal ice cream. The use of CSM can serve as an acceptable replacement for commercial stabilisers such as guar gum, carrageenan, and locust bean gum. Several researchers reported the impact of varying levels of dietary fibre on ice creams containing plant-based milk ingredients, such as hemp milk and almond milk. Nevertheless, there is a lack of research regarding the impact of different hemp milk amounts on the characteristics of ice cream. Hence, the aim of this research is to develop a novel ice cream product that combines hemp milk and CSM while ensuring it has optimal physicochemical and sensory characteristics, as well as an appropriate level of storage durability.

Chapter 3 Materials and Methods

3.1 Introduction

This chapter describes the materials and methods used in the experiment. The study was divided into three phases. Phase one describes the extraction and dehydration of chia seed mucilage used to prepare ice cream. The water activity of the dried chia seed mucilage was measured to standardise the dried material. Hemp seed milk added to the ice cream mix was prepared in the laboratory, and subsequent analysis was conducted to determine the content of fat, protein, and sugar. This is because the carbohydrate, fat, and protein composition of hemp seeds could be influenced by various factors, including origin, variety, pre-treatment method, storage conditions, and other relevant factors. Consequently, it is imperative to establish standardised protocols for measuring the constituents of hemp milk.

Phase two consisted of the evaluation and analysis of five ice cream samples with varying proportions of hemp and cow milk content. A focus group was the primary method employed for screening. Additionally, meltdown test, overrun and pH measurements were utilised as supplementary criteria in the screening process.

Phase three comprised a comprehensive analysis of the most promising ice cream samples, which were selected based on the feedback obtained from the focus groups. The physical characteristics of ice cream were measured, including hardness, fat particle distribution, viscosity, colour, meltdown, and overrun. A proximate analysis of ice cream was conducted to determine the fat, protein, ash, carbohydrate, dietary fibre, and calorie content of the ice cream. Due to the extensive involvement of sensory participants in the consumer sensory evaluation of ice cream, stringent measures were taken to assure the food safety of this product. Therefore, microbial analyses were conducted on the ice cream samples used for consumer sensory evaluation, including coliforms and total plate count. Also, testing of ice cream samples for *Listeria monocytogenes* was necessary because the ice cream samples were stored refrigerated.

The phase four consisted of analysing the storage stability of the most promising ice cream samples, which were stored in a freezer at -19 °C during the entire test period. The samples were tested for pH, colour, hardness, and sensory attributes (colour, aroma, texture, taste, mouthfeel, and sweetness) on days 1, 7, 14, and 21, respectively, and analysed to determine the storage stability of the best formulation ice cream sample.

3.2 Materials

Chia seeds (Ceres, New Zealand) for mucilage preparation were obtained from a local super market. Hemp seeds (Green Brother Ltd., New Zealand) and sunflower lecithin (Symbiota Ltd., New Zealand) for hemp milk preparation were obtained from an online store. Skim milk (Meadow Fresh, New Zealand), fresh cream (Meadow Fresh, New Zealand) , and sugar (Chelsea, New Zealand) for the ice cream preparation were obtained from a local super market. Emulsifier (MDG 0069) (Hawkins Watts Ltd., New Zealand), sodium chloride (Baxter, New Zealand), and calcium phosphate (Eisen-Golden Laboratories, New Zealand) was obtained from the Massey University laboratory.

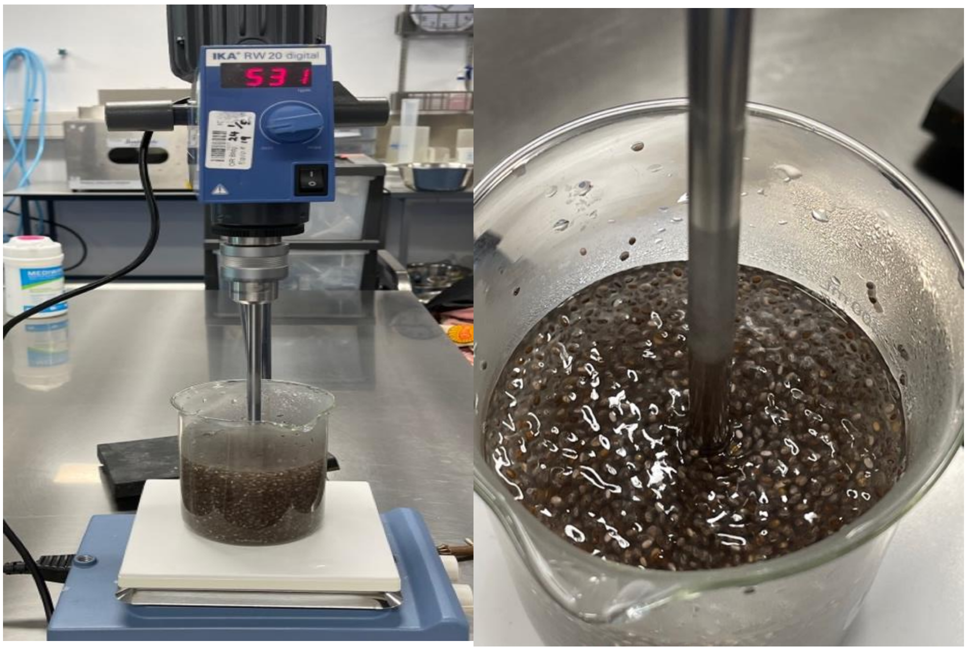
3.3 Phase 1: Preparation of chia seed mucilage and hemp seed milk

3.3.1 Preparation of chia seed mucilage

The preparation of chia seed mucilage involved extracting the mucilage from the seeds, drying, and storing it, and determining its water activity before incorporation into the ice cream samples. Details are provided below.

3.3.1.1 Extraction of chia seed mucilage

The methodology for extracting mucilage from chia seeds was based on the conclusions of Campos et al. (2016), with several adjustments included in this study. Chia seeds were performed to a soaking process in hot distilled water at a temperature of 80 °C, using a ratio of 1:20 (w/w). The purpose of this process was to promote the absorption of water by the mucilage present in the outer layer of the seeds. To accelerate the advancement of this process, the mixture was performed stirring at a rate of 550 rpm for 20 minutes, using an overhead stirrer (RW20.n, Labortechnik, Malaysia). Subsequently, in order to remove the mucilage layer adhered to the swelled seeds, the stirring speed was increased to 1680 rpm, and the stirring process persisted for 2 hours. After that, the mixture was performed to centrifugation using a centrifuge (6e16 KS, Sigma, Germany) at 12,800g, 20°C for 40 min to separate mucilage, and this centrifugation step was employed to facilitate the separation of mucilage, seeds, and water components. The process used to extract chia seeds mucilage was shown in Figure 3.1.

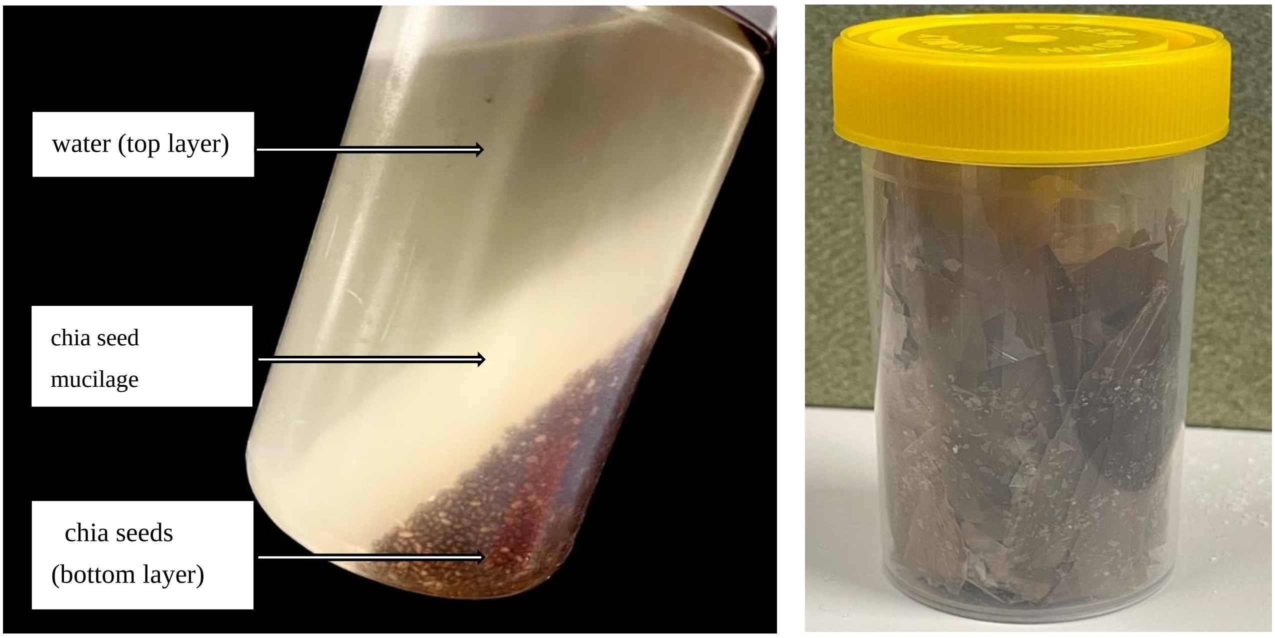


(a) (b)

Figure 3.1 Mixing process (a) and chia seed mucilage (b).

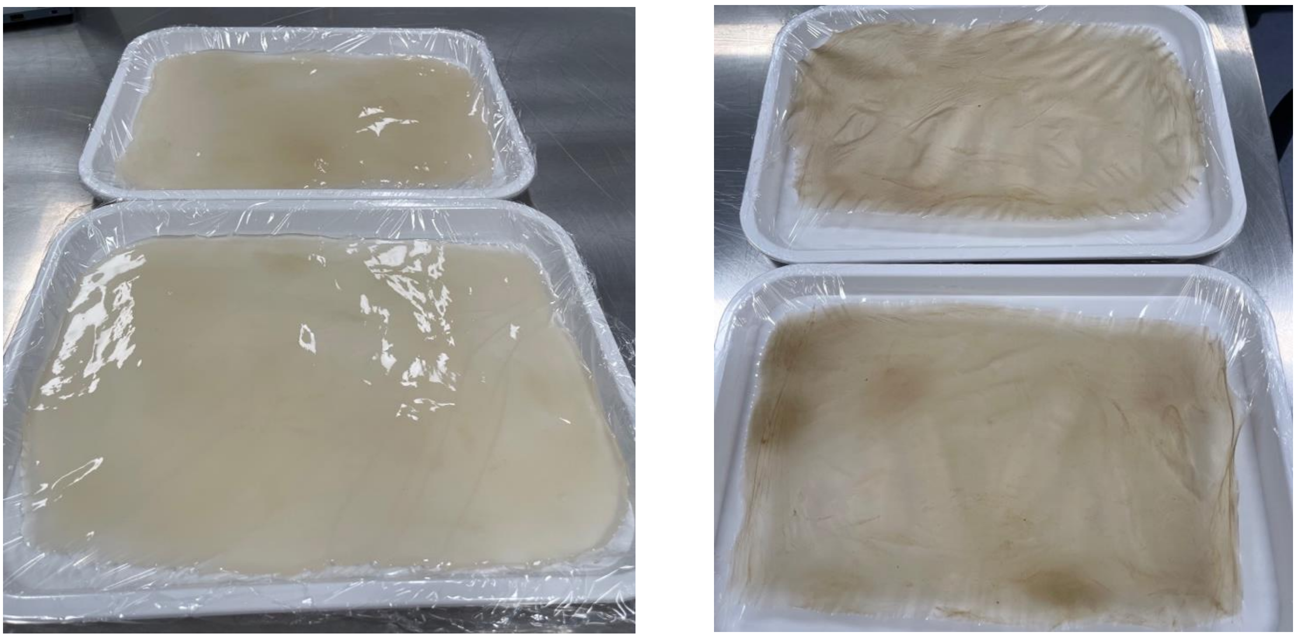
3.3.1.2 Drying and storage of chia seeds mucilage

Following the completion of the centrifugation procedure, the water (located in the upper layer) and chia seeds (located in the lower layer) were extracted. Subsequently, the chia seed mucilage (found in the middle layer) was carefully transferred onto a tray. The layered state of CSM after centrifugation and dried CSM chips was shown in Figure 3.2. The tray containing the mucilage was then performed to an air-dry process at an incubator with 50°C for 24 hours. The dried mucilage was placed in plastic containers and stored under optimal conditions of low temperature and low humidity until it was required. The comparison of chia seed mucilage before and after drying was shown in Figure 3.3.



(a) (b)

Figure 3.2 Centrifuged chia seed mucilage (a) and stored dried chia seed mucilage chips (b).



(a) (b)

Figure 3.3 wet chia seed mucilage (a) and dried chia seed mucilage flakes (b).

3.3.1.3 Determination of water activity of chia seeds mucilage

The CSM chips stored in an airtight container was transferred to a plastic container designed for the water activity meter (4TEV, AQUA LAB, USA), then the entire container loading CSM was inserted into the water activity meter. Upon closure and locking of the machine cover, the measurement was run automatedly by water activity meter.

3.3.1.4 Determination of moisture content of chia seeds mucilage

The moisture content of CSM chips was determined using the air oven method, as stated in AOAC Official Method 941.08. A 10 g portion of the CSM sample stored in an airtight container was transferred to an aluminium can. Subsequently, the sample was heated in a ventilated oven (32max, Moffat, Australia) at 100°C for 3.5 h. The container and sample were placed in a desiccator to cool to room temperature and quickly weighed to prevent any potential absorption of moisture.

3.3.2 Preparation of hemp milk

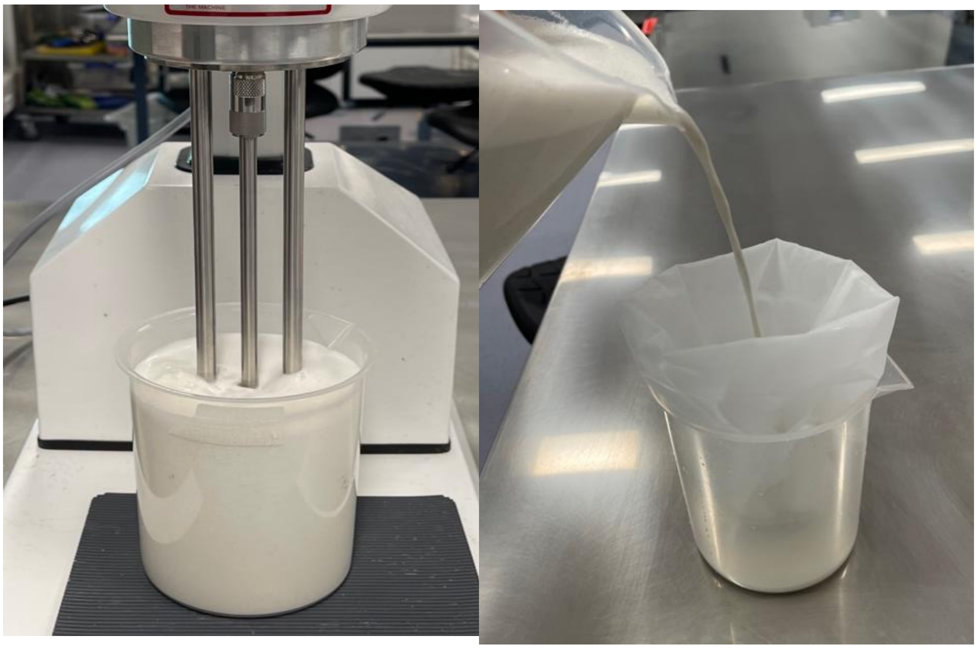
The preparation of hemp milk involved soaking, extracting the milk from the hemp seeds with a high-shear blender, homogenising, filtrating, and storing it. Before incorporating the hemp milk into the ice cream samples, its total solids, dissolved sugar, protein content, and fat content were determined. Details are provided below.

3.3.2.1 Extraction of hemp milk from whole hemp seeds

The hemp milk used in this experiment was produced in a laboratory setting, with a concentration of 7%, which Naylor (2021) determined as the optimum. Hulled hemp seeds were soaked in distilled water at 22°C for 10 h. To extract hemp milk, the rehydrated hemp seeds were combined with distilled water in a ratio of 7:100 (g/v). Additionally, sunflower lecithin, calcium phosphate, and salt were added to the formulation, as shown in Table 3.1. The mixture was placed in a high shear blander (PC-UM 1006, Proficook, Germany) operating at maximum speed for 5 minutes. After that, the homogenization process for mix was carried out using a Silverson Mixer (L5M-A, Chesham, England) at speed of 3000 rpm for 30 minutes. The mixed liquid was filtrated using a nut milk filter bag with a mesh size of 200, aiming to eliminate particulate matter and fibrous components. Ultimately, the filtrated hemp seed milk was carefully preserved in containers, poised for use in the production of ice cream. The process used to prepare hemp milk was shown in Figure 3.4 and the flow chart was shown in Figure 3.5 .

Table 3.1 Formulation of hemp seed milk preparation (Naylor, 2021).

Ingredient	**% of formulation**
Water	91.87
Hemp seeds	7.00
Sunflower lecithin	0.65
$Ca_3(PO_4)_2$	0.30
Salt	0.18



(a) (b)

Figure 3.4 Homogenizing of HSM (a), filtration of HSM (b)

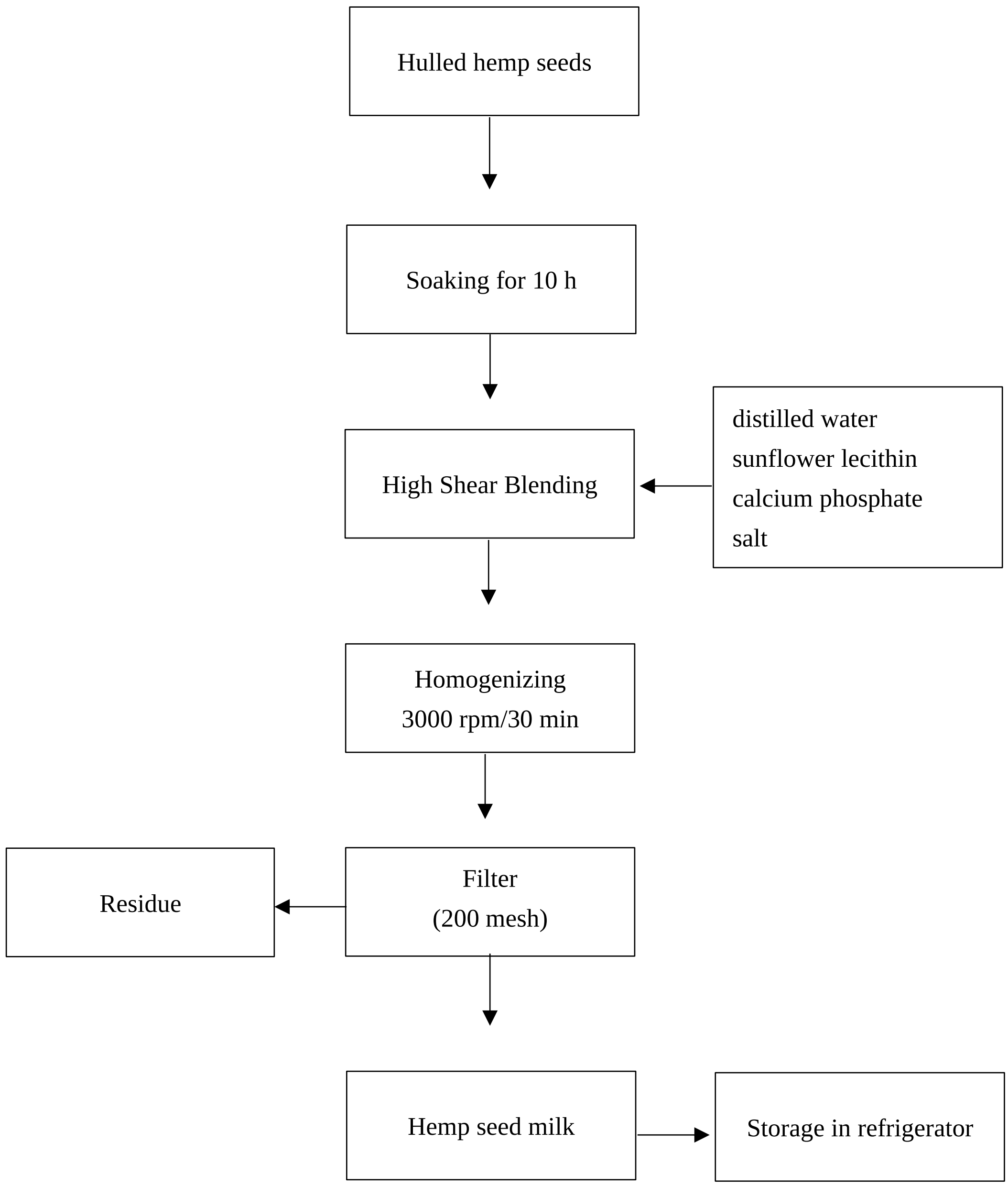


Figure 3.5 The flow chart of HSM preparation.

3.3.2.2 Determination of total solids of hemp milk

The analysis of total solids in hemp milk was conducted using the air oven method, as stated in the AOAC Official Method 941.08. 10 g portion of the well-mixed hemp milk sample was transferred to an aluminium round box. Subsequently, the sample was heated in a ventilated oven (32max, Moffat, Australia) at 100°C for 3.5 h. The container was placed for cooling within an actively controlled desiccator and quickly weighed to prevent any potential absorption of moisture.

