BSc THESIS

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Development of fermented and non-fermented beverages by Buttermilk

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1) Introduction and Objectives

This thesis, titled "Development of Beverages by Buttermilk," highlights the growing demand in the dairy business to introduce new products while integrating sustainable techniques. Buttermilk is a dairy by-product that is often neglected despite its great nutritional value and potential for a variety of uses in the food sector **(Ali, 2018).** An excellent starting point for the development of functional beverages is buttermilk, a by-product of the production of butter that is high in proteins, minerals, and phospholipids. In this study, buttermilk is considered as a resource for making value-added drinks that can satisfy customer desires for wholesome and environmentally friendly food options, rather than only as a by-product.

Waste management and environmental sustainability are major issues for the dairy industry globally, especially when it comes to handling by-products like whey, skim milk, and buttermilk. These resources are frequently assigned to low value uses or waste due to traditional procedures, which ignores their potential to contribute to culinary innovation. This thesis addresses this issue by examining how buttermilk might be used to make nutrient-dense, refreshing drinks, establishing it as a valuable ingredient rather than just a byproduct. This investigation is in line with industry trends that seek to optimize resource use, reduce environmental effect, and satisfy a growing consumer demand for sustainable and health-conscious food items.

This study also assesses the effects of fermentation and the addition of beneficial ingredients like inulin on the quality and acceptance of buttermilk-based drinks. Prebiotic fiber inulin provides health advantages, such as better gut health, in addition to adding nutritional value to the beverage. By introducing advantageous microbial cultures, fermentation improves flavor, texture, and shelf life.

As customers' awareness of environmental issues grows, this study demonstrates how the dairy industry may meet market demand through sustainable innovation by transforming by-products into appealing, health-conscious beverages. This study supports a circular approach that minimizes waste and environmental effect by optimizing the usage and value of raw materials, offering a workable model for the dairy industry to adopt.

The primary objective of this thesis is to use buttermilk, a nutrient-dense dairy by-product, to make functional beverages. We created three distinct varieties of buttermilk beverages: plain, apricot-flavored, and strawberry-flavored, all with the addition of inulin. Each beverage was made using both fermentation and non-fermentation methods to directly analyze their concentration effects on physiochemical and rheological properties such dry matter content, pH, color measurement, and viscosity. The study evaluated the effects of various inulin levels on texture, viscosity, and sensory attributes to identify the optimal formulation that appeals to consumers.

2) Literature review

2.1) Introduction to milk

Milk is a foodstuff, typically considered as the most perfect food item and it is consumed directly by the consumers without any further processing. Worldwide nutritionists and dietitians agree on the fact that milk is of great significance in terms of promoting the growth and development of bones and body mass in infants and children **(Kailasapathy, 2015**). Although milk plays a central role in young ones' feed, it is also considered a vital pillar in our diet during our whole life due to its ingredients. A lot of uses milk in the diet including infant feeding, as a stabilizer and taste improver in tea, coffee, and beverages, an element for manufactured foods such as bakery (bread, sweets, pastry or cake) and confectionery items, and a prime component for processed dairy products (yogurt, cheese, ice cream, and butter) **(Fox, 2003**). Milk can be processed and stored in different forms e.g., powdered milk, condensed milk, and other fortified milk forms to overcome milk's perishable nature in its ordinary state (**Kailasapathy, 2009**).

Milk, a complex biological liquid comprised chiefly of water, proteins, liquids, carbohydrates, and a range of bioactive factors **(Dayon et al., 2021)**, and is secreted by mammary glands of all female mammals to feed their young for a specific period starting right after birth. The lacteal secretion that is obtained for 15 days before and after parturition may not be included in the term milk as it may practically contain colostrum and not contain the minimum approved percentage of milk solids-not-fat (SNF) and milk fat. The milk from domesticated animals like cows, water buffalo, sheep, goats and camel is an essential source of food for humans either consumed as fresh milk or processed food into a category of dairy products such as yogurt, cheese, and butter **(Kailasapathy, 2015).** Another term "market milk" denotes liquid whole milk that is consumed directly by the consumers excluding the milk quantity used on the farms to produce other dairy products. At present there are several thousand milk products are made from natural milk, these principal groups include cheese (35%), beverage milk (40%), milk powder (15%) butter (30%), concentrated and fermented milk (2% each), and some other food products such as dairy cream, lactose, infant formula, and some protein-rich food items **(Fox, 2008).**

2.2) Composition of milk

Milk is a very complex lacteal secretion obtained by the milking of healthy domestic mammals e.g., cows & buffaloes. However, there are several minor molecular species or trace elements such as minerals, hormones, enzymes, vitamins, and many other miscellaneous compounds. The milk chemistry is similar in the majority of mammals' species but sometimes there is a structural difference of compounds noted in different species that reflects evolutionary variations among them **(Fox, 2008).**

The concentration of the main elements of milk fluctuates widely among mammal species containing lipids (2-55%), lactose sugar (0-10%), and proteins (1-20%), showing mainly the energy requirement of young ones by milk sugar and milk fat, and proteins for the growth of neonate. The minor or trace components of milk also vary commonly and give milk of different concentrations. Within a species, the milk composition may vary amongst individuals, between breeds, health, and feed of the animal, number of parturition by the animal, time of milking, stage of the lactation, and many other factors of significance **(Petit et al., 2001).**

The lipid content of bovine milk gives an idea of wide inter-breed variation, and between two species a large inter-species fat and protein content for discrete animals, with similar changes noted among sheep, goat, cow, and buffalo milk which reflects the milk constituent's variation. This molecular variation of milk for different species indicates the nutritional and functional necessities of the neonate. Table 1 displays cow milk's physicochemical properties, such as fat globule size, pH, viscosity, density, surface tension, acidity, phosphatase units, and casein micelle concentration **(Fox, 2013).**

