Using Freshly-Extracted Chia Seeds Mucilage as a Stabiliser/Emulsifier for Gelato Ice Cream

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Abstract

Plant-derived hydrocolloids contain polysaccharides that can help improve food quality by improving food structure and texture and emulsifying and thickening properties. Seed plants that produce mucilage constituents, including flax, psyllium, yellow mustard, basil, and chia seeds, are among the most promising sources of hydrocolloids. Due to its unique physiochemical and conformational aspects from the hydrophobic and hydrophilic side groups, plant seed mucilage has a great water and oil-holding capacity.

This study introduces a novel approach to gelato formulation, utilising the unique properties of freshly extracted chia seed mucilage. Notably, the mucilage contains uronic acid that can retain water up to 27 times its weight, demonstrating its potential for stabilising the mix and emulsifying activity. The mucilage was extracted at 80°C for 2.5 hours with a 1:20 seed-to-water ratio and was evaluated for its ability as a stabiliser/emulsifier in gelato. With an average extracted mucilage of 4.61 ± 0.39 g (w/w) and an average pH of 6.8 ± 0.07, the mucilage was used in various concentrations: 1.75%, 2%, 2.25%, and 2.5%.

The gelato formulations made with chia mucilage in place of commercial stabiliser were characterised. The pH, fat globule size distribution, overrun, meltdown rate, rheological properties, and texture were determined. The microbiological activity of *Listeria* and viable microbes from the product were checked. The gelato samples were evaluated and discussed through focus groups. The chosen gelato formulation was put through a consumer sensory evaluation to affirm its acceptance.

The best formulation contained 2.25% (w/w) mucilage, which was incorporated into a premade gelato mix at 4000 rpm and mixed for 10 min. Its pH was 6.51 ± 0.03, with an overrun of 24.77 ± 0.94 (v/v), a total meltdown rate of 0.38 ± 0.004 g/min, the fat globule size in the gelato mix was 0.28 ± 0.081 mm while in the gelato melt it was 0.49 ± 0.08 mm. The frozen gelato with 2.25% chia seed mucilage had a hardness of 65.59 ± 8.91 N. No *Listeria* was detected, and <10 viable microbe colonies were detected in the gelato, indicating a hygienic and clean manufacturing, handling, and storage process. The focus group agreed that the best formulation had a creamy consistency without ice crystals. This result was supported by the consumer sensory evaluation that deemed the attributes to be Just-About-Right without any off flavour and off textures from the mucilage. Overall, using chia mucilage as an emulsifier/stabiliser for gelato has shown potential and possibility for further evaluation and uses.

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Chapter 1. Introduction

Chia (*Salvia hispanica* L.) seeds are a superfood because they are rich in antioxidant compounds and essential fatty acids and have a good amount of dietary fibre, proteins, minerals, and vitamins (Ürkek, 2021). Chia seed mucilage extracted from chia seeds can be an alternative to replace the common stabilisers such as locust bean gum and guar gum. When placed in water, it can form a polysaccharide-based gel made from 7-15% soluble fibre (Kulczyński et al., 2019). The gel mainly comprises crude fibre and carbohydrates but also contains uronic acid, which can hold water up to 27 times its weight in water. In addition, the mucilaginous gel formed can be used as an alternative to emulsifying agents, binding agents, thickening agents, and stabilisers (Timilsena et al., 2016; Ürkek, 2021). The fibre in chia seeds is more abundant than other dried fruits, nuts, and cereals (Kulczyński et al., 2019). It has a higher protein content than brown and gold flax seeds (Nitrayová et al., 2014). For this reason, chia seed mucilage has been reported to stabilise ice cream type food systems because of its water-holding and absorption capacities (Timilsena et al., 2016).

Ice cream is a widely popular dessert, and its consumption usually peaks during the summertime (Motyl et al., 2019). New Zealand is the world's number one country with the most ice cream consumption per capita, consuming 28.4 L per year in 2023, and consumers in the USA consumed 20.8 L per year per capita (World Population Review, 2024). Ice cream is a frozen dairy product made by freezing a pasteurised mixture of dairy and other ingredients and agitating it to incorporate air (Arbuckle, 2013). The ice cream mix is usually made with milk products, fat, and sugar in dry or liquid forms; it may or may not contain eggs, flavourings, and often stabilisers and emulsifiers (Arbuckle, 2013; Velotto et al., 2021). The combination of ingredients and the ice cream processing forms the microstructure of the ice cream, which is built from ice crystals, air bubbles, and fat droplets. The microstructure is a crucial part of the ice cream as it contributes to its texture, density, and meltdown rate (Clarke, 2015). A colloidal system is created when the fat droplets, air bubbles, and ice crystals are connected in a continuous phase, making ice cream the most complex food colloid (Clarke, 2015; Motyl et al., 2019). The addition of food additives such as stabilisers and emulsifiers to the ice cream mix are required to improve smoothness, decelerate melting, and regulate the growth of ice crystals during storage (Ürkek, 2021l; Motyl et al., 2019).

Emulsifiers in ice cream are used to improve air incorporation during whipping and reduce the whipping time (Arbuckle, 2013; Goff et al., 2013). Traditionally, egg yolk was used due to its lecithin content. However, soy lecithin is commonly used nowadays as it has a similar composition and lower cost (Goff et al., 2013; Clarke, 2015). Mono/di-glycerides are also used, which promote finer air bubble distribution and enhance ice crystal smoothness (Clarke, 2015; Goff et al., 2013). Stabilisers facilitate air incorporation and help stabilise the foam. It also helps during pumping and filling for more effortless flow, prevents shrinkage, slows down moisture migration during storage, produces smoothness, reduces melt rate, and masks the detection of ice crystals during consumption (Clarke, 2015). Although most emulsifiers/stabilisers are sourced from natural sources, European law still considers stabilisers as additives and are listed as an "E-number" on labels (European Commission, 2008).

Consumers have become more aware of the ingredients in their food and tend to avoid food with additives. This new food trend under the so-called clean labels started when consumers avoided "E-numbers" (Asioli et al., 2017). They correlate the absence of "E-numbers" with clean labels, which means there are fewer or no food additives such as preservatives, flavours, and colours, especially when the additives are artificially made (Osborn, 2015). However, in some foods such as ice cream, the presence of food additives such as stabilisers and emulsifiers, which fall inside the "E-numbers", help improve the textures, structures, mouthfeel, shelf-life, and even improve the stability of the ice cream during storage. Proper emulsification and stabilisation are needed to maintain the right consistency of the ice cream (Motyl et al., 2019). Therefore, using much more familiar ingredients available in a home kitchen, such as chia (*Salvia hispanica*) seeds, may help to fulfil the demand for more natural food stabilisers and emulsifiers (Ürkek, 2021).

There is still a gap in the market because chia seed gum powder as a stabiliser is not available commercially, unlike locust bean gum and guar gum. Feizi et al. (2021) stated that using 0.2% chia seed gum showed comparable results or superior characteristics to guar gum. Previous published research has used dried gum powder. However, the drying process for the gum is costly and takes a long time, no matter the drying method (Chiang et al., 2021; Feizi et al., 2021; Velotto et al., 2021). Therefore, this research opted for the non-drying option and utilised the freshly extracted or wet chia mucilage.

Although research on chia seed mucilage is relatively new, previous research on basil seed gum, which has similar functionality and properties as chia seed mucilage, has been conducted (BahramParvar & Goff, 2013). However, less research has been conducted on gelato than regular ice cream. Gelato is an Italian-style ice cream with milk, cream, sugar, and often egg yolk as its core ingredients. In general, gelato has a lower fat content, around 4-8%, compared to regular dairy ice cream, which has 14-16% fat and a higher sugar content, up to 25%. It is hypothesised that chia seed mucilage will provide stabilisation and emulsification in gelato comparable to commercial stabilisers while providing a new natural source of stabilisers/emulsifiers. This study aims to expand the knowledge of chia seed gum as a stabiliser/emulsifier for gelato and to develop a new gelato stabiliser that is more natural and cheaper without changing the desirable gelato mouthfeel, textures, and structures. This study will only focus on one standard commercial gelato formulation, and churning will be carried out in a lab-scale ice cream maker.

The objectives of the study were:

1. To develop acceptable sensory attributes in gelato formulations with fresh chia seed mucilage through focus groups.

2. To determine the physical and chemical properties and the microbiological content of the gelato ice cream.
3. To determine the consumer acceptability of gelato stabilised with chia seed mucilage.

Chapter 2. Literature Review

2.1 Chia (*Salvia hispanica*) Seeds

Chia seeds come from an annual herbal plant and originates from Southern Mexico and Northern Guatemala (De Falco et al., 2017). They have been commonly cultivated in Mexico, and the two most widely cultivated species are *Salvia* and *Hyptis* from the *Labiatae* family (Weber et al., 1991). The *Salvia hispanica* shrubs (Figure 1) usually grow in the summer and produce schizocarps (fruits) that contain seeds, around 2 mm long that are mottle-coloured with black, white, grey, and brown (Marcinek & Krejpcio, 2017). The chia seed coat secretes a gelatinous polysaccharides mixture known as mucilage that helps the seed disperse in soil, protecting the seed from animals, helping the seed to float or sink in the water, and maintaining hydration and nutrients when water is scarce (Tsai et al., 2021)

Figure 1. Chia (*Salvia hispanica* L.) shrub (Carwood, 2024).

Chia seeds (*Salvia hispanica* L.) have been consumed for centuries due to their medicinal properties (De Falco et al., 2017; Marcinek & Krejpcio, 2017). Research has shown that chia seeds have excellent nutritional value; therefore, they are known as a superfood (Shrestha et al., 2022). Chia seeds are high in antioxidant compounds and essential fatty acids and have high dietary fibre, healthy oils, proteins, minerals, and some vitamins (Ürkek, 2021).

Nowadays, chia seeds are utilised in various ways as they can be consumed as is or added as an ingredient in baked products, muesli, dairy drinks, smoothies and salads, as well as thickeners in soups and sauces (Kulczyński et al., 2019).

2.1.1 Composition and Polysaccharides Content of Chia Seeds

Chia seeds contain high dietary fibre, approximately 30% of its weight (Table 1). The dietary fibre in the chia seeds is divided into insoluble and soluble fibre, with approximately 32.9–34.9 g and 6.2–6.8 g per 100 g, respectively (Reyes-Caudillo et al., 2008). The fibre present in chia seeds is more abundant compared to other dried fruits, nuts, and cereals (Kulczyński et al., 2019). Chia seeds are a promising plant protein, containing 25.3% of protein by weight. This percentage is higher than brown and gold flax seeds, containing 23.5% and 21.2% protein by weight, respectively (Nitrayová et al., 2014).

Table 1. The chemical composition of chia seeds

Components	Nutrients content in chia seeds (g /100g)			
	Weber et al. (1991)	Ixtaina et al. (2011)	Felisberto et al. (2015)	Romankiewicz et al. (2017)
Moisture	-	-	6.5 ± 0.1	8.9 ± 0.0
Protein	20.9	15-25	24.4 ± 0.2	25.7 ± 0.4
Lipids	29.3	30-33	34.1 ± 0.0	31.4 ± 0.1
Ash	4.9	4-5	3.7 ± 0.1	4.05 ± 0.1
Total Dietary Fiber	-	18-30	14.8 ± 0.7	42.9 ± 0.9
Carbohydrates	-	26-41	16.6	-

'-': not given / no data

Chia seeds contain a high quantity of polyunsaturated fatty acids, with around 60% comprising α-linolenic acid (ALA), commonly known as omega-3, and around 19.63% comprising linoleic acid, commonly known as omega-6 (Table 2). The percentage of ALA is higher than that found in flaxseed, with around 15.72% ALA and 57.29% linoleic acid (Ciftci et al., 2012; Nitrayová et al., 2014).

Table 2. Comparison of fatty acid content in chia and flax seeds (Ciftci et al., 2012; Nitrayová et al., 2014).

Fatty Acids		Chia Seeds (%)	Flax Seeds (%)
Saturated Fatty Acid (SFA)	Lauric acid	0.03	0.03
	Myristic acid	0.06	0.06
	Pentadecanoic acid	0.04	0.05
	Palmitic acid	7.07	5.25
	Margaric acid	0.06	0.08
	Stearic acid	3.04	3.24
	Arachidic acid	0.13	0.17
	Behenic acid	0.08	0.14
	Lignoceric acid	0.1	0.09
Monounsaturated Fatty Acid (MUFA)	Palmitoleic acid	0.12	0.06
	Margaric acid	0.06	0.08
	Oleic acid	8.92	18.4
	Eicosenoic acid	0.16	0.2
Polyunsaturated Fatty Acid (PUFA)	Linoleic acid (omega-6)	19.63	15.72
	Linolenic acid (omega-3)	61.78	57.29
	Eicosadienoic acid	0.08	-
Saturated Fatty Acid		9.32	8.33
Monounsaturated Fatty Acid		9.14	18.61
Polyunsaturated Acid		81.54	73.07
Ratio of omega-6 fatty acid / omega-3 fatty acid		0.32	0.27

'-': not given / no data.

In addition, chia seeds are rich in amino acids such as aspartic and glutamic acid, threonine, serine, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and proline (Gómez-Favela et al., 2017; Melo-Ruíz et al., 2016). Chia seeds are also rich in vitamins and minerals such as phosphorus, potassium, calcium, magnesium, sodium, iron, zinc, and copper (Melo-Ruíz et al., 2016), as well as vitamins A, C, E, B1, B2, B3, B9, and B12 (Motyka et al., 2023). Chia seeds are also rich in phytocompounds such as carotenoids, phytosterols, tocopherols, isoflavones, polyphenols, and phenolic compounds, such as rutin, quercetin, and caffeic acid (Ali et al., 2021; Rajput et al., 2021).

Chia seeds have exceptional mucilaginous properties and have been deemed a potential polysaccharide gum source by FAO (Muñoz et al., 2012). Their ability to retain water due to uronic acid in the mucilage makes them a potential ingredient for different applications such as thickening and gelling agents, emulsion stabilisation, and stabiliser (Muñoz et al., 2012; Timilsena et al., 2016). Thus far, there is no evidence of allergenicity caused by chia seeds and their derived products (EFSA, 2009). Chia seeds are also naturally gluten-free (Muñoz et al., 2012; Kulczyński et al., 2019).

2.2 Chia Seeds Mucilage

Chia seed gum is the coat surrounding the chia seed (Figure 2); when hydrated, the gum swells and creates a polysaccharide-based gel layer made from 5% soluble fibre, which appears clear when dissolved in water (Muñoz et al., 2012; Timilsena et al., 2016). This fibre corresponds to a high molecular weight (800-2000 kDa) polysaccharide gum (Xiao et al., 2023).

Figure 2. Chia seeds (Morris & Morris, 2013)

It was proposed that the mucilage is a tetrasaccharide with a main chain consisting of units of (1→4)-β-d-xylopyranosyl-(1→4)-α-d-glucopyranosyl-(1→4)-β-d-xylopyranosyl with 4-O-methyl-α-d-glucuronic acid ramifications in the O-2 position of β-d-xylopyranosyl in the main chain (Figure 3) (Lin et al., 1994). The ratio of β-d-xylose to α-d-glucose monosaccharides to 4-O-methyl-α-d-glucuronic acid is 2:1:1 (Lin et al., 1994). In addition, the mucilage has monosaccharide units such as xylose, glucose, arabinose, galactose, and uronic acid (Timilsena et al., 2016). The uronic acid is attached to position 2 or 4 of the d-xylosyl residue of the chia mucilage (Lin et al., 1994). The uronic acid offers a unique quality as it can hold water up to 27 times its weight once hydrated (Ürkek, 2021). Timilsena et al. (2016) found surface activity and emulsifying properties, which were superior if not comparable to other common gums such as guar and xanthan gum.

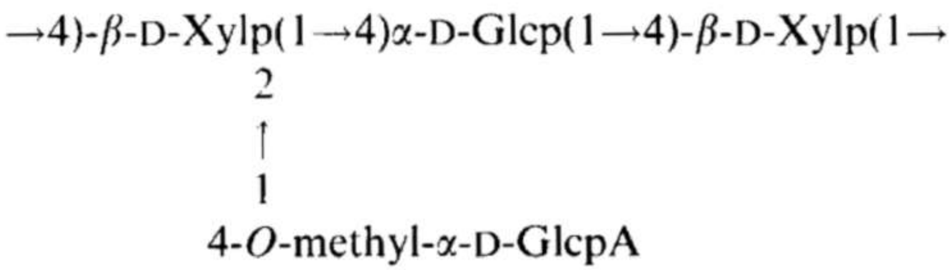

Figure 3. Proposed repeating chia seed mucilage unit structure (Lin et al., 1994).

2.2.1 Extraction Methods and Conditions for Recovering Chia Seed Mucilage

Several people have used aqueous extraction to extract chia seed mucilage over the years, altering the conditions slightly each time. The extraction method involves the seed-to-water ratio, hydration time, temperature conditions, and sometimes water pH.

Muñoz et al. (2012) extracted the mucilage by running three centre points for each condition evaluated, at high and low temperatures (4, 40, and 80 ± 1.5°C), pH (4, 6, and 8), and seed-to-water ratio (1:20, 1:30, and 1:40). The chia seeds were placed in 1 L beakers and distilled water was added in 1:20, 1:30, and 1:40 ratio. The pH was adjusted and maintained during extraction using 0.2 M sodium hydroxide (NaOH) or hydrochloric acid (HCl) solutions. The temperatures were maintained using a temperature controller whilst constantly stirring using a magnetic stirrer for two hours. The study concluded that a temperature of 80°C, water with pH 8, and a 1:40 seed-to-water ratio were the best variables for extracting the mucilage. The extracted mucilage that was still attached to the seeds was dried at 50°C for 10 h. The dried mucilage was separated from the seed using a 40-mesh screen (0.47 mm aperture size).