3.3.2.3 Measurement of dissolved sugar of hemp milk

Three drops of hemp milk were taken out from a well-mixed sample using a dropper. These drops were afterwards placed in the central region of the test lens of the refractometer (PR-101, palette, ATAGO). The resulting data was carefully read and recorded.

3.3.2.4 Determination of fat content of hemp milk

The determination of the fat content in the samples was conducted using the Mojonnier fat extraction method, as stated in the AOAC official method 989.05. An amount of 10 g of hemp seed milk was placed into a Mojonnier flask. The flask then be supplemented with 1.5 mL of ammonium hydroxide (NH4OH) solution, ensuring full mixing. Following this, three drops of phenolphthalein indicator need to be added. To finalize the pre-treatment of the sample, 10 mL of 95% ethanol was added to the flask and mixed sufficiently. In order to separate the fat content from the samples, a mixture of 25 ml diethyl ether and 25 ml petroleum ether was added into the Mojonnier flask. Following the addition of the two solvents, the flask continued vigorous shaking for 1 min. The second extraction was conducted using a mixture of 5 mL of 95% ethanol, 15 mL of diethyl ether, and 15 mL of petroleum ether. Then, the third extraction was carried out using only 15 mL of diethyl ether and 15 mL of petroleum ether. The centrifugation process was conducted at 600 rpm for 30 s using a centrifuge (Sigma 6e16 KS centrifuge), Following complete separation of the aqueous phase and non-polar solvent phase, the solvent ether phase, which contained the extracted fat, was transferred into a ceramic dish that had been pre-weighed. Subsequently, the solvent was evaporated from the dish on a steam bath located within a fume hood. Following the evaporation of the solvent for a duration of 15 minutes, the fat sample was transferred to a dish and air-dried by oven at 100° ± 1°C for 5 minutes, ensuring complete removal of the solvent. After that, the dish was placed to a desiccator in order to facilitate the cooling process until it reached the room temperature. The quantity of extracted fat was measured which can be calculated the fat content of sample. The determination of fat content of hemp milk was performed by Massey Nutrition Lab (Palmerston North campus, New Zealand).

3.3.2.5 Determination of protein content of hemp milk

Protein content analysis was performed according to AOAC official method 968.06 using the Dumas method for nitrogen determination. An empty aluminium boat was weighed and recorded weight, and then 5 g hemp seed milk was placed in aluminium boat. After that, the aluminium boat loaded with samples was transfer to autosampler tray of Dumas nitrogen analyser(IL, Oak Brook, USA) to determine protein content. The determination of protein content of hemp milk was performed by Massey Nutrition Lab (Palmerston North campus, New Zealand).

3.4 Phase 2: Selection of the most promising ice cream formulation

3.4.1 Preparation of ice creams

The ratios of skim milk and hemp milk utilised in the preparation of ice cream samples for this study were determined based on the findings of Bekiroğlu and Özdemir (2020) and Bekiroglu et al. (2022). The five ratios of skim milk and hemp milk are 100%:0%, 75%:25%, 50%:50%, 25%:75%, 0%:100% in five ice cream samples (C, S1, S2, S3, S4). A commercial emulsifier (MDG 0069) was applied in ice cream mixes. The quantity of chia seeds mucilage employed as a stabilizer in the ice cream formulation was determined according to the research carried out by Feizi et al. (2021). The ice cream mixture was prepared following the methods described by Homayouni et al. (2018) and Marshall et al. (2003) with certain adjustments, and the ice cream content percentage of ingredient formulation was shown in Table.3.2 and ice cream formulation was shown in Table.3.3.

Table.3.2 Ice cream content percentage of ingredient formulation.

	Content percentage (%)				
Ingredient	**Control**	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
Cream[a]	20.00	20.00	20.00	20.00	20.00
Sucrose[b]	15.00	15.00	15.00	15.00	15.00
Chia mucilage[b]	0.25	0.25	0.25	0.25	0.25
MDG (0069)[b]	0.20	0.20	0.20	0.20	0.20
Skim milk[c]	64.55	48.41	32.8	16.14	0.00
hemp milk[c]	0.00	16.14	32.8	48.41	64.55
Total	100.00	100.00	100.00	100.00	100.00

[a]Marshall et al. (2003); [c]Bekiroğlu and Özdemir (2020) and Bekiroglu et al. (2022);
[b]Feizi et al. (2021).

Table 3.3 Formulation for preparation of ice cream samples.

Experiment	**Code**	**Hemp milk (g)**	**Skim milk (g)**	**Cream (g)**	**Sucrose (g)**	**CSM (g)**	**MDG (0069) (g)**
Control	C	None	322.8	100	75	1.25	1
Sample 1	S1	80.7	242.1	100	75	1.25	1
Sample 2	S2	161.4	161.4	100	75	1.25	1
Sample 3	S3	242.1	80.7	100	75	1.25	1
Sample 4	S4	322.8	None	100	75	1.25	1

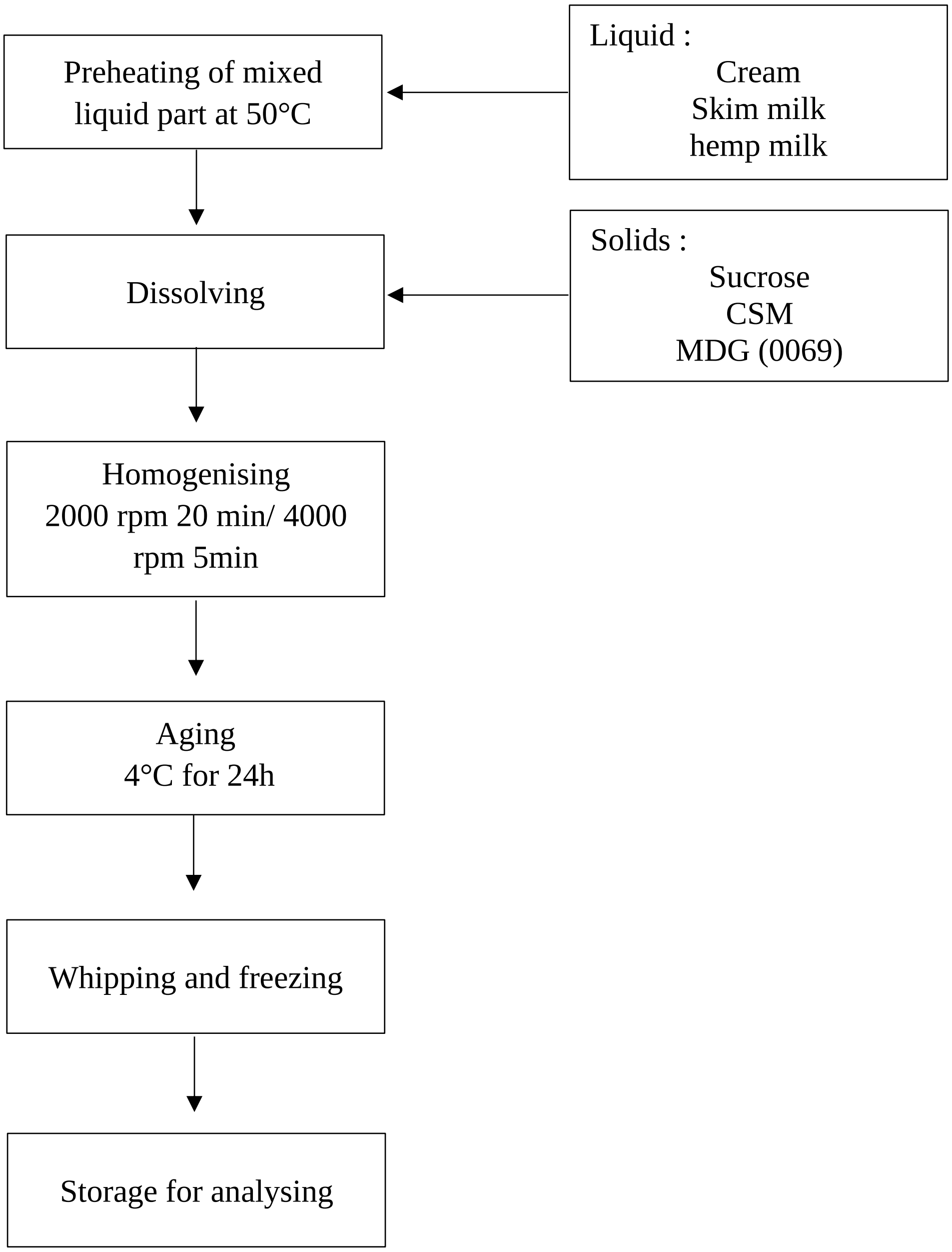


Figure 3.6 Flow chart of ice cream preparation

Initially, a combination of liquid parts including hemp seed milk, skim milk, and fresh cream were mixed. Subsequently, a container loaded mixture was transferred into a water bath (TC120 AUS, Grant Instruments Ltd, UK) and was heated at 50°C. Following this, the solid parts, including chia seed mucilage, MDG 0069, and sugar, were weighed and added to the mixture, and the entire material was stirred continuously until all the solid parts were completely dissolved. After that, the mixture was firstly treated to low-speed homogenising at 2000 rpm for 20 min, using a Silverson mixer (L4RT, Chesham, England), followed by an increase in speed to 4000 rpm for 20 minutes to achieve homogenisation. The ice cream mixture was cooled and aged overnight at 4°C. After aging, the ice cream mixture was frozen and whipped in an ice cream machine (MagiMix, Gelato Expert, Italy), and then was hard at -20°C for 24 hours. The process used to prepare ice cream was shown in Figure 3.5.

3.4.2 Measurement of pH of ice cream mix

The pH value of the ice cream mixture was measured using a pH meter (3540 pH meter, Jenway, UK). 20g of five ice cream samples were completely melted at 22°C. The electrode of the pH meter was first calibrated in the standard buffer solution, and then the electrode was inserted into the sample for one minute to measured pH value. When data from meter has stabilised, it was recorded. (Nooshkam et al., 2023)

3.4.3 Determination of overrun of ice cream

The measurement of overrun was done according to the procedure used by Liu et al. (2023). The aged ice cream mixture was placed into a container and weighed to get the m_{mix}. After the freezing process was completed, the frozen and whipped ice cream was placed into the same container and weighed to obtain the $m_{ice\ cream}$. The overrun of ice cream was calculated by comparing the weight using the Equation (3):

$$\text{Overrun\%} = \frac{m_{mix} - m_{ice\ cream}}{m_{ice\ cream}} \quad \text{---------------------------- Equation 1}$$

where m_{mix} is the mass of ice cream mix (g) and $m_{ice\ cream}$ is the mass of ice cream (g).

3.4.4 Determination of melt rate of ice cream

This experimental procedure followed the methodology utilised by Xiao (2020). The ice cream cubes were removed from circular containers and were placed on a steel net in order to determine the meltdown rate of the ice cream. The shape of ice cream was composed of a top diameter of 55mm, a bottom diameter of 45mm, and a height of 35mm. Besides, the net consisted of stainless steel material with a mesh size of 1 cm, and the net was set up at a height of 20 cm above the desktop. Plastic containers were placed under the ice cream cubes, to collect any liquid from the melting process. To mitigate potential instrument mistakes, only one balance was used for the weighing process, and plastic containers were weighed accurately at 5-minute intervals, and the first dripping time of each sample was recorded. The test process was shown in Figure 3.6.

Figure 3.7 Setup of the meltdown test of ice cream at 22 °C.

3.4.5 Evaluation of ice cream by a focus group

The focus group meeting was conducted following the strategy and methodology described by Sathyanarayanan (2022) with the aim of obtaining the perspectives and attitudes of the participants (n = 10) on four different types of hemp seed milk ice cream. The participants who were in the age range of 18 to 35 recruited from the staff and students at Massey University. The participants were allocated four ice cream samples. They were given a brief introduction to the samples, without disclosing any specific details, in order to minimise any biases. The perspectives and opinions of participants were summarised to facilitate comprehension of the overall consensus regarding the sensory attributes of the samples.

This is because ice cream lovers could offer valuable insights, especially personal viewpoints and emotional responses, derived from their own experiences with consuming ice cream. The participants, of whom 80% were affiliated with Massey University and 33% with the University of Auckland, were requested to taste the ice cream and subsequently complete a questionnaire to obtain their perspectives and evaluations of the product. The focus group guide is provided in Appendix A1.

To ensure the gathering of accurate and authentic focus group data, meticulous and objective judgement was essential. Therefore, it was crucial to focus on the main purpose of the focus group, which was to explore topics thoroughly and comprehensively. In order to evaluate ice cream samples, a simple description was typically adequate. Unless a specific research object was required, providing an excessively thorough description would be neither efficient nor productive. Consequently, the initial stage in the analysis of the data from focus groups was the transcription of discussions that occurred during the focus group.

The responses provided by the participants were categorised according to the specific questions covered. The pertinent responses were organised into the same category, whereas the irrelevant responses were also grouped together. This strategy helped establish connections between the responses provided by the participants, leading the focus group participants to collectively reach an agreement regarding the essential sensory qualities of these samples.

Each sample was assigned a unique 3-digit code obtained from Random Number Generator (https://www.calculator.net/about-us.html) before being provided to participants. This procedure achieves the purpose of ensuring that participants were unaware of the details of the recipes. The 3-digit random codes and corresponding ice cream sample numbers are shown in Appendix A2.

To understand the perspectives of participants regarding the "likelihood of consumption" of the four ice cream samples, including whether they were likely, neutral, or unlikely to consume ice cream again, a 3D bar chart was then constructed utilising information from the focus group.

After collecting the entirety of the data, it was essential to summarise the consumer opinions regarding the four samples. This involves collecting descriptive statements and comments, both positive and negative, from the participants. These comments were grouped into a table, allowing for a distinction between general descriptions and personal quotes exhibited by the participants regarding the various ice cream samples.

To summarise the findings obtained from the focus groups into a comprehensive resource of descriptive comments for consumer sensory evaluations, another table was generated. This table comprised the extraction and summary of participants' comments relating to the sensory attributes of the four ice cream samples.

3.5 Phase 3: Overall characteristics of the best formulation of ice cream

3.5.1 Physical properties

3.5.1.1 Measurement of pH of ice cream mix

pH of ice cream mix was measured using the same method as described in Section 3.3.2 in Chapter 3, which was based on the methods reported by Nooshkam et al. (2023).

3.5.1.2 Measurement of colour of ice cream mix

The ice cream mixture was placed in a circular transparent plastic container. The colorimeter (CR-300, Konica Minolta, Japan) was calibrated with standard colour tile, and then the container loading the ice cream sample was positioned on the probe of the colorimeter for analysis. The entire probe was enclosed within a paper box in order to prevent the entry of light. the whiteness index (WI) of each sample was calculated as following Equation (4)

$$WI = 100 - \sqrt{(100 - L*)^2 + (a*)^2 + (b*)^2} \text{ ------------------------------ Equation 2}$$

3.5.1.3 Determination of density of ice cream mix

The density of the ice cream mix was determined by the study of Nooshkam et al. (2023), a known volume (20 mL) of the ice cream mix was weighed and then the density of the ice cream mix was calculated by the following Equation (5):

$$\text{Density (g/cm}^3) = \frac{m}{V} \text{ -- Equation 3}$$

where m and V are the mix weight and volume, respectively.

3.5.1.4 Determination of overrun of ice cream

The overrun of ice cream sample was determined using the same method as described in Section 3.4.3 in Chapter 3, which was based on the methods reported by Feizi et al. (2021)

3.5.1.5 Determination of meltdown of ice cream

The meltdown rate and first dripping time of the best formulation ice cream was determined using the same method as described in Section 3.3.4 in Chapter 3, which was based on the methods reported by Xiao (2020).

3.5.1.6 Measurement of texture of ice cream

The hardness and stickiness of the ice cream was analysed using a texture analyser (TA.XT.Plus Texture Analyzer, UK). A 10 mm stainless steel cylindrical probe with a penetration speed of 0.5 mm/s was employed for this analysis, and the pre-test speed and post-test speed were set to 5 mm/s and 10.0 mm/s, respectively, according to Feizi et al. (2021). Following the fit of the probe and connection of the load cell (50 kg), the force applied by the equipment was calibrated using a standard weight of 200g. The height between the sample and the probe was calibrated with a ruler. The insertion depth of the probe was set to 25mm. All measurements were performed at 20 °C, and samples were immediately tested during the experiment in order to minimise errors resulting from changes in the environment because they could potentially affect the texture of the ice cream. The data was recorded utilising the measurement of gravity (g) by the exponential program, and it would be subsequently converted to force (N) by the utilisation of equation (7). The parameters in the experiment were shown with the Table 3.5.

$$\text{Hardness}=\frac{\text{Hardness(g)}}{1000}\times 9.81$$ ------------------------ Equation 4

$$\text{Stickiness}=\frac{\text{Stickiness(g)}}{1000}\times 9.81$$ -------------------- Equation 5

Table 3.5 The parameters for texture analyser.

Parameters	**Value**
Probe	10 mm stainless steel cylindrical probe
Load cell	50 kg
T. A. sequence	Return to start
Original probe distance	75mm
Test mode	Hardness
Pre-test speed	5 mm/sec
Test speed	0.5 mm/sec
Post-test speed	10 mm/sec
Target mode	Distance
Distance	25 mm

3.5.1.7 Measurement of rheological measurements of ice cream mix

The viscosity of the aged ice cream mixture was determined using the method by Feizi et al. (2021) using a rheometer (TA, AR-550, UK) loaded with cone (angle 2°) steel geometry (diameter 60mm). The samples were placed on the lower plate of the rheometer, which was accompanied by a cooling water bath (FT200, Julabo, Germany) to maintain temperature (5°C). Following the addition of the sample to the lower plate of the rheometer using a dropper, it was equilibrated for 2 minutes, which made the sample mix well. After the pre-shear rate parameter ($1000s^{-1}$, with a duration of 10s) was set, an automated shearing process would be initiated before testing, with the objective of mitigating the effects of thixotropy. The shear rate in the viscosity test was kept within a predetermined range ($0.1–100s^{-1}$). Data would be obtained from the Rheology Advantage program (TA Instruments Ltd.) plotting viscosity as a function of shear rate on a logarithmic scale.

3.5.1.8 Measurement of particle size distribution of ice cream

The Mastersizer (3000, Malvern Panalytical, UK) was used to measure the fat particle distribution. The measurements were carried out on both the unfrozen ice cream mix and the melted ice cream separately. About 50 g of unfrozen ice cream mixture and 50 g of melted ice cream were placed in separate beakers and allowed to stand for 10 minutes at 5°C to eliminate some of the foam, and the samples were added with the drip irrigation reaching as far as possible below the liquid surface to avoid inhaling the foam. After the cleaning procedure was completed and the measurements indicated that a stable level had been reached, the sample was added drop by drop until the laser mask value fell within the specified range of 10% to 20%.After the measurement process had finished, the average diameter (d4,3) of the fat particles within the sample was obtained. The degree of fat agglomeration could be determined through a comparative analysis of the particle size distribution between the unfrozen ice cream mix and the melted ice cream.

3.5.2 Proximate analysis of ice cream

3.5.2.1 Protein content

The Protein content of ice cream was determined using the same method as described in Section 3.2.2.5 in Chapter 3, which was based on the standard methods (AOAC, 2000).

3.5.2.2 Fat content

The fat content of ice cream was determined using the same method as described in Section 3.2.2.4 in Chapter 3, which was based on the standard methods (AOAC, 2000).

3.5.2.3 Ash content

The ash content of ice cream samples was analysed using the AOAC official method 940.26. Five grams of ice cream samples was placed in a pre-weighed crucible. After the sample was charred on a Bunsen burner, the entire crucible was placed in a muffle furnace (A-550, Vulcan, USA) at 550°C for 4 hours until the sample was completely burned. Then the crucible was weighed after cooling to room temperature in a desiccator. The ash content was calculated as the percentage of ash in the sample.

3.5.2.4 Moisture content

The moisture content of the best formulation ice cream was determined using the same method as described in Section 3.2.2.2 in Chapter 3, which was based on the standard methods (AOAC, 2000).

3.5.2.4 Insoluble, soluble, and total dietary fibre content

The analysis of the insoluble, soluble, and total dietary fibre content in the best formulation ice cream samples was performed using the AOAC official method 991.43. In the case of products that contained high levels of sugar, fat, and protein, the sample was defatted three times using a 25mL/g test sample with petroleum ether before the measurement of dietary fibre. Subsequently, the sample was extracted using 85% ethanol at 10 mL/g in order to eliminate sugars. Following this, the sample was dried overnight at 40°C. A 1 ± 0.05 g test portion was weighed and transferred to a beaker and 40 mL of MES-TRIS buffer solution (pH 8.2) was also added into the beaker. 50 µL of thermostable α-amylase solution was added and then incubated in a boiling water bath for 15 min with low-speed stirring to remove carbohydrates. After that, 100 µL of protease solution was added to the beaker and incubated at 60 °C for 30 min to remove proteins. Following this step, 300 µL of amyloglucosidase solution was added and incubated at 60 °C for 30 min with stirring.

The above pre-treated digestion liquid was filtered through the crucible with a celite bed, and the residue was washed twice with 10 mL of 70°C distilled water. The filtrate was transferred to a beaker and used to measure the soluble dietary fibre content. The residue was then washed twice with 15 ml each of 78% ethanol, 95% ethanol and acetone. The precipitate was thoroughly dried and weighed to determine the amount of insoluble dietary fibre. 160 mL of 95% ethanol at 60°C of the filtrate described above was added to a beaker. The filtrate was stood at room temperature for one hour to allow the insoluble dietary fibre to settle. This was followed by filtration and drying. The weight of the precipitate was used to determine the amount of soluble dietary fibre. The total dietary fibre was calculated by summing insoluble dietary fibre and soluble dietary fibre.