The milk composition changes are mostly assessed during the early days after parturition of the milking animal, specifically the fraction of immunoglobulin proteins. For instance, in marsupials, the high-content milk carbohydrate (oligosaccharides) secretion down to a high-fat secretion and compels the leaving of the neonate from the mother pouch and live without milk thereafter. During the mid-lactation, the milk composition relatively remains constant but it gradually changes at the end of the lactation period, presenting the fact of mammary gland tissue involution and the greater invasion of blood ingredients in the mammary tissue of the animal **(Hurley, 2003).**

Table 1: Physicochemical Characteristics of Cow Milk

(Fox, 2013)

Characteristics	**Cow Milk**
Fat globule size (μm)	3.85
pH	6.60
Viscosity (cP)	1.86
Density at 20 ℃	1.02
Surface tension	5.59
Acidity (%)	0.14
Phosphatase units	83.0
Casein micelles (micelles/ mL of milk)	10^{14} to 10^{16}

2.3) Milk constituents

The principal components of milk include water, proteins, sugar, and milk fats as can seen in table 2.

Table 2: Chemical Composition of Cow Milk (% Composition)

(Fox, 2013; Anon,2005)

Component	**% Fraction**
Water	85.3-88.7
Fat	2.5-5.5
Lactose	3.8-5.3
Protein	2.3-4.4
Ash	0.5-0.9

2.3.1) Water

Water is considered the principal component of milk in cow's milk. In neonates, milk water meets the requirement of water of the young one. Water present in milk serves as the solvent for milk proteins, salts, and lactose, and affects their stability and properties. Milk water controls the reactions happening in milk such as microbial growth, enzyme activity, lipid oxidation, and Maillard browning reaction and thus disturbs the stability of milk and its products **(Fox, 2013)**

2.3.2) Milk Fats

Milk lipids are commonly comprised of fats or oils which are solids or liquids at ambient temperature. Conventionally, milk fat was regarded as the most valuable constituent of milk, and milk is valued based on its fat content. Milk lipids are commonly distributed into three classes including neutral, polar, and miscellaneous lipids. Neutral lipids (esters of glycerol and simple fatty acids) are the dominant group of lipids present in milk making 98.5% of total lipids. Polar lipids are another class of lipids, made from a mixture of fatty acid esters of sphingosine and glycerol, some may have nitrogen-containing compound choline, serine, or ethanolamine, and others may contain oligosaccharide and phosphoric acid. Polar lipids make up 1% of milk lipids and play a very good role in milk products as they are very good emulsifiers that maintain discrete fat globules and ensure physicochemical stability of milk. Miscellaneous lipids are a heterogeneous group of lipids including cholesterol, carotenoids, and fat-soluble vitamins (A, E, D, and K), present in a very minute quantity in milk and are responsible for milk and milk products color **(Fox, 2008).**

Milk lipids contain a variety of fatty acids nearly 400 fatty acids have been reported in milk with some present in trace amounts. The cow milk lipids are unique as they are natural lipids having butyric acid, and some substantial fatty acids including hexanoic acid, octanoic and decanoic acid. Butyric acid is synthesized by ruminant microbiota, and it is the natural milk fat present in cow's milk. There are certain polyunsaturated fatty acids (PUFAs) present in the milk of cows, and they are hydrogenated by ruminant bacteria. Milk enriched with PUFAs has been perceived to have improved nutritional value and better spreadability of butter and ghee **(Fox, 2003).**

2.3.3) Milk carbohydrates

2.3.3.1) Lactose

Milk sugar is comprised mainly of lactose, a reducing disaccharide carbohydrate, which is composed via β 1-4 glycosidic linkage between two monosaccharides glucose and galactose. Lactose concentration varies from zero to ten percent and the only known source of lactose is milk **(Fox, 2013)**. Lactose synthesis mostly occurs in epithelial cells of mammary glands from the absorption of two molecules of glucose from the bloodstream. Phosphorylation of one molecule of glucose happens there and is converted into galactose-P molecule by the Leloir pathway, a widespread pathway in bacterial and animal tissues. This galactose-P gets condensed with another glucose molecule through a unique enzyme lactose synthetase and lactose forms. Lactose has an inverse relation with the concentration of casein protein in milk. After the formation of lactose, water enters into the Golgi vesicles osmotically and consequently affects the milk volume and concentration of casein protein enveloped in the Golgi vesicles **(Jenness & Holt, 1987)**. Lactose works as a complete source of vitality for the neonates by offering 30% of the calories in cattle milk and acting as a substitute for the more compact energy lipids. Milk, having a high content of lactose tends to be the food with a lower percentage of lipids **(Jenness, 1970).**

Oligosaccharides are another group of sugars most commonly present in milk and synthesized in the mammary glands by special transferase of milk. **(Mehra & Kelly, 2006).**. These milk sugars also play an imperative role in the synthesis of glycoproteins and glycolipids, important for the development of the brain in neonates **(Kunz & Rudloff, 2006).**

2.3.4) Proteins

There is a variety of milk proteins present in bovine milk, and these proteins greatly affect the properties of milk and products made from milk. The major portion of milk proteins is made from casein, other proteins including lactalbumin, and lactoglobulin are lower than casein. Serum (whey) proteins make up 20 of the total proteins of milk. In the milk of a mature cow, about 0.6-1 g of immunoglobulins (Igs)/L is present. There are many minor proteins, vitamin-binding proteins, β- microglobulin, glycoproteins, growth hormones, osteopontin, angiogenins, and kininogen make a certain portion of cow's milk inhabiting specific properties **(Fox et al., 2015).**

2.3.5) Vitamins

Milk contains a range of vitamins making it a necessary vitamin-rich diet for its consumers. Vitamins including biotin, riboflavin, and cobalamin are very significant vitamins present in cow's milk. Other than these vitamins, vitamin A, vitamin C, vitamin E, vitamin D and carotenoids are responsible for maintaining milk stability and milk properties (**Fox, 2003)**

2.3.6) Minerals

There is a range of milk salts present in cow's milk rendering specific nutritional and technological functions to the milk. The most common salt in milk is sodium which gets lost during evaporation **(Fox, 2008)**. The concentration and distribution of salts of different metals is as follows in table 3.