Campos et al. (2016) used response surface methodology to determine the best extraction variables. In this study, the temperature was maintained using a water bath, and the seeds were stirred constantly. pH was not one of the variables; however, hydration time was. The study concluded that a temperature of 80°C, a hydration time of 4 hours, and a 1:30 seed-to-water ratio were the best conditions for extracting the mucilage. The extracted mucilage was dried at 50°C, the same temperature as the previous study, using an oven with forced air circulation for overnight before separating the gum using a 30 mm screen. However, the use of this screen is highly unlikely as chia seeds are around 2 mm in size (Marcinek & Krejpcio, 2017).

Timilsena et al. (2016) used a 1:20 seed-to-water ratio and a hydration time of 2 hours without any mention of water pH and temperature. They used a freeze drier and 200 µm sieves to separate the seed from the gum. However, this study used a series of procedures to recover purified gum by first removing any lipids using the Soxhlet method as well as the cellulose, hemicellulose, lignin, and protein present in the mucilage by washing the gum with ethanol, acetone, and diethyl ether.

Feizi et al. (2021) combined previous research and used a 50°C extraction temperature with a 1:20 seed-to-water ratio, stirring constantly using an overhead stirrer for 20 min before changing to a Silverson mixer. The mucilage was separated from the seed using a centrifuge. Therefore, the different components; the seed, the mucilage, and the water, were separated by centrifugal force. The mucilage sat between the seed and the water and was transferred to a stainless-steel tray to be freeze-dried.

2.2.2 Characteristics, Properties, and Potential Use of Chia Seeds Mucilage

Chia seed mucilage has much potential to be utilised in the food industry as it is abundant (Katunzi-Kilewela et al., 2021). The mucilage can be used as a fat/oil replacer as it can establish bonds with water molecules, resulting in excellent water-holding capacity (Chiang et al., 2021). It can be used in cookies, pound cake, and bread, as it helps to hydrate, develop viscosity, and maintain freshness in these products (Felisberto et al., 2015; Fernandes & Salas-Mellado, 2017; Punia & Dhull, 2019). It can also be used in dairy products such as ice cream (Muñoz et al., 2012; Campos et al., 2016; Feizi et al., 2021), as well as mayonnaise (Fernandes & Mellado, 2018). The lipids in the mucilage show emulsifying properties that can interact with the hydrophobic portion in ice cream and mayonnaise (Chiang et al., 2021). When combined with other hydrophobic moieties, the lipids in the mucilage adsorb the oil droplets and act as an anchor. However, the proteins are preferentially adsorbed on the surface of oil droplets (Fernandes & Mellado, 2018). The mucilage can also stabilise the protein gel networks in emulsion-based products such as sausages (Jiménez Colmenero, 2000).

The mucilage can also be used as an egg replacer as the water-holding capacity can also increase moisture without competing with gluten or starchy polysaccharides in products such as cakes, including cupcakes (Chiang et al., 2021; Chavan et al., 2017; Gallo et al., 2020) and can be used as egg replacers in mayonnaise (Fernandes & Mellado, 2018).

The chia seed mucilage can mimic the viscoelastic properties of gluten; therefore, it can be used as a gluten replacement in gluten-free products such as pasta and cake (Menga et al., 2017; Hargreaves & Zandonadi, 2017). Steffolani et al. (2014) reported that prehydrated chia seeds and chia seed flour positively impacted the texture of the gluten-free bread. However, Zettel et al. (2015) reported that excessive chia seed mucilage negatively affected the texture of the bread as it produced a soft and sticky bread.

With its high viscosity and good emulsion stability properties, chia seed mucilage can be utilised in ice cream manufacturing, where it shows potential as an emulsifier and stabiliser in the formulation (Campos et al., 2016). Previous research reported that adding 0.1-0.2% (w/w) of chia seed mucilage in the ice cream formulation showed comparable results and even superior to commonly used gums such as guar gum (Campos et al., 2016; Feizi et al., 2021). Feizi *et al*. (2021) reported that with the increase of the chia seed gum concentration in the formulation resulted in decreasing overrun and meltdown rate in ice cream. The excessive amount of chia seed gum resulted in an insufficient amount of air incorporation during freezing due to the low viscosity of the ice cream mix. Although adding 2% (w/w) of chia seed gum decreased the meltdown rate, it was significantly slower compared to ice cream without added chia seed gum. But counter to this, the increase of chia seed gum concentration in the formulation increased the fat globule size distribution and destabilised the fat index. The increase in chia seed gum concentration also increased the viscosity of the ice cream mix, increasing the emulsion droplet size. The increase in the fat globule was due to fat droplet coalescence and/or aggregation during aeration or due to the interaction between the polysaccharide in the chia seed gum and the protein in the ice cream via bridging flocculation (BahramParvar & Goff, 2013). The increase in the destabilised fat index was affected by the surface-active properties in the chia seed gum, which became more apparent with the increase of the chia seed gum concentration. The adsorption of the polysaccharide chain at the air-water interface decreased the surface tension (Timilsena et al., 2016). The combination of mucilage and other hydrophobic moieties adsorb the oil droplets as well as proteins adsorption on the surface of oil droplets, possibly influencing the fat destabilisation.

2.3 Ice Cream

Ice cream, a popular frozen dessert, is usually sweetened and flavoured (Clarke, 2015). Legally, ice cream is defined as a sweet frozen food that is made from cream or milk products or both, and other foods, and is generally aerated (FSANZ, 2015), while in general, frozen dessert is a dessert that is consumed in the frozen state, with or without milk-derived ingredients in its composition, such as sorbets, and water ices (Goff, 2011). Ice cream is a mixture of water, fats, milk solids-not-fat, sweeteners, stabilisers, emulsifiers, and flavours, and when combined, form the ice cream mix. Partially crystalline fat globules and casein micelles are present in the ice cream mix (Figure 4) (Goff et al., 2013). The ice cream mix before freezing is pasteurised and aged, and then air is incorporated by agitation (Goff et al., 2013; Arbuckle, 2013). Ice cream is a colloidal system in a frozen state where the partially coalesced fat globules, air molecules, and ice crystals are connected (Figure 4) (Motyl et al., 2019; Goff et al., 2013).

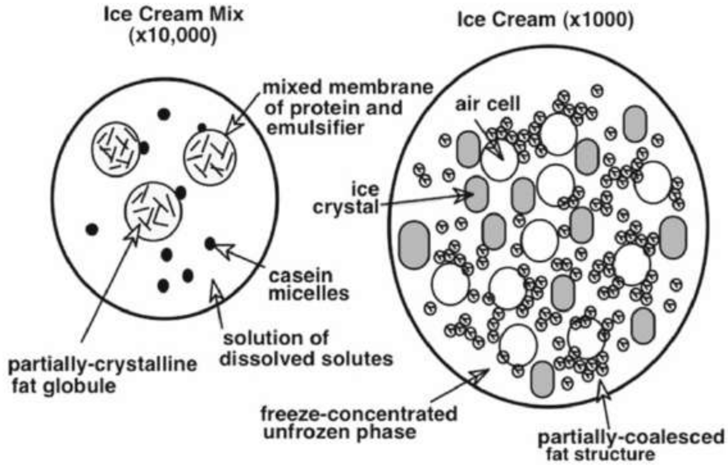

Figure 4. Illustration of the structure of ice cream mix and ice cream (Goff et al., 2013)

The partially coalesced fat globules tend to agglomerate and adsorb to the air cells, surrounded by emulsifiers and proteins (Goff et al., 2013). The air is dispersed in the matrix phase with embedded ice crystals (Crilly et al., 2008). The matrix phase, also known as the liquid phase, contains milk proteins, fat globules, stabilisers, sugar, and, in some cases, lactose crystals, as well as soluble salts in the solution (Crilly et al., 2008; Arbuckle, 2013).

2.3.1 Difference Between Ice Cream and Gelato

Although ice cream and gelato are made with similar ingredients, they are different in their compositions (Shingh et al., 2020). A gelato is an Italian-style ice cream produced in small batches, naturally low-fat, with a soft and scoopable texture (Conficoni et al., 2017; Goff et al., 2013). Gelatos have a lower fat and milk solids-not-fat content and a higher sugar and total solids content compared to ice cream (Table 3) (Clarke, 2015; Shingh et al., 2020).

Table 3. Comparison of the ingredients (%) in ice cream and gelato (Liu et al., 2022, Clarke, 2015; Shingh et al., 2020)

Frozen Desserts	Fat (% w/w)	Milk Solids-Not-Fat (%)	Sweeteners (%)	Total Solids (%)	Overrun (% w/w)
Ice Cream	10-16	10-12	15-17	32-36	25-120
Gelato	4-8	9-12	15-25	32-42	20-40

Storage of ice cream and gelatos can differ as the storage of ice cream and gelato also contributes to their textures (Rinaldi et al., 2014; Shingh et al., 2020). Gelatos have lower fat content and less air incorporated during aeration compared to ice cream. The ice cream overrun ranges from 25-120%, whereas gelato is denser, ranging from 20-40% (Clarke, 2015). Gelato is typically more viscous and has a semi-frozen consistency when made fresh, whereas ice cream has a harder consistency and needs hardening, usually overnight, before serving (Shingh et al., 2020). Gelato is typically made in a batch freezer, drawn into shallow tubs and not hardened as much, allowing it to be scooped when served at a higher temperature of -11°C (Rinaldi et al., 2014). However, when manufactured in bigger batches or on an industrial scale, gelato can be stored at -18 to -22°C, the same temperature as ice cream storage (Shingh et al., 2020). The difference comes in serving temperature; ice cream is usually served at a colder temperature, around -15°C, whereas gelato is served at a warmer temperature, around -13°C (Thompson et al., 2009)

2.3.2 Ingredients in Ice Cream and Gelato

The ingredients in ice cream and gelato are similar and serve the same purpose. Their biggest difference lies within the levels of ingredients (Table 3) (Arbuckle, 2013; Thompson et al., 2009). The composition of ice cream usually starts with the same ingredients: milk and cream (contributing water and fat), sweetener, emulsifier, and stabiliser (Silva Junior & Lannes, 2011). Flavours, colours, and components such as fruits, chocolate, and nuts are often added to ice cream (Clarke, 2015). Sucrose and corn syrup are the most common sweeteners in ice cream making (Miller-Livney & Hartel, 1997). Combining both not only sweetens the ice cream but also enhances the creaminess and the flavour of added ingredients, such as the fruits added to the final product (Syed et al., 2018). Egg yolks are often used in premium ice cream; while they add value and delicate flavour, they also contain a natural emulsifier called lecithin in the lecithin-protein complex (van Nieuwenhuyzen & Szuhaj, 1998). Another common ingredient used in place of egg lecithin is soy lecithin, which comes from soybeans (List, 2015). Gelatine has been used as a stabiliser to prevent large ice crystals from forming. Plant-derived stabilisers, such as carrageenan, agar powder, locust gum, and xanthan gum produced by bacteria, are also commonly used as alternatives to animal-derived stabilisers (Goff et al., 2013; Arbuckle, 2013).

Gelato is an Italian-style ice cream that is naturally low-fat, as it has lower fat content than ice cream (Goff et al., 2013; Shingh et al., 2020). Its low overrun gives it a distinctive body, texture, and desirable flavour release (Alfaifi & Stathopoulos, 2010).

Milk plays an important role in gelato as it is essential due to the milk protein present in casein and whey protein (Arbuckle, 2013). Milk is a complex physiochemical system where the components are dispersed in a true solution consisting of lactose, whey proteins, and some minerals (Musallam et al., 2017). Structurally, milk comprises fat globules, plasma, serum, and some minerals (Figure 5). Milk is mainly made up of fat globules and plasma. The fat globules have a membrane that comprises 2% of the total weight of milk fat, and the inside is not always liquid and consists of numerous components that differ depending on the species and diet (Mulder & Walstra, 1974). The plasma comprises casein micelles and serum proteins. Casein micelles are composed of proteins, minerals, and water. The protein is present in the form of casein and usually occurs as caseinate, which is combined with calcium phosphate. The casein and calcium phosphate complex is known as casein micelles (Musallam et al., 2017; Dalgleish & Corredig, 2012). The serum proteins comprise globular proteins that are considered as macromolecules, usually known as β-lactoglobulin, α-lactalbumin, blood serum albumin, and immunoglobulins, as well as lipoprotein particles that contain phospholipids and other glycerides, cholesterol, protein and nucleic acids, and several enzymes. The mineral constituents in the milk are bound to the protein and this is colloidal phosphate, which is bound to the casein micelles complex (Mulder & Walstra, 1974).

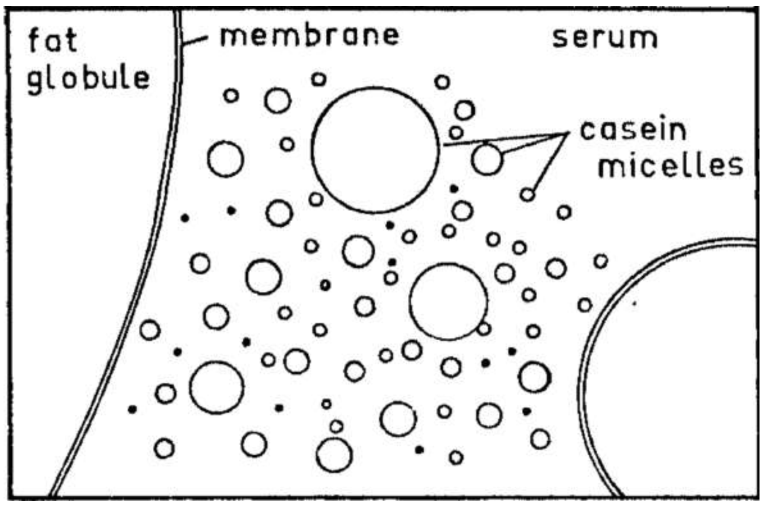

Figure 5. Milk viewed at x500000 magnification (Mulder & Walstra, 1974).

The presence of milk protein helps to stabilise the water-continuous emulsion and foams. They stabilise due to the hydrophilic amino acids present on the surface of the casein micelles (Singh, 2011). The milk proteins are an excellent stabiliser of an oil-in-water emulsion; during homogenisation, the protein stabilises the emulsion by covering the oil droplets, which prevents coalescence (Singh, 2011). Another component that plays a significant part in gelato composition is lactose. Lactose is a disaccharide sugar made up of glucose and galactose that is dissolved in milk. The presence of lactose increases the dissolved solids and reduces the freezing point of the solution (Herrington, 1934). Water is a significant component as it makes up 60-65% of the ice cream mix and can be obtained from milk, cream, and even liquid sweeteners (Goff et al., 2013). It is the medium in which all the other ingredients are dissolved or dispersed, and during freezing, it is converted to ice (Clarke, 2015).

Fat is one of the components in gelato and frozen desserts that helps give the right texture and mouthfeel and results in desirable melting properties (Ohmes et al., 1998). When used in gelato manufacturing, milk fat allows the gelato to achieve a smooth, creamy texture that melts slowly when eaten while delivering any fat-soluble flavours (Zhao et al., 2023). A fat emulsion exists in the gelato mix, and as it freezes, the emulsion will destabilise. The fat destabilisation happens during air incorporation and ice crystallisation as some fat in the ice cream mix aggregates and provides structural integrity for the ice cream. This partial coalescence is essential as it will set up the structure of the ice cream by air incorporation (Goff et al., 2013).

Milk Solid-Not-Fat (MSNF) are the ice cream ingredients including lactose, proteins, minerals, water-soluble vitamins, and enzymes (Chandan, 2011). MSNF can be found in cream and milk used in gelato production. Milk powder is the primary source of MSNF used for gelato manufacturing (Goff et al., 2013). Adding MSNF increases the viscosity of the mix and slows the meltdown rate (Arbuckle, 2013). However, higher-fat gelato usually requires less MSNF to reduce any potential sandiness in the final frozen product as a result of the crystallisation of excess lactose in the gelato mix. The protein in MSNF helps with the emulsification of fat, stabilises the foam formation by the air bubbles, enhances the viscosity of the gelato mix, and gives the gelato its body and smooth texture. The mineral salts in MSNF carry a slightly salty flavour that rounds the gelato flavour profile (Goff et al., 2013).

A sweetener is an important ingredient in gelato as it has several functions (Syed et al., 2018). For one, it adds sweetness to gelato, increasing its acceptance. It also enhances the creamy flavour (Stampanoni Koeferli et al., 1996). The lack of sweetness in gelato may lead to a flat taste and make the product undesirable (Arbuckle, 2013). The sweetener in gelato can also determine the softness of the gelato as it can lower the freezing point of the ice cream, leading to decreased ice crystals (Clarke, 2015). The total solid and sugar content of the gelato should not exceed 42% and 16% (w/w), respectively. Therefore, the viscosity of the gelato will increase, resulting in a better body and texture of the ice cream. When these amounts are exceeded, it will produce a sticky and soggy gelato due to the depression of the freezing point that requires a longer time and lower temperature for hardening (Arbuckle, 2013). It can also produce gelato that melts quickly and interferes with the aeration of the gelato. Therefore, an appropriate amount of sweetness will lead to a balanced, sweet gelato with the proper amount of total solids and good hardness (Goff et al., 2013). Common sugars, including dextrose, fructose, sucrose, and corn syrup, are commonly used (Clarke, 2015).

The plant-based frozen dessert is a new type of frozen dessert, and it is currently in demand as it is considered a healthier and more ethical choice than regular dairy ice cream (Bullock et al., 2020). Plant-based ice cream is usually made with the same ingredients as dairy ice cream. Plant-based milk, such as almond, coconut, soy, oat, or cashew, is used instead of dairy milk (Velotto et al., 2021). Compared to plant-based milk, dairy milk naturally has a higher fat content (Bekiroglu et al., 2022). The lack of fat is considered a manufacturing challenge (Leahu et al., 2022). Therefore, manufacturers sometimes replace the lack of fat with cheaper alternatives like vegetable oil, resulting in a frozen dessert with total calories similar to dairy ice cream (Bullock et al., 2020). Bekiroglu et al. (2022) found that using walnut milk improves the physicochemical properties of the plant-based frozen dessert due to the high-fat content that walnut milk has. Another manufacturing challenge is a less viscous mix that leads to ice needles. Therefore, additional ingredients, such as psyllium or pectin fibres, can help to increase the viscosity, consistency and density of the ice cream (Leahu et al., 2022).