3.5.2.5 Total carbohydrate content

The calculation of the total carbohydrate content of the samples was performed using the method of difference, according to (Nooshkam et al., 2023). The carbohydrate content of the ice cream samples was calculated by deducting the mass of water, protein, fat, and ash from the total mass.

3.5.2.6 Calculation of caloric value

The caloric value of ice cream was calculated based on the energy supply ratio of the three major nutrients and their mass. The calculation of Equation (8) was provided by Nooshkam et al. (2023).

$$\text{Calorie value (kcal/100 g)} = [(4\times\text{Protein}) + (4\times\text{Carbohydrate}) + (9\times\text{Fat})] \text{ ------- Equation 6}$$

3.5.3 Consumer sensory evaluation of ice cream

3.5.3.1 Sensory Evaluation for ice cream

This sensory evaluation was carried out in a purpose-built sensory laboratory in IC building at Massey University, and this sensory testing had been approved by the Massey University Ethics Committee with a low-risk approval number of 4000027703. A total of 35 participants aged 18–60 recruited from the Massey University campus participated in the consumer sensory evaluation of the ice cream. The questionnaire (Appendix B) was created on the questionnaire website (https://redjade.net/) and all data was collected electronically. Each participant was also asked to sign a paper consent form.

35 best formulation ice cream samples, each weighing 15g, were carefully packaged in transparent plastic cups labelled with 3-digit random codes. The participants were instructed to evaluate the product based on seven attributes (colour, aroma, texture, taste, mouthfeel, sweetness, and overall). The acceptability score for each attribute was determined using a 9-point hedonic scale. The scoring system utilised in this study varied from 1, indicating ('dislike very much'), to 9, indicating ('like

very much'). A score of 5 represented a neutral stance, indicating neither liking nor disliking the product.

3.5.3.2 Just-about-right (JAR) evaluation of ice cream

The JAR scale combines strength and acceptability to relate the perceived strength of a particular attribute to the theoretical optimum intensity for the participants. Participants chose from five options: 'much too weak', 'little too weak', 'just about right', 'little too strong', and 'much too strong' to indicate their opinion about the intensities of the attributes. The mean score of JAR and the corresponding overall liking score was used to calculate a penalty score to determine the drivers of liking for the sample.

3.5.4 Microbiological of ice cream

3.5.4.1 Total plate count of ice cream

The total plate count of ice cream was determined by aerobic plate count (APC) method according to AOAC official methods 996.23. 25g of ice cream samples were placed into the stomacher bag, and 225g of 0.1% w/v peptone water was added to it to dilute the sample to 10^{-1}. After the air was vented and the bag was sealed, the sample was homogenised by using a paddle blender (IUL Instruments, Spain) for 2 minutes. One ml of the 10^{-1} dilution sample was transferred to a sterilised glass bottle containing 9 ml of 0.1% w/v peptone water to dilute the sample to 10^{-2}, and then the glass bottle was shaken in the Vortex mixer (Velp Scientica, Italy) for 30 seconds.

One ml of the 10^{-2} and 10^{-1} dilution samples was transferred to a clean, sterile, labelled Petri dish to prepare 10^{-2} and 10^{-1} count plates, and 10 ml of the 10^{-1} dilution sample was transferred to three Petri dishes to prepare 10^{0} count plates. After that, 12–15 ml of count plate agar was added to each petri dish, and each plate was rotated and moved gently and immediately. The plates were then incubated at 35°C for 48 hours. All the above operations were performed near the Bunsen burner flame to reduce microbial contamination in the environment.

The plate with a colony forming unit (CFU) range of 25–250 was selected to count the number of bacterial colonies with a marker pen, and the following formula (9) was used to calculate the APC.

$$N = \frac{\sum c}{[(1\times n1)+(0.1\times n2)\times d]} \text{ ----------------------- Equation 7}$$

where N = number of colonies per ml or g of sample
$\sum c$ = sum of all colonies on all plates counted.
n_1 = number of plates in first dilution counted
n_2 = number of plates in second dilution counted
d = dilution from which the first counts were obtained

3.5.4.2 *Listeria monocytogenes* of ice cream

The Listeria monocytogenes in ice cream were determined by the FDA method according to FDA laboratory methods in Chapter 10: Detection of Listeria monocytogenes in Foods. 25g of the ice cream sample was placed in the stomacher bag, and 225g of Listeria Selective Enrichment Supplement (LSES) was added to dilute the sample. The digestion bag was sealed and shaken 10 times to fully mix the sample and LSES, and then it was incubated at 30°C for 48 h. After 48 hours of selection and enrichment, the sample was streaked onto PALCAM and Oxford agar plates by using an inoculation ring, and then these plates were incubated at 35°C for 48 h.

If colonies grow on PALCAM and Oxford agar plates, five suspicious strains should be streaked onto TSAYE plates, and then these plates were incubated at 30°C for 48 h.

Once colonies have grown on TSAYE plates, confirmatory experiments were required to determine the identity of the colonies.

3.5.4.3 Coliforms of ice cream

The Coliforms of ice cream were determined by the solid medium method according to FDA laboratory methods in Chapter 4: Enumeration of *Escherichia coli* and the coliform bacteria. 25g of ice cream sample was operated on by the same procedures described in Section 3.4.4.1. 12–15 ml of Violet Red Bile Agar (VRBA) was added into each Petri dish, and then each plate was rotated and moved gently and immediately. After the mixture of sample and agar solidifies, 5 ml of extra VRBA needs to be added to cover the mixture to prevent the growth and spread of colonies on the surface.

The plates were incubated at 35°C for 48 h. All the above operations are performed near the Bunsen burner flame to reduce microbial contamination in the environment.

If colonies with red surroundings and bile acid precipitation were grown on the VRBA plate, then 10 representative colonies were transferred to Brilliant Green Lactose Bile (BGLB) broth test tubes and incubated at 35°C. After that, gas production at 24 h and 48 h was examined. If the gas was positive and the BGLB showed film production, a Gram stain verification was required to ensure that the gas production was not caused by Gram-positive lactose-fermenting bacilli.

The number of coliforms per g or ml of sample could be calculated by multiplying the number of colonies by the percentage confirmed in the BGLB and then multiplying by the dilution factor.

3.6 Phase 4: Storage stability of the best formulation of ice cream at -19 °C for 21 days

3.6.1 Changes in pH of best formulation ice cream during storage at -19 °C for 21 days

The pH of the best formulation ice cream was determined on days 1, 7, 14 and 21. Determination process using the same method as described in Section 3.2.2 in Chapter 3.

3.6.2 Changes in colour of best formulation ice cream during storage at -19 °C for 21 days

The colour of the best formulation ice cream was determined on days 1, 7, 14 and 21. Determination process using the same method as described in Section 3.4.1.2 in Chapter 3.

3.6.3 Changes in hardness of best formulation ice cream during storage at -19 °C for 21 days

The hardness of the best formulation ice cream was determined on days 1, 7, 14 and 21. Determination process using the same method as described in Section 3.4.1.6 in Chapter 3.

3.6.4 Changes in sensory attributes of best formulation ice cream during storage at -19 °C for 21 days

The 20 best formulation ice cream samples (each weighing 15 g) were carefully packaged in clear plastic cups and stored together in a freezer at -19 °C. The ice cream samples were removed on days 1, 7, 14, and 21 for sensory evaluation and participants were asked to rate the performance of the product based on seven attributes (colour, aroma, texture, taste, sweetness, mouthfeel, and overall acceptability). The acceptability score for each attribute was determined using the 9 hedonic scale. The scoring system used in this study ranged from a score of 1 indicating a strong aversion (‘dislike very much’) to a score of 9 indicating a strong preference (‘like very much’). A score of 5 represents a neutral liking, indicating that the product is neither liked nor disliked (Appendix C).

3.7 Statistical analysis

All measurement or determination of samples described in the above were carried out from at least duplicate experiments, and data were analysed using Minitab version 21 statistical software for Windows Inc. (Minitab Inc., PE, USA). One-way analysis of variance (ANOVA) was used to analyse the mean results to identify any significant differences ($p < 0.05$) between the formulations. The data related to the property analysis was analysed using Microsoft Excel 2023.

Chapter 4 Results and Discussion

4.1 Phase 1 : Physiochemical properties of chia seed mucilage and hemp seed milk extracts

4.1.1 Moisture content of chia seeds mucilage

Recently, there has been a significant global rise in the number of individuals who prioritise food ingredient identification, with most customers now checking food product labels first while shopping (Galanakis, 2022a). Clean labelling claims aim to use natural alternatives for food additives and to specify defined materials on the label instead of utilising electronic numbering (Feizi et al., 2021). The preparation process in this experiment focused on preparing chia seed mucilage (CSM) obtained from seeds as a replacement for a commercial stabiliser for direct addition to ice cream and further usage in food applications.

It is crucial for identifying the water absorption characteristics of CSM in the food industry because of its significant economic potential and requirement for long-term storage (Moreira et al., 2010). Understanding the water absorption characteristics is also important to determining the correct water content during drying and for designing equipment used in airflow, storage, and transportation (Chavan et al., 2017). Standardising the amount of any material added to an ice cream formulation is crucial for the ice cream manufacturing industry(Clarke, 2004). This experiment tested and recorded the moisture of the CSM because water content is the main variable affecting its weight. In this experiment, the water activity of the CSM was 0.4881 ± 0.004. Although the results differed slightly from previous studies, it is important to note that when the moisture activity is below 0.6, most microorganisms are unable to survive and grow(Arnak & Tarakçi, 2021). Thus, the CSM utilised in this experiment was considered to be storage safe. The water content of CSM was 2.96% ± 0.05%, which closely matches the equilibrium moisture content forecasted in the water adsorption isotherms of CSM in the paper by Moreira et al. (2010). However, Campos et al. (2016) argues that water adsorption equilibrium equations are effective for forecasting the water adsorption characteristics of food products but offer limited insight into the interactions between water and food components. In addition, the water content of CSM determined by Bahramparvar and Mazaheri Tehrani (2011) was slightly lower than this experiment, possibly caused by the difference between air-drying and freeze-drying.

4.1.2 Physicochemical analysis of hemp milk

Several factors, including the pre-treatment of hemp seeds, the treatment methods, and the variety and origin of the seeds, affect the nutrient content in hemp milk (BeŞİR et al., 2022). The nutrient content of the hemp milk used in this experiment was measured in order to standardise the formulation. The physicochemical analysis results of hemp milk are presented in Table 4.1.

Table 4.1 Physicochemical analysis results of hemp milk

Component	Amount
Protein	1.8%
Fat	3.5%
Total fibre	0.2%
- Soluble fibre	0.2%
- Insoluble fibre	<0.1%
Total solids	9.4%
Dissolved sugar	4.43°Brix

According to the nutrition information label of skim milk from Meadow Fresh used in this experimental, hemp milk in this study has a protein content of 1.8%, which is slightly lower than skim milk (3%), a fat content of 3.5%, which is higher than skim milk (0.1%) and a total solids content of 9.4%. In previous research by BeŞİR et al. (2022), results comparable to this study were reported, with hemp milk containing 1.23% protein, 1.34% fat, and 7.15% total solids. The difference in results might result from the short duration of grinding and homogenization treatments. The protein content in this experiment might be mainly affected by the inherent properties of the hemp seed rather than the method used to prepare the hemp milk, because hemp milk is also reported many times for its low protein content (Leonard et al., 2020; Martin et al., 2023; Xu et al., 2021). However, hemp seed protein has a rich amino acid profile, having all nine essential amino acids necessary for humans, and its amino acid profile is typified by high quantities of arginine and glutamic acid (Pham, 2021).

Hemp seed has a higher fat content than cow's milk, and it also has a high percentage of mono-unsaturated and poly-unsaturated fatty acids (Tura et al., 2023). The sugar content of hemp milk was 4.43 °Brix, showing a similarity to the sugar content of cow's milk, which is 5°Brix. Only one critical review by Paul et al. (2020) has reported a carbohydrate range of 2.50–20 (g/100g) for hemp milk, which is consistent with the results of this study. The hemp milk in this experiment contained 0.8% total fibre, and no previous study on hemp milk fibre has been reported. Hemp milk has a higher fibre content compared to other plant milks such as soy milk (0.14 g/100g) and oat milk (0.13g/100g) (Paul et al., 2020).

4.2 Phase 2 : Selection of the most promising ice cream formulation

4.2.1 pH of ice cream mix

The pH of five ice cream mixes containing different levels of hemp milk were measured. Cow milk added to the ice cream formulations was replaced with hemp milk at different levels (C:0%; S1:25%; S2:50%; S3:75%; and S4:100%). The pH of the mixes increased with increased amount of hemp milk added (Figure 4.1). The pH of the formulations ranged from 6.68±0.03 to 6.94±0.03 ($p<0.05$).

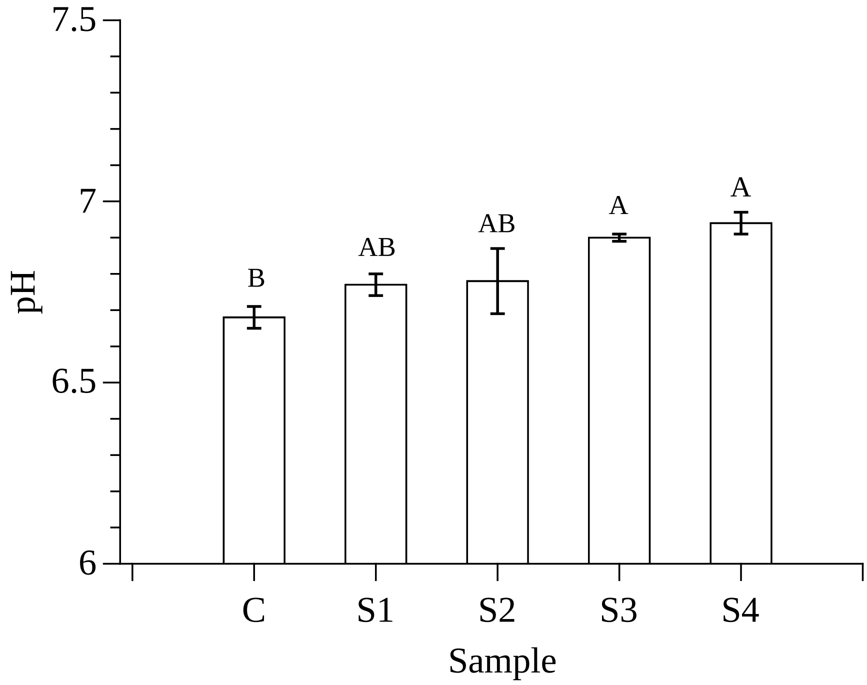


Figure 4.1 pH of five ice cream mixes

C = control; S1 = ice cream mix containing 25% hemp milk; S2 = ice cream mix containing 50% hemp milk; S3 = ice cream mix containing 75% hemp milk; S4 = ice cream mix containing 100%

hemp milk; Error bars mean ± standard deviations; Different letters on each bar indicate significant differences ($p<0.05$) ($n=3$).

Increase in pH of the ice cream mixes with added hemp milk can be attributed to its chemical and naturally existing alkali component (Xiao, 2020). Leahu et al. (2022) reported a correlation between the pH of ice cream and titratable acidity, independent of the protein and fat content of the system. The authors generated a matrix by analysing the measured quantities and variables of the ice cream. It was observed that the pH of the ice cream showed a closer relationship with titratable acidity, while the pH was more distant from protein and fat in the matrix. Naylor (2021) used similar concentrations of hemp milk in ice cream formulations as those used in this study, which resulted in a comparable pH range of 6.70 to 6.96. Nooshkam et al. (2023) used similar formulations as the control sample of this experiment to prepare ice cream mix and obtained pH ranging from 6.32 - 6.65, which is consistent with the results of this study.

Some other studies have shown that the effect of adding different types of plant milks on the pH of ice cream depends on the chemical composition of the plant milk contain (Leahu et al., 2022). The study by Wang et al. (2022) on ice cream with soy ingredients were similar to the pH range obtained from our research, which ranged between 6.8 and 6.9. However, some previous studies on plant-based milk ice cream reported a lower pH than cow milk ice cream. The pH of ice cream developed with walnut milk was 6.33, and the pH of ice cream with coconut oil by-products was 5.84. The probable reason for the lower pH was the presence of naturally occurring acidic compounds in the plant-based milks (Bekiroglu et al., 2022; Kasapoglu et al., 2023).

4.2.2 Overrun of ice cream

Overrun is an essential characteristic of ice cream since it indicates the volume of air pumped into the ice cream, which impacts on melting, texture, and other sensory properties (Wang et al., 2022). A low level of overrun leads to a hard ice cream texture and weak thermal shock resistance. Conversely, a high level of overrun not only reduces the saturation of colour but also produces a thin texture that negatively impacts on the sensory characteristics of the ice cream (Arslaner & Salik, 2022).

Results show that hemp milk added into ice cream affected the overrun. The addition of hemp milk into ice cream formulation significantly increased the overrun of the ice cream, which ranged from 25.04% ± 1.365% to 41.32% ± 1.17% ($p<0.05$). Furthermore, as the level of hemp milk increased, there was a distinct increase in overrun of ice cream (Figure 4.2)

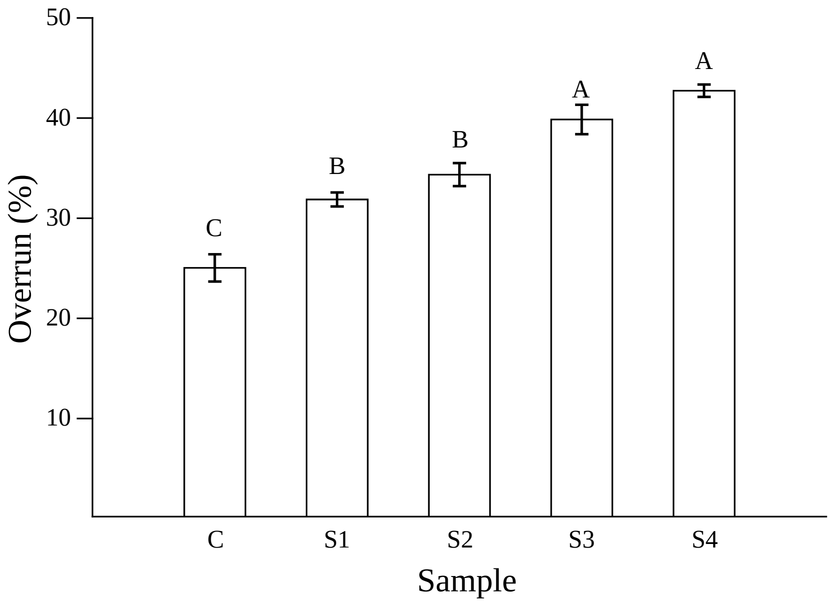


Figure 4.2 Overrun of five ice cream samples

C = control; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk; Error bars mean ± standard deviations; Different letters on each bar indicate significant differences ($p<0.05$) between the samples (n=3).

The main factor affecting overrun of ice cream is the fibre content in the plant-based milk (Narala et al., 2022). The fibre increases the viscosity of the ice cream, thereby enhancing its ability to incorporate and strengthen the structure of air bubbles (Clarke, 2005). Leahu et al. (2022) used almond milk and hemp milk in ice cream and found that ice cream samples containing the two plant-based milk showed higher overrun compared to ice cream containing only cow milk only. Bekiroglu et al. (2022) added walnut milk produced from both fresh and dried walnuts to the ice cream, resulting in a significantly higher overrun compared to ice cream produced using cow milk only. The overrun levels for ice cream with both forms of walnut milk were 48.13% and 49.83%, whereas the overrun for ice cream with cow milk was 19.47%, which showed a high similarity to our present results. In contrast, Bekiroğlu and Özdemir (2020) used buffalo milk and cow milk in the production of ice cream separately, the results showed no difference ($p>0.05$) in the overrun of each product. The use of inulin by Akbari et al. (2019) resulted in a significant increase in the overrun of ice cream. In addition to inulin, the addition of bacterial cellulose and maltodextrin to ice cream also significantly ($p<0.05$) improved the overrun of the ice cream (Xavier & Ramana, 2021).

Hemp seed is naturally high in fat and protein, both components can synergistically affect overrun (Leonard et al., 2020). Despite the slightly lower protein level of hemp milk (1.8%) compared to cow's milk (3%) used in our experiment, all the samples containing hemp milk showed a significantly higher overrun than the control ($p<0.05$). This may be attributed to the structural and technological properties of plant proteins that lead to their superior emulsification and foaming properties compared to whey proteins (Akalın et al., 2007). The natural foaming and emulsifying properties of the proteins may improve the overrun of the ice cream (Clarke, 2005). Pontonio et al. (2022) added pea protein and soy protein to the ice creams separately, the overrun of ice cream containing pea protein or soy isolate protein was both higher than that of ice creams containing cow milk only. The fat content (3.5%) of hemp milk used in the present experiment was higher than that of cow milk (3%), which can contribute to the increased overrun of the hemp milk ice cream in comparison to the control. During the mixing and whipping process of ice cream, the fat droplets are stabilised by absorbing to the surface of the bubbles through the Pickering-type mechanism (Leonard et al., 2020). During the Pickering mechanism, a portion of the fat undergoes partial coalescence which prevents the retention of a stable pore structure in the ice cream. Previous studies reported a positive correlation between the fat content and the overrun of ice cream. Xiao (2020) added non-homogenised cream to ice cream, which increased the overrun of the ice cream. When soybean oil emulsion (Wang et al., 2022) and coconut oil in water (Kasapoglu et al., 2023) were added to ice cream formulations, the overrun of both ice creams increased ($p<0.05$). This may be attributed to the presence of long-chain fatty acids in the saturated fats, which exhibit surface activity similar to that of emulsifiers like monoglycerides (Akalın et al., 2007).