Table 3 : Concentration of different salts in milk

(Fox, 2008)

Species of salt	**Concentration (mg/L) %**
Sodium	92
Potassium	92
Chloride	100
Sulfate	100
Phosphate	43
Calcium	34
Magnesium	67
Citrate	94

2.4) Dairy by products

Milk stands out as an exceptionally versatile food material, as illustrated in Table 4, showcasing various milk-based food categories, each encompassing numerous distinct products. The inherent disparity in composition and quantity between these primary products and raw milk results in the generation of byproducts requiring processing. The primary goal in processing and fractionating milk is to derive commercially viable, nutritionally rich, and techno-functional ingredients and products while minimizing side stream and waste production. In the dairy industry, the substantial volume, composition, and chemical/biological oxygen demand of side streams and byproducts necessitate processing, even when they possess a negative value **(De Boer, 2014).**

In manufacturing, a byproduct is a secondary outcome arising from a production process or chemical reaction. Its significance is minor in terms of quantity and/or net realizable value compared to the main products in a joint production process. The utility and marketability of a byproduct vary, contingent upon factors such as composition, volume, quality, and ease of processing **(O'Mahony & Fox, 2014)**. In the milk processing domain, the classification of byproducts can vary depending on the specific context. For instance, in the production of butter or butter oil, byproducts such as buttermilk and butter serum are readily identifiable. In this discourse, cream and skimmed milk are delineated as such. In butter manufacturing, butter constitutes the principal output, while buttermilk emerges as a secondary byproduct.

Table 4*:* Diversity of dairy products

(Oliveira et al., 2019)

Process	**Primary Product**	**Further Products**
Centrifugal separation	Cream	Butter, butter oil, ghee, anhydrous milk fat; creams of various fat content (coffee creams, whipping creams, dessert creams; cream cheeses)
	Skim milk	casein, cheese, protein concentrates and infant formulae
Thermal processing		HTST or super-pasteurization, UHT-sterilized or in-container sterilized
Concentration, thermal evaporation or membrane filtration		Evaporated or sweetened condensed milk
Enzymatic coagulation	Cheese	1000 varieties; further products, e.g., processed cheese, cheese sauces, cheese dips
	Rennet casein	Cheese analogues
	Whey	Whey powders, demineralized whey powders, whey protein concentrates, whey protein isolates, individual whey proteins, whey protein hydrolysates, nutraceuticals. Lactose and lactose derivatives
Acid coagulation	Cheese	Fresh cheeses and cheese-based products
	Acid casein	Functional applications, e.g., coffee creamers, meat extenders; nutritional applications, cream liquors
Fermentation		Various fermented milk products, e.g., yogurt, buttermilk, acidophilus milk, bio-yogurt
Freezing		Ice-cream (numerous types and formulations)
Miscellaneous		Chocolate products

2.4.1) Composition and nutritional value of dairy by-products

2.4.1.1) Buttermilk

The chemical composition of buttermilk determines the nutritional and flavor properties of the product **(Gebreselassie et al., 2016)**. A dairy by-product rich in nutrients, buttermilk has a high protein content that can range from roughly 19% to over 33%, depending on the kind. Also, it contains beneficial fats like phospholipids that promote the integrity of cell membranes and food product emulsification. Milk sugar lactose gives you energy, and vital minerals like calcium and potassium help maintain healthy bones and electrolyte balance. The comparatively low pH of buttermilk also makes it appropriate for fermented items, improving their digestibility and possibly providing microbial advantages as we can see the compositional values of buttermilk in table 5.

Table 5: Chemical composition of dry matter of buttermilk (% of composition)

(Ali, 2018)

Components	**% fraction**
Protein	26
Fat	14
Phospholipids	4
Ash (Mineral)	30
Lactose	50
pH	6.1

Buttermilk is a versatile component and a healthy complement to a balanced diet because of its creamy texture and slightly sour flavor as illustrated in table 6.

Table 6: Nutritional value of low fat, cultured buttermilk

(Ali, 2018)

Nutrients	**Amount per 1 cup(237ml)**
Calories	98 kcal
Total fat	2.16 g
Saturated fat	1.34 g
Cholesterol	10 mg
Sodium	257 mg
Potassium	370mg
Total carbohydrates	11.74 g
Sugars	11.74 g
Protein	8.11 g
Vitamin A	1%
Vitamin C	4%
Calcium	28%
Iron	1%

2.4.1.2) Whey

The source of the milk, the kind of feed given to dairy animals, the stage of lactation, and the processing technique are some of the factors that determine the composition and quality attributes of whey and whey proteins. The enzymes employed in the production of cheese and the type of cheese can affect the whey's characteristics, causing it to be classified as either sweet or acidic **(Soltani et al., 2017).** The composition of sweet and acidic whey is illustrated in table 7.