2.3.3 Stabilisers and Emulsifiers in Ice Cream and Gelato

Ice cream and gelato manufacturers use a blend of stabilisers and emulsifiers in the formulation. Each ingredient has a different purpose. Emulsifiers are typically used to improve the whipping quality of the ice cream and gelato. In contrast, stabilisers are typically used to increase the viscosity of the ice cream and gelato to create a desirable body and texture (Goff et al., 2013).

Emulsifiers are substances used to reduce the surface tension in the system and can have a minor effect on acidity (Arbuckle, 2013). Emulsifiers in ice cream and gelato are used to improve air incorporation during whipping and reduce the whipping time. Adding emulsifiers produces ice cream with a stiffer body and smoother texture and gives a uniform whipping quality to the mix (Syed et al., 2018). In older ice cream and gelato formulations, egg yolk was used as an emulsifier as the yolk contains lecithin (Alfaifi & Stathopoulos, 2010). However, nowadays, egg yolk is used for different purposes, such as adding colour and flavour, and mostly premium products use high concentrations of egg yolk (Goff et al., 2013; Clarke, 2015). Egg yolk has the approximate composition of 51.1% water, 3.6% lipids, 16% proteins, 0.6% carbohydrates, 1.7% minerals, and 9% lecithin (Anton, 2007; Clarke, 2015). The lipids comprise 62% triglycerides, 33% phospholipids, and <5% cholesterol (Anton, 2007). The lecithin in egg yolk consists of phosphatides and phospholipids with emulsifying properties that help achieve a smoother, creamier texture and incorporates more air into the mix. In traditional recipes, fresh egg yolk is used. However, modern-day manufacturing uses processed egg yolk, such as pasteurised fresh egg yolk, frozen sugared egg yolk (with 10% sucrose as protection during freezing), and dehydrated egg yolk powder (Clarke, 2015). Due to the high cost of egg yolk lecithin, soy lecithin is used in its place as it also contains phospholipids and has shown to provide comparable attributes to ice cream and gelato with mono-/diglycerides (Goff et al., 2013; Rinaldi et al., 2014). However, Baer et al. (1997) reported that soy lecithin may affect the taste profile of ice cream and gelato when used in a high concentration, producing a soy-like off-flavour. Mono-/diglycerides are a type of emulsifier that ice cream and gelato manufacturers commonly use (Arbuckle, 2013). They naturally occur from plant oils and animal fats or can be made synthetically by chemical reaction or enzymatically using direct esterification or transesterification (Kara & Bor, 2019). Monoglycerides comprise esters of glycerol and one fatty acid molecule (Figure 6a). In contrast, diglycerides comprise esters of glycerol with two fatty acid molecules (Figure 6b) (Clarke, 2015). They are listed in Annex II of Regulation (EC) No 1333/2008 as additives, according to the European Commission (2008).

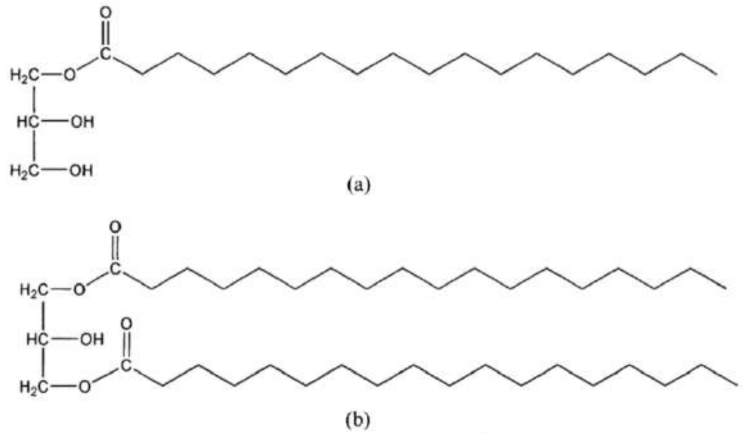

Figure 6. Structure of (a) monoglycerides and (b) diglycerides (Clarke, 2015).

However, emulsifiers with a high monoglyceride content (>90%) are more challenging to disperse as they can become highly viscous and form a gel in an aqueous system. In some cases, such as manufacturing a very low-fat ice cream and gelato, this gelling property may be advantageous as it can promote finer air bubble distribution and enhance ice crystal smoothness (Clarke, 2015; Goff et al., 2013). Although the emulsifier helps to create a smoother texture and stiffer body, using an excessive amount of emulsifier, especially the mono-/diglycerides, can lead to body and textural defects as well as a slowdown of the melting rate and they create a curdy meltdown of the ice cream (Arbuckle, 2013). A curdy meltdown happens due to the lack of uniformity between the ice cream and gelato, which may be influenced by the emulsifier and stabiliser type and concentration (Alvarez, 2009).

Stabilisers, a group of water-soluble or water-dispersible biopolymers, are commonly derived from various sources and are also a source of soluble fibre (Young, 2014). These include seaweed (carrageenan and alginate), tree seeds (locust bean gum and guar gum), fruit (pectin), cotton (sodium carboxymethyl cellulose), bacteria (xanthan), and polypeptides from animals (gelatine) (Goff et al., 2013; Arbuckle, 2013; Clarke, 2015). Despite originating from a natural source, these biopolymers are considered food additives by European law and are associated with ‘E’ numbers on food labels (Motyl et al., 2019). They are responsible for increasing the viscosity of the ice cream and gelato mix (Alfaifi & Stathopoulos, 2010). Each polymer chain forms a separate coil, which can move without disturbing other coils at a very low concentration (Figure 7a). However, when the concentration reaches a certain level, the coils will overlap and become entangled with each other (Figure 7b). When a high-shear is applied, this entanglement can lead to a decrease in viscosity as it can be pulled apart and disentangled, known as shear-thinning (Clarke, 2015).

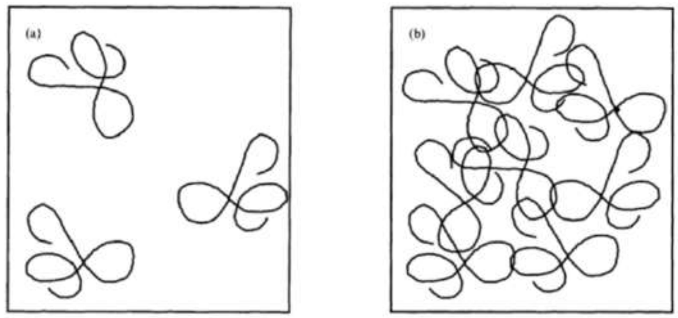

Figure 7. Biopolymers coil at (a) low and (b) high concentrations (Clarke, 2015).

Stabilisers in ice cream and gelato are used to prevent large ice crystals from forming in ice cream and provide a uniform product that is resistant to melting (Bahramparvar & Mazaheri Tehrani, 2011). They are used in low concentrations as they can lead to shear-thinning and may influence the flavour of the ice cream and gelato (Arbuckle, 2013). Therefore, stabilisers must have a neutral flavour and not interfere with the ice cream and gelato flavour (Goff et al., 2013). Stabilisers generally have a high water-holding capacity, which can help give a smooth texture and body to the ice cream and gelato (Bahramparvar & Mazaheri Tehrani, 2011). Vegetable gums such as carrageenan, alginate, and carboxymethyl cellulose are known to help prevent sandiness (Syed et al., 2018). As mentioned, stabilisers help to increase the viscosity without affecting the freezing point of the ice cream and gelato (Arbuckle, 2013). Excessive use of stabilisers can lead to undesirable melting characteristics, heavy body, and limited air incorporation into the ice cream and gelato (Goff et al., 2013; Arbuckle, 2013). Stabilisers are crucial in ice cream and gelato making as they benefit manufacturing, storage, and consumption. Stabilisers can facilitate air incorporation and help stabilise the foam, especially when gelato has a lower fat content. It also helps during pumping and filling for better flow, prevents shrinkage and slows down moisture migration during storage, promotes a smooth texture, reduces melt rate, and masks the detection of ice crystals during consumption (Clarke, 2015).

Research has shown that plant polysaccharides hydrocolloids can offer structuring, texturing, emulsifying, and thickening properties (Soukoulis et al., 2018). One of the plant polysaccharides that shows great potential is mucilaginous constituents of seed coats (Liu et al., 2021). Seed plants that can produce mucilage include flax, psyllium, yellow mustard, basil, and chia seeds (Eghbaljoo et al., 2022). Proteinaceous moieties and hydrophobic side chain groups are responsible for plant seed mucilage's surface and interfacial activity (Naji-Tabasi et al., 2016). The molecular weight, chain flexibility, and surface charge of the biopolymer molecules influence the interfacial activity. The concentration increases alongside high backbone chain flexibility and low uronic acid support adsorption to the air-water or lipid-water interfaces, enhancing emulsifying capacity and stability (Soukoulis et al., 2018). Increasing the concentration of the plant-based stabiliser will affect the viscoelasticity in the lipid-water interface and increase the repulsive forces between lipid droplets, causing bridging flocculation and impeding coalescence (Avila-de la Rosa et al., 2015). Plant seed mucilage is known to have great water and oil-holding capacity due to its unique physicochemical and conformational aspects from the presence of hydrophobic and hydrophilic side groups. In addition, the uronic acid content, pH, ionic strength and polysaccharide co-structure such as micro-gelled particles, fibrils, and protein micro-aggregates make plant seed mucilage water and oil holding capacity comparable, if not higher, than commercial gums such as guar gum and gelatine (Soukoulis et al., 2018).

Velázquez-Gutiérrez et al. (2015) reported the use of chia seed mucilage in food products. Chia seed mucilage exhibits type-II water sorption with 6 to 11% (w/w) bound to the monolayer of the chia seeds, a higher amount than water sorption by commercial gums such as pectin and carrageenan. Depending on the amount of chia mucilage dispersed in the continuous phase, it can provide an oil-in-water emulsion with a stabilising action. This ability is likely due to the formation of a viscoelastic structure composed of protein and polysaccharides, as well as the microstructural diversity of the mucilage (Capitani et al., 2016).

Previous studies by BahramParvar and Goff (2013) used basil seed gum as a stabiliser in ice cream. Ürkek (2021) and Velotto et al. (2021) used chia seed powder as a stabiliser in ice cream. Campos et al. (2016) used oven-dried chia seeds, and Feizi et al. (2021) used freeze-dried chia seeds mucilage as stabilisers and emulsifiers in ice cream. All these studies concluded that the use of plant seed gum, such as from basil and chia seeds, had comparable results to commercial emulsifiers and stabilisers such as lecithin, guar and xanthan gum, or improved the quality of the ice cream.

2.3.4 Ice Cream and Gelato Characterisation Techniques

The overrun of the ice cream can determine the type and the quality of ice cream (Syed et al., 2018). Premium ice cream and gelato tend to have a lower overrun of around 20-40%, whereas cheaper ice cream has a higher overrun of around 100-120% (Clarke, 2015). Emulsifiers are known to improve the whipping ability of the mix, lowering surface tension and producing smaller air bubbles (Goff et al., 2013). Adding sugar can affect the whipping ability; however, homogenisation of the mix will increase the overrun. Therefore, calculating overrun will determine whether the amount of emulsifier is sufficient and if the homogenisation is adequately achieved (Arbuckle, 2013).

Viscosity for a Newtonian fluid is the resistance of a liquid to flow (Goff et al., 2013). However, ice cream type, including gelato is a non-Newtonian pseudoplastic fluid where the relationship between the shear stress and shear rate is a nonlinear relationship. It is generally expressed as (Equation 1) where τ is the shear stress (Pa), K is the consistency index (Pa.sn), $\dot{\gamma}$ is the shear rate (s−1), and n is flow behaviour index (dimensionless) (Bahramparvar &

Mazaheri Tehrani, 2011). Measuring the viscosity of the ice cream mix is essential, as a higher viscosity contributes to a desirable mouthfeel (Amador et al., 2017). However, ice cream mixes that are too viscous will result in less desirable ice cream and gelato (Amador et al., 2017). Stabilisers, when used appropriately, can increase the viscosity of the mix, leading to a more acceptable product (Mahdian & Karazhian, 2013). Measuring viscosity is crucial in ensuring sufficient stabiliser usage, as too high or too low viscosity will indicate a problem (Goff et al., 2013). Measuring the viscosity of ice cream and gelato can be carried out by using a rheometer, as reported by Velotto et al. (2021).

$$\tau = K\gamma\, \dot{n} \qquad \text{(Equation 1)}$$

The diameter of the fat globule in the mix provides the degree of fat aggregation or partial coalescence that occurs in ice cream and gelato. The ideal diameter of the fat globule size should be less than one μm, and the maximum size should be 2-3 μm (Goff et al., 2013). The fat globule size can be measured using a laser light scattering technique with equipment such as a Mastersizer (Malvern, UK) with a fat globule absorbance value of 0.001. The droplet size (d 4,3) is defined as $S_{ni}\ D_{4i}$ / $S_{ni}\ D_{3i}$, where ni is the number of particles with diameter D_i (Goh et al., 2008).

The hardness of ice cream and gelato is a crucial aspect of its quality and is influenced by its composition, such as sugar, total solids, overrun, and the concentration of stabilisers (Kurultay et al., 2010). Stabilisers, when used appropriately, can prevent large ice crystals from forming, thereby enhancing the quality of the product. The amount of sugar is also crucial as it might alter the freezing point of the ice cream (Goff et al., 2013). Measuring the hardness of the ice cream and gelato can be carried out by using a texture analyser, as reported by Velotto et al. (2021).

The meltdown rate can impact how the ice cream and gelato are consumed (Wu et al., 2019). If the ice cream melts too fast, it tends to make the product undesirable. Therefore, stabilisers are crucial as they can stabilise the foam structure and help the ice cream melt more slowly (Bahramparvar & Mazaheri Tehrani, 2011). The meltdown rate can be determined by measuring the drips from the product through a mesh screen within a certain amount of time at room temperature (Goff et al., 2013).

Sensory attributes in ice cream and gelato are important, as most people enjoy ice cream with a smooth and creamy texture (Beegum et al., 2022). The ingredients, process used, and handling affect the sensory properties of the ice cream and gelato (Genovese et al., 2022). Therefore, a sensory analysis is required to determine whether ice cream is acceptable. A high-quality, unflavoured ice cream should have a balanced flavour and be firm to give an abundant solid matter sensation, yet not so firm during serving (Alvarez, 2009). It should have a smooth, creamy texture with no easily detected large ice crystals (Goff et al., 2013). It should melt slowly, give a pleasant mouthfeel, and have a natural colour (Alvarez, 2009). Sensory analysis can be carried out in a focus group to collect subjective information (Goff et al., 2013). If a more significant number of consumers is used, then the data can be analysed using a Just-About-Right method (Li et al., 2014). The panellist should be given a sample of about 60 mL using a metal or plastic spoon as it does not interfere with the flavours (Goff et al., 2013).

2.4 Clean Label Foods

The food label is an important tool for customers to communicate with manufacturers as it can inform the content of the food product. Clean label foods are an emerging concept where food products have less artificial additives, less processed, and with simpler and shorter ingredient lists (Cao & Miao, 2023). However, it still needs to be clarified what a clean label exactly is as there are no clear definitions from any regulations and legislation (Asioli et al., 2017). A clean label product may use fewer ingredients, with no artificial or synthetic ingredients (Chen et al., 2022). It may refer to a sustainable, eco-friendly, minimally processed food (Soukoulis et al., 2018). Consumers are more interested in the origin of their food, the method of manufacture and components present (Asioli et al., 2017). The European Food Safety Authority described E-numbers as used to identify permitted food additives that have passed safety tests and have been approved for use (EFSA, 2024). Consumers started to avoid the E-numbers and deemed some food processing methods less natural when they first appeared. They labelled unfamiliar ingredients, such as additives, as unhealthy and associated them with adverse health effects, such as allergies (Asioli et al., 2017). Therefore, the demand for food products with fewer or no additives increased (Rinaldi et al., 2014). Additives such as emulsifiers and stabilisers can be derived from plants (carrageenan, guar gum, pectin, et cetera) and animals (egg lecithin, whey proteins, gelatine, et cetera) and are labelled with E-numbers. These coded additives create a misinterpretation by consumers, perceived as unsafe, unhealthy, and artificial or synthetic (Loffredi & Alamprese, 2023). Therefore, food with familiar and simpler ingredients is their choice as they appear to have fewer potential harmful effects on their health that could contribute to chronic illness (Chen et al., 2022).

While the clean label trend has gained momentum, it has posed significant challenges for food manufacturers, particularly in producing products like ice cream and gelato (Motyl et al., 2019). The absence of E-numbers, especially those in the E400-E499 group, can lead to issues such as improper emulsification, product instability, and an inability to maintain the desired consistency (Motyl et al., 2019; Loffredi et al., 2021). In response, formulators and researchers are striving to identify natural food ingredients that can mimic the functions of these E-numbers (Euston & Goff, 2019).

2.5 Conclusions

Chia seeds are an excellent source of antioxidants, essential fatty acids, dietary fibre, oil, and proteins that can be added to various food products. They also have exceptional mucilaginous properties made of polysaccharides that have the potential to be used as thickening and gelling agents, emulsifiers, and stabilisers. The mucilage contains uronic acid and has a unique quality that allows it to retain water up to 27 times its weight once hydrated. The mucilage also has emulsifying properties comparable to common gums such as guar and xanthan gum. The mucilage has much potential as it can be used as a fat/oil replacer in baked products, gluten replacer in pasta, egg replacer in mayonnaise, emulsifier and stabiliser in ice cream.