4.2.3 Meltdown of ice cream

The melting properties of ice cream are closely associated with the release of taste and flavour, resulting in a significant factor that influences consumer impressions (Bekiroğlu &

Özdemir, 2020).

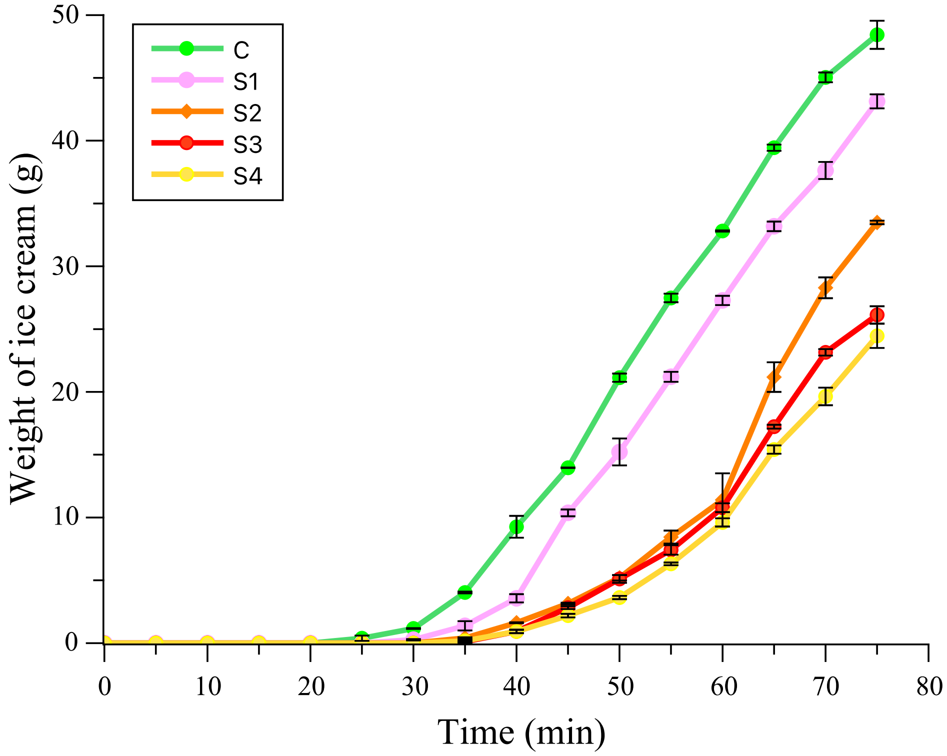


Figure 4.3. The melting curves of five ice cream sample

C = control; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk; Error bars means ± standard deviations (n=2).

Figure 4.3 shows the correlation between the amount of melting measured in five ice cream samples over time. No melting of ice cream was observed during the time range of 0 to 20 minutes. After 20 minutes, the control sample (C) started melting and exhibited a consistent rate of melting throughout the entire process. The remaining four ice cream samples containing hemp milk exhibited a slower rate of melting compared to the control sample. Furthermore, the ice cream samples with higher amounts of hemp milk showed a correspondingly lower melting rate. The melting curves of S1 samples showed a steady gradient between 20 and 55 minutes, then increased after 55 minutes. Conversely, the melting curves of the samples S2, S3, and S4 showed increased gradients after 60 minutes. Additionally, the gradient of the melting curves for four samples containing hemp milk were similar.

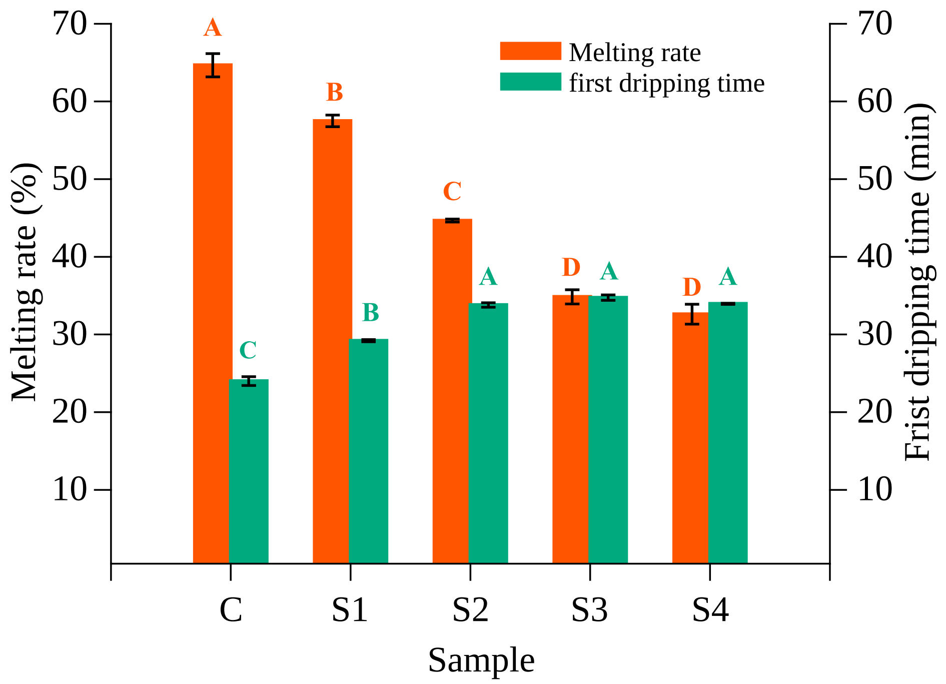


Figure 4.4 The melting rate and first dripping time of five ice cream samples

C = control; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk; Error bars mean ± standard deviations; Different letters on each bar indicate significant differences ($p<0.05$) between the samples (n=2); First dripping time indicates the time recorded when the melted ice cream first dripped.

Figure 4.4 shows the melting rates of five ice cream samples. All the samples exhibited significant differences in melting rate ($p<0.05$). The control group (C) had the highest melting rate of 65 %, while group S4 had the lowest melting rate of 33 %. The melting rate of the ice cream decreased as the amount of hemp milk in the ice cream increased. The first dripping in all five ice cream samples occurred after 20 minutes. The time of the first drop ranged between 24 and 33 minutes, and it was delayed as the amount of hemp milk in the ice cream increased.

The viscosity of ice cream is one of the factors affecting the melting rate, and previous studies have shown that an increase in apparent viscosity enhances the melting resistance and smoothness of ice cream (Clarke, 2005; Narala et al., 2022). Increase in the viscosity of the serum takes longer to diffuse from the inside to the outside of the ice cream. The melting rate of the control sample (65%) in this experiment was similar to the range of melting rates of ice cream samples containing chia seed mucilage reported by Feizi et al. (2021). Furthermore, the ice cream samples containing hemp seed melted at a slower rate compared to the control group. This may be attributed to the higher viscosity of the ice cream mix caused by the added hemp milk, which is abundant in fat and dietary fibre, according to Table 4.1 (Akalın et al., 2007).

Several studies indicated a clear correlation between the overrun and the melting rate (Campos et al., 2016; Leahu et al., 2022; Wang et al., 2022). Due to the low thermal conductivity of air, the process of melting experiences a decrease in heat transfer (Bekiroglu et al., 2022). The increase in overrun is positively correlated with the size of the air bubbles in the ice cream, and the air bubbles mixed into the ice cream enhance the thermal resistance of the ice cream and the stability of the fats in the ice cream, which play a key role in reducing the melting rate of the ice cream (Akbari et al., 2019). Results of melting rate in this section and the overrun discussed in Section 4.2.2 was consistent with the descriptions of these previous studies. As the overrun of the ice cream samples increased, the melting rate of the ice cream decreased.

4.2.4 Colour of ice cream

The colour of ice cream is an essential characteristic that significantly impacts the consumer's perspective and serves as the first sensory attribute that is easily observed (Ferdouse et al., 2024). The results of colour measurement are expressed in CIE L*, a*, and b* Hunter values, which correspond to the lightness/darkness, redness/greenness, and yellowness/blueness, respectively (Hu, 2021). The whiteness index (WI) is a key visual indicator for dairy products that impacts consumer choice of food products (Kurt & Atalar, 2018).

All five ice cream samples in the experiment had a pale yellow tint with low saturation. The ice cream became slightly lighter and lighter on visual observation as the amount of hemp milk in it increased. The visual observation is consistent with the L* and WI values measured from the colorimeter. The L* value increased from 66.81 to 77.23, while the WI value rose from 65.05 to 75.33. Table 4.2 shows that as the amount of hemp milk in the ice cream samples increased, there was a corresponding increase in WI value, L* value (brightness), and a* value (more negative, greenish) of ice cream samples ($p<0.05$), and an insignificant fall in b* values of ice cream samples ($p>0.05$).

Table 4.2 The colour of five ice cream samples

Colour	Sample				
	Control	S1	S2	S3	S4
Lightness (L*)	66.81 ± 2.08^{c}	70.26 ± 3.82^{cb}	71.43 ± 2.41^{abc}	73.85 ± 0.81^{ab}	77.23 ± 0.2^{a}
Redness (a*)	-3.27 ± 0.02^{d}	-2.84 ± 0.13^{c}	-2.08 ± 0.04^{b}	-1.98 ± 0.05^{b}	-1.38 ± 0.02^{a}
Yellowness (b*)	10.45 ± 0.48^{a}	10.23 ± 0.05^{a}	9.31 ± 0.19^{a}	9.09 ± 1.23^{a}	9.39 ± 0.5^{a}
Whiteness index	65.05 ± 2.01^{c}	68.40 ± 3.59^{bc}	69.88 ± 2.31^{abc}	72.23 ± 0.85^{ab}	75.33 ± 0.35^{a}

C = control; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk; Values with the different letter within the same line are significantly different ($p < 0.05$). Results are mean ± SD of three determinations.

Five ice cream samples showed a lighter yellow colour with a reddish-yellow tint, leading to relatively low L* and a* values. The low L* value may be attributed to the use of chia seed mucilage as a stabiliser in the experiment, which becomes brown during the air-drying process. The increase in a* value of the ice cream is likely caused by the chlorophyll from the seed coat of hemp seed(Xu et al., 2021). The b* value of the ice cream samples did not indicate a notable difference, perhaps due to the fact that the b-values of hemp milk and milk ranged around a comparable range, consistent with results from previous research by Naylor (2021). The whiteness index values were also positively correlated with the amount of hemp milk in the ice cream. As the amount of hemp milk increased, the whiteness values also increased significantly ($p < 0.05$). This relationship may be attributed to the chromaticity of the proteins present in hemp milk (Martin et al., 2023). Furthermore, Gram and Mortas (2023) suggested that the homogenization procedure during sample preparation impacts the particles in the ice cream mix in terms of number and size, leading to improved light reflection and a notable increase in the whiteness index (Kurt & Atalar, 2018).

4.2.5 Evaluation of ice cream by a focus group

The likelihood of consumers repurchasing hemp milk ice cream is similar to some extent, as shown in Figure 4.5. All five samples exhibited a consistent trend of being "neutral to unlikely to consume," with a slight decrease observed in samples S1 and S2. Furthermore, a notable difference could be found in the 'like' reaction of sample S1, which clearly indicated that ice cream containing 25% hemp milk was preferred compared to ice cream with either no hemp milk or 100% hemp milk.

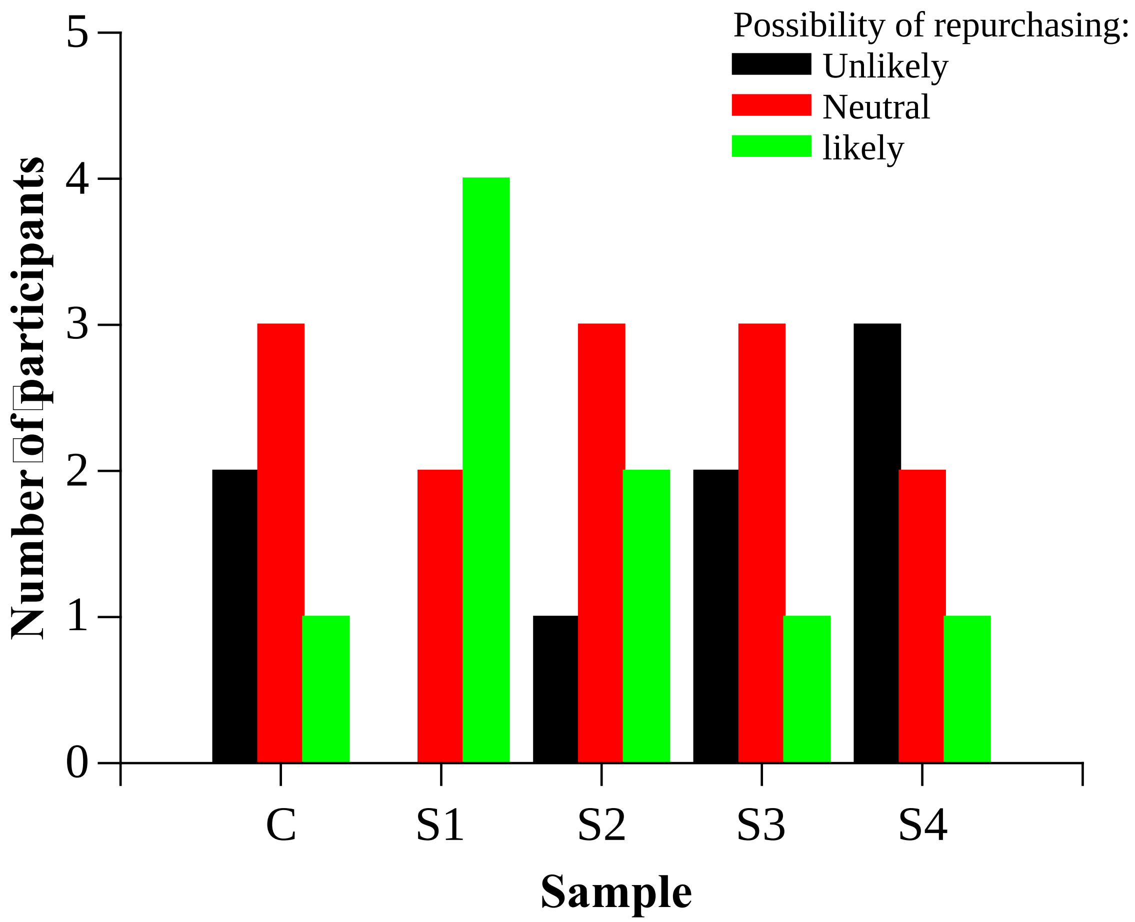


Figure 4.5 Possibility of repurchasing of five ice cream samples

C = ice cream sample with no hemp milk; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk; total number of participants = 6.

To obtain more detailed and comprehensive understanding of consumer responses in the focus groups, Table 4.3 containing general comments and individual quotes from the participants was generated. it shows an overview of the positive and negative descriptions associated with the sensory attributes of ice cream. Hemp milk ice creams were evaluated by considering these comments, quotes, and the general responses.

Table 4.3 Summarised comments from the focus group for five ice cream samples

Sample	General comments		Individual quotes
	Positive attributes	Negative attributes	
C	• Milky taste and flavour • Thick mouthfeel • Creamy for mouthfeel • Sweet	• Fast melting speed • Oily • Too sweet	'Milky taste good but strong, melting speed is more faster than others' 'Milk smell far more than milk taste'
S1	• Balanced plant-based flavours • Attractive colour • Nice creamy smell • Creamy texture	• Slightly hard at first • Lack of sweetness	'The texture is smoother than sample S4' 'Not too creamy or nutty'
S2	• Rich taste • Increased sweetness and rich flavour	• Not too much odour • A little strange taste • Strong plant milk flavour • Not too sweet	'Not as sweet as the S1 sample' 'Strong plant-based flavour' 'nuttiness and milkiness are lacking'
S3	• Smells great • Suitably sweet	• Excessive nutty flavour • Beany flavour • Hard texture	'barely taste the milk flavour" "hard and icy'
S4	• Soy or oat milk like flavour • Taste good • Lasting aftertaste	• Hard • Not fine and smooth enough Too icy • Not sweet	'Too hard for ice cream' 'mouthfeel like shelved ice' 'lack of creaminess'

C = ice cream sample with no hemp milk; S1= ice cream sample containing 25% hemp milk; S2 = ice cream sample containing 50% hemp milk; S3 = ice cream sample containing 75% hemp milk; S4 = ice cream sample containing 100% hemp milk;

4.2.6 Summary

There is a lack of information on the optimisation of ice cream that uses hemp milk instead of non-fat milk solids and GSM instead of stabilisers. This research aims to develop an ice cream containing hemp milk with clean labels. The findings from the second phase indicated that ice cream samples containing different amounts of hemp milk (C:0%; S1:25%; S2:50%; S3:75%; and S4:100%) had various physicochemical characteristics. Additionally, the pH, overrun, first dripping time, lightness value, and whiteness value of the samples increased relative to the amount of hemp milk contained in the ice cream formulation, whereas the melting rate of the samples showed a significant decrease. Furthermore, the ice cream sample that contained 25% hemp milk (S1) obtained the highest average score and the largest percentage of likelihood for repurchase throughout the focus group evaluation.

The pH, overrun, colour, meltdown rate, and first dripping time of the sample S1 were changed and exhibited significant differences ($p<0.05$) when compared to the same characteristics of the control sample (C) through turkey analysis. Hence, the ice cream sample (S1) that contains 25% hemp milk shows the most promising formulation.

4.3 Phase 3: Overall characteristics of the best formulation of ice cream

4.3.1 Physical properties

4.3.1.1 Some physical properties of the best formulation ice cream

Some physical properties of the best formulation ice cream are shown in Table 4.4. The mean pH of the best formulation ice cream sample was 6.79 ± 0.04, which is in a neutral pH range and matches with the pH range of 6.32–6.65 reported by many studies on ice cream (Bekiroglu et al., 2022; Kurt & Atalar, 2018; Leahu et al., 2022). The reason for the pH of the best formulation ice creams to be slightly higher than this range could be due to the presence of hemp milk since hemp milk has a higher pH than cow milk, according to a study by Naylor (2021). The best formulation ice cream had a density of 0.86 ± 0.025 g/cm^3 and an overrun of 30.65 ± 0.448%. The CSM and hemp milk in the ice cream formulation both significantly increased the amount of air, which causes the ice cream to have a lower density and a higher overrun than milk ice cream (Chavan et al., 2017). In the study by Nooshkam et al. (2023), the foam system was introduced as a novel structure, and this system significantly decreases the calorie density of ice cream due to its lower density. Additionally, a thinner mix will result from some extent of density reduction and higher overrun, which will improve the rheological, textural, and sensory properties (Chalupa-Krebzdak et al., 2018). In contrast to some previous overrun results of plant-based ice creams, 29.74% (Wang et al., 2022), 34.8% (Pontonio et al., 2022), and overrun results of stabiliser replacements, 14% (Utpott et al., 2020), and 22.02% (Kasapoglu et al., 2023), none of their overrun results were as good as the performance of this experiment. The best formulation ice cream showed excellent resistance to melting, with a melt rate of 44.1 ± 0.74% and a first dripping time at 28 ± 1.27 minutes. James (2020) obtained a melt rate of nearly 100% by adding basil seed flour to the ice creams, whereas Wang et al. (2022) and Arnak and Tarakçi (2021) reported similar melt rates to this experiment, but the first drop times all occurred before 20 minutes. These previous studies showed ordinary resistance to melting compared to this experiment.

Table 4.4 Some physical properties of the best formulation ice cream

Physical properties	**Results**
pH	6.79 ± 0.04
Density (g/cm^3)	0.86 ± 0.025
Lightness(L*)	72.37 ± 0.11
Redness(a*)	-2.71 ± 0.03
Yellowness(b*)	9.17 ± 0.16
Whiteness	70.77 ± 0.15
Overrun (%)	39.65 ± 0.448
Melting rate (%)	54.10 ± 0.74
First dripping time (min)	28 ± 1.27
Hardness (N)	34.92 ± 0.52
Stickiness (N)	-2.86 ± 0.01

The best formulation ice cream had a white to yellowish colour visible to the human eye, as shown in Figure 4.6. The L* value was 72.37 ± 0.11, the a* value was -2.71 ± 0.03, and the b* value was 9.17 ± 0.16. Previous research by Chavan et al. (2017) on milk ice creams showed similar colour values to the result obtained in this experiment, which indicates that the best formulation ice cream had a common and acceptable colouration. Studies by Bekiroglu et al. (2022) using walnut milk for ice cream resulted in dark hues and high brown index values, which may be attributed to the dark pigment found in the seed coat of walnuts. This means that dairy products have a fixed range of colour values, and the factors that significantly affect the colour values can be mostly attributed to the introduced plant products.

Figure 4.6 Appearance of the best formulation ice cream

It is necessary to consider the hardness of the product to get the desired consistency when scooped or dipped at a particular temperature (Marshall et al., 2003). Analysing the hardness and stickiness of ice creams is valuable for evaluating their ‘scoopability’ (Feizi et al., 2021). Typical measurements involve putting a cylindrical probe into the ice cream with a texture analyser. Varying probe diameters and test conditions may lead to multiple results. Hardness is determined using a specialised measurement procedure, unlike Young's modulus, which is an intrinsic characteristic of a material (Clarke, 2004). The hardness of the best formulation ice cream was 34.92 ± 0.52N, falling within the range of 30 – 40 N reported by (Feizi et al., 2021), so the addition of hemp milk did not have a negative effect on the ice cream's texture. Meanwhile, the authors used the same texture analyser, probe, and parameters as in the present experiment, so they were comparable.