Table 7: Chemical composition of Whey

(Ganju and Gogate 2017)

Constituents	Sweet Whey	Acidic whey
Water	93-94%	94-95%
Dry matter content	5-6%	5-6%
Lactose	4.5-5.0%	3.8- 4.3%
Lactic acid	Traces	Up to 0.8%
Total protein	0.8- 1.0%	0.8-1.0%
Whey protein	0.60-0.65%	0.60-0.65%
Citric acid	0.1%	0.1%
Minerals	0.5-0.7%	0.5-0.7%
pH	6.2-6.4	4.6-5.0

2.4.2) Utilization of milk by-products in dairy industry

Buttermilk is used in preparing sports drinks and brews and whey is utilized in the synthesis of a special type of cheese called ricotta cheese. Skim milk is a dairy by-product obtained during the preparation of cream and has been used to give flavor in most of the flavored milks and in the manufacturing of cheeses like quark and cottage cheese. During the manufacturing of ghee, ghee residues are obtained that are used in the preparation of cookies, sweets, and chocolates. Furthermore, caseins and whey proteins are also used in the packaging of films **(Rafiq & Rafiq, 2019).**

Globally, there are many attempts that have been taken to utilize dairy industry by-products in an effective way due to their high nutritive profile. In India, dairy plants are still challenged with the problem of efficient use of dairy by-products as there is a lack of suitable technology and high cost of new expertise. However, countries are making improvements in this technology. Whey, the key by-product of dairy industry is a valuable reserve of nutrients covering almost 50% of the solids of milk **(Kunte & Patankar, 2015)**. The production of whey is going on steadily, and its high organic content is an important issue for environment and public health **(Macwan et al., 2016)**. Therefore, appropriate management of dairy waste is required for One health stability. Whey, just like milk may have different animals of origin (e.g., cattle, buffalo, sheep, and goat), but the best applicable in terms of economical and volume of production is obtained from the processing of cattle milk. It is rich in solid not fat (SNF) content with beneficial effect in nutrition and has been used in the manufacturing of a few dairy products in powder form. Buttermilk is a dairy by-product of butter and has been used in its original dried form.**(Rafiq & Rafiq, 2019).**

2.4.2.1) Whey utilization

Whey is a by-product of cheese processing and has almost 7% dry matter with 13% proteins **(Kumar et al., 2018)**. It commonly signifies a volume portion of 90% in milk and is categorized into acid and sweet whey Whey protein is used to increase nutritional profile of many products such as yogurt, infant feed formulas, and energy bars. Lactose, a milk sugar is also used as a sweetener **(Hettinga, 2019).**

The sweetened whey comes from cheese or it originates from casein produced in industry where casein is coagulated by rennet at a range of pH 6.0-6.5 **(Guinee, 2021).** While the acidic whey having pH below 5.0 is produced in the result of coagulation of casein via fermentation or addition of mineral or organic acids in it (e.g., formation of quark or cottage cheese) or strained or Greek style yogurt. The main components for both sweet and acidic whey are listed in Table 5. Water makes around 93% of the whey while lactose makes 70-72%, minerals 12-15%, and whey proteins 8-10% of the total solids fraction **(Kilara, 2015)**. The main difference between sweet and acid whey is the mineral portion, composition of protein, and the acidity. Calcium fraction is higher in acidic whey as at low pH, the colloidal calcium in the casein micelles in raw milk is solubilized and segregated into the whey. In sweet whey, the composition of whey protein is different as it contains fragment of casein molecule called glycomacropeptide, formed during rennet clotting (rennet-based whey) and it makes 20% of whey protein giving its sweet taste **(Glass & Hedrick, 1977)**. Acidic whey is mainly used in beverages manufacturing units due to its nutritive potential. The application of acid whey bids an interesting tactic as here is no requirement for consuming complex machinery other than conventional method of pasteurization **(Mulvihill, 1992).**

Further processing of liquid whey will separate and purify either concentrate of whey protein isolate (almost 80% protein in dry matter) or whey protein isolate having 90% protein in dry matter in it **(Wong et al., 1978)**. Ultrafiltration protocol is performed for cheese milk to preserve whey protein to increase the production of product. Additionally, the nutritional profile of cheese is increased via adding whey proteins in it. Various pre-treatments have been established to treat cheese milk with the incorporation of innate or denatured whey proteins in the cheese matrix

(Tarapata et al., 2022). These options have unlocked new opportunities for advancement in the cheese industry. It has multiple uses in animal feed and human feed formulation and shares a bioactive peptide source **(Ismail, 2012).**

2.4.2.2) Utilization of ghee residue

Ghee is an important component of meals consumed by the public and it is prepared by different methods. It is simplified form of milk fat with unique organoleptic properties and qualities, which makes it an important element in an inclusive range of diet applications. It is particularly highly manufactured in countries where its use in traditional cooking is more including Middle East, South Asia, and the Africa **(Wani et al., 2022)**. The annual estimation of precise volume of milk altered into ghee can be perplexing due to fluctuating production practices, local intake patterns, and alterations in the accessibility of consistent data. However, few common figures and drifts can offer perceptions into ghee making. In India, it is projected that about 30-35% of the entire milk produced in country is converted into different milk products, with ghee sharing the major ratio among the products. In India, annually 22 million tons of ghee is produced which requires a big amount of milk for its conversion to ghee. Usually it takes about 15 to 20 liters of milk to produce 1 kg of ghee, highlighting that for annual production of ghee, hundreds of millions of milk will be required alone just in one country India **(Hae-Soo et al., 2013)**. Similarly other countries including Pakistan (2^{nd} major producer of ghee), Middle East, Bangladesh, Nepal, Sri Lanka, and Africa are also producing valuable amount of desi ghee more confined for domestic intake with an overall 30 million tons of ghee from overall globe **(Wani et al., 2022).**

Ghee residues (GR) are obtained during the ghee manufacturing process and their amount depends upon the method of ghee preparation. In the direct creamy (DC) method of ghee, the maximum amount of ghee residues is achieved (12%) followed by direct creamy butter method (3.7%). Ghee residues mainly contain milk proteins with a little quantity of lactose and minerals. These residues are used in food industry for making bakery products, biscuits, cookies, sweets, and as a flavor improver **(Sojan et al., 2019)**. In the country where protein and nitrogenous sources are maximum, will give an appreciable sum of residues. In sweet items, GR is being used for making burfi, khoa, halwa, sugar, chocolate, candies, toffees, etc via mixing skim milk or powder milk with GR and other food elements **(Rafiq & Rafiq, 2019)**. The utilization of this dairy by-product is an important part of the dairy and food industry making it more attractive.