Chia mucilage has shown potential and has the characteristics of emulsifiers and stabilisers in gelato. Previous research demonstrated that it is comparable to commercial gum, such as guar and xanthan gum, in ice cream. Therefore, in this study, freshly extracted mucilage is utilised as an emulsifier and stabiliser for gelato.

Chapter 3. Materials and Methods

3.1 Experimental Design

This project was divided into five parts.

Part 1. Chia seed mucilage was extracted using previously published methods.

Part 2. The gelato base was incorporated with different concentrations of chia seed mucilage extracted (Part 1). The various concentrations, F1 - 1.75% w/w, F2 - 2% w/w, F3 - 2.25% w/w, and F4 - 2.5% w/w of chia seed mucilage, was used.

Part 3. The gelato mix and frozen gelato were characterised by measuring the pH, fat globule distribution, overrun, meltdown rate, viscosity, and texture of the gelato as well as microbiological analyses for *Listeria monocytogenes* and total plate count. Formulations were screened using a focus group discussion to find the best gelato base to mucilage ratio to move forward with.

Part 4. From those screened in Part 3, the gelato was reformulated as F5 – 2% w/w and F6 2.25% w/w of chia seed mucilage and assessed by characterising and screening by a focus group once more.

Part 5. The final selected formulation was assessed with consumer sensory evaluation.

3.2 Materials

Certified organic black chia seeds (Pro Earth, New South Wales, Australia) were purchased in September 2023, from a local supermarket in Auckland, New Zealand. The chia seeds were stored at room temperature (20°C ± 2°C) in a sealed container. Little Lato™ (Auckland, New Zealand) supplied a base formulation of commercial gelato. To prolong the shelf life of the gelato base, once received, the base was immediately frozen at -20 ± 2°C. The gelato cream base consisted of whole milk, sugar, dairy cream, glucose, and skim milk solids.

3.3 Extraction of Chia Seed Mucilage

The mucilage was extracted based on a previous study by Feizi et al. (2021) with slight modifications, as shown in Figure 8.

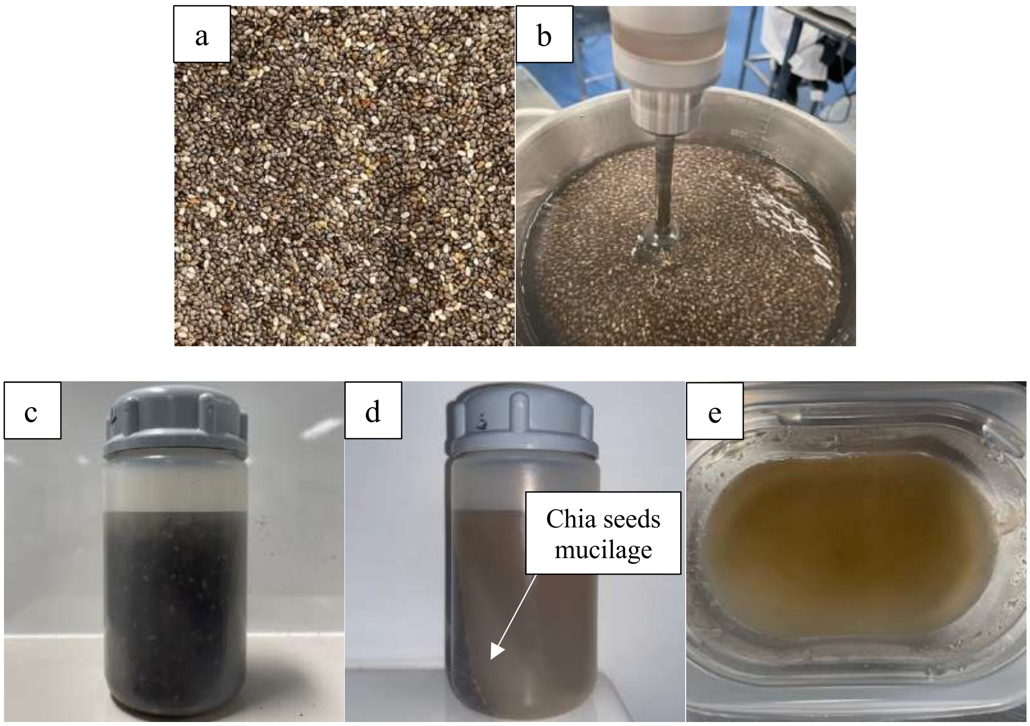

Figure 8. Process for extracting chia seed mucilage: (a) dry chia seeds; (b) hydrating chia seeds with warm water and overhead stirrer; (c) fully hydrated chia seeds in a centrifuge tube; (d) separated chia suspension in a centrifuge tube; (e) freshly extracted

The dry chia seeds were hydrated in warm water (80°C ± 1°C) at a ratio of seed-to-water of 1:20 (w/w). The mixture was stirred for 30 min at 1000 rpm using an overhead stirrer (IKA Labortechnik, RW 20 digital, Malaysia) while maintaining the temperature at 80°C using a hotplate (CH2092-001, Industrial Equipment & Control, Australia). After 30 min, the speed was increased to 1600 rpm and stirred for another 2 h while being maintained at 80°C to facilitate swelling. The mucilage had swelled to the maximum possible, and then the mucilage could be easily separated from the seeds. The aqueous suspension was centrifuged

(SIGMA 6-16KS, Germany) for 40 min at 12800 g, at 20°C. The centrifuge separated the suspension into three layers, the middle being the mucilage, with an aqueous layer on the top. The freshly extracted mucilage was collected in an airtight plastic container and stored in the refrigerator at 4 ± 2°C (Chiang et al., 2021). The yield of the freshly extracted mucilage was calculated using Equation 2.

$$\text{Yield}(\%) = \frac{\text{Weight of freshly extracted mucilage (g)}}{\text{Weight of chia seeds+water (g)}} \text{x}100\%$$

(Equation 2)

The moisture content of the chia seed mucilage was measured by evaporating the water in the mucilage using an oven at 50 ± 1°C for 48 hours (Nielsen, 2010). The percentage of the moisture content was calculated using Equation 3.

$$Moisture(\%) = \frac{Weight\ of\ wet\ mucilage\ (g) - Weight\ of\ dry\ mucilage\ (g)}{Weight\ of\ wet\ muclage\ (g)}\ x\ 100\%$$

(Equation 3)

The chia mucilage that was used to make gelato was freshly extracted. However, an additional test was carried out to determine its stability and shelf life. The freshly extracted mucilage was stored in the refrigerator at 4 ± 2°C in an airtight container for three or six months. During storage, the pH of the chia seed mucilage was monitored.

3.4 Preparation of Gelato

The gelato base, which consisted of pasteurised whole milk, sugar, pasteurised dairy cream, glucose, and skim milk solids without any stabilisers and emulsifiers, was prepared by pre-homogenising and pasteurising by Little Lato™ in their facility in Auckland, New Zealand, for use in this study. Once received, the prepared gelato base was subsampled into one-litre portions and frozen at -20 ± 2°C to prolong the shelf life of the gelato base. When required, the gelato base was thawed overnight in the refrigerator at 4 ± 2°C. Once thawed, the gelato base was immediately used at 4 ± 2°C and combined with the freshly extracted chia seed mucilage in different concentrations. The extracted chia seed mucilage was used in the gelato to act as a stabiliser and emulsifier in place of a commercial stabiliser and emulsifier. Various concentrations of chia seed mucilage were determined by referring to previous experiments by Campos et al. (2016), where 1-2% of dried mucilage was used as a stabiliser and emulsifier of ice cream. The concentration was adapted to take into account the moisture content in the chia seed mucilage. Four different gelato mixes were formulated with different concentrations of chia seed mucilage, according to Table 4.

Table 4. Formulations of chia seed mucilage gelato prepared

	F1	F2	F3	F4	F5	F6
Gelato cream base (g)	500	500	500	500	500	500
Concentration of chia seed mucilage (% w/w)	1.75	2	2.25	2.5	2	2.25
Added chia seed mucilage (g)	8.75	10	11.25	12.5	10	11.25
Chia seeds mucilage on a dry weight basis	0.053	0.060	0.068	0.075	0.060	0.068

The gelato preparation was adapted from the preparation of ice cream with chia seed mucilage by Feizi et al. (2021) and Campos et al. (2016). The gelato was prepared by weighing the gelato and the mucilage following the procedure outlined in Figure 9.

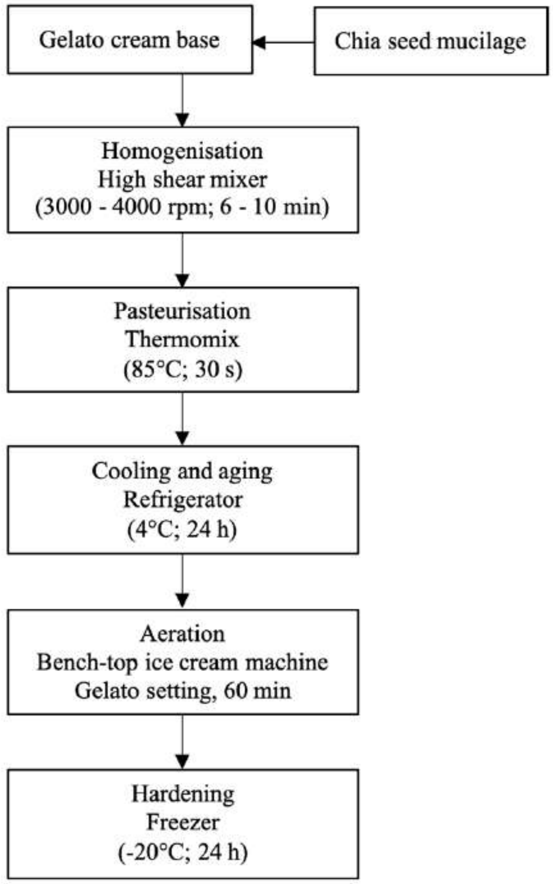

Figure 9. Procedure for laboratory-scale gelato production with added chia seed mucilage.

A high-shear mixer (Silverson L5M-A1, USA) was used at 3000 rpm for 6 minutes to ensure the mucilage was incorporated fully into the gelato. Once the ingredients were thoroughly mixed, the gelato mixes were pasteurised using a Thermomix (TM 5, Vorwerk, Germany), which allowed the mix to be heated and mixed simultaneously (Vorwerk, 2023). Gelato mixes were heated to 85°C and held at this temperature for 30 s. The gelato mixes were transferred to plastic containers to cool down at room temperature (25 ± 1 °C). After cooling down to 25°C, the gelatos were moved to a refrigerator to age at 4 ± 2 °C for 24 h. The last step in the gelato preparation was aerating the aged gelato. The aeration process was carried out with a bench-top ice cream machine (Gelato Expert, MagiMix, Italy) using the pre-programmed gelato settings, which ran for 60 min. The ice cream machine was equipped with a plastic paddle that ensured homogenous mixing and aeration during the freezing of the gelato mixes. After approximately 60 min, the aerated gelatos were transferred to laminated ice cream cardboard cups for storing and allowed to harden in a freezer at -20 ± 1 °C for 24 h. All gelato formulations were produced in duplicate.

For formulations F5 and F6 (Table 1), the gelatos were prepared in the same manner with a slight modification during mixing; the speed and time were increased to 4000 rpm for 10 minutes during the homogenisation process using the same Silverson high-shear mixer. These modifications were made based on feedback from the first focus group to achieve a gelato with less of an icy texture and not buttery by improving the homogenisation of the gelato and chia seed mucilage with increased homogenisation shear rate and with the addition of more chia seed mucilage.

3.5 Characterisation of Gelato Mix

During the gelato production, approximately 25 mL of gelato mix samples were collected to analyse the pH of the mix and the fat globule size distribution. Each duplicate batch was tested.

3.5.1 Measurement of pH

The pH of the chia seed mucilage and gelato mixes were determined using a pH meter with a glass electrode (Accumet AB150, Fisher Scientific, US) and calibrated using pH standard buffer solutions (pH 4 and 7, LabChem, Thermo Fisher Scientific, US). The measurements were carried out in triplicate. In addition, the pH of the chia seed mucilage stored for three and six months in the refrigerator (4 ± 2°C) was also measured in triplicate (Srinu et al., 2022).

3.5.2 Fat Globule Size Distribution

The fat globule size distribution of the gelato mixes was determined using a Malvern Mastersizer 3000 (Malvern, UK). The samples tested were the gelato cream base, the gelato mixes with added chia seed mucilage and the melted frozen gelato with incorporated chia seed mucilage. The parameters were set to have a ratio of the refractive index of the emulsion droplets (1.456) to the dispersion medium (1.33) at 1.095 (Goh et al., 2008). The small volume sample suspension unit tank (Hydro SM, Malvern, UK) was rinsed three times with RO water to remove the ethanol residue and then filled with RO water. The instrument was ready once the system showed the maximum energy in light scattering of less than 100, and the detector number was at 20. Through the software program, the system was initialised until it was stabilised. Once stabilised, the sample was added slowly, one drop at a time, using a pipette until the laser obscuration of 10-15% was reached. Once the sample was within the obscuration range, the test was run to obtain the volume-average mean diameters (d 4,3) of the fat globules. The readings on each sample were carried out in triplicate. A representative output from the mastersizer is shown in Table 5.

Table 5. Example of a mastersizer output.

Sample Name	Measurement Date	D [3,2]	D [4,3]	D [5,0]	Specific Surface Area	Dx (10)	Dx (50)	Dx (90)	Laser Obscuration

3.6 Characterisation of Frozen Gelato

The overrun was measured using the freshly aerated gelato. In contrast, the melted frozen gelato was used to determine the viscosity and the frozen gelato was used to measure the meltdown rate and hardness of the samples.

3.6.1 Overrun

The gelato overrun was calculated using Equation 4 (Velotto et al., 2021) by weighing the gelato mix with added chia seed mucilage and the freshly aerated gelato with incorporated mucilage in identical 40-ml cups. The measurement was carried out in triplicate.

$$\text{Overrun}(\%) = \frac{\text{Weight of gelato mix (g)} - \text{Weight of aerated gelato (g)}}{\text{Weight of aerated gelato (g)}} \text{ x } 100 \qquad \text{(Equation 4)}$$

3.6.2 Meltdown Rate

The meltdown rate of the gelato was determined by following the method outlined in Feizi et al. (2021) with some modifications. Instead of placing the gelato in a 250 mL container, the frozen gelato samples were transferred to a 40 ml plastic cup, ensuring no air bubbles were trapped inside the cups, and stored at –20 ± 2°C for 24 h (Chaves et al., 2018). The gelato samples were withdrawn from the freezer, removed from the containers, and placed on a stainless-steel mesh wire (1 cm x 1 cm aperture size) at a height of 20 cm above bench-top scales (S-4002, Denver Instrument, Germany) with the help of a Burette stand (Figure 10). The gelatos were placed strategically so each sample was directly above the containers on top of the bench-top scales. The gelatos were allowed to melt at 20 ± 2°C, and the weight of the melted gelato was recorded at 5 min intervals for 70 min. The time and weight of the first drop of the melted gelato were recorded. The data were used to generate a plot of the weight of melted gelato against time (minutes).

Figure 10. Equipment set up for meltdown rate of gelato using mesh screen and bench-top scale.

3.6.3 Rheological Measurements

The rheological properties of the gelato were performed using a rheometer (TA Instrument AR550, UK) with a cone and plate geometry, 60 mm geometry, cone angle 2°. The temperature of the plate was maintained at 5 ± 1°C, the temperature was controlled at this temperature by the rheometer, and assisted with an external waterbath (Julabo FT200, Germany). About 1 g of melted frozen gelato was loaded onto the plate, ensuring no excess amounts between the rheometer plate and the plate geometry. The program was run, and the samples were equilibrated for 2 min. The thixotropy in the samples was erased by subjecting the samples to a pre-shear rate of 1000 s^{-1} for 10 s. The apparent viscosity of the gelato samples was measured over a range of shear rates of 0.1 to 100 s^{-1}. The data were used to generate a plot of apparent viscosity against shear rates (Syed et al., 2018; Feizi et al., 2021).

3.6.4 Texture

The frozen gelato texture was measured according to Velotto et al. (2021) using a texture analyser (TA-XT plus, Stable Micro Systems, UK). The conditions and probe used on the texture analyser are listed in Table 6.

Table 6. Conditions used on the TA-XT plus texture analyser

Parameters	Specifications
Probe	2 mm cylindrical
Depth of penetration	20 mm
Cross-head speed	10 mm / m
Preload	0.01 N
Cell load	50 N
Measurements recorded	Hardness, stickiness, and stringiness

The samples were removed from the freezer (-20 ± 2°C) and left out for approximately 5 minutes at room temperature to reach the gelato serving temperature (-10 ± 2°C) (Shingh et al., 2020) before being measured at room temperature (20 ± 2°C). The parameters recorded were hardness (N), stickiness (N), and stringiness (mm) (Feizi et al., 2021; Velotto et al., 2021). The measurements of each sample were carried out in triplicate. The output is shown in Table 7.

Table 7. Example of TA-XT plus texture analyser output

Test ID	Batch	Hardness (g)	Stickiness (g)	Stringiness (mm)

3.7 Microbiological Analyses of Frozen Gelato

Microbiological analyses were carried out to determine product microbial safety. Two assays were completed in this study. Firstly, the detection of *Listeria monocytogenes* was carried out to ensure *Listeria* was not present in the products, as it is commonly found in dairy products. Secondly, a total plate count was carried out to ensure the cleanliness of the gelatos production.