Stickiness can be defined as a force that overcomes the attraction between the food surface and tableware (Marshall et al., 2003). The ice cream in this experiment showed appropriate stickiness with a value of -2.86 ± 0.01, while Kozłowicz et al. (2021) reported a far larger stickiness than this experiment (-15.72 – -44.47 N). High stickiness may negatively affect the quality of the ice cream product. At the appropriate dipping temperature, the spoon should easily move across the product and scoop the ice cream without excessive stickiness or gumminess (Marshall et al., 2003). Arnak and Tarakçi (2021) found the results similar to the results of this study and suggested stickiness was influenced by the amount and type of stabilisers and sugars in the formulation.

4.3.1.2 Rheological measurements of ice cream mix

The rheological qualities of ice cream mixes have an impact on the sensory properties of ice cream, as well as the process design and equipment selection (Feizi et al., 2021). Figure 4.7 shows the rheological parameters of the ice cream mixtures through the measurement of apparent viscosity. The measured viscosity of the best formulation ice cream sample exhibited a reduction from 56.21 to 0.32 Pa·s as the shear rate increased, indicating a shear-thinning behaviour (non-Newtonian pseudoplastic flow behaviour).

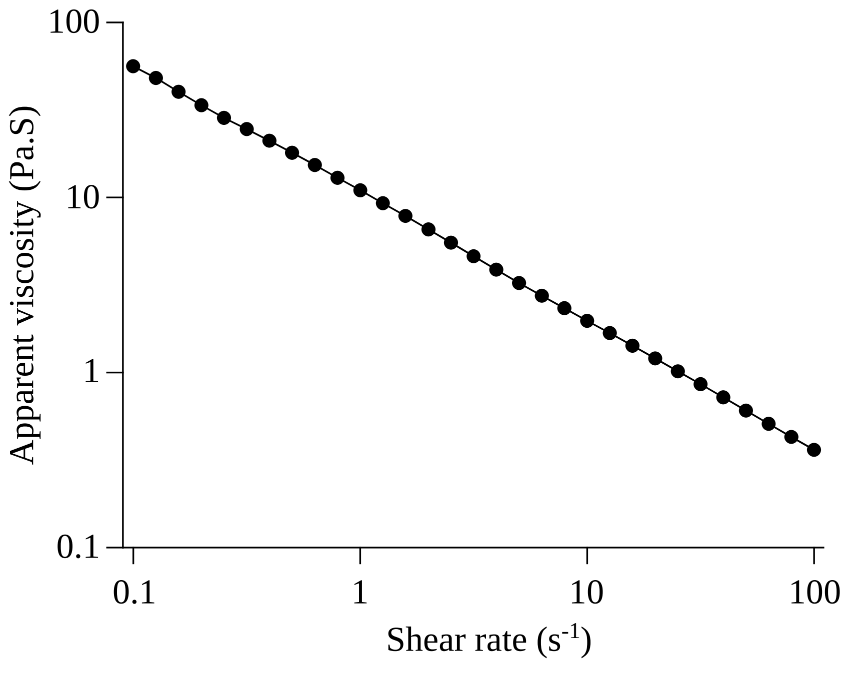


Figure 4.7 Apparent viscosity of ice cream mix.

Viscosity is a crucial attribute of ice cream since it plays an essential role in catching air within the ice cream mix. Consequently, the ice cream mix must have a specific viscosity value (Nooshkam et al., 2023). The elevated viscosity of the best formulation ice cream sample indicates an improved capacity to combine and hold air bubbles. Previous sections have addressed the overrun and sensory characteristics associated with the samples in this study. The primary factor for the increase in viscosity of the mix is mostly due to the stabiliser present in the formulation (Arnak & Tarakçi, 2021). The use of the CSM in this experiment increased the viscosity of the system, which is consistent with the results reported by Feizi et al. (2021). Hydrocolloids in the system not only increase viscosity and limit molecular mobility but also regulate the development of ice crystals during storage (Marshall et al., 2003). Furthermore, the presence of protein and fibre in hemp milk may have contributed to improved foaming and emulsifying characteristics.

4.3.1.3 Particle size of the best formulation ice cream mix

The particle size distributions of the aged mix and frozen melt of the best formulation ice cream is displayed in Figure 4.8. The freezing of the ice cream resulted in obvious changes in the particle size distribution, which included an increased number of peaks. Marshall et al. (2003) reported that this change in particle size is attributed to the instability of the fat during freezing and storage, leading to a multi-peak distribution in the particle size distribution of the ice cream.

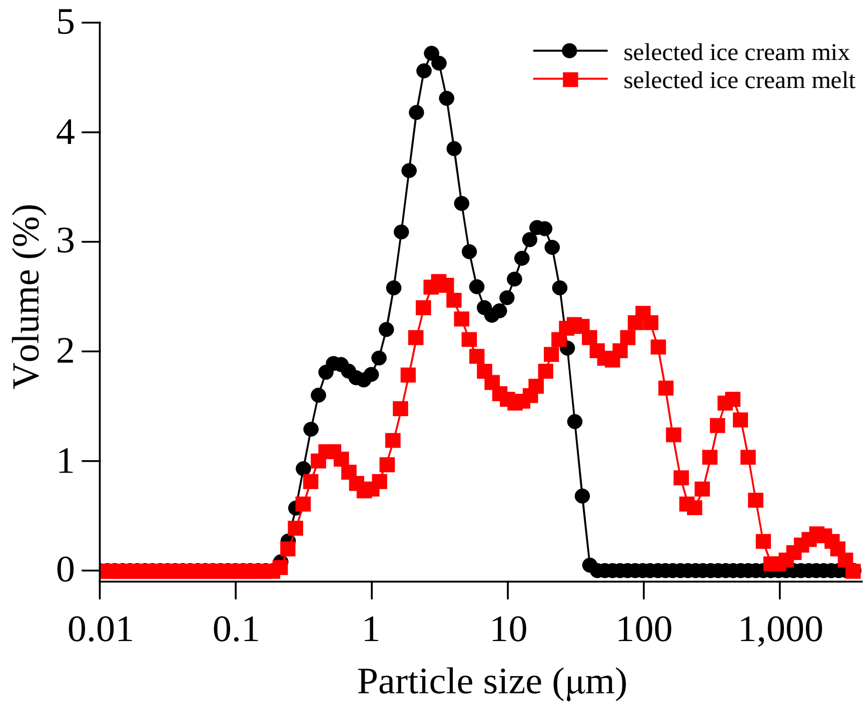


Figure 4.8 the particle size distribution of the best formulation ice cream aged mix and melt.

The D[4, 3] values of the frozen ice cream samples significantly increased, indicating the formation of large aggregates after freezing. This led to a significant increase in the volume mean diameter of the particles, as well as the median diameter increasing from 3.66 to 22.50. Additionally, the range of boundary particle size increased from 0.66–20.90 to 1.58–312, indicating the existence of the fat agglomeration phenomenon (Table 4.5).

Table 4.5 Particle size of ice cream mix and ice cream.

Parameter	**Aged mix (μm)**	**Ice cream melt (μm)**
D [3,2]	1.90	3.75
D [4,3]	7.49	91.50
Dx (10)	0.66	1.58
Dx (50)	3.66	22.50
Dx (90)	20.90	312.00

Following the freezing of the ice cream and the agglomeration of fat, the three peaks present in the small particle size range did not disappear but rather experienced a reduction in their height. It is possible that the particle size distribution shown in Figure 5 may not only indicate the particle size of fat globules but also include proteins and fibres that occur in hemp milk. Previous research by Wang et al. (2023) has shown that edestin has a propensity to form aggregations near a neutral pH. This may lead to a decrease in dissolvability in this pH range, resulting in the proteins separating from the surface of the fat globules. Due to this reduction in steric stabilisation between proteins, the fat was promoted to become unstable (Martin et al., 2023). Furthermore, ice cream with larger particle sizes creates stronger gel structures. This is mainly because the gelation behaviour of CSM can create network structures in ice cream through interactions with proteins or fats. As a result, these ice creams exhibit a wider range of particle size distribution and a slower rate of melting, also leading to increased hardness and viscosity (Nooshkam et al., 2023). Ice cream is a type of foam, melted ice cream samples inevitably contain some air bubbles during the measurement process. If air bubbles are not eliminated, the change in particle size between aged and melted ice cream samples may be potentially affected.

4.3.2 Proximate analysis of the best formulation ice cream

The nutrient contents of the best formulation ice cream are shown in Table 4.6, with the total solids fitting within the recommended range of 28–40% (Clarke, 2004). Marshall et al. (2003) argued that ice cream must contain a minimum of 19.2% total solids, and increased total solids can allow a higher overrun. Besides, a formulation with high total solids leads to high-quality ice cream (Clarke, 2004). However, if the total solids content exceeds 40–42%, it can lead to a heavy, sticky texture of the product (Marshall et al., 2003).

Table 4.6 Proximate analysis results of ice cream

Parameter	Amount
Moisture	71.2%
Protein	2.3%
Fat	9.8%
Ash	0.5%
Total dietary fibre	0.2%
Total carbohydrate	16%
Caloric value	161.4 kcal/100 g

The protein content of the best formulation ice creams was 2.3%, similar to the result of Bekiroglu et al. (2022) for walnut milk ice cream (2.55%), and both of them slightly lower than common cow milk ice creams (Chavan et al., 2017). This could be because both hemp seed and walnut are low in protein content. Nonetheless, the amino acid profile of the protein in hemp seed is wide and includes multiple amino acids lacking in milk (Martin et al., 2023). However, this phenomenon does not exist in all plant-based ice creams. According to Wang et al. (2022), soy milk ice cream contains 6.01% more protein than cow milk ice cream because the type of plant and its own protein content may have an impact on this.

The best formulation ice cream had a fat content of 9.8%, which was within the typical range (7–15%) of ice cream fat content reported by Clarke (2004). The result was higher than the 5.6% fat content of soy milk ice cream, as reported by Wang et al. (2022). However, the fat content of walnut milk ice cream (21%) reported by Bekiroglu et al. (2022) was significantly higher than the best formulation ice cream in this study due to the fact that walnut is a high-oil-content nut. In addition, the best formulation ice cream samples had a lower fat content, which imposes less burden and calories on the consumer. Hemp seeds also contain multiple kinds of unsaturated fatty acids, which offer cardiovascular health advantages (Leonard et al., 2020).

The ash content of the best formulation ice cream sample was 0.5%, probably due to the mineral-rich content of hemp seeds (Ferdouse et al., 2024). BeŞİR et al. (2022) and Chavan et al. (2017) reported a common ash content range of 0.5-0.85, which agreed with the results of this experiment. The best formulation ice cream had a carbohydrate content of 16%, which is lower than the results of all previous research on ice cream, ranging from 18.14% to 21.68% (Chavan et al., 2017; Wang et al., 2022). Sugar in ice cream has two main purposes: controlling sweetness and influencing the amount of ice crystals, which affects the texture of the ice cream (Marshall et al., 2003). Low-carbohydrate ice cream offers health benefits but may lack a positive mouthfeel (Campos et al., 2016). On the other hand, excessive carbohydrate content may lead to obesity and diabetes risks, as well as an unpleasant mouthfeel due to the excessive viscosity(Clarke, 2004).

The ingredients of different ice creams vary widely, so the calorie value of each product will also be different (Marshall et al., 2003). Therefore, it is impractical to establish calorie standards for each product. The ice cream in this study had a calorie content of 161.4 kcal/100 g, which agrees with the results of (Nooshkam et al., 2023). If the calorie value of a product was stated, consumers could consider it when making a purchase.

4.3.3 Consumer sensory evaluation of ice cream

4.3.3.1 Sensory Evaluation for ice cream

The objective of the consumer sensory evaluation was to evaluate each sensory characteristic of the best formulation ice cream, as well as the overall score, using consumers (n = 35). This section discussed the results of the best formulation ice cream sample, which was presented to the participants for evaluation. The results of the consumer sensory assessment on the colour, aroma, texture, sweetness, taste, mouthfeel, and overall liking of the sample are shown in Figure 4.9.

Figure 4.9 Radar plot for liking scores for sensory attribute and overall liking of the best formulation ice cream sample

The radar plot illustrates that the sensory evaluation scores of the ice cream containing 25% hemp milk were located around 6. The average overall score for the samples was 6.75. The mouthfeel received the highest score of 6.85. Combined with the comments from the previous focus groups, this could be attributed to its refreshing and non-oily mouthfeel. On the other hand, aroma received the lowest score of 6.5. When analysed in combination with the comments from the previous focus groups, it was found that the low scores were probably due to the fact that some people thought the cannabis flavour was too strong, while others thought it was lacking. Regardless, On a nine-point scale, an average liking score of 7 or higher usually indicates highly acceptable sensory quality (Everitt, 2009), while there were no sensory liking scores for the best formulation ice cream that exceeded score of 7, all of the indicators were very close to score of 7, especially the mouthfeel. Thus, it can be considered a promising achievement in the field of plant milk ice cream technology and new product development. Similar to a previous study by Bekiroglu et al. (2022), adding walnut milk to the production of ice cream enhanced the acceptability of the product compared to cow milk ice cream. But it is worth noting that the overall scores were lower than the results of this study, which did not exceed a score of 6. However, the study reported by Nooshkam et al. (2023) on ice cream containing licorice extract showed lower sensory scores, ranging from 2 to 4.

4.3.3.2 Just-about-right (JAR) for ice cream sensory attributes

Just-about-right (JAR) combines intensity and acceptability to relate the sensory strength of a specific attribute to the theoretical best value of participants (Rothman & Parker, 2009). Thirty-five participants provided their JAR evaluations of the characteristics associated with the best formulation ice cream. As shown in Figure 4.10, participants evaluated the characteristics of the best formulation ice cream for six attributes: colour, aroma, taste, sweetness, texture, and mouthfeel. According to Figure 4.9, over 70% of the participants found the colour, sweetness, texture, and mouthfeel of the samples to be satisfactory, while the aroma and taste were rated below 70%.

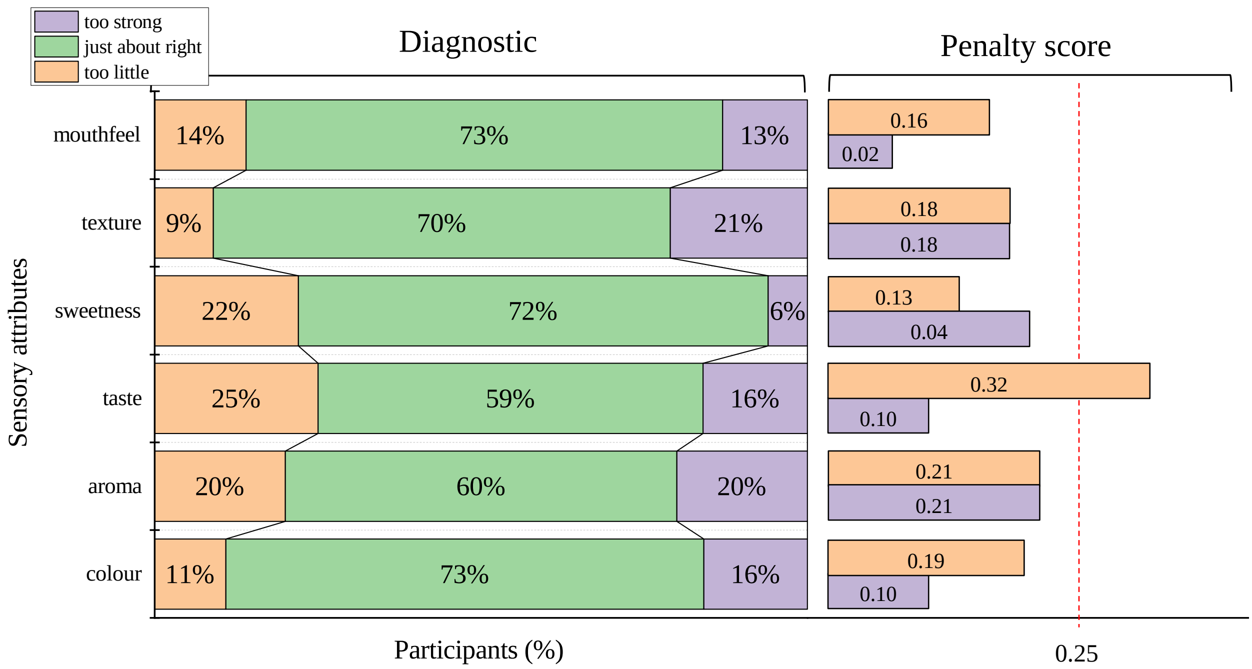


Figure 4.10 Percentage of participants for the just-about-right ("JAR") level and their penalty score of the mouthfeel, texture, sweetness, taste, aroma, and colour of the best formulation ice cream

Hu (2021) states that if 70% of the participants in the sensory test respond at the JAR level, then this attribute can be identified as the optimal level. Hence, the colour, sweetness, texture, and mouthfeel in this study could be considered to have optimal performance. Rothman and Parker (2009) reported that over 20% of the non-JAR categories will be candidates for penalty analysis, and this analysis will determine if the outcomes of the non-JAR options are worthy of consideration. Based on the results of the study, while some non-JAR categories for aroma, taste, and sweetness of ice cream exceeded 20%, the results of the analysis showed only 'too little' of a penalty score about taste exceeding 0.25, which falls within the noteworthy range but not in the high impact (>0.5) range. Meanwhile, Narayanan et al. (2014) reports that these results indicate the specific attributes that require adjustment and the direction in which they should be adjusted. However, penalty analysis was not able to provide a comprehensive description of the extent of change required to achieve the JAR property accurately. Furthermore, it is not known whether modifying the level of the target attribute would impact the overall properties of the product due to the interactions between variables. For instance, increasing the amount of hemp milk to improve flavour might have negative effects on properties like the colour and hardness of the ice cream. Therefore, it can be concluded that only a minority of consumers regard the taste of hemp milk as somewhat lower than their expectations, and this comment is noteworthy (Schraidt, 2009).

4.3.4 Microbiological of ice cream

Ice cream, while not sterile, is safe to consume if it is made in a clean environment and follows an authorised production process, as it does not contain harmful microorganisms. Furthermore, when ice cream includes ingredients derived from soil, such as nuts or seeds, the microorganisms that can potentially be harmful are *Listeria monocytogenes*. Hence, the ice cream samples were examined to determine the total number of aerobic microorganisms, the presence of *E. coli* bacteria, and the existence of *Listeria monocytogenes* before being used with sensory evaluation in this experiment. The results are shown in Table 4.7. The ice cream samples met the sensory testing requirements according to the New Zealand Ministry of Primary Industries (MPI) standards.

Table 4.7 Microbiological test result of the ice cream sample

Microbiological test	**Result (CFU)**
Total plate count	4.6×10^{-2}
Listeria monocytogenes	Not detected
Coliforms	Not detected

4.4 Phase 4: Storage stability of the best formulation of ice cream at -19 °C for 21 days

4.4.1 Changes in pH of the best formulation ice cream during storage at -19 °C for 21 days

Figure 4.11 shows the pH values of the best formulation ice cream sample that was stored at -19 °C for 21 days. As expected, the pH of the best formulation ice cream was unchanged from day 1 to day 21 ($p > 0.05$), staying within the range of 6.71-6.74. The result was similar to that observed by Murtaza et al. (2004) who found the pH of ice cream remained stable between 6.7 and 6.8 after 40 days of storage. A typical pH of ice cream is around 6.3 (Marshall et al., 2003).

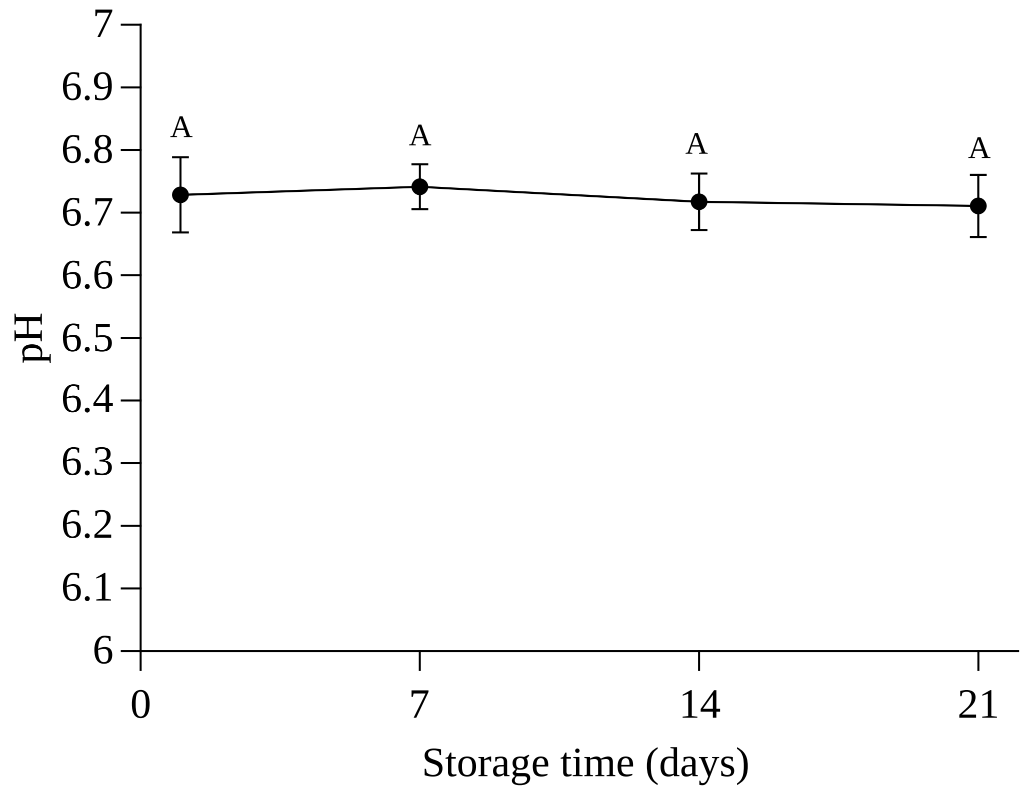


Figure 4.11 Mean pH of the best formulation ice cream during the storage (-19 °C) /21 days
Error bars means ± standard deviations; Capital letters on each symbol indicate significant differences ($p>0.05$) between the samples ($n=3$).