2.4.2.3) Buttermilk

Butter milk is a by-product achieved during butter production; it is rich in proteins and phospholipids. It is used in food industry for baking goods, giving aroma, and in other confection products **(Skryplonek et al., 2019).** Buttermilk serves as a natural emulsifier in the food and beverage industry, enhancing the flavor, texture, and stability of items like sauces, dressings, and processed foods. Because of buttermilk emulsifying property, It has best use in yogurt, kefir, cheese, ice cream and beverages **(Nyulas-Zeke et al., 2024).** It has unique functional properties and in functional drinks, frequently mixed with fruit juices and carbonation to enhance flavor and nutrient content. Certain elements in buttermilk, like calcium, protein, and B vitamins, become more bioavailable because of fermentation, improving the body's ability to absorb them **(Vidhauliya et al., 2024).** Additional health advantages can be obtained by fermenting buttermilk to improve its antioxidant concentration and activity **(Gebreselassie et al., 2016)**. Aloe vera or dietary fiber fortification improves its nutritional profile and makes it a popular option for health-conscious beverages **(Ali 2018).**

2.4.2.4) Butter fat

Butter fat is extracted from cream, and it is used in different products of dairy including ghee, butter, and clarified butter. It also has used in pharmaceuticals and cosmetics industry **(Kumbhare et al., 2021).**

2.4.2.5) Milk permeates

Milk permeate is a dairy by-product rich in lactose and achieved after the ultrafiltration of milk. It is used in beverage and food industry to replace content of artificial sweeteners and decrease the quantity of salt **(Menchik et al., 2019).**

2.4.3) Uses of dairy by-products in other industries

Milk by-products can also be used in animal feed such as whey is used in animal feed formulation **(Ahmedsham et al., 2018)** as they provide essential proteins and nutrients due to their high nutritive value **(Ajila et al., 2012).**

Many uses of dairy by-products are used in medicine and nutraceutical industry. These are used mainly in the formulation of dietary supplements and protein powders **(Pawar et al., 2023).** This highlights the importance of dairy raw products in industry other than food **(Meisel, 1997).** Because of its anti-inflammatory and cholesterol-lowering properties, buttermilk's bioactive components are valued in the pharmaceutical sector and can be used to create medicines that promote immune and cardiovascular health.

The cosmetics industry also uses milk by-products such as lactic acid (derived from milk) in the manufacturing of skincare or makeup products. This is due to the moisturizing and exfoliating properties of these products **(Kazimierska & Kalinowska-Lis, 2021).**

Dairy by-product such as lactic acid finds its application in industry other than food such as it acts as a chemical precursor in plastic industry **(Leong et al., 2021)** and also used in the manufacturing of biodegradable plastics **(Sar et al., 2022).**Dairy by-products and waste material can also be used in agriculture such as fertilizers due to their high organic content that will aid in sustainable agriculture **(Leong et al., 2021).**

2.4.4) Ecofriendly and commercial considerations regarding dairy by-products

Dairy by-products also help to decrease waste, expand sustainability, and improve the economic feasibility of the dairy industry. The use of by-products in several industries also reduces the environmental influence of dairy production, endorsing an improved circular economy. Scientific and industrial advancements in handling can expand the efficacy of extracting and consuming these by-products, leading to additional and advanced uses. Innovative uses of milk by-products via increasing functional foods formulations fortified through whey proteins and bioactive complexes derived from milk **(Leong et al., 2021).**Biodegradable plastics are also an application of milk by-products via consuming milk proteins, milk sugars, and lactic acid **(Leong et al., 2021).** Via using advanced purification and extraction procedures, nutrients from dairy waste water can be recovered and hold back for other industrial use **(Rust, 1991).**

Modern research on milk by-products such as milk proteins gives idea of bioactive compounds that can be used in pharmaceutical industry, prominently in the manufacturing of drugs such as anti-inflammatory, antihypertensive, and antimicrobial or antibiotics **(Dziuba & Dziuba, 2014).**

The effective use of milk and milk by-products can considerably increase the sustainability and productivity of the dairy industry, subsidizing novelties in diet and technology, sustenance, and ecological management.