3.7.1 *Listeria monocytogenes* detection

The detection of *Listeria monocytogenes* in the product using the analysis protocol by ISO Standard No. 11290-1:2017 (ISO, 2018). To prepare the *Listeria* broth (Listeria Enrichment Broth Base, Oxoid, UK), 18 g of the broth base was added to 500 mL of distilled water and was sterilised by autoclaving the media at 121°C for 15 minutes. The broth was cooled prior to usage. Gelato samples (25 g) were added to 225 mL of previously prepared *Listeria* broth and sample of gelato in *Listeria* broth was homogenised using a masticator homogeniser (IUL instruments, Spain). Once fully homogenised, the samples were incubated using an orbital shaker waterbath (Orbital Shaker, OHAUS, US) for 48 h at 37 ± 2°C. The presence of *L. monocytogenes* was confirmed by streaking the samples onto Oxford agar plates (1450, Fort Richard, NZ) and onto Palcam agar plates (1440, Forth Richard, NZ) and incubating for 24 h at 37°C (ISO, 2018).

3.7.2 Total plate counts

Estimating bacteria content in the sample was carried out using the total plate count method (ISO, 2018). Gelato samples (25 g) were added to 0.1% peptone water (Universal Peptone M66, Merck, Germany) to achieve a 10^{-1} dilution and homogenised using a masticator homogeniser (IUL instruments, Spain). A serial dilution was carried out for each sample. To make a 10^{-2} concentration, 1 mL of 10^{-1} sample was transferred to a 15 mL glass dilution bottle filled with 9 mL of 0.1% peptone water. The exact process was repeated until 10^{-2}, 10^{-}

^{3}, and 10^{-4} dilution were obtained. Each dilution bottle was homogenised using an orbital motion vortex mixer (Wisemix VM-10, Witeg, Germany). Plate count media (Standard Plate Count Agar CM0463, Oxoid, UK) was prepared by mixing 17.5 g of the media with 1 L of distilled water and sterilised by autoclaving the media at 121°C for 15 minutes. Each dilution (1 mL) was placed in a petri dish, and plate count media (15 mL) at approximately 28°C was poured into the same dish. A circular motion was applied to mix the diluted sample and the media. The plates were incubated for 24 h at 37°C (ISO, 2018).

3.8 Sensory Evaluation

Part 5 of this study focused on choosing the optimal concentration of chia seed mucilage to incorporate into the gelato base to provide an acceptable gelato with chia seed mucilage formulation with a smooth and soft texture. Focus group sessions were run to narrow down the acceptable formulations. Two focus group sessions were conducted, and the feedback from the first focus group session were used to improve the homogenisation process. The formulations were changed in an attempt to produce a second set of gelato samples that was more desirable, according to the discussion. Using the new formulations (F5 and F6), the second focus group session was conducted to define the most acceptable concentration of chia seed mucilage. The results from the second focus group resulted in the selection of the gelato with the optimal concentration of chia seed mucilage to present for consumer sensory.

3.8.1 Focus Group and Screening

A preliminary tasting was carried out using a sensory focus group of five Food Technology postgraduate students at Massey University (Feizi et al., 2021). Upon starting, the participants were asked to sign a consent form and information regarding the product (Appendix A1). Rice crackers (Pams Original Rice Crackers) and water were provided to cleanse the palate before the evaluation and between samples. Four gelato samples (40 mL each) containing chia seed mucilage (1.75%, 2%, 2.25%, and 2.5%) were randomly labelled with 3-digit codes and presented to the group. The panellists were asked to taste the samples. A discussion was started, and the panellists commented on the colour, taste, sweetness, texture, mouthfeel, and overall opinion of each gelato. They also stated what they liked and disliked most about each sample. Lastly, they were asked to rank the 'most liked' to 'least liked' gelatos. The goal was to narrow down the formulations to a single preferred sample.

The second sensory focus group session was conducted with a different group of five Food Technology, Massey University postgraduate students. The same procedure as described previously was used to conduct this focus group session.

3.8.2 Consumer Sensory Evaluation

The final formulation selected from the second focus group session was subjected to consumer sensory evaluation (n = 30). The consumer test was conducted in a sensory laboratory at Massey University, Auckland. The consumers were asked to walk inside the sensory booth and were given information regarding the sample, as it may contain allergens such as dairy (Appendix A5). The palate cleanser and samples were given from the sensory preparation room (Figure 11).

Figure 11. Sensory preparation room during the consumer sensory evaluation, Massey University, Auckland.

The consumers were given a tray with rice crackers (Pams Original Rice Crackers) and water to cleanse their palate and the sample (approximately 40 mL) (Figure 12). A specific QR code generated by Red Jade was also given to access the assessment questionnaire (Appendix A6). The evaluation was started once the consumer agreed to the evaluation by signing the consent form (Appendix A7). The JAR scale was used to assess specific attributes (Li et al., 2014). The attributes evaluated include colour, aroma, flavour, sweetness, meltdown rate, mouthfeel, texture, and overall opinion towards the sample (Appendix A8) (Velotto et al., 2021).

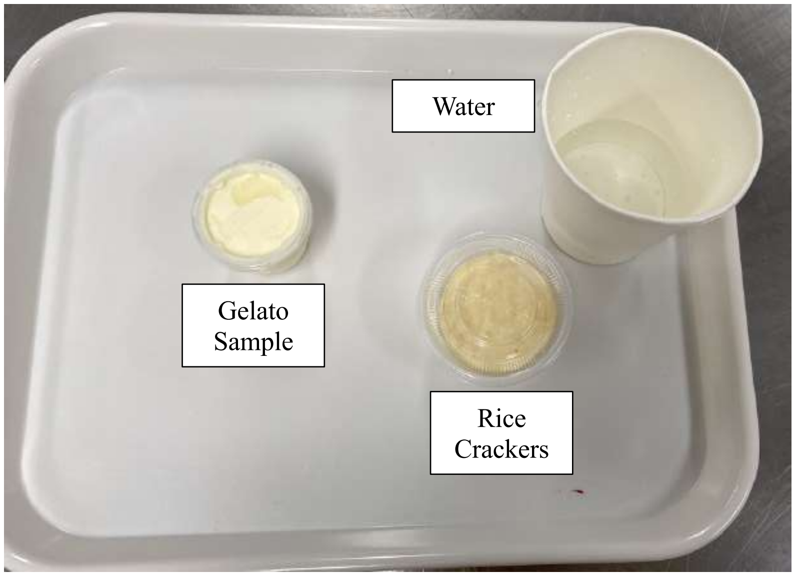

Figure 12. Sensory tray during the consumer sensory evaluation.

3.9 Statistical Analyses

Duplicate gelato were each tested in triplicate, providing six results for each formulation. All data collected throughout the process was analysed using Minitab (version 21.3.1) and Microsoft Excel (version 16.82). A one-way analysis of variance (ANOVA) was used to compare data between the different gelato concentrations. Post hoc means comparison with Tukey's HSD ($p < 0.05$) was applied to all the data to determine whether each sample differed significantly from the others.

Chapter 4. Results and Discussion

4.1 Chia Mucilage Extraction Yield and Moisture Content

Chia seeds were extracted with a 1:20 seed-to-water ratio at 80°C, which resulted in an average mucilage extraction yield of 4.61 ± 0.39 g wet chia seeds mucilage / g dry chia seeds. The mucilage was found to have an average moisture content of 99.40 ± 0.04% w/w. Therefore, assuming 0.65 total solids, the mucilage extraction yield was approximately 2.8 g total solids/100 g chia seeds.

No published studies reported the yield of freshly extracted mucilage, as most used dried chia seeds mucilage (Feizi et al., 2021; Muñoz et al., 2012; Campos et al., 2016) or chia seeds powder (Velotto et al., 2021). Muñoz et al. (2012) reported their highest extraction yield at 6.97 g dried chia mucilage/100 g chia seeds. Campos et al. (2016) reported their highest extraction yield at 5.09 g dried mucilage/100 g chia seeds, and Feizi et al. (2021) reported their extraction yield at 3.4 g dried mucilage/100 g chia seeds. The different results may vary depending on the variety of chia seeds, hydration rate, pH, temperature, and seed-to-water ratio. A previous study reported that mucilage extraction could be done at pH of 4, 6, and 8, at temperatures of 4, 40, and 80°C, and with a seed-to-water ratio of 1:20, 1:30, and 1:40 (Muñoz et al., 2012). Koocheki et al. (2009) reported that a higher extraction yield could be obtained with a higher temperature and longer extraction time. Higher temperatures helped the seeds to be less sticky, and the mucilage easily detached from the seeds, increasing the yield (Koocheki et al., 2010). The extracted mucilage can be used as an emulsifier and stabiliser in food products such as ice cream (Campos et al., 2016). Previous research has shown that using chia mucilage in ice cream is comparable to commercial emulsifiers and stabilisers while maintaining the quality of the product (Campos et al., 2016; Feizi et al., 2021).

4.2 pH of Mucilage and Gelato

The pH of the mucilage was measured to ensure its addition would not affect the pH of the gelato, and the pH of the freshly extracted mucilage was measured. The pH of the mucilage was monitored over a six-month period during storage at 4°C to determine if the pH would change over this period (Table 8). This stored mucilage was not used for any gelato formulations as its microbiological safety was not determined.

Table 8. pH of chia seeds mucilage during different storage periods.

Storage Period	pH
Freshly extracted (0 months)	6.87 ± 0.01^a
3 months	6.86 ± 0.01^a
6 months	6.85 ± 0.00^a

n = 3. Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different according to Tukey's HSD comparison ($p < 0.05$).

The freshly extracted chia seed mucilage had an average pH of 6.87 ± 0.01; after 3 and 6 months of storage, the chia seed mucilage had an average pH of 6.86 ± 0.01 and 6.85 ± 0.01 (Table 8), respectively. After storage, there was no significant shift in the pH of the mucilage ($p > 0.05$).

The pH of all gelato formulations prepared ranged from 6.49 to 6.55, with an average of 6.52 ± 0.03 (Table 9). The presence of the different concentrations of mucilage in the gelato did not significantly affect the pH of each formulation ($p > 0.05$). The pH of the gelato was similar to that of ice cream formulations using dried chia seeds gum, which ranged from 6.50 to 6.59 (Feizi et al., 2021) and was similar to gelato formulations using fish gelatine with a pH of 6.6 (Alika & Atma, 2018).

Table 9. pH, overrun, and meltdown rate of the gelato formulations.

Formulation	Chia seeds mucilage added (%)	pH	Overrun (%)
F1	1.75	6.49 ± 0.04^{a}	$34.13\% \pm 1.74^{a}$
F2	2	6.52 ± 0.03^{a}	23.35 ± 1.67^{b}
F3	2.25	6.49 ± 0.05^{a}	24.63 ± 0.99^{b}
F4	2.5	6.55 ± 0.03^{a}	25.52 ± 2.07^{b}
F5	2	6.50 ± 0.05^{x}	22.68 ± 1.06^{y}
F6	2.25	6.51 ± 0.03^{x}	24.77 ± 0.94^{y}

n = 6. Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different according to Tukey's HSD comparison ($p < 0.05$). F1, F2, F3, and F4 were compared while F5 and F6 were compared separately.

4.3 Overrun

The gelato structure was affected by the air incorporation during gelato making (Figure 13) (Shingh et al., 2020). In this study, the overrun achieved in each formulation is presented in Table 9. Formulation F1 had a 34.13 ± 1.74% overrun, significantly different from the other formulations. The overrun in the remaining formulations were: F2 had 23.35 ± 1.67%, F3 had 24.63 ± 0.99%, and F4 had 25.52 ± 2.07% overrun (Table 9). However, F1 was significantly different compared to F2, F3, and F4 ($p < 0.05$). The high overrun in F1 may be due to a lack of mucilage, where the mix was less stable, resulting in higher air incorporation than other formulations (Shingh et al., 2020).

After focus group evaluation and after narrowing down and changing some parameters during mixing, such as extending the homogenisation time and increasing the agitation speed, F5 had a final overrun of 22.68 ± 1.06%, and F6 had 24.77 ± 0.94% (Table 9). The overrun in F5 and F6 were not significantly different ($p > 0.05$). However, all the formulations were still within the expected overrun for a gelato, around 20-40% (Clarke, 2015). Due to the lower fat and higher sugar content, along with a slower aeration rate compared to ice cream, gelato has a lower overrun compared to results reported by Feizi et al. (2021). The addition of chia mucilage as an emulsifier or stabiliser also helps to maintain a higher overrun, as the uronic acid in the mucilage helps to stabilise the mix to hold more air and to provide emulsifying activity (Timilsena et al., 2016).

Figure 13. Gelato after being aerated for 60 min in a bench-top gelato machine.

4.4 Meltdown Rate

The meltdown rate of the gelato samples was carried out at room temperature (20°C), and weights were recorded at intervals of 5 min for 70 min. The rate of melting can be affected by various reasons. A low fat/water interfacial tension can reduce the stability of the fat emulsion and displace protein from the surface of the fat globule (Goff et al., 1989). When these events take place, this can lead to a partial coalescence during aeration and freezing and, therefore, a too-fast or too-slow meltdown where the gelato does not melt to a smooth consistency and leaves a structure behind (Arbuckle, 2013). When the ice crystals melt, they dilute the liquid phase of the gelato. The liquid phase of the gelato needs to be viscous to slow down the meltdown rate (Wu et al., 2019). Therefore, stabilisers in ice cream are used to prevent large ice crystals from forming in ice cream (Arbuckle et al., 2013). The addition of chia seed mucilage can act as a stabiliser to reduce the meltdown rate of the gelato. These results aligned with those reported by Feizi et al. (2020) for ice cream with dried chia seed gum added as stabilisers. The gelato meltdown rate is usually divided into 3 phases: lag, fast-melting, and plateau. The lag phase ended when the first drip happened. The fast-melting phase is the meltdown at the maximum rate. The plateau phase happened when the meltdown slowed and became static (Koxholt et al., 2001). The maximum weight loss rate happened during the fast-melting phase, and the total meltdown rate was the overall weight loss from all three phases (Table 10).

Table 10. Weight loss at maximum rate and total meltdown rate of gelato formulations

Formulation	Weight loss at maximum rate (g / min)	Total meltdown rate (g / min)
F1	0.81 ± 0.01^{a}	0.38 ± 0.01^{g}
F2	0.81 ± 0.00^{a}	0.39 ± 0.01^{g}
F3	0.78 ± 0.03^{a}	0.36 ± 0.01^{g}
F4	0.82 ± 0.03^{a}	0.36 ± 0.00^{g}
F5	0.78 ± 0.02^{m}	0.44 ± 0.00^{x}
F6	0.53 ± 0.05^{n}	0.38 ± 0.00^{y}

n = 6. Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different according to Tukey's HSD comparison ($p < 0.05$). F1, F2, F3, and F4 were compared while F5 and F6 were compared separately.

The weight loss meltdown rate of the gelatos with chia seed mucilage are shown in Table 10. Gelatos formulation for batch 1 (F1 – F4) showed a maximum weight loss rate in F3 (0.78 ± 0.03 g /min), followed by F1 (0.81 ± 0.01 g/min) and F2 (0.81 ± 0.00) and F4 (0.082 ± 0.03). The maximum total meltdown rate for formulation F2 (0.39 ± 0.01 g/min), followed by F1 (0.38 ± 0.01 g/min) and the slowest were F3 (0.36 ± 0.01 g/min) and F4 (0.36 ± 0.008 g/min) (Table 10). The weight loss and total meltdown rate of F1, F2, F3, and F4 were not significantly different ($p > 0.05$). Whereas, the weight loss and total meltdown rate of F5 and F6 were significantly different ($p < 0.05$).

As seen in Figures 14, gelato with higher concentrations of the mucilage was more resistant to melting. These results align again with a study by Feizi et al. (2021), where the parameters were improved by extending the homogenisation time while preparing the mix and increasing the agitation speed.

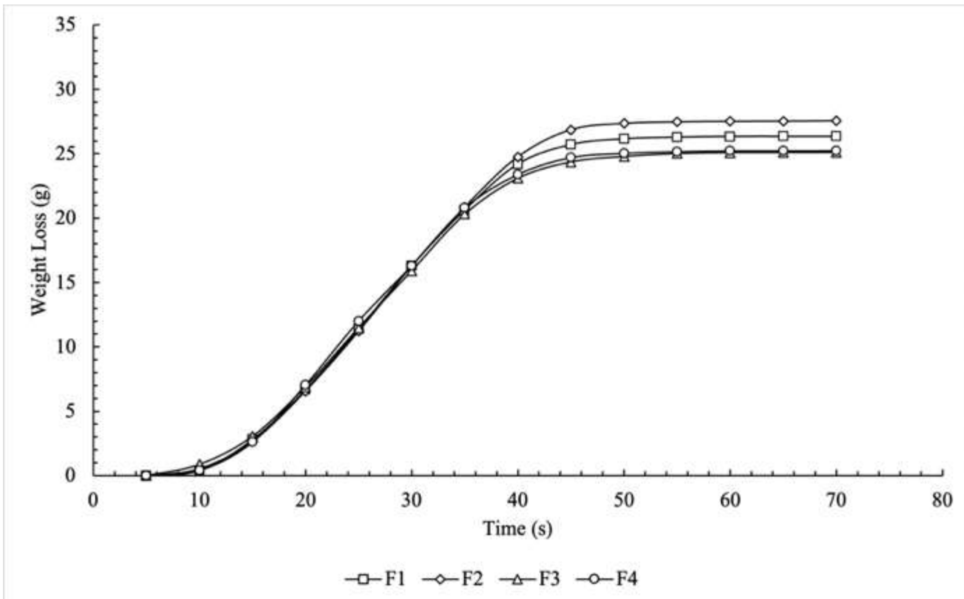

Figure 14. Gelato batch 1 of total meltdown rate for 70 min with maximum rate from 10 to 35 min. F1 (□) - 1.75% mucilage, F2 (◇) - 2% mucilage, F3 (△) - 2.25% mucilage, F4 (○) - 2.5% mucilage. Data were generated from two replications.

As shown in Figures 15, F5 has a maximum weight loss rate of 0.78 ± 0.02 g/ min and a total meltdown rate of 0.44 ± 0.00 g/min, and F6 has a maximum weight loss rate of 0.53 ± 0.05 g/ min and a total meltdown rate of 0.38 ± 0.00 g/min (Table 10). These results align again with a study by Feizi et al. (2021) and reflect the same with batch 1 (F1, F2, F3, and F4).