In general, non-fermented or non-probiotic ice cream formulations should not experience significant pH changes before the expiration date. This is because the apparent or natural acidity of ice cream mixes is caused by the existence of NMS, mineral salts (mostly phosphates and citrates), and dissolved carbon dioxide (Clarke, 2004). In non-fermented ice cream, pH variations in the short term could be a sign of product instability or microbiological contamination risk. Apart from maintaining a stable pH, a product with a low pH is also not recommended since it may result in an unstable mix, a low whipping rate, high viscosity, and poor flavour (Murtaza et al., 2004). Low-pH ice cream may "cook on" during the pasteurisation process because heat and acidity accelerate the denaturation of proteins (Marshall et al., 2003).

4.4.2 Changes in colour of the best formulation ice cream during storage at -19 °C for 21 days

The changes in colour of the best formulation ice creams stored at -19 °C for 21 days are shown in Figure 4.12. The lightness of the ice cream (L*) decreased from 72.37±0.11 (day 1) to 70.13±0.04 (day 21) ($p<0.05$). The redness of the ice cream (a*) increased from -2.87 ± 0.01 to -2.71 ± 0.03 from days 1 to 21 ($p<0.05$). Although the changes were not substantial, they were statistically significant. During storage, the yellowness (b*) of the ice cream did not change ($p > 0.05$).

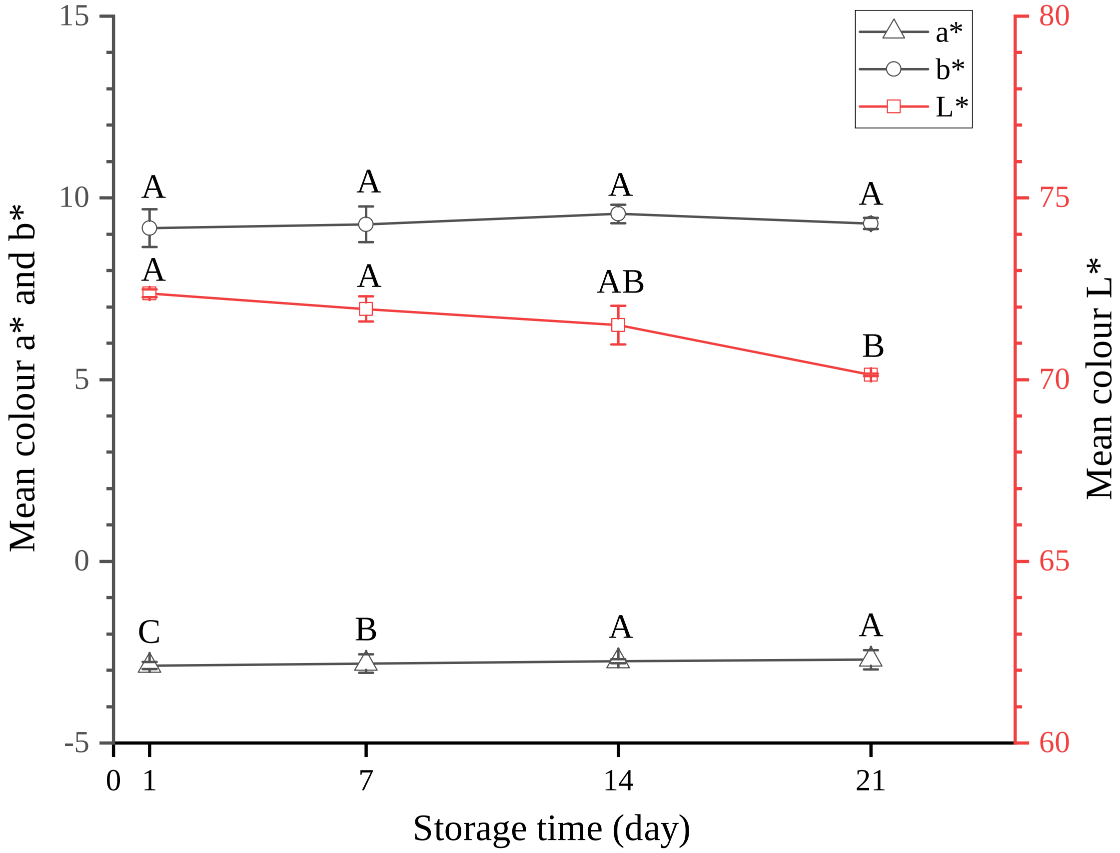


Figure 4.12 Mean colour values of best formulation ice cream during the storage (-19 °C) /21 days
Error bars means ± standard deviations; Different letters on each symbol indicate significant differences ($p>0.05$) between the samples ($n=3$).

The lightness (L*) of ice cream decreased significantly ($p<0.05$) in the research by Soukoulis et al. (2008) as well. Bekiroglu et al. (2022) also reported similar results regarding the L* value decreasing during the storage of ice cream containing walnut milk. Conversely, Alfaifi and Stathopoulos (2010) reported that the L* value of the egg yolk-based ice cream sample rose noticeably following four weeks of storage. Thus, the ingredient in the ice cream formulation may have a significant impact on the change in L* value during ice cream storage. For instance, certain unique components will experience enzymatic browning and oxidation (Utpott et al., 2020). Furthermore, a decrease in lightness may result from drying at the sample surface caused by the low humidity in the refrigerator (Alfaifi & Stathopoulos, 2010). The increased a* value of the best formulation ice cream indicates a decline in greenness, which could be caused by the gradual decomposition of green organic matter and chlorophyll in hemp milk (Leonard et al., 2020). The yellowness (b*) of the ice cream sample was not changed. According to Marshall et al. (2003), the natural yellow colour of milk fat is the main source of the yellow hue in ice cream, and under stable conditions, this colour will not change significantly.

4.4.3 Changes in hardness of the best formulation ice cream during storage at -19 °C for 21 days

The growth of ice crystals during the storage of ice cream is accompanied by high values of hardness as determined by the equipment. The measurement of ice cream hardness can be used as an indicator of the extent of ice crystal growth during storage. The changes in hardness values of the best formulation ice creams stored at -19 °C for 21 days are shown in Figure 4.13. The hardness of the ice cream increased from 34.22 ± 0.17 N (day 1) to 35.27 ± 0.14 N (day 14) ($p<0.05$) and then remained stable after Day 14 ($p>0.05$).

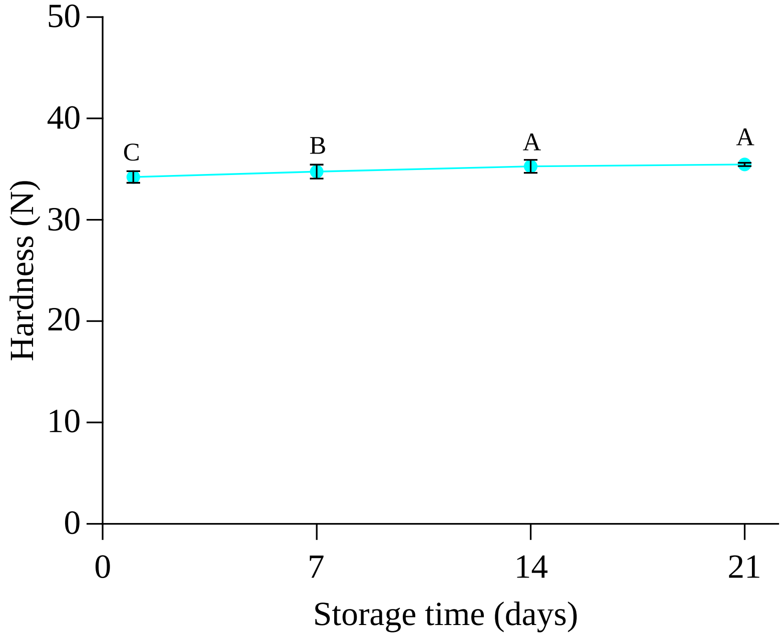


Figure 4.13 Mean hardness of the best formulation ice cream during the storage (-19 °C) /21 days
Error bars means ± standard deviations; Different letters on each symbol indicate significant differences ($p < 0.05$) between the samples (n=3).

The reason for this phenomenon may be due to the CSM used in this experiment combined with water in the system, causing it to transform into a cryogel when under freezing conditions. This cryogel formation effectively regulates the process of recrystallization (Soukoulis et al., 2008). Furthermore, milk proteins such as whey and casein proteins have the ability to interact with water. This interaction changes the rheological properties of the system, which affects the formation and stability of ice crystals as well as the movement of solutes (Marshall et al., 2003).

4.4.4 Changes in sensory attributes of the best formulation ice cream during storage at -19 °C for 21 days

The results of the consumer sensory scores (n = 20) for the best formulation ice cream during storage are shown in Figure 4.14. The average sensory scores for colour and overall acceptability showed very slight changes ($p > 0.05$) during the 21 days storage period of the samples. The scores for the texture and mouthfeel of the best formulation ice cream showed a significant decreasing trend while the time of storage increased ($p < 0.05$). Nevertheless, scores of the aroma, taste, and sweetness of the best formulation ice cream did not exhibit an obvious trend, although they were statistically different ($p < 0.05$), implying that these three sensory characteristics are more influenced by the subjective preferences of participants (Appendix E 2.4). Therefore, despite slight changes in some sensory scores over the three-week storage period at -19 °C, the best formulation ice cream samples were still considered acceptable by consumers.

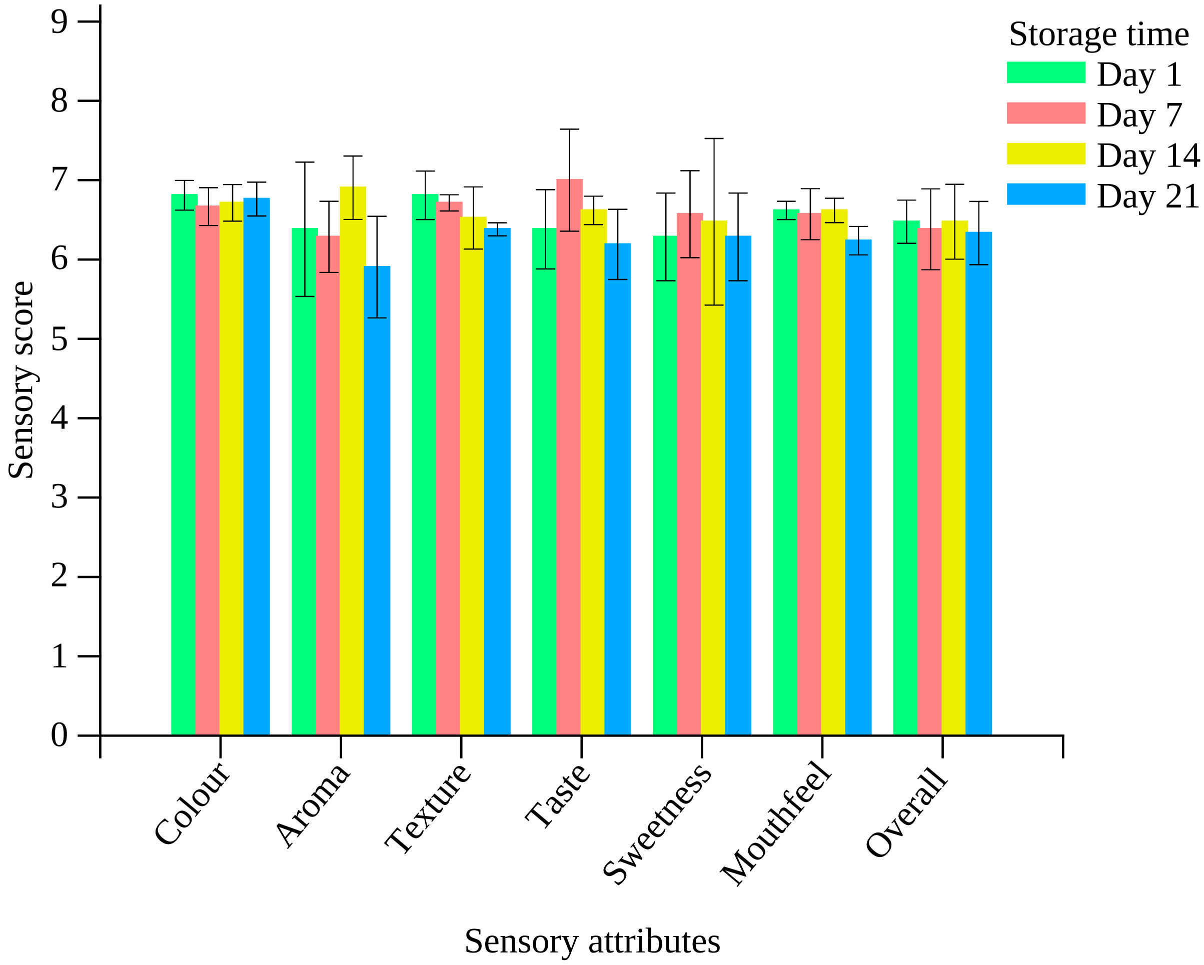


Figure 4.14 Mean sensory attributes score of the best formulation ice cream during the storage (-19 °C) /21 days

Error bars means ± standard deviation (n=20); A 9-point hedonic scale with descriptive anchors was used to evaluate each parameter (1= dislike extremely; 9 = like extremely).

The sensory evaluation results in this experiment agree with the results of Murtaza et al. (2004), who reported that storing the ice creams at -25°C for 40 days did not have a significant impact on the taste and overall scores. However, there was a decline in the colour and texture of the ice cream. Soukoulis et al. (2008) reported a significant decrease in the sensory scores for the colour and texture of ice cream samples stored for 16 weeks. This decrease was attributed to the fact that the texture attributes of ice cream will deteriorate as time passes, with negative impacts on properties such as viscosity, hardness, and mouthfeel. Consequently, the occurrence of ice cream deteriorating during the entire storage process for each sensory characteristic was unavoidable (Alfaifi & Stathopoulos, 2010). By analysing the current data, it was determined that the best formulation ice creams exhibited storage stability according to sensory evaluation.

4.4.5 Summary

Analysis of the best formulation ice creams indicated that the samples exhibited excellent storage stability throughout the storage period. Although the L* a* values in colour and hardness of the ice cream showed statistically significant changes ($p<0.05$), the b* values in the colour, pH, and all the sensory characteristics of the ice cream remained stable ($p>0.05$).

Chapter 5 Overall Conclusions & Recommendations

5.1 Research Outcomes

The objective of this research project was to create a novel plant-based ice cream using hemp milk and stabilised by CSM. The emphasis was on examining how the addition of hemp milk affects the physiochemical characteristics of the ice cream. The researchers used different amounts of hemp milk in the ice cream mix to identify the optimal amount required to prevent unpleasant sensations such as excessive hardness, strong smell, and taste, which was to ensure that the quality and texture of the hemp milk ice cream met the expectations of customers. This study offers crucial insights for the development of novel plant-based ice cream products containing hemp milk, fulfilling specific market needs. This experiment aimed at exploring the impact of replacing cow milk with different amounts of hemp milk (0%, 25%, 50%, and 100%) on the physicochemical and sensory attributes of ice cream. Furthermore, this study also examined the storage stability of the best formulation ice cream.

This experiment acknowledges that the equipment used in this experiment included laboratory grade equipment such as the Silverson mixer and MagiMix ice cream machine. Therefore, the formulation in this study might not be fully representative of commercial products. Also, the amount of hemp milk utilised in this experiment is the amount of liquid hemp extract, which depends on the formulation used to prepare the hemp milk, resulting in specific outcomes of this experiment.

Different amounts of hemp milk in the ice cream had significant effects on the characteristics of the ice cream. The addition of hemp milk influenced the pH, colour, overrun, and melting characteristics of the ice cream, which are important factors in ice cream quality. The study found that as the amount of hemp milk added increased, the pH, whiteness index, brightness, redness, overrun, and first dripping time of the ice creams increased, whereas the melting rate decreased. However, there was no significant change in the yellowness value.

Despite the increase in the addition of hemp milk, each of the above characteristics of the ice cream showed an increase. Nevertheless, descriptions from the focus group revealed that as the amount of hemp milk increased, there was negative sensory feedback for the ice cream samples, particularly in terms of texture, flavour, and mouthfeel. Consequently, the focus group concluded that the ice cream sample with 25% hemp milk received the highest score for the possibility of repurchasing.

The ice cream with 25% hemp milk showed similar shear-thinning flow properties to cow milk ice cream, along with substantial fat agglomeration during the freezing process. Furthermore, when compared to several previous studies, the best formulation ice cream as the most promising also performed better than other plant-based milk ice cream in terms of physical and nutritional characteristics (Bekiroglu et al., 2022; Pontonio et al., 2022). Two connected sensory evaluations have been carried out in this project to analyse and describe the sensory attributes of the best formulation ice cream. The first consumer sensory test showed scores ranging from 6.5 to 6.85 for all sensory attributes, indicating that the best formulation ice cream achieved high consumer acceptability, as an average liking score of 7 or higher on a nine-point scale usually indicates that the samples have high sensory quality (Everitt, 2009). In the second JAR test, 25% of the participants considered that "the ice cream taste was too weak" and that the penalty score for this option was exceeding 0.25, suggesting that this result is worth considering in future optimisations (Schraidt, 2009). The rest of the ice cream attributes resulted in " just about right".

To evaluate the storage stability of the novel 25% hemp milk ice cream, the results of the analyses performed throughout the storage period indicated that the yellowness, pH, and sensory attributes of the ice cream were unchanged ($p > 0.05$). However, there were slight changes found in the hardness, brightness, and redness of the product. In general, hemp milk ice cream exhibited storage stability at -19 °C for 21 days.

5.2 Recommendations

The objective of this study was to develop a clean-label plant-based ice cream product containing hemp milk that would improve the purchasing experience while also offering diversified product needs for consumers. The experimental purpose of this project was to examine the impact of adding hemp milk on the quality and sensory characteristics of the ice cream.

In addition, the physicochemical characteristics of hemp milk ice cream during storage were determined and evaluated, such as pH, colour, and texture features during storage. Furthermore, this study included a comprehensive sensory evaluation. However, this study applied only a single method to extract raw hemp milk. The protein content of hemp milk (1.8%) prepared in this experiment is slightly lower than skim milk (3%). This is directly related to the amount of water added in the formulation, but the extraction methods also affect the protein content of the prepared hemp milk. Hence, the use of other pre-treatments (such as baking or boiling), different extraction techniques (such as dry or wet milling), and improved extraction conditions (such as high pressure, ultrasound, or enzymes) in future studies to figure out their impact on the different characteristics of ice creams are recommended. The addition of protein, fat, or dietary fibre in hemp milk may have a considerable impact on the essential physicochemical and sensory features of ice cream. This experiment aimed to analyse the melting characteristics of hemp milk ice cream by examining its melting rate and the first dripping time. Unfortunately, the limitations of the experimental equipment at the university hindered us from exploring the impact of heat shock on the ice cream samples. Therefore, future research should be focused on changes in both the physicochemical and microscopic characteristics of hemp milk ice cream due to heat shock. Further research is also needed to understand the effect of CSM by comparing its impact with commonly used stabilisers in ice cream.

Furthermore, this study conducted a complete sensory evaluation that included multiple sensory tests. However, as a result of the COVID-19 pandemic, both sensory tests were only able to collect fewer than 40 individuals each for sensory testing. Therefore, it cannot be assumed that there were a sufficient number of participants for sensory evaluation. Hence, it is imperative to suggest future work to gather more participants for sensory evaluation. Increasing the number of participants in this study might reveal particular trends in the feedback regarding the sensory characteristics of hemp milk ice cream, such as its aroma, flavour, and sweetness, which are significantly influenced by individual preferences.

Another objective of this work was to develop a plant-based ice cream product with a clean label, and naturally obtained CSM was used in this experiment as a replacement for commercial stabilisers. However, using naturally obtained CSM as an alternative to commercial stabilisers is just one of the ingredients that can be replaced. Due to the limitations on the number of experiments required for a master's thesis, the emulsifier in the ice cream was not replaced by a natural ingredient, so a commercial emulsifier, MDG (0069), was used in this study. Hence, it is essential to suggest that future research apply natural components as replacements for commercial emulsifiers in the present formulation in order to finish the development of a completely clean-labelled hemp milk ice cream.

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Appendices

Appendix A: Focus group conference

Appendix A1: The description of the whole focus group conference

1. Reception: Provide water and snacks in advance at the location where the focus group will be held (IC Building 2.06B meeting room). After the arrival of all participants, it was imperative to ensure that the participants had an adequate understanding of the conference and that their participation was entirely voluntary. Before the start of the discussion, participants were requested to provide their approval by signing a formal consent document. The previously mentioned requirement was an authority issued by the ethics association, aimed at enhancing their familiarity with focus groups.

2. Introduction: This stage begins after everyone was settled in the room. Some basic rules were stated, according to focus group methods, and then the purpose and background of the focus group were briefly introduced, but without revealing too much detail. Make sure that the audio recording was turned on before the focus group discussion started, as some missed details cloud be discovered in the recording during review.

3. Introduction: During this stage, all participants in the conference were given the opportunity to express their viewpoints and preferences regarding ice cream. The purpose of this task was to collect comments from the participants on various aspects, including trends, modifications, and innovations related to ice cream brands, flavours, packaging, and other relevant factors.