3) Materials and Methods

3.1) Materials

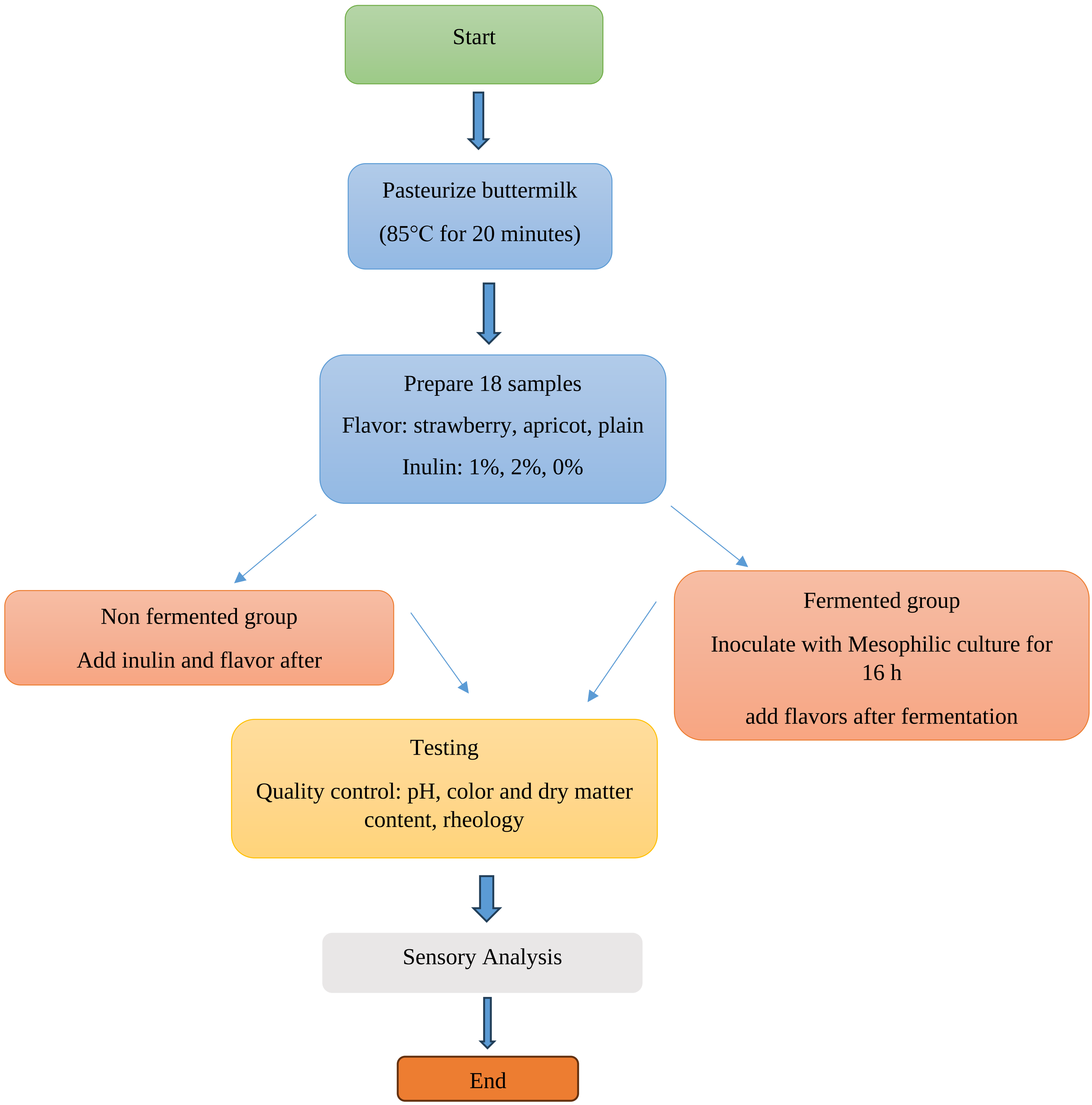
The experiments were conducted at The Hungarian University of Agriculture and Life Sciences (MATE), Buda Campus. Budapest, Hungary. For the experiment we used buttermilk, and the source is the following: Alföldi Tej Kft. Hungary. The two fruit pulps used were the following: Apricot, Strawberry, and source was: Alföldi Tej Kft. Hungary.

Inulin was purchased from BIORGANIK.HU. For fermentation we used Mesophilic culture, and the source was the following: CHR HANSEN, Denmark.

3.2) Formulation of beverages

The production of beverages is illustrated in figure 1. The experiment began with pasteurizing buttermilk at 85°C for 20 minutes to ensure microbiological safety. After pasteurization, we prepared 18 bottles samples with the 100 ml volume each by using different flavors such as strawberry, apricot, and plain with varying inulin levels (0%, 1%, 2%). Samples were divided into fermented and non-fermented groups. Fermented samples were inoculated with 0.02 g of mesophilic culture per the total weight of all fermented samples and incubated for 16 hours to allow for proper fermentation, with flavors added afterward. Non-fermented samples had flavors added immediately after pasteurization and inulin incorporation.

Figure 1: The experimental design's flow chart illustrates the various samples and the beverages production procedure



3.3) Methods

3.3.1) Dry matter content

The dry matter content was determined according to AOAC (1995). Approximately 1 to 2 grams of each sample were weighed using an analytical balance and placed in pre-weighed Petri dishes. Three replicates per sample were prepared. The dishes were then dried in an oven at 105°C for 24 hours, cooled in a desiccator, and re-weighed using the analytical balance. The dry matter content was subsequently calculated by using the following equation.

$$DM = \frac{Wd}{Wi} \times 100 \qquad (1)$$

Where:

DM: Dry matter content (100%)

Wd: weight of dry sample (g)

Wi: weight of initial sample (g)

3.3.2) pH measurement

A portable digital pH meter type (206-pH2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany). was used to measure the pH of beverages from each sample at room temperature, three replicates have been done.

3.3.3) Color measurement

The different beverage samples were each placed in transparent bags and carefully distributed to ensure even measurement. The samples were then measured using a CR-400 chromameter (Konica Minolta Co., Japan) at room temperature. Five replications were carried out for each sample.

The CIE-LAB color model was employed to assess color attributes, with L*, a*, and b* values quantified. Positive a* values indicate red hues, negative a* values signify green hues, positive b* values denote yellow hues, and negative b* values reflect blue hues. The L* value, ranging from 0 to 100, measures the lightness of the sample.