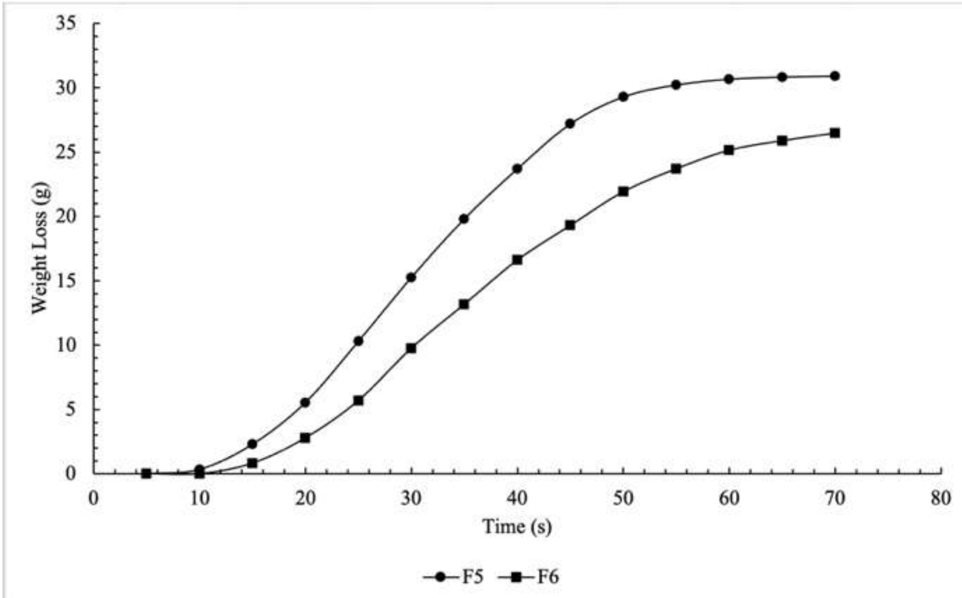

Figure 15. Gelato batch 2 of total meltdown rate for 70 min with maximum rate from 10 to 35 min. F5 (●) - 2% mucilage, and F6 (■) - 2.25% mucilage. Data were generated from two replications.

A desirable meltdown rate is when the serum phase is viscous, leading to a slower drainage process and a low meltdown rate (Wu et al., 2019). The presence of the mucilage caused the slower meltdown rate as it helps to incorporate air and slowed the heat transfer rate due to the volume of air cells that act as insulators and helped prevent heat from penetrating quickly through the gelato (Chiang et al., 2021). A desirable meltdown rate can be achieved as the mucilage increases the resistance to shrinkage due to their interaction with water by entangling and interacting with each other (Goff et al., 2013), and the high dietary fibre helped improve water holding capacity (Ürkek, 2021). Gelato has a higher sugar content and can help improve the meltdown rate as it reduces the freezing point of the ice cream (Silva Junior & Lannes, 2011).

4.5 Fat Globule Size Distribution

The fat globule size distribution is important in gelato as larger fat globules can affect the meltdown rate as they retain the structure of the foam, this leads to a poor meltdown where it leaves a structure behind (Koxholt et al., 2001). Larger-sized fat globules also tend to aggregate, making them less stable; however, stable fat globules are needed as they stabilise air cells and prevent them from recombining and forming air bubbles, which will accelerate the growth of ice crystals during storage and decrease the shelf life of the gelato (Silva Junior & Lannes, 2011). Fat destabilisation is generally used to describe the occurrence of coalescence, partial-coalescence, and aggregation in ice cream (Goh et al., 2008). The $d_{3,2}$ value is the volume-surface mean diameter (Meynier et al., 2005). The $d_{3,2}$ value of the fat globule in the gelato mix, which was the mixture of gelato base and chia mucilage, was found to be lower than in the gelato melt, which was aerated and frozen then melted gelato, the $d_{3,2}$ value was the highest in the gelato mix without mucilage (1.12 ± 0.091) (Table 11). The peak shown in the gelato mix and melt may be attributed to the fat droplet coalescence and/or fat droplet aggregation that might form during aeration and freezing (Feizi et al., 2021).

Table 11. Fat globule size distribution of gelato mix and gelato melt

Formulation	Chia seeds mucilage (%)	$d_{3,2}$ particle size (μm)	
		Gelato Mix	Gelato Melt
F1	1.75	0.39 ± 0.18^{a}	0.33 ± 0.09^{c}
F2	2	0.25 ± 0.08^{b}	0.29 ± 0.05^{c}
F3	2.25	0.19 ± 0.06^{b}	0.41 ± 0.04^{d}
F4	2.5	0.28 ± 0.05^{b}	0.55 ± 0.08^{d}
F5	2	0.21 ± 0.07^{w}	0.36 ± 0.06^{y}
F6	2.25	0.28 ± 0.08^{x}	0.49 ± 0.08^{z}

n = 3. Data expressed as the mean ± standard deviation. In each column, values followed by different letters are significantly different according to Tukey's HSD comparison ($p < 0.05$). F1, F2, F3, and F4 were compared while F5 and F6 were compared separately.

During freezing, fat destabilisation may occur and lead to the formation of larger diameter fat globules (Alvarez et al., 2005). There was a significant difference in the fat globule size in F1 gelato mix compared to gelato mixes F2, F3, and F4 ($p < 0.05$) (Figure 16). There was also a significant difference between fat globule size in gelato mixes F5 and F6 ($p < 0.05$) (Figure 17).

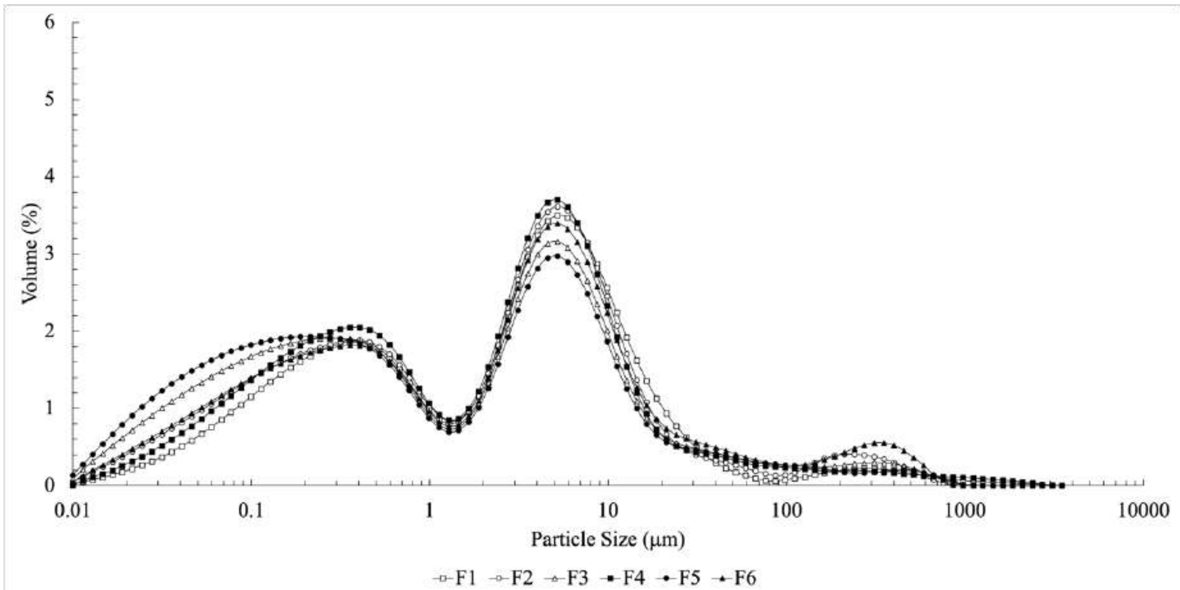

Figure 16. Average particle size distribution in gelato mix. F1 (□) - 1.75% mucilage, F2 (○) - 2% mucilage, F3 (△) - 2.25% mucilage, F4 (■) - 2.5% mucilage, F5 (●) - 2% mucilage, and F6 (▲) - 2.25% mucilage. Data were generated from two replications. F1, F2, F3, and F4 were compared while F5 and F6 were compared separately.

However, the gelato melts with F1 and F2 significantly differed from F3 and F4 ($p < 0.05$) (Figure 17), which may have been caused by a lower emulsifying capacity with less mucilage in F1 and F2, resulting in a less stable emulsion formation (Alvarez et al., 2005). Upon narrowing down the parameters, both the gelato mix and the gelato melt of F5 and F6 were significantly different from each other ($p < 0.05$), where the formulation with a higher percentage of mucilage had a larger fat globule size, which was consistent with results obtained by Feizi et al. (2021). A limitation of this research was confocal microscopy, which would have helped identify which components belong to each peak, as there will be a combination of fat globules and other particulates in the mix.

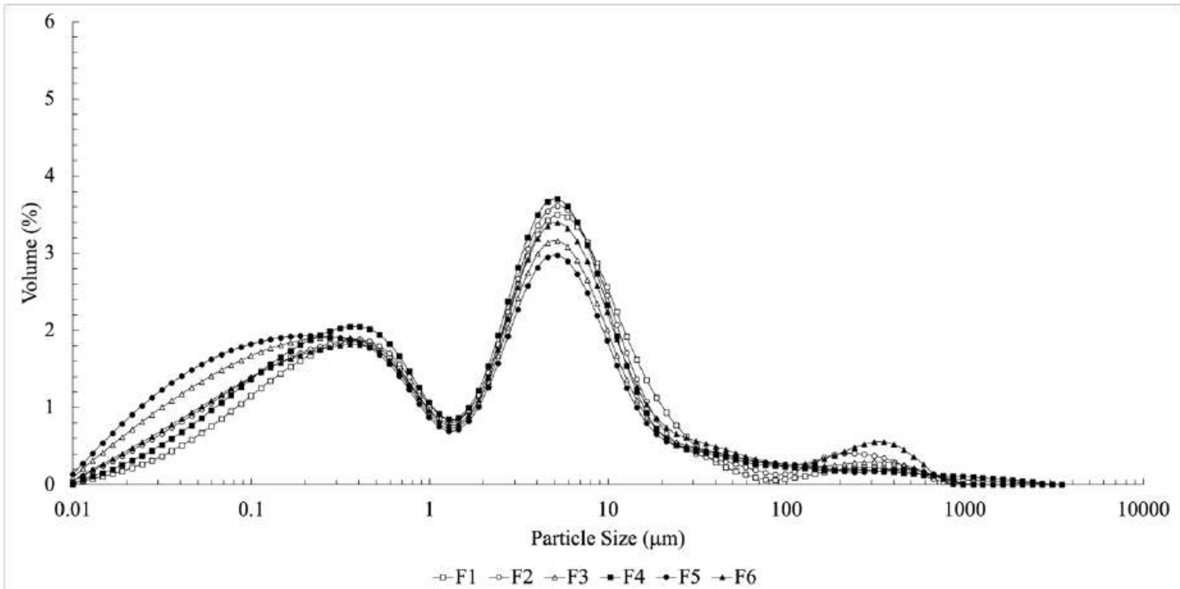

Figure 17. Average particle size distribution in gelato melt. F1 (□) - 1.75% mucilage, F2 (○) - 2% mucilage, F3 (△)- 2.25% mucilage, F4 (■) - 2.5% mucilage, F5 (●) - 2% mucilage, and F6 (▲) - 2.25% mucilage. Data were generated from two replications. F1, F2, F3, and F4 were compared while F5 and F6 were compared separately.

4.6 Rheological Properties

The rheological properties of the gelato melt, which was aerated, frozen, and thawed gelato, were determined by measuring the apparent viscosity, as shown in Figures 18 and 19. The highest apparent viscosity was observed in F1, which had the least mucilage (Figure 18). The hydrocolloids in the mucilage should increase the viscosity and control the growth of ice crystals (BahramParvar & Goff., 2013). The decrease in viscosity and ice crystal growth occurs due to improper emulsification (Motyl et al., 2019). Adding chia seed mucilage should increase the apparent viscosity of the gelato mix (Feizi et al., 2021).

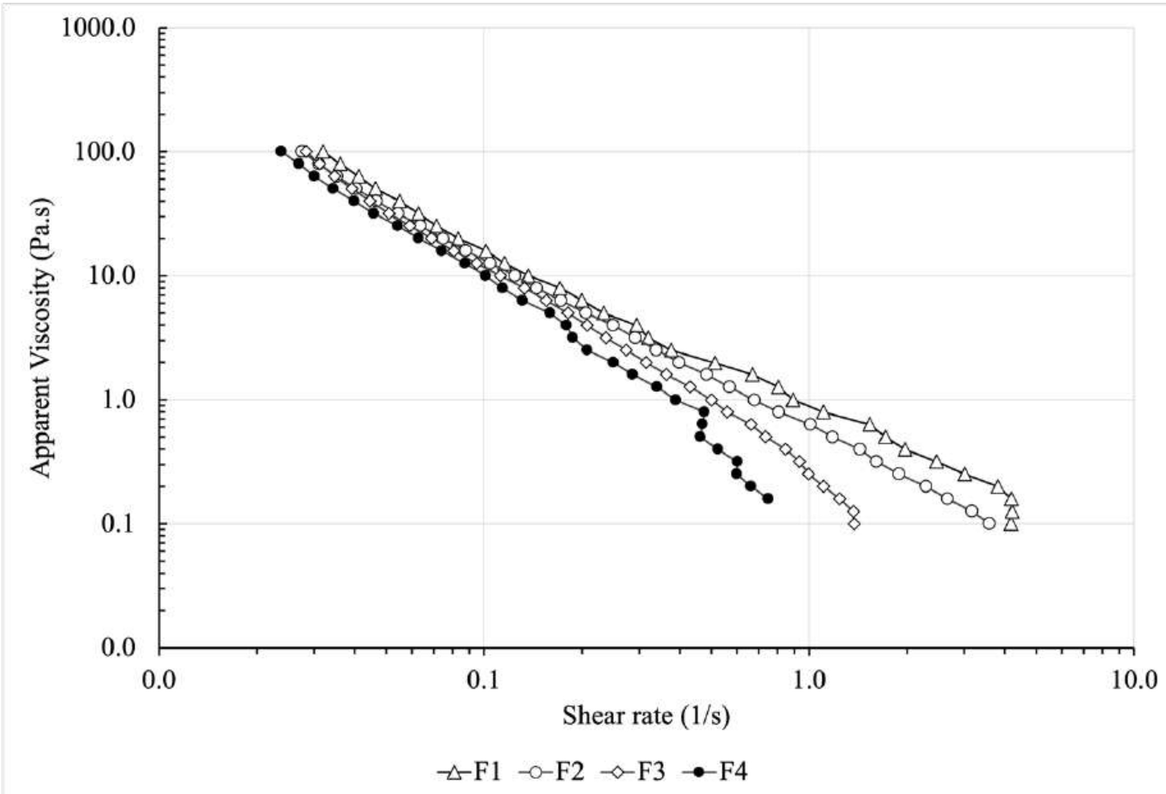

Figure 18. Apparent viscosity of gelato melt. F1 (△) - 1.75% mucilage, F2 (○) - 2% mucilage, F3 (□) - 2.25% mucilage, and F4 (●) - 2.5% mucilage. Data were generated from two replications.

However, in the second batch of improved gelato, F6, gelato melt with more mucilage, had a higher apparent viscosity than F5 (Figure 19). These results showed that adding mucilage with proper emulsification resulted in a higher apparent viscosity of the gelato mix and contributed to the creaminess and texture of the final gelato. Chia mucilage has shown the potential to exert interfacial activity to stabilise the air-water and water-oil interfaces and stabilise the foams in gelato (Soukoulis et al., 2018). The stabilisation of these interfaces increased the viscosity and enhanced the air lamellae; therefore, foam drainage may be prevented (Soukoulis et al., 2018). Figures 18 and 19 show that the decrease in apparent viscosity aligned with the increase of the shear rate from 0.1 to 100 s^{-1}. This behaviour happened because ice cream, in general, exhibited the behaviour of non-Newtonian pseudo-plastic flow. The chia mucilage contributed to the elastic properties and affected the apparent viscosity of the gelato mix (Goh et al., 2016).

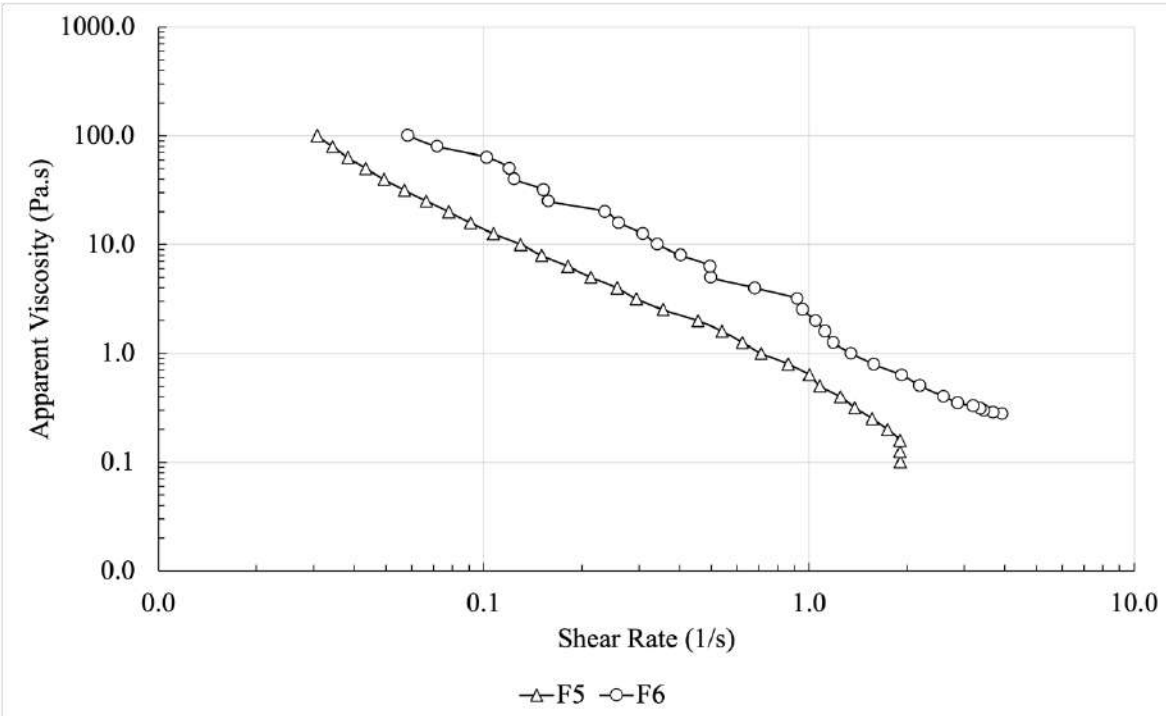

Figure 19. The apparent viscosity of gelato melt. F5 (△) – 2% mucilage and F6 (○) – 2.25% mucilage. Data were generated from two replications.