4. Test Procedure: During this stage, four ice cream samples were sequentially shown to the participants. Following the taste of each ice cream sample, the participants were instructed to complete a brief sensory questionnaire designed to collect data related to the sensory attributes associated with the ice cream.

5. Discussion: This stage entails the examination and analysis of the questions presented in the questionnaire. All participants collectively provided comments to each question and afterwards verified the consistency of their answers. Additionally, any disagreements were properly recorded. Furthermore, this stage will also address any queries or concerns regarding the products, as well as adjustments, recommendations, and satisfaction with the objective of obtaining more insightful data.

6. Conclusion: Following the conclusion of the discussion stage, express appreciation to the participants for their active participation. Additionally, it would be beneficial to provide them with small gifts of appreciation.

Appendix A2: 3-digit random codes and corresponding ice cream sample numbers

ice cream sample number	The random number of the sample
S1	507
S2	899
S3	642
S4	271

Appendix A3: the questionnaire for focus group evaluation

ICE CREAM SENSORY TEST

MASSEY UNIVERSITY TE KUNENGA KI PŪREHUROA UNIVERSITY OF NEW ZEALAND	**Focus Group Model** **Sensory Evaluation of Hemp Ice Cream**
	information sheet

Researcher Information

Researcher: Weilun Zhang	supervisor: Dr. Tony Mutukumira
contact details:0272574145	contact details: Weilun.Zhang.1@uni.massey.ac.nz

We invite you to participate in an informal sensory evaluation.
You will have approximately 30 minutes to participate in this activity.
The type of food you will taste is: Ice cream.
The ice cream you are about to taste contains the following ingredients, which may be harmful or cause allergic reactions in certain groups of people. You will be excluded if you are allergic, or may be adversely affected by any of the following:

- Dairy
- Sugar
- Hemp Seeds
- Chia Seeds

The information gathered in this study will be used to complete part of the dissertation thesis project in partial fulfilment of the Masters in food technology. No personally identifiable data is collected.

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Refuse to answer any specific questions
- Withdraw from the study at any time
- Ask any questions about the study at any time during participation
- Provide information that your name will not be used unless you agree to the researcher

Consent Form

Project: A novel ice cream containing hemp milk and chia seed mucilage

- I have read and understood the information provided to me. Any questions about the study have been addressed and I know I can always ask any further questions.
- I understand that I may withdraw from this study at any time and may refuse to answer any specific questions.
- I have discussed with the researcher any cultural, religious or ethical beliefs that might prevent me from consuming the food.
- By signing this form, I voluntarily agree to participate in this study under the conditions stated in the information sheet. I am also not responsible for anything that happens to me during or after my participation in this research.

Signature: ________________________Date: ______________________ Ethic number____________________________

Introduction and Questions

ICE CREAM SENSORY TEST

Introduction

Five types of ice cream (Sample 485, Sample 507, Sample 899, Sample 642, Sample271) consisting of slightly different recipes participated in this sensory experience.

This study will analyse the sensory characteristics of ice cream such as:

- appearance
- colour
- taste
- Mouth feel
- Aroma
- ...

The sensory objective was to evaluate the differences between 5 ice creams with similar ingredients. This sense is also being used to gain insight into consumers' overall acceptance of plant-based ice cream.

Follow the instructions to score the ice cream accordingly. Drink water and sniff the coffee powder after each tasting to flush your taste buds and reset your sense of smell.

Questions

Sample271

Properties/features/characteristics of **sample271** that you like

____

Properties/features/characteristics of **sample271** that you dislike, and why?

____

How likely are you to consumer this ice cream again?

Unlikely / neutral / more likely

Numerical Descriptive Scale for Sensory:

Please rate the intensity of each sub-sensory characteristic of the ice cream using a shaded number from 0-10, where: (0-very weak & 10-very strong)

Colour • Whiteness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Yellowness ⓪①②③④⑤⑥⑦⑧⑨⑩	Smell • Nutty smell ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky smell ⓪①②③④⑤⑥⑦⑧⑨⑩
Texture • Hardness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Stickiness ⓪①②③④⑤⑥⑦⑧⑨⑩	Mouthfeel • Creaminess ⓪①②③④⑤⑥⑦⑧⑨⑩ • Ice crystals ⓪①②③④⑤⑥⑦⑧⑨⑩
Taste • Sweetness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Nutty taste ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky taste ⓪①②③④⑤⑥⑦⑧⑨⑩	Overall performance (9-Point Hedonic method) **1**=Dislike Extremely **2**= Dislike Very Much **3**=Dislike Moderately **4**= Dislike Slightly **5**= Neither Like nor Dislike **6**= Like Slightly **7**= Like Moderately **8**= Like Very Much **9**= Like Extremely your score__________

ICE CREAM SENSORY TEST

Sample507

Properties/features/characteristics of **sample507** that you like

Properties/features/characteristics of **sample507** that you dislike, and why?

How likely are you to consumer this ice cream again?

Unlikely / neutral / more likely

Numerical Descriptive Scale for Sensory:

Please rate the intensity of each sub-sensory characteristic of the ice cream using a shaded number from 0-10, where: (0-very weak & 10-very strong)

Colour • Whiteness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Yellowness ⓪①②③④⑤⑥⑦⑧⑨⑩	Smell • Nutty smell ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky smell ⓪①②③④⑤⑥⑦⑧⑨⑩
Texture • Hardness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Stickiness ⓪①②③④⑤⑥⑦⑧⑨⑩	Mouthfeel • Creaminess ⓪①②③④⑤⑥⑦⑧⑨⑩ • Ice crystals ⓪①②③④⑤⑥⑦⑧⑨⑩
Taste • Sweetness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Nutty taste ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky taste ⓪①②③④⑤⑥⑦⑧⑨⑩	Overall performance (9-Point Hedonic method) **1**=Dislike Extremely **2**= Dislike Very Much **3**=Dislike Moderately **4**= Dislike Slightly **5**= Neither Like nor Dislike **6**= Like Slightly **7**= Like Moderately **8**= Like Very Much **9**= Like Extremely your score__________

Sample899

Properties/features/characteristics of **sample899** that you like

Properties/features/characteristics of **sample899** that you dislike, and why?

How likely are you to consumer this ice cream again?

Unlikely / neutral / more likely

Numerical Descriptive Scale for Sensory:

Please rate the intensity of each sub-sensory characteristic of the ice cream using a shaded number from 0-10, where: (0-very weak & 10-very strong)

ICE CREAM SENSORY TEST

Colour • Whiteness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Yellowness ⓪①②③④⑤⑥⑦⑧⑨⑩	Smell • Nutty smell ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky smell ⓪①②③④⑤⑥⑦⑧⑨⑩
Texture • Hardness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Stickiness ⓪①②③④⑤⑥⑦⑧⑨⑩	Mouthfeel • Creaminess ⓪①②③④⑤⑥⑦⑧⑨⑩ • Ice crystals ⓪①②③④⑤⑥⑦⑧⑨⑩
Taste • Sweetness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Nutty taste ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky taste ⓪①②③④⑤⑥⑦⑧⑨⑩	Overall performance (9-Point Hedonic method) **1**=Dislike Extremely **2**= Dislike Very Much **3**=Dislike Moderately **4**= Dislike Slightly **5**= Neither Like nor Dislike **6**= Like Slightly **7**= Like Moderately **8**= Like Very Much **9**= Like Extremely your score__________

sample485

Properties/features/characteristics of **sample485** that you like

____

Properties/features/characteristics of **sample485** that you dislike, and why?

____

How likely are you to consumer this ice cream again?

Unlikely / neutral / more likely

Numerical Descriptive Scale for Sensory:

Please rate the intensity of each sub-sensory characteristic of the ice cream using a shaded number from 0-10, where: (0-very weak & 10-very strong)

Colour • Whiteness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Yellowness ⓪①②③④⑤⑥⑦⑧⑨⑩	Smell • Nutty smell ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky smell ⓪①②③④⑤⑥⑦⑧⑨⑩
Texture • Hardness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Stickiness ⓪①②③④⑤⑥⑦⑧⑨⑩	Mouthfeel • Creaminess ⓪①②③④⑤⑥⑦⑧⑨⑩ • Ice crystals ⓪①②③④⑤⑥⑦⑧⑨⑩
Taste • Sweetness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Nutty taste ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky taste ⓪①②③④⑤⑥⑦⑧⑨⑩	Overall performance (9-Point Hedonic method) **1**=Dislike Extremely **2**= Dislike Very Much **3**=Dislike Moderately **4**= Dislike Slightly **5**= Neither Like nor Dislike **6**= Like Slightly **7**= Like Moderately **8**= Like Very Much **9**= Like Extremely your score__________

ICE CREAM SENSORY TEST

Sample642

Properties/features/characteristics of **sample642** that you like

____

Properties/features/characteristics of **sample642** that you dislike, and why?

____

How likely are you to consumer this ice cream again?

Unlikely / neutral / more likely

Numerical Descriptive Scale for Sensory:

Please rate the intensity of each sub-sensory characteristic of the ice cream using a shaded number from 0-10, where: (0-very weak & 10-very strong)

Colour • Whiteness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Yellowness ⓪①②③④⑤⑥⑦⑧⑨⑩	Smell • Nutty smell ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky smell ⓪①②③④⑤⑥⑦⑧⑨⑩
Texture • Hardness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Stickiness ⓪①②③④⑤⑥⑦⑧⑨⑩	Mouthfeel • Creaminess ⓪①②③④⑤⑥⑦⑧⑨⑩ • Ice crystals ⓪①②③④⑤⑥⑦⑧⑨⑩
Taste • Sweetness ⓪①②③④⑤⑥⑦⑧⑨⑩ • Nutty taste ⓪①②③④⑤⑥⑦⑧⑨⑩ • Milky taste ⓪①②③④⑤⑥⑦⑧⑨⑩	Overall performance (9-Point Hedonic method) **1**=Dislike Extremely **2**= Dislike Very Much **3**=Dislike Moderately **4**= Dislike Slightly **5**= Neither Like nor Dislike **6**= Like Slightly **7**= Like Moderately **8**= Like Very Much **9**= Like Extremely your score__________

Appendix B : the 9-point hedonic scale questionnaire (a) and JAR questionnaire (b) for consumer sensory evaluation

COLLAPSE ALL

Please select the following phrases to describe the **Colour** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Aroma** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Texture** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Sweetness** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Taste** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Mouthfeel** of this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Please select the following phrases to describe the **Overall Liking** for this product.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Page 1

(a)

COLLAPSE ALL

Please indicate your opinion about the **Colour** of this product

Much Too White	Slightly Too White	Just About Right	Slightly Too Yellow	Much Too Yellow
○	○	○	○	○

Please indicate your opinion about the **Aroma** of this product

Much Too Hemp Seed Aroma	Slightly Too Hemp Seed Aroma	Just About Right	Slightly Too Milk Aroma	Much Too Milk Aroma
○	○	○	○	○

Please indicate your opinion about the **Taste** of this product

Much Too Hemp Seeds Flavour	Slightly Too Hemp Seed Flavour	Just About Right	Slightly Too Milk Flavour	Much Too Milk Flavour
○	○	○	○	○

Please indicate your opinion about the **Sweetness** of this product

Not At All Sweet Enough	Not Quite Sweet Enough	Just About Right	Slightly Too Sweet	Much Too Sweet
○	○	○	○	○

Please indicate your opinion about the **Texture** of this product

Much Too Soft	Slightly Too Soft	Just About Right	Slightly Too Hard	Much Too Hard
○	○	○	○	○

Please indicate your opinion about the **Mouthfeel** of this product

Much Too Creamy	Slightly Too Creamy	Just About Right	Slightly Too Thin	Much Too Thin
○	○	○	○	○

Page 2

(b)

Appendix C : the 9-point hedonic scale questionnaire for storage stability sensory evaluation

COLLAPSE ALL

How much you like or dislike the **Colour** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Aroma (smell)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Texture** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Taste (Flavour)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Sweetness** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Mouthfeel** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

How much you like or dislike the **Overall of this product?**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
○	○	○	○	○	○	○	○	○

Page 1

Appendix D : Raw data

D.1 Raw data phase 1: CSM and hemp milk preparation

D.1.1 Moisture content and water activity of CSM

1/1

MASSEY UNIVERSITY
COLLEGE OF SCIENCES

Nutrition Laboratory
MASSEY UNIVERSITY
T: +64 **6 9517579**
Email: F.S.Jackson@massey.ac.nz
http://nutritionlab.massey.ac.nz

TO:	**Waylon Zhang**	**AT:**	**Massey University**		
SUBJECT:	**Final Report**	**DATE:**	**15/08/23**	**ON:**	
TRIAL:	**TN23-582**	***SAMPLES RECEIVED:***	**4/08/23**		

Number of pages in this report: 1 **Client Reference:** NP96312ZHAWE

Testing initiated: *4/08/23* **Testing completed:** *15/08/23*

TN23-582 **Hemp Milk** Results are on an as received basis

NutLab ID	Sample Name	Crude Protein %	Fat %
TN23-582-01	Hemp Milk	1.8	3.5

Methodology

Crude protein : AOAC 968.06 (Dumas method). N-P = 5.3
Fat : (Mojonnier) Acid, (Flour, Baked, extruded products) AOAC 922.06

WDesai.
Wibha Desai
IANZ Key Technical Person

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Fliss Jackson
Manager, Nutrition Laboratory
School of Food and Advanced Technology
Massey University, Private Bag 11222
Palmerston North 4442, New Zealand
DDI 06 9517579
Email. F.S.Jackson@massey.ac.nz

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ilac-MRA ACCREDITED IANZ TESTING LABORATORY Nº 1363, 926, 927

School of Food and Advanced Technology
Private Bag 11222, Palmerston North 4442, New Zealand

D.1.2 Moisture content and water activity of CSM

	Replication	Raw data
Water activity	1	0.4927
	2	0.4843
	3	0.4872
Moisture content (%)	1	3.01
	2	2.95
	3	2.91

D.1.3 Total solids of hemp milk

	Replication	Raw data
total solids (%)	1	9.68
	2	9.38
	3	9.08

D.1.4 Dissolved sugar of hemp milk

	Replication	Raw data
Dissolved sugar (°Brix)	1	4.46
	2	4.45
	3	4.39

D.2 Raw data phase 2: Selection of the most promising ice cream formulation

D.2.1 pH of ice cream mix

Sample	Replication	pH
C	1	6.66
	2	6.70
S1	1	6.75
	2	6.79
S2	1	6.85
	2	6.72
S3	1	6.90
	2	6.91
S4	1	6.94
	2	6.92

D.2.2 Overrun of ice cream

Sample	Replication	Overrun
C	1	25.50%
	2	26.11%
	3	23.50%
S1	1	31.69%
	2	31.28%
	3	32.64%
S2	1	36.11%
	2	33.76%
	3	33.19%
S3	1	39.16%
	2	38.87%
	3	41.55%
S4	1	42.56%
	2	43.43%
	3	42.22%

D.2.3 Meltdown of ice cream

Sample	Replication	Melting rate	First dripping time
C	1	0.6563	24.4
	2	0.6351	23.6
S1	1	0.5803	29.1
	2	0.5697	29.3
S2	1	0.4452	33.6
	2	0.4479	34.0
S3	1	0.3419	35.0
	2	0.3549	34.5
S4	1	0.3171	34.0
	2	0.3352	33.9

D.2.4 The melting curves data of five ice cream samples

Sample C

Time	Replication 1	Replication 2
0min	0.00	0.00
5min	0.00	0.00
10min	0.00	0.00
15min	0.00	0.00
20min	0.00	0.00
25min	0.24	0.53
30min	1.14	1.18
35min	4.08	3.98
40min	9.87	8.64
45min	13.97	13.94
50min	20.90	21.36
55min	27.24	27.72
60min	32.77	32.84
65min	39.61	39.26
70min	45.31	44.76
75min	49.22	47.63

Sample S1

Time	Replication 1	Replication 2
0min	0.00	0.00
5min	0.00	0.00
10min	0.00	0.00
15min	0.00	0.00
20min	0.00	0.00
25min	0.00	0.00
30min	0.31	0.24
35min	1.64	1.12
40min	3.80	3.34
45min	10.56	10.18
50min	15.98	14.46
55min	21.48	20.91
60min	27.54	27.01
65min	33.45	32.91
70min	38.11	37.15
75min	43.52	42.73

Sample S2

Time	Replication 1	Replication 2
0min	0.00	0.00
5min	0.00	0.00
10min	0.00	0.00
15min	0.00	0.00
20min	0.00	0.00
25min	0.00	0.00
30min	0.00	0.00
35min	0.37	0.45
40min	1.65	1.59
45min	3.10	3.20
50min	5.04	5.35
55min	8.07	8.81
60min	9.90	12.90
65min	20.34	22.01
70min	27.70	28.87
75min	33.39	33.59

Sample S3

Time	Replication 1	Replication 2
0min	0.00	0.00
5min	0.00	0.00
10min	0.00	0.00
15min	0.00	0.00
20min	0.00	0.00
25min	0.00	0.00
30min	0.00	0.00
35min	0.06	0.16
40min	1.02	0.83
45min	2.74	2.89
50min	4.89	5.33
55min	7.15	7.69
60min	11.03	10.53
65min	17.33	17.12
70min	23.33	22.96
75min	25.64	26.62

Sample S4

Time	Replication 1	Replication 2
0min	0.00	0.00
5min	0.00	0.00
10min	0.00	0.00
15min	0.00	0.00
20min	0.00	0.00
25min	0.00	0.00
30min	0.00	0.00
35min	0.11	0.24
40min	0.83	1.03
45min	2.09	2.29
50min	3.54	3.72
55min	6.23	6.39
60min	9.38	9.84
65min	15.64	15.18
70min	19.14	20.13
75min	23.78	25.14

D.2.5 Colour of ice cream

Sample	Replication	L*	a*	b*	WI
C	1	65.58	-3.29	+10.97	63.72
	2	65.65	-3.26	+10.04	64.06
	3	69.21	-3.27	+10.33	67.36
S1	1	66.05	-2.69	+10.19	64.45
	2	73.51	-2.91	+10.21	71.46
	3	71.21	-2.91	+10.29	69.29
S2	1	72.19	-2.05	+9.46	70.55
	2	68.74	-2.06	+9.36	67.30
	3	73.37	-2.12	+9.10	71.78
S3	1	74.36	-2.03	+7.90	73.09
	2	74.28	-1.94	+10.36	72.20
	3	72.92	-1.98	+9.01	71.39
S4	1	77.46	-1.38	+9.10	75.65
	2	77.17	-1.36	+9.09	75.39
	3	77.06	-1.39	+9.97	74.95

D.2.6 Possibility of repurchasing five ice cream samples

sample	Possibility of repurchasing	participants
C	unlikely	2
	neutral	3
	likely	1
S1	unlikely	0
	neutral	2
	likely	4
S2	unlikely	1
	neutral	3
	likely	2
S3	unlikely	2
	neutral	3
	likely	1
S4	unlikely	3
	neutral	2
	likely	1

D.2.7 Sensory evaluation scores of five ice cream samples

Sample	participant	colour	smell	texture	mouthfeel	flavour
C	1	6	7	3	6	7
	2	6	6	6	5	7
	3	7	5	4	4	6
	4	6	5	5	4	5
	5	8	4	5	4	5
	6	5	7	6	7	7
S1	1	6	7	8	7	6
	2	8	7	7	7	7
	3	7	4	7	5	6
	4	6	4	7	6	6
	5	6	6	5	5	5
	6	7	8	5	7	7
S2	1	4	6	7	4	4
	2	5	5	8	6	4
	3	4	5	8	6	5
	4	5	4	4	5	3
	5	4	4	5	4	3
	6	6	6	5	6	6
S3	1	7	6	7	4	5
	2	6	6	8	6	6
	3	4	4	4	5	5
	4	4	4	4	5	5
	5	4	4	4	4	3
	6	5	6	4	6	6
S4	1	4	6	4	5	5
	2	6	5	6	6	2
	3	5	4	5	5	5
	4	6	5	3	4	3
	5	5	3	3	4	3
	6	4	7	2	6	6

D.3 Raw data phase 3: The best formulation ice cream

D.3.1 Physical properties of the best formulation ice cream

Parameters	Replication	Data
pH	1	6.77
	2	6.82
	3	6.75
Density (g/cm^3)	1	0.844
	2	0.891
	3	0.853
L*	1	72.31
	2	72.31
	3	72.5
a*	1	-2.73
	2	-2.72
	3	-2.68
b*	1	9.24
	2	9.28
	3	8.99
WI	1	70.68
	2	70.67
	3	70.94
Overrun (%)	1	39.64
	2	39.93
	3	39.79
Hardness (N)	1	35.51
	2	34.92
	3	34.32
Stickiness (N)	1	-2.86
	2	-2.87
	3	-2.85
Melting rate	1	0.535
	2	0.541
	3	0.546
First dripping time (min)	1	26.5
	2	28.2
	3	29

D.3.2 Particle size of ice cream mix and ice cream

Sample	Replication	D [3,2]	D [4,3]	Dx (10)	Dx (50)	Dx (90)
Aged mix (µm)	1	1.81	6.5	0.64	3.43	18.1
	2	1.87	7.14	0.653	3.57	20
	3	1.91	7.59	0.663	3.69	21.2
	4	1.95	8.04	0.672	3.81	22.3
	5	1.97	8.17	0.677	3.88	22.3
Ice cream melt (µm)	1	4.01	97.5	1.77	25.1	322
	2	3.89	102	1.68	25.9	351
	3	3.68	86.3	1.54	21.6	288
	4	3.64	90	1.51	21.5	314
	5	3.56	81.2	1.45	20	277