3.3.4) Rheological measurement

Based on **(Hidas et al., 2021**), samples' rheological characteristics were evaluated using an MCR 92 rheometer (Anton Paar, France). The dimensions of this rheometer's spinning cylinder and concentric cylinder arrangement were as follows: 40.003 mm for the bob length, 26.651 mm for the bob diameter, 28.920 mm for the cup diameter, 72.5 mm for the positioning length, and 120.2 mm for the active length. All measurements were carried out at a consistent temperature of 20°C, and the apparatus was managed by Anton Paar RheoCompass software.
The shear rate was varied logarithmically from 10 to 1000 1/s over 31 measurement points, with data being taken every 3 seconds, to quantify the shear stress.

3.3.5) Sensory analysis

Nine volunteers in a panel of 20 to 30 years old participated in a sensory analysis, rating each sample on a scale of 1 to 9 (1 being the least pleasant, and 9 being the best). The samples were taken out of the refrigerator and served immediately so that the panelists could enjoy the best possible serving circumstances. Panelists washed their lips with water in between tastings to avoid flavor interference. Aspects such as flavor, texture, color, scent, and general acceptance were assessed. For impartial evaluation, a distinct three-digit code was given to every sample.

4) Results and Discussions

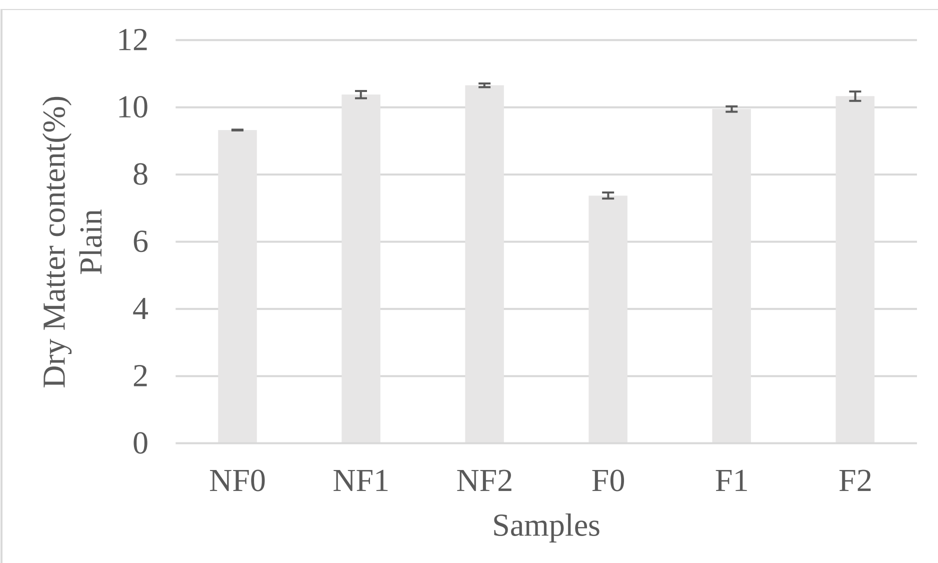
4.1) Dry matter content of buttermilk beverages

4.1.1) Dry matter content of plain beverages

In Figure 2, the dry matter content trend for fermented and non-fermented plain beverages with varying inulin concentrations was similar. In non-fermented and fermented plain beverages, the 2% inulin has highest values of 10.65333± 0.055076% and 10.33± 0.14% respectively. While inulin-free samples in both cases had the lowest dry matter concentration of 9.3233± 0.0115% in non fermented and 7.3733 ± 0.090% in fermented beverages

These results are in keeping with earlier findings in functional food research on the dry matter content of fermented and non-fermented beverages. Research has demonstrated that by binding water and increasing the viscosity of the beverage matrix, inulin fortification typically raises the dry matter content **(Pinto et al., 2022).** Additionally, as seen in dairy-based beverages, fermentation tends to significantly reduce dry matter content because microorganisms consume carbohydrates **(Avîrvarei et al., 2023).**

Figure 2: DMC of plain beverages

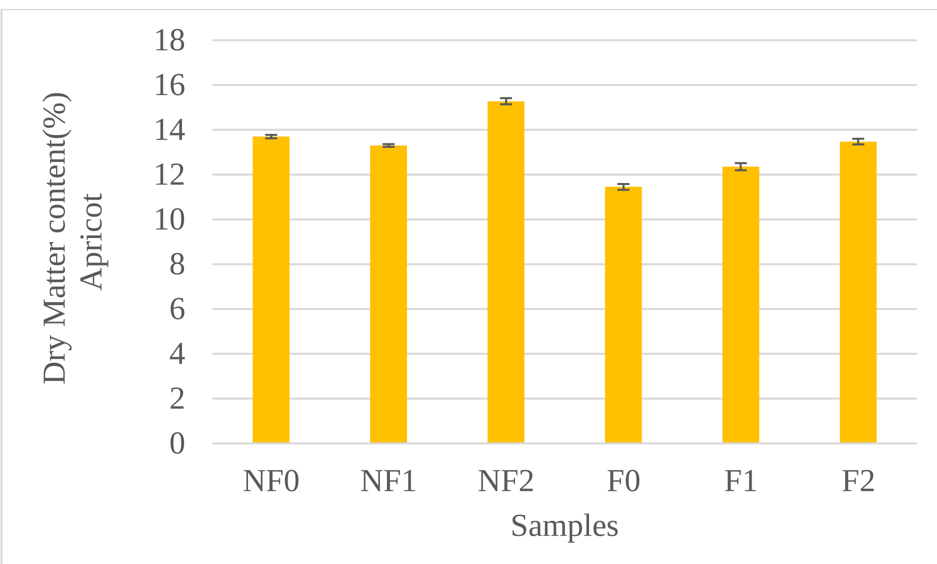


4.1.2) Dry matter content of apricot beverages

Figure 3 illustrates the difference in dry matter content between fermented and non-fermented samples with varying inulin concentrations in buttermilk-based apricot beverages. The sample with 2% inulin had the highest dry matter concentration of any non-fermented apricot beverage, averaging 15.28 ± 0.14%. The sample without inulin came in next, at 13.70 ± 0.08%. The non-fermented sample with 1% inulin had the lowest dry matter content, measuring 13.30 ± 0.06%. The pattern was a little different for fermented apricot drinks. With an average dry matter content of 13.47± 0.12%, the 2% inulin sample had the highest dry matter content, whereas the 1% inulin sample had a moderate value of 12.35 ± 0.15%. With a dry matter content of 11.45 ± 0.13%, the fermented apricot beverage without inulin had the lowest value.