4.7 Texture

The hardness of the frozen gelatos was measured, and this relates to their ability to be scooped (Goh et al., 2008). The ice crystal size, fat destabilisation, and viscosity of the mix influence the hardness of gelato. Figure 20 shows the force needed (N) to penetrate each frozen gelato. In this study, there were no significant differences in hardness between F1, F2, F3, or F4 ($p < 0.05$). It followed the trend where the increase of the mucilage also increased the hardness (Campos et al., 2016). However, F3 did not follow this trend as it was the softest sample among the four (25.69 ± 16.76 N), and it contained 2.25% of chia seed mucilage, the formulation with the second highest mucilage. The formation of large crystals might influence the texture of gelato and produce a harder gelato; this may occur due to improper homogenisation (Soukoulis et al., 2009). Properly homogenised gelato has a uniformly dispersed fat, resulting in a more stable emulsion and a smoother texture (Schmidt & Smith, 1988).

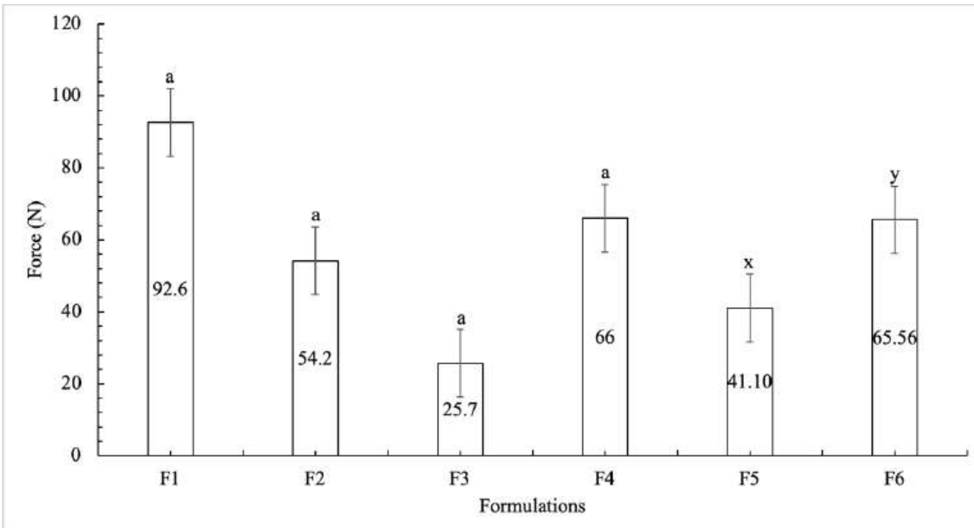

Figure 20. The hardness of gelato sample (measured at (-10 ± 2°C) : F1 – 1.75% mucilage, F2 – 2% mucilage, F3 – 2.25% mucilage, F4 – 2.5% mucilage, F5 – 2% mucilage, and F6 – 2.25% mucilage. F1, F2, F3, and F4 were compared, and *a* represents the samples are not significantly different after Tukey's HSD comparison ($p < 0.05$). F5 and F6 were compared separately, and *x* and *y* represent the samples are significantly different after Tukey's HSD comparison ($p < 0.05$).

After the focus group, where formulations were narrowed down and some parameters were changed, formulations F5 and F6 were significantly different to each other ($p < 0.05$), with F6 being the hardest (65.59 ± 8.91 N) compared to F5. Compared to a study by Velotto et al. (2021), gelato with chia mucilage was softer than gelato with chia seeds powder and stevia, with their lowest value at 1025.3 ± 68.2 N and their highest value at 1486.2 ± 68.2 N. There could be several reasons why the texture was different, such as the air, fat, and ice crystal size distribution, which can influence the hardness of ice cream. Destabilised fat could provide a network to bridge air cells in the gelato that influence the hardness of the ice cream (Rinaldi et al., 2014). However, Muse and Hartel (2004) found that the low overrun value in gelato did not influence the hardness of the samples.

4.8 Microbiological Analyses

Dairy products such as gelato have the potential to be exposed to *Listeria* (Ribeiro et al., 2023). Although pasteurising the dairy product can eliminate the presence of *Listeria*, sometimes post-pasteurisation contamination may occur (Kozak et al., 1996). A total plate count was conducted to determine if there were any viable microbiological populations. The presence of the colonies reflects the hygiene and cleanliness of the manufacture, handling, and storage (Barman et al., 2017).

Although gelato with formulations F1–F4 were not tested for microbiological activity, all care during preparation, handling, and mixing was carried out under food-safe hygienic conditions and were immediately used for sensory evaluation through focus groups.

Table 12. Microbiological assay on gelato

Assay		F5 (CFU/mL)	F6 (CFU/mL)
Listeria	Oxford Medium	ND	ND
	Palcam Agar	ND	ND
Total Plate Count	10^{-1}	2	1
	10^{-2}	ND	1
	10^{-3}	ND	ND
	10^{-4}	ND	ND
	10^{-5}	ND	ND

F5 and F6 were tested for microbiological assays, as both samples were intended to be consumed by many people during consumer sensory evaluation. *Listeria* was not detected in F5 and F6 after testing on Oxford Medium and Palcam Agar. The total plate count showed viable growth on the 10^{-1} dilution with F5 (2 CFU/mL) and F6 (1 CFU/mL) as well as viable growth on F6 10^{-2} dilution (1 CFU/mL) (Table 12). These numbers are perceived as safe as the microbiological standards stated by MPI (2017). The standard plate count for pasteurised milk products should not exceed 50,000 colonies/ml. The low growth is reportedly due to pasteurisation, and no ingredients were added after pasteurisation (Kozak et al., 1996; Barman et al., 2017). Any contamination may have come from the containers used to store the gelato (Barman et al., 2017).

4.9 Sensory Evaluation

Preliminary sensory evaluation was carried out with focus groups of postgraduate students of Food Technology at Massey University (Feizi et al., 2021). Two sensory focus groups were conducted to evaluate the gelato formulations. The first focus group was conducted to evaluate formulations F1, F2, F3, and F4. This batch of gelato was not tested for microbiological activity but it was severed immediately after the hardening process was completed.

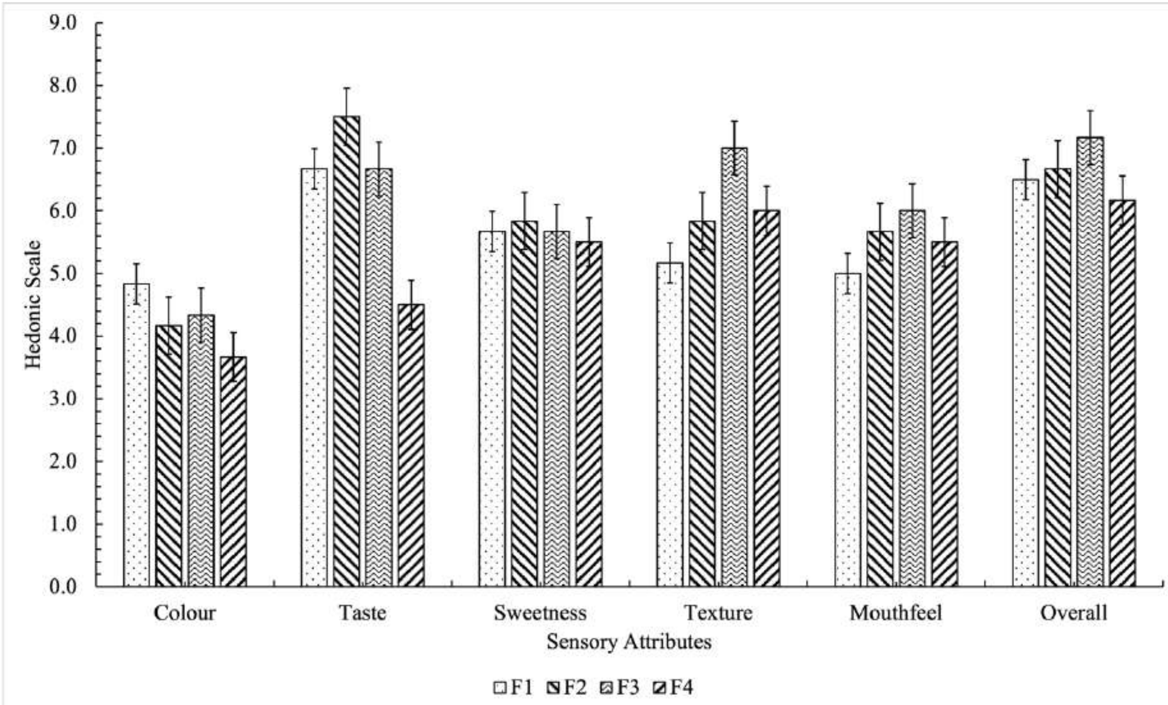

Figure 21. The results of the first focus group evaluation (n=6).

The discussion in the first focus group concluded that F2 and F3 had the most desirable characteristics as they were the creamiest texture and the least icy mouthfeel among the four samples (Figure 21). F1 was described as not melting quickly, and the ice crystals were very noticeable, whereas F4 had some inconsistencies due to butter-like mouthfeel after eating. Therefore, the panellists in the focus group agreed that F2 and F3 were the best formulations among the four samples based on sensory evaluation. However, flaws were mentioned, such as the presence of ice crystals that were not pleasant during eating. Some parameters were tweaked with the feedback by extending the homogenisation time while preparing the mix and increasing the agitation speed.

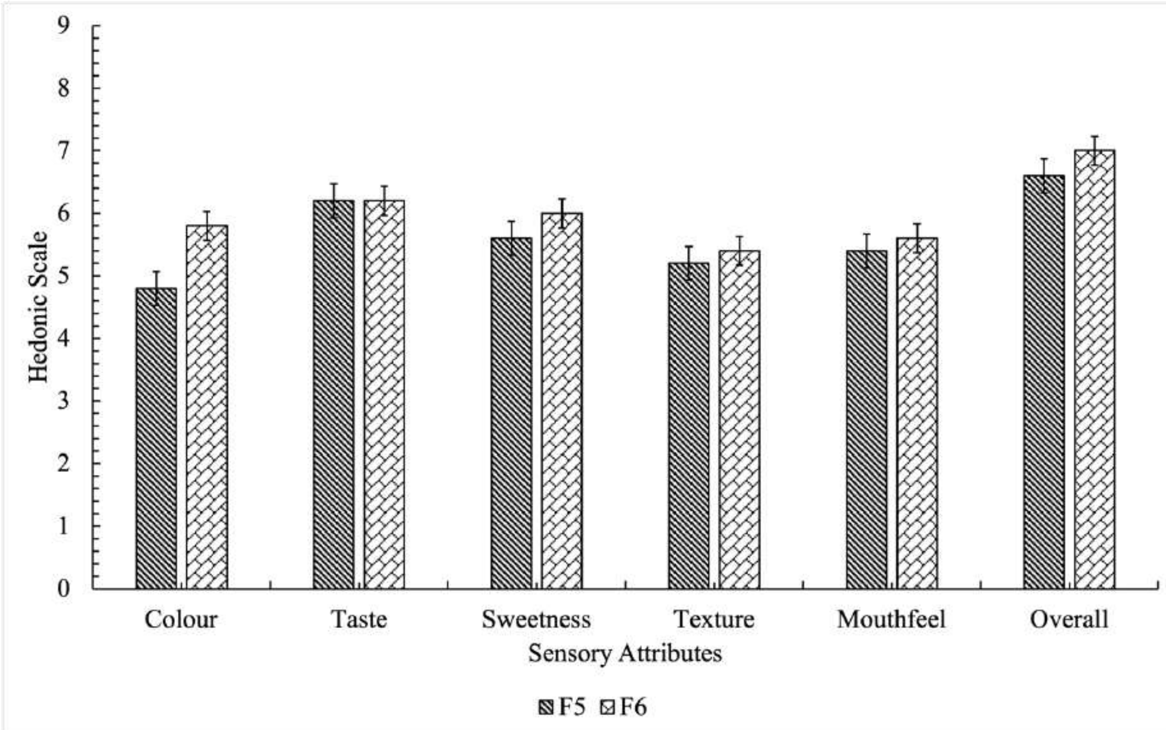

Figure 22. The results of the second focus group evaluation (n=5).

The second focus group was conducted after narrowing down the formulations to F5 and F6, which were evaluated (Figure 22). The focus group discussion concluded that overall, F6 had a creamier consistency and was described as not having any ice crystals, whereas F5 had a similar texture; however, some ice crystals were easily noticed. Therefore, they agreed that F6 was the most preferred formulation among the two samples. Formulation F6 was then produced on a larger scale and presented to consumer sensory evaluation. Repeating the focus group using a larger panel with more consumers would be recommended as the sensory for F1-4 and F5-6 were conducted at different times. The separate evaluation might influence the unexpected sensory evaluation, resulting in a lower gelato score for the improved formulations.

4.10 Consumer Sensory Evaluation

The consumer sensory evaluation was carried out to see if the gelato made with chia mucilage was acceptable to a larger group of consumers (n= 33). A compilation of just-about-right (JAR) scales was designed to fit the evaluated attributes. Five descriptive anchors were provided: much too weak on the leftmost end, slightly too weak on the second from the left end, just about right at the centre, slightly too strong on the second from the right, and much too strong on the rightmost end (Li et al., 2014). Each anchor was represented from 1 to 5 from the left end to the right end. The attributes scored were the same as reported by Velotto et al. (2021): colour, the aroma of milk, milky flavour, nutty flavour, sweetness, meltdown rate, mouthfeel (iciness), mouthfeel (creaminess), and hardness.

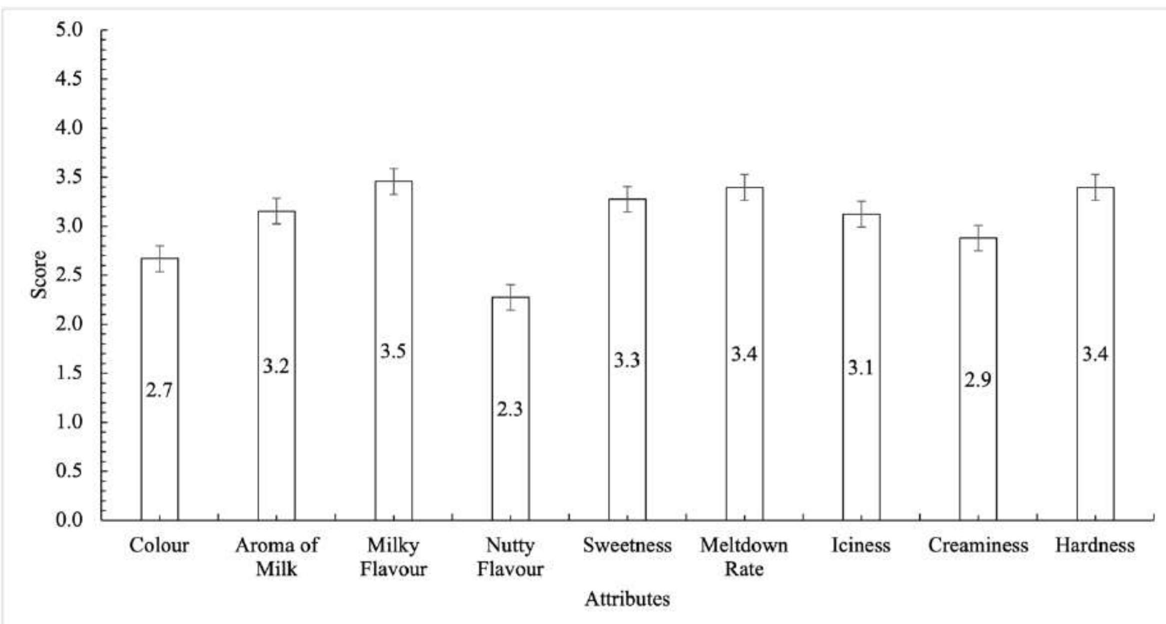

Figure 23. JAR attributes response for various attributes for formulation F6: colour, aroma of milk, milky flavour, nutty flavour, sweetness, meltdown rate, iciness, creaminess, and hardness, and likeness score for the overall taste. The averages were generated from n = 33.

The majority of scores were around 3, translating to just about right (Figure 23). The lowest score was in the nuttiness attributes, indicating that the chia seed mucilage did not affect the flavour of the gelato, as chia seeds have a mild, nutty flavour (Subhashree & Krishnamoorthy, 2019). The attribute scoring the highest was in the milky flavour attributes, which was expected as the product did not have any flavour. The consumer judged the meltdown rate by judging the longevity of the gelato during consumption. The gelato should not melt too fast or too slow. The consumers were also asked to score their overall experience with the product on a scale of 1 to 9 (1 – dislike extremely and 9 – like extremely). The product scored an average score of 6.8 ± 1.6, translating to like slightly (score of 6) and leaning towards like moderately (score of 7). One of the significant dislikes mentioned by the consumers was that the product was too milky, which is understandable as there was no flavouring in the product.