D.3.3 Apparent viscosity of ice cream mix and ice cream

Replication 1		Replication 2	
Shear rate (1/s)	**Viscosity (Pa.s)**	**Shear rate (1/s)**	**Viscosity (Pa.s)**
0.09976	56.22	0.09976	56.2
0.1257	48.33	0.1257	47.97
0.1583	40.21	0.1583	40.11
0.1996	33.73	0.1995	33.55
0.2508	28.97	0.2509	28.01
0.3161	25.36	0.316	23.8
0.398	21.79	0.3975	20.4
0.5008	18.55	0.5009	17.46
0.6313	15.63	0.6302	15.02
0.7941	13.3	0.794	12.6
1.002	11.28	0.9995	10.7
1.258	9.479	1.258	9.062
1.583	7.967	1.582	7.722
1.999	6.609	1.997	6.523
2.509	5.542	2.512	5.492
3.159	4.659	3.159	4.57
3.979	3.928	3.974	3.808
5.012	3.309	5.007	3.179
6.315	2.802	6.309	2.686
7.94	2.378	7.936	2.279
10	2.026	9.993	1.92
12.59	1.721	12.58	1.638
15.84	1.459	15.84	1.386
19.95	1.236	19.95	1.17
25.12	1.043	25.11	0.9875
31.62	0.88	31.61	0.834
39.81	0.7399	39.79	0.7014
50.11	0.6217	50.1	0.5893
63.09	0.5222	63.07	0.4952
79.42	0.4383	79.4	0.4184
99.99	0.3693	99.95	0.3533

D.3.4 Proximate analysis results of ice cream

MASSEY UNIVERSITY
COLLEGE OF SCIENCES

Nutrition Laboratory
MASSEY UNIVERSITY
T: +64 **6 9517579**
Email: F.S.Jackson@massey.ac.nz
http://nutritionlab.massey.ac.nz

TO:	Waylon Zhang	**AT:** Massey University	
SUBJECT:	Final Report	**DATE:** 7/12/23	**ON:**
TRIAL:	TN23-772	***SAMPLES RECEIVED:*** 27/10/23	

Number of pages in this report: 1 **Client Reference:** cardlock account number: NP96312ZHAWE

Testing initiated: *27/10/23* **Testing completed:** *7/12/23*

TN23-772 Plant based ice cream Results are on an as received basis

NutLab ID	Sample Name	Moisture %	Ash %	Crude Protein %	Fat %	IDF %	SDF %	TDF %
TN23-772-01	Ice Cream	71.2	0.5	2.3	9.8	<0.1	0.2	0.2

NB: Sample arrived defrosted, at room temperature

Methodology

Ash : Furnace 550°C AOAC 942.05 (Feed, meat)

* *Moisture : AOAC 925.45A, 934.01, Vacuum oven*

Crude protein : AOAC 968.06 (Dumas method). N-P = 6.25

Fat : (Mojonnier) Acid, (Flour, Baked, extruded products) AOAC 922.06

* *TDF + Sol + Insol fibre : Megazyme, AOAC 991.43*

*Tests marked with an asterix are currently outside the scope of the Nutrition Laboratory's accreditation

Karl Dale
IANZ Key Technical Person

Wibha Desai
IANZ Key Technical Person

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Fliss Jackson
Manager, Nutrition Laboratory
School of Food and Advanced Technology
Massey University, Private Bag 11222
Palmerston North 4442, New Zealand
DDI 06 9517579
Email. F.S.Jackson@massey.ac.nz

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Test results indicated as not accredited are outside the scope of the laboratory's accreditation

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Nº 1363, 926, 927

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Private Bag 11222, Palmerston North 4442, New Zealand

D.3.5 consumer sensory attribute scores of ice cream sample

Replication 1

participant	Colour	Aroma	Texture	Taste	Sweetness	Mouthfeel	Overall
1	8	8	8	9	7	8	8
2	7	8	7	9	7	8	8
3	9	7	9	9	9	9	9
4	9	7	9	9	9	7	9
5	8	7	9	9	9	7	9
6	8	8	8	8	7	8	8
7	5	5	5	5	5	5	9
8	6	7	7	6	5	6	4
9	5	5	7	4	4	6	5
10	6	6	5	7	9	8	7

Replication 2

participant	Colour	Aroma	Texture	Taste	Sweetness	Mouthfeel	Overall
1	5	5	4	3	6	4	5
2	7	6	5	4	7	6	5
3	5	6	4	6	4	6	4
4	7	5	6	5	4	9	5
5	4	5	7	5	7	8	4
6	7	7	8	6	4	6	6
7	6	8	8	9	8	8	8
8	5	6	5	5	5	8	5
9	8	7	6	9	9	8	8
10	8	8	5	8	7	6	8

Replication 3

participant	Colour	Aroma	Texture	Taste	Sweetness	Mouthfeel	Overall
1	7	8	8	7	8	7	8
2	8	6	6	6	6	6	7
3	7	6	5	5	5	5	5
4	5	6	6	6	7	6	8
5	8	6	6	6	6	5	6
6	8	5	5	9	9	6	5
7	8	5	7	8	6	5	8
8	5	7	7	7	7	7	7
9	6	8	8	8	8	8	8
10	5	7	8	7	7	9	6

D.3.5 participant of ice cream sample in JAR test

	participant					
	Colour	Aroma	Texture	Taste	Sweetness	Mouthfeel
Too little	3	6	3	8	7	4
Just about right	22	18	22	17	21	22
Too strong	5	6	5	5	2	4

D.4 Raw data phase 4: Storage stability of the best formulation of ice cream

D.4.1 Changes in pH of the best formulation ice cream during storage at -19 °C for 21 days

Days	Replication	pH
1	1	6.75
	2	6.605
	3	6.83
7	1	6.643
	2	6.78
	3	6.801
14	1	6.676
	2	6.65
	3	6.826
21	1	6.801
	2	6.68
	3	6.651

D.4.2 Changes in colour of the best formulation ice cream during storage at -19 °C for 21 days

Sample	Replication	L*	a*	b*
C	1	72.31	-2.73	9.24
	2	72.31	-2.72	9.28
	3	72.5	-2.68	8.99
S1	1	71.08	-2.75	9.25
	2	72.35	-2.76	9.24
	3	72.39	-2.75	9.33
S2	1	72.16	-2.79	9.69
	2	72.15	-2.81	9.72
	3	70.19	-2.84	9.26
S3	1	70.17	-2.87	9.29
	2	70.13	-2.86	9.28
	3	70.1	-2.88	9.31
S4	1	72.31	-2.73	9.24
	2	72.31	-2.72	9.28
	3	72.5	-2.68	8.99

D.4.2 Changes in hardness of the best formulation ice cream during storage at -19 °C for 21 days

Days	Replication	Hardness (N)
1	1	34.0403
	2	34.2183
	3	34.3910
7	1	34.5593
	2	34.7522
	3	34.9366
14	1	35.1252
	2	35.2840
	3	35.4013
21	1	35.4439
	2	35.4482
	3	35.4737

Appendix E : Statistical output

E.1 Statistical output phase 1: Selection of the most promising ice cream formulation

E.1.1 pH of ice cream mix

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.08494	0.021235	10.31	0.012
Error	5	0.01030	0.002060		
Total	9	0.09524			

Means

Sample	N	Mean	StDev	95% CI
C	2	6.6800	0.0283	(6.5975, 6.7625)
S1	2	6.7700	0.0283	(6.6875, 6.8525)
S2	2	6.7850	0.0919	(6.7025, 6.8675)
S3	2	6.90500	0.00707	(6.82250, 6.98750)
S4	2	6.9300	0.0141	(6.8475, 7.0125)

Pooled StDev = 0.0453872

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping	
S4	2	6.9300	A	
S3	2	6.90500	A	
S2	2	6.7850	A	B
S1	2	6.7700	A	B
C	2	6.6800		B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
S1 - C	0.0900	0.0454	(-0.0920, 0.2720)	1.98	0.386
S2 - C	0.1050	0.0454	(-0.0770, 0.2870)	2.31	0.276
S3 - C	0.2250	0.0454	(0.0430, 0.4070)	4.96	0.022
S4 - C	0.2500	0.0454	(0.0680, 0.4320)	5.51	0.014
S2 - S1	0.0150	0.0454	(-0.1670, 0.1970)	0.33	0.997
S3 - S1	0.1350	0.0454	(-0.0470, 0.3170)	2.97	0.139
S4 - S1	0.1600	0.0454	(-0.0220, 0.3420)	3.53	0.080
S3 - S2	0.1200	0.0454	(-0.0620, 0.3020)	2.64	0.196
S4 - S2	0.1450	0.0454	(-0.0370, 0.3270)	3.19	0.111
S4 - S3	0.0250	0.0454	(-0.1570, 0.2070)	0.55	0.977

Individual confidence level = 98.98%

E.1.2 Overrun of ice cream

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.057809	0.014452	99.00	0.000
Error	10	0.001460	0.000146		
Total	14	0.059269			

Means

Sample	N	Mean	StDev	95% CI
C	3	0.25037	0.01365	(0.23482, 0.26591)
S1	3	0.31870	0.00698	(0.30316, 0.33424)
S2	3	0.34353	0.01548	(0.32799, 0.35908)
S3	3	0.39860	0.01471	(0.38306, 0.41414)
S4	3	0.42737	0.00624	(0.41182, 0.44291)

Pooled StDev = 0.0120821

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping		
S4	3	0.42737	A		
S3	3	0.39860	A		
S2	3	0.34353		B	
S1	3	0.31870		B	
C	3	0.25037			C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
S1 - C	0.06833	0.00987	(0.03590, 0.10077)	6.93	0.000
S2 - C	0.09317	0.00987	(0.06073, 0.12560)	9.44	0.000
S3 - C	0.14823	0.00987	(0.11580, 0.18067)	15.03	0.000
S4 - C	0.17700	0.00987	(0.14456, 0.20944)	17.94	0.000
S2 - S1	0.02483	0.00987	(-0.00760, 0.05727)	2.52	0.162
S3 - S1	0.07990	0.00987	(0.04746, 0.11234)	8.10	0.000
S4 - S1	0.10867	0.00987	(0.07623, 0.14110)	11.02	0.000
S3 - S2	0.05507	0.00987	(0.02263, 0.08750)	5.58	0.002
S4 - S2	0.08383	0.00987	(0.05140, 0.11627)	8.50	0.000
S4 - S3	0.02877	0.00987	(-0.00367, 0.06120)	2.92	0.089

Individual confidence level = 99.18%

E.1.3 First dripping time of ice cream

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	165.494	41.3735	376.12	0.000
Error	5	0.550	0.1100		
Total	9	166.044			

Means

Sample	N	Mean	StDev	95% CI
C	2	24.000	0.566	(23.397, 24.603)
S1	2	29.200	0.141	(28.597, 29.803)
S2	2	33.800	0.283	(33.197, 34.403)
S3	2	34.750	0.354	(34.147, 35.353)
S4	2	33.9500	0.0707	(33.3471, 34.5529)

Pooled StDev = 0.331662

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping		
S3	2	34.750	A		
S4	2	33.9500	A		
S2	2	33.800	A		
S1	2	29.200		B	
C	2	24.000			C

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
S1 - C	5.200	0.332	(3.870, 6.530)	15.68	0.000
S2 - C	9.800	0.332	(8.470, 11.130)	29.55	0.000
S3 - C	10.750	0.332	(9.420, 12.080)	32.41	0.000
S4 - C	9.950	0.332	(8.620, 11.280)	30.00	0.000
S2 - S1	4.600	0.332	(3.270, 5.930)	13.87	0.000
S3 - S1	5.550	0.332	(4.220, 6.880)	16.73	0.000
S4 - S1	4.750	0.332	(3.420, 6.080)	14.32	0.000
S3 - S2	0.950	0.332	(-0.380, 2.280)	2.86	0.156
S4 - S2	0.150	0.332	(-1.180, 1.480)	0.45	0.989
S4 - S3	-0.800	0.332	(-2.130, 0.530)	-2.41	0.250

Individual confidence level = 98.98%

E.1.4 Melting rate of ice cream

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.155823	0.038956	365.54	0.000
Error	5	0.000533	0.000107		
Total	9	0.156356			

Means

Sample	N	Mean	StDev	95% CI
C	2	0.6457	0.0150	(0.6269, 0.6645)
S1	2	0.57500	0.00750	(0.55624, 0.59376)
S2	2	0.44655	0.00191	(0.42779, 0.46531)
S3	2	0.34840	0.00919	(0.32964, 0.36716)
S4	2	0.32615	0.01280	(0.30739, 0.34491)

Pooled StDev = 0.0103233

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping			
C	2	0.6457	A			
S1	2	0.57500		B		
S2	2	0.44655			C	
S3	2	0.34840				D
S4	2	0.32615				D

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
S1 - C	-0.0707	0.0103	(-0.1121, -0.0293)	-6.85	0.005
S2 - C	-0.1991	0.0103	(-0.2405, -0.1578)	-19.29	0.000
S3 - C	-0.2973	0.0103	(-0.3387, -0.2559)	-28.80	0.000
S4 - C	-0.3195	0.0103	(-0.3609, -0.2782)	-30.95	0.000
S2 - S1	-0.1284	0.0103	(-0.1698, -0.0871)	-12.44	0.000
S3 - S1	-0.2266	0.0103	(-0.2680, -0.1852)	-21.95	0.000
S4 - S1	-0.2488	0.0103	(-0.2902, -0.2075)	-24.11	0.000
S3 - S2	-0.0982	0.0103	(-0.1395, -0.0568)	-9.51	0.001
S4 - S2	-0.1204	0.0103	(-0.1618, -0.0790)	-11.66	0.000
S4 - S3	-0.0222	0.0103	(-0.0636, 0.0191)	-2.16	0.325

Individual confidence level = 98.98%

E.1.5 Colour of ice cream

(1)L*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	4	183.05	45.762	9.01	0.002
Error	10	50.78	5.078		
Total	14	233.83			

Means

sample	N	Mean	StDev	95% CI
C	3	66.81	2.08	(63.91, 69.71)
S1	3	70.26	3.82	(67.36, 73.16)
S2	3	71.43	2.41	(68.53, 74.33)
S3	3	73.853	0.809	(70.954, 76.752)
S4	3	77.230	0.207	(74.331, 80.129)

Pooled StDev = 2.25344

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping		
S4	3	77.230	A		
S3	3	73.853	A	B	
S2	3	71.43	A	B	C
S1	3	70.26		B	C
C	3	66.81			C

Means that do not share a letter are significantly different.

(2)a*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	4	6.71296	1.67824	418.17	0.000
Error	10	0.04013	0.00401		
Total	14	6.75309			

Means

sample	N	Mean	StDev	95% CI
c	3	-3.27333	0.01528	(-3.35483, -3.19184)
s1	3	-2.8367	0.1270	(-2.9182, -2.7552)
s2	3	-2.0767	0.0379	(-2.1582, -1.9952)
s3	3	-1.9833	0.0451	(-2.0648, -1.9018)
s4	3	-1.37667	0.01528	(-1.45816, -1.29517)

Pooled StDev = 0.0633509

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping			
s4	3	-1.37667	A			
s3	3	-1.9833		B		
s2	3	-2.0767		B		
s1	3	-2.8367			C	
c	3	-3.27333				D

Means that do not share a letter are significantly different.

(3)b*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	4	4.389	1.0973	2.69	0.093
Error	10	4.073	0.4073		
Total	14	8.463			

Means

sample	N	Mean	StDev	95% CI
c	3	10.447	0.476	(9.626, 11.268)
s1	3	10.2300	0.0529	(9.4090, 11.0510)
s2	3	9.307	0.186	(8.486, 10.128)
s3	3	9.090	1.232	(8.269, 9.911)
s4	3	9.387	0.505	(8.566, 10.208)

Pooled StDev = 0.638232

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping
c	3	10.447	A
s1	3	10.2300	A
s4	3	9.387	A
s2	3	9.307	A
s3	3	9.090	A

Means that do not share a letter are significantly different.

(4)White index

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
sample	4	180.97	45.242	9.78	0.002
Error	10	46.25	4.625		
Total	14	227.22			

Means

sample	N	Mean	StDev	95% CI
c	3	65.05	2.01	(62.28, 67.81)
s1	3	68.40	3.59	(65.63, 71.17)
s2	3	69.88	2.31	(67.11, 72.64)
s3	3	72.227	0.850	(69.460, 74.993)
s4	3	75.330	0.354	(72.563, 78.097)

Pooled StDev = 2.15070

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping		
s4	3	75.330	A		

s3	3	72.227	A	B	
s2	3	69.88	A	B	C
s1	3	68.40		B	C
c	3	65.05			C

Means that do not share a letter are significantly different.

E.2 Statistical output phase 4: Storage stability of the best formulation of ice cream

E.2.1 Changes in pH of the best formulation ice cream during storage at -19 °C for 21 days

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage day	3	0.001622	0.000541	0.06	0.979
Error	8	0.071453	0.008932		
Total	11	0.073075			

Means

Storage day	N	Mean	StDev	95% CI
D1	3	6.7283	0.1141	(6.6025, 6.8542)
D14	3	6.7173	0.0950	(6.5915, 6.8432)
D21	3	6.7107	0.0796	(6.5848, 6.8365)
D7	3	6.7413	0.0858	(6.6155, 6.8672)

Pooled StDev = 0.0945071

Grouping Information Using the Tukey Method and 95% Confidence

Storage day	N	Mean	Grouping
D7	3	6.7413	A
D1	3	6.7283	A
D14	3	6.7173	A
D21	3	6.7107	A

Means that do not share a letter are significantly different.

E.2.2 Changes in colour of the best formulation ice cream during storage at -19 °C for 21 days

(1)L*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
storage	3	8.470	2.8234	6.09	0.018
Error	8	3.711	0.4639		
Total	11	12.181			

Means

storage	N	Mean	StDev	95% CI
1	3	72.3733	0.1097	(71.4666, 73.2801)
7	3	71.940	0.745	(71.033, 72.847)
14	3	71.500	1.135	(70.593, 72.407)
21	3	70.1333	0.0351	(69.2266, 71.0401)

Pooled StDev = 0.681078

Grouping Information Using the Tukey Method and 95% Confidence

storage	N	Mean	Grouping	
1	3	72.3733	A	
7	3	71.940	A	
14	3	71.500	A	B
21	3	70.1333		B

Means that do not share a letter are significantly different.

(2)a*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
storage	3	0.043933	0.014644	39.94	0.000
Error	8	0.002933	0.000367		
Total	11	0.046867			

Means

storage	N	Mean	StDev	95% CI
1	3	-2.87000	0.01000	(-2.89549, -2.84451)
7	3	-2.8133	0.0252	(-2.8388, -2.7878)
14	3	-2.75333	0.00577	(-2.77883, -2.72784)
21	3	-2.7100	0.0265	(-2.7355, -2.6845)

Pooled StDev = 0.0191485

Grouping Information Using the Tukey Method and 95% Confidence

storage	N	Mean	Grouping		
21	3	-2.7100	A		
14	3	-2.75333	A		
7	3	-2.8133		B	
1	3	-2.87000			C

Means that do not share a letter are significantly different.

(3)b*

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
storage	3	0.2441	0.08136	3.48	0.070
Error	8	0.1872	0.02340		
Total	11	0.4313			

Means

storage	N	Mean	StDev	95% CI
1	3	9.1700	0.1572	(8.9663, 9.3737)
7	3	9.2733	0.0493	(9.0697, 9.4770)
14	3	9.557	0.257	(9.353, 9.760)
21	3	9.29333	0.01528	(9.08967, 9.49699)

Pooled StDev = 0.152971

Grouping Information Using the Tukey Method and 95% Confidence

storage	N	Mean	Grouping
14	3	9.557	A
21	3	9.29333	A
7	3	9.2733	A
1	3	9.1700	A

Means that do not share a letter are significantly different.

E.2.3 Changes in hardness of the best formulation ice cream during storage at -19 °C for 21 days

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Storage time	3	2.7992	0.93307	43.51	0.000
Error	8	0.1716	0.02144		
Total	11	2.9708			

Means

Storage time	N	Mean	StDev	95% CI
1	3	34.217	0.175	(34.022, 34.411)
7	3	34.749	0.189	(34.554, 34.944)
14	3	35.2702	0.1385	(35.0752, 35.4651)
21	3	35.4553	0.0161	(35.2603, 35.6502)

Pooled StDev = 0.146438

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
21	3	35.4553	A		
14	3	35.2702	A		
7	3	34.749		B	
1	3	34.217			C

Means that do not share a letter are significantly different.

E.2.4 Changes in sensory of the best formulation ice cream during storage at -19 °C for 21 days

Colour

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.81	A		
7	20	6.67	A	B	
14	20	6.71	A	B	
21	20	6.76	A	B	

Means that do not share a letter are significantly different.

Aroma

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.38	A		
7	20	6.29	A		
14	20	6.90		B	
21	20	5.90			C

Means that do not share a letter are significantly different.

Texture

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.81	A		
7	20	6.71	A	B	
14	20	6.52		B	
21	20	6.38			C

Means that do not share a letter are significantly different.

Taste

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.38	A		
7	20	7.00		B	
14	20	6.62		B	C
21	20	6.19			C

Means that do not share a letter are significantly different.

Sweetness

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.29	A		
7	20	6.57		B	
14	20	6.48		B	
21	20	6.29	A		

Means that do not share a letter are significantly different.

Mouthfeel

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.62	A		
7	20	6.57	A		
14	20	6.62	A		
21	20	6.24		B	

Means that do not share a letter are significantly different.

Overall

Grouping Information Using the Tukey Method and 95% Confidence

Storage time	N	Mean	Grouping		
1	20	6.48	A		
7	20	6.38	A		
14	20	6.48	A		
21	20	6.33	A		

Means that do not share a letter are significantly different.