4.11 Overall discussion

Freshly extracted chia seed mucilage in gelato was evaluated using different concentrations as an emulsifier/stabiliser in gelato. It was found to produce results comparable to those reported by of Campos et al. (2016) and Feizi et al. (2021). The chia seed mucilage after extraction was stable during storage in the refrigerator based on the pH which did not change after three and six months. The addition of chia mucilage did not affect the pH of the gelato as was found to have a similar pH to ice cream made with chia seeds by Feizi et al. (2021) and ice cream with fish gelatine by Alika & Atma (2018). The overrun increased with the increase of chia mucilage concentration due to the uronic acid in the mucilage, as mentioned by Timilsena et al. (2016). The fat globule size distribution increased with the increase of chia mucilage concentration, which was consistent with the results reported by Feizi et al. (2021). The increase in concentration of chia seed mucilage created a more stable condition, as mentioned by Alvarez et al. (2005). The viscosity increased with the increase of chia mucilage concentration and helped control the growth of ice crystals, creating a more desirable gelato texture (BahramParvar & Goff., 2013). The meltdown rate decreased with increased chia mucilage concentration as the presence of chia mucilage increased the viscosity of the serum phase and prevented large ice crystals from forming, leading to a lower meltdown rate (Wu et al., 2019; Arbuckle et al., 2013). A relatively low overrun resulted in a softer texture that was achieved using the mucilage as the emulsifier/stabiliser (Campos et al., 2016). The microbiological assessment of the gelato showed low to no growth of viable bacteria colonies, which were within the acceptable range as mentioned in MPI (2017), reflecting the hygienic and clean manufacturing, handling, and storage process. During the focus group sensory evaluation, a consensus was reached that adding 2.25% mucilage in gelato produced the best overall experience compared to the other formulations using 1.75%, 2%, and 2.5% mucilage. This evaluation was also reflected during a consumer sensory evaluation, in which the consumer liked the texture and meltdown rate of the gelato. They also found that the gelato did not have a nutty flavour from the chia seeds, indicating that adding chia seeds did not affect the flavour of the gelato. The relatively low overrun, slow meltdown rate, high-fat globule distribution, and acceptable sensory properties showed that freshly extracted chia mucilage has potential as an emulsifier/stabiliser replacement for gelato.

There were some flaws in this study, such as the comparison with gelato without the addition of chia seed mucilage as a control for the other formulations was not carried out. Some methods can be added to improve gelato production, such as using a two-staged homogenisation method instead of a Silverson mixer. A larger quantity can also be produced using a professional ice cream maker. Therefore, the process will be streamlined as the pasteurisation and homogenisation can be carried out together one after the other. Comparison with readily available commercial gums, such as guar gum and locust bean gum, could have been conducted to ensure the chia seed mucilage is suitably good stabiliser/emulsifier. A more extensive focus group and sensory evaluation may be conducted by comparing more than one formulation and commercial gelatos available in the market.

Chapter 5 Conclusions and Recommendation

5.1 Conclusions

- The addition of 2.25% chia seed mucilage showed the best overall experience compared to the other formulations.
- The addition of chia seed mucilage did not affect the pH and flavour of the gelato.
- Gelato made with chia seed mucilage has a relatively low overrun, high fat globule distribution, slow meltdown rate, and soft texture.
- Gelato made with chia seed mucilage has acceptable sensory properties.
- Gelato made with freshly extracted or wet chia mucilage has the potential to be an emulsifier/stabiliser replacement.

5.2 Recommendation

- More comprehensive studies on the properties of freshly extracted chia mucilage, including its chemical and nutritional compositions—like fats, protein, fibre, and vitamins—along with microbial and shelf-life analyses.
- Purifying the chia seed mucilage from fat, as it has 29-34 g /100 g chia seeds, could be done to concentrate the emulsifying and stabilising activity.
- A comparison of dried chia seed mucilage to the fresh mucilage should also be conducted.
- A comparison of gelato without and with chia seed mucilage, as well as with other natural and commercial stabilisers/emulsifiers, should be investigated.
- Extended research can also be done by upsizing the production size and using a proper gelato machine to homogenise, pasteurise, and aerate the gelato.

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APPENDICES

Appendix 1. Consent form for focus group.

SENSORY EVALUATION FOR GELATO ICE CREAM

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Focus Group Model

Focus Group Sensory Evaluation of Gelato Ice Cream Stabilised by Chia Seed Gum

Information sheet

Consent Form

Project: Gelato Ice Cream Stabilised by Chia Seed Gum.

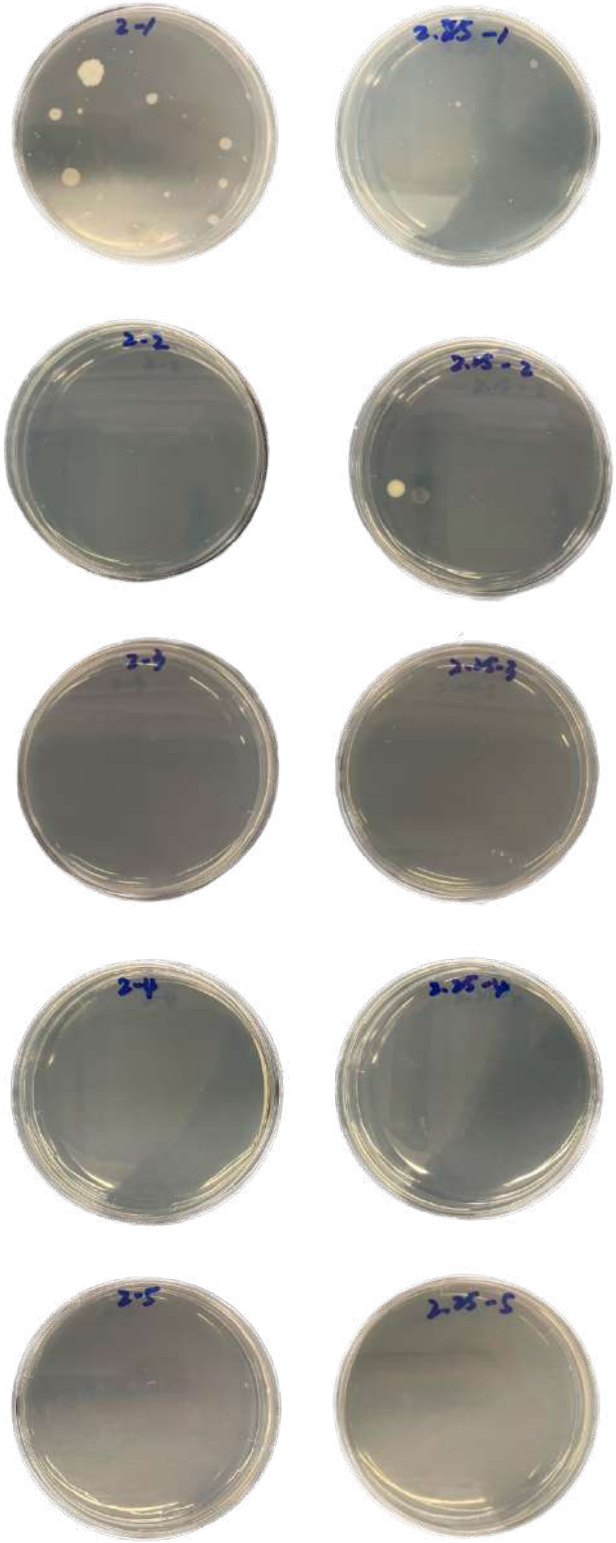
- I have read and understood the information provided to me. Any questions about the study have been disclosed or given and I know I can always ask any further questions.
- I understand that I may withdraw from this study at any time and may refuse to answer any specific questions.
- I have discussed with the researcher about 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 responsible for anything that happens to me during or after my participation in this research.

Signature: ______________________ Date: ____________________

Ethics number: 4000028338

Appendix 2. Flyers for the mass sensory evaluation.

Appendix 3. Total plate count on F5 and F6.



Appendix 4. Listeria detection on F5 and F6 using Oxford Medium and PALCAM Agar.

Appendix 5. Information about the evaluation and potential allergen for consumer sensory evaluation.

GELATO SENSORY TEST

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Mass Sensory Evaluation

Sensory Evaluation of Gelato with Chia Seeds Gum

Information sheet

Researcher Information

Researcher: Josephine Graciella	Supervisor: Dr. Tony Mutukumira
Contact details: 027 2993477	E-mail: Josephine. Graciella.1@uni.massey.ac.nz

We invite you to participate in a consumer sensory evaluation.

You will have approximately 15 minutes to participate in this activity.

The type of food you will taste is **Gelato**

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
- Chia Seeds

The information gathered in this study will be used to complete part of the dissertation thesis project in partial fulfilment of the Master 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

1

Appendix 6. QR Code for consumer sensory evaluation.

GELATO SENSORY TEST

The questionnaires are to be completed by you only.

No other person is allowed to participate in the test.

Please keep all instructions until you have completed the online questionnaires.

To start the questionnaire, enter the following site into the address bar of the web browser: https://app.redjade.net/surveys/masu/gelatotasting

QR Code:

Confirm the **Sample Code 989** to start your questionnaire.

Thank you in advance for your cooperation.

Appendix 7. Consent form for consumer sensory evaluation.

GELATO SENSORY TEST

Consent Form

Project: A Novel Gelato Ice Cream using Chia Seeds Gum as a Stabiliser.

• 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.

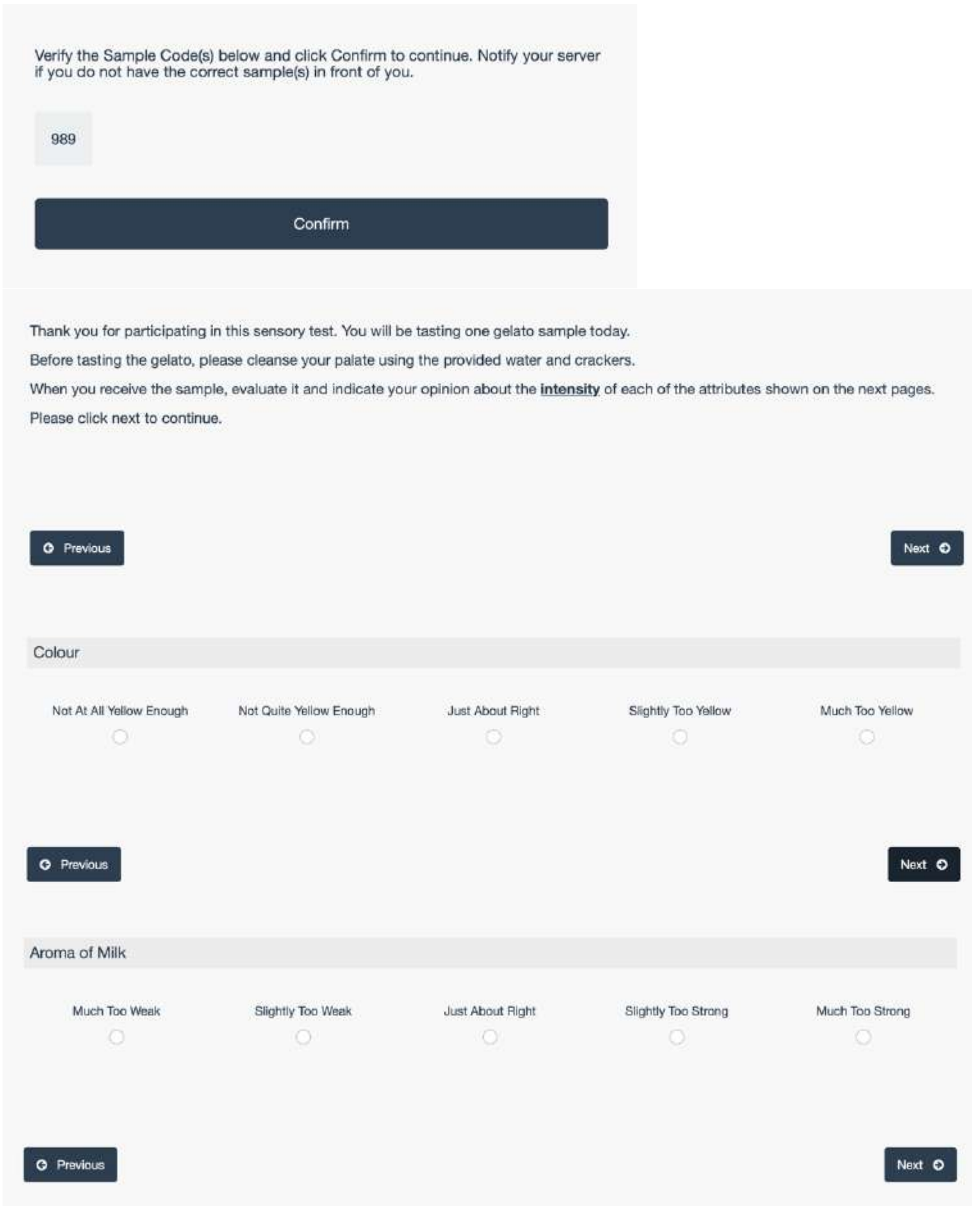
• I understand that I may withdraw from this study at any time and may refuse to answer any specific questions.

• 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: **4000028338**

Appendix 8. Assessment form from Red Jade.



Verify the Sample Code(s) below and click Confirm to continue. Notify your server if you do not have the correct sample(s) in front of you.

989

Confirm

Thank you for participating in this sensory test. You will be tasting one gelato sample today.

Before tasting the gelato, please cleanse your palate using the provided water and crackers.

When you receive the sample, evaluate it and indicate your opinion about the **intensity** of each of the attributes shown on the next pages.

Please click next to continue.

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Colour

Not At All Yellow Enough | Not Quite Yellow Enough | Just About Right | Slightly Too Yellow | Much Too Yellow

Previous | Next

Aroma of Milk

Much Too Weak | Slightly Too Weak | Just About Right | Slightly Too Strong | Much Too Strong

Previous | Next

Milky Flavour

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

Nutty Flavour

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

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Sweetness

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

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Meltdown Rate

Much Too Slow	Slightly Too Slow	Just About Right	Slightly Too Fast	Much Too Fast
○	○	○	○	○

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Mouthfeel (Iciness)

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

Mouthfeel (Creaminess)

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

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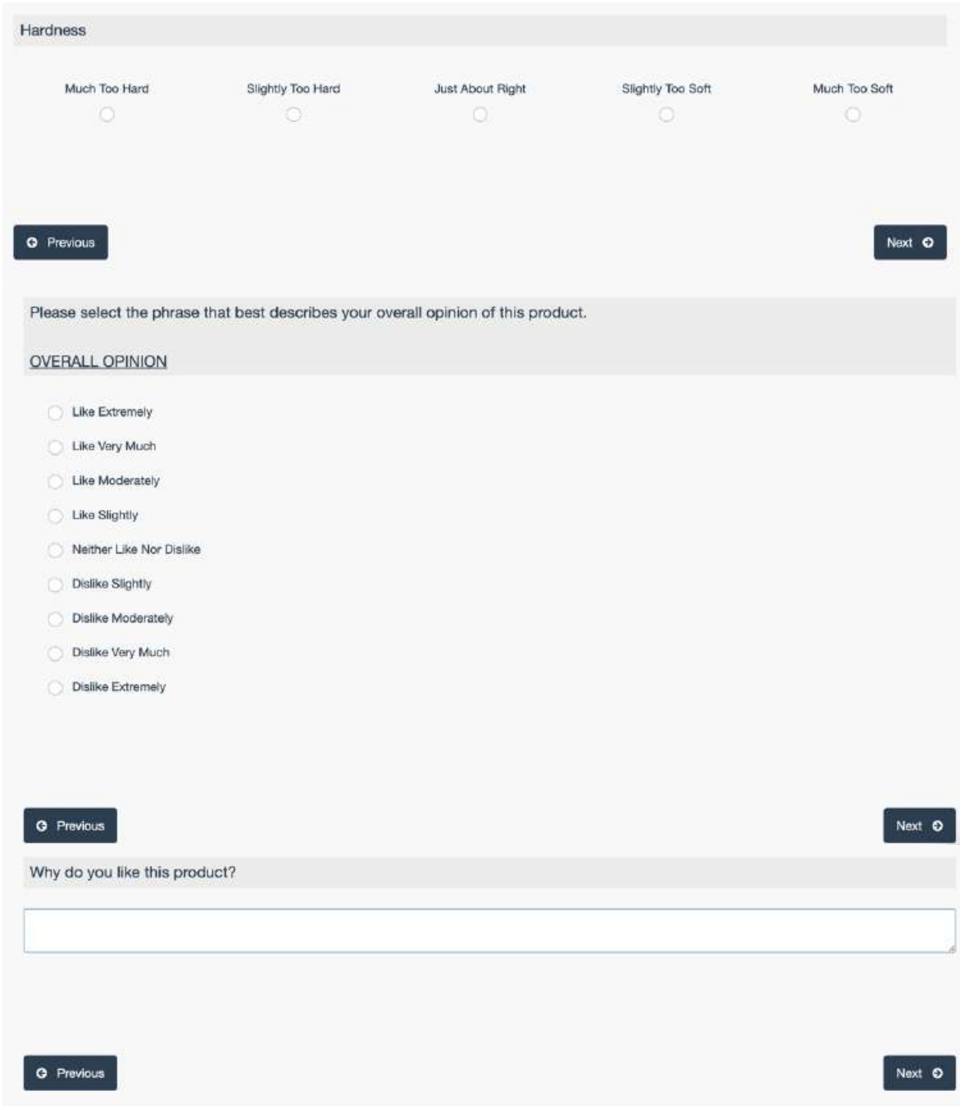
Hardness

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

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Please select the phrase that best describes your overall opinion of this product.

OVERALL OPINION

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

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Why do you like this product?

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