When we compared apricot beverages trend with the plain beverages which Is controlled sample, we found that non ferementeded with varying inulin percentage followed a slightly different trend due to apricot inulin's hygroscopic (water-binding) nature and its impact on texture **(Anon,2010)**

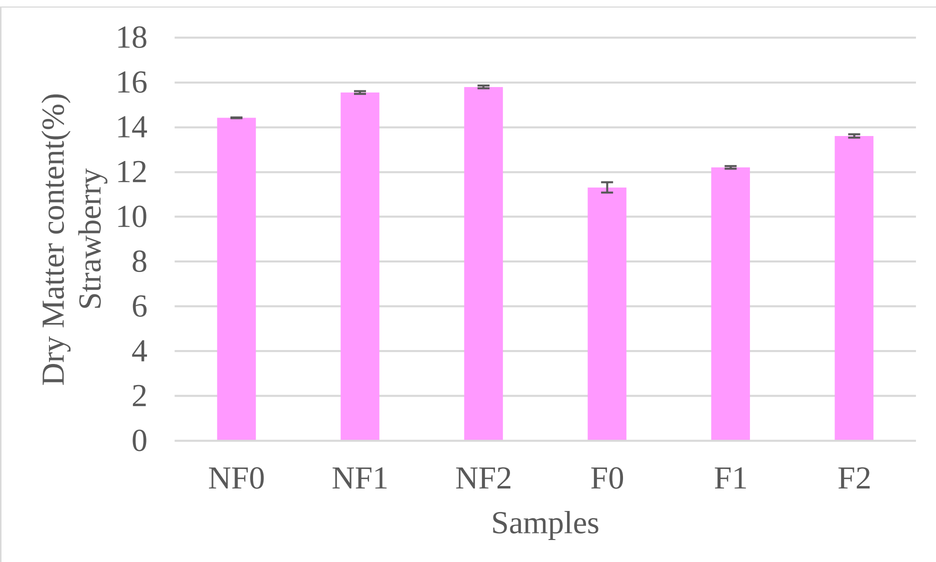
Figure 3 : DMC of apricot beverages



4.1.3) Dry matter content of strawberry beverages

The non-fermented strawberry beverages containing 2% inulin had the highest dry matter concentration 15.77 ± 0.01% in Figure 4, closely followed by the beverage containing 1% inulin 15.62 ± 0.06%. The inulin-free sample showed a lower value of 14.42 ± 0.02%. The trend in fermented strawberry beverages was likewise. At an average of 13.61 ± 0.07%, the 2% inulin sample again had the greatest dry matter content, followed by the 1% inulin beverage at 12.21 ± 0.05%. The inulin-free sample had the lowest dry matter concentration, measuring 11.31 ± 0.23%. Due to their naturally thick consistency, strawberries can improve the dry matter content of buttermilk by increasing its viscosity when mixed with it **(Özcan and Hacıseferoğulları 2006).**

Figure 4: DMC of strawberry beverages



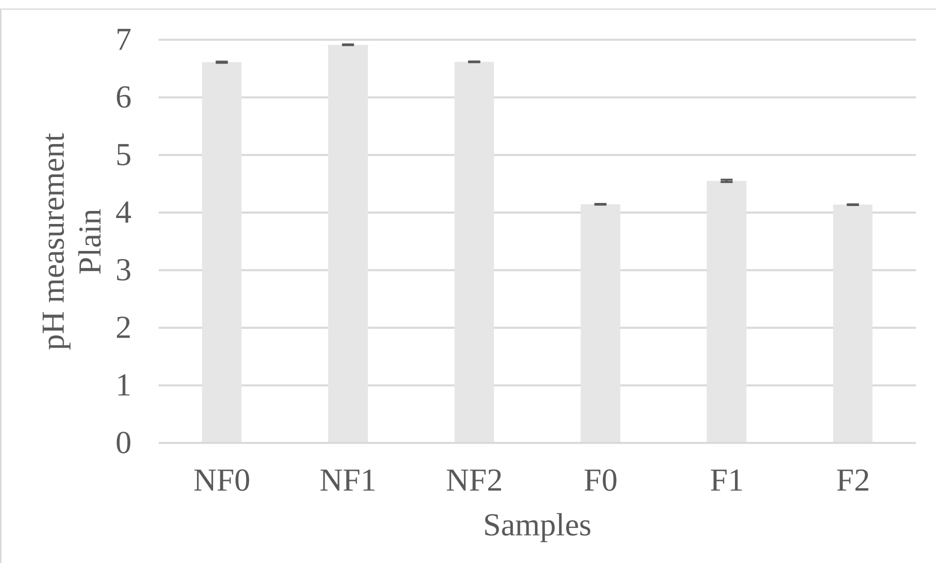
4.2) pH measurements of buttermilk beverages

4.2.1) pH of plain beverages

The pH values for both fermented and non-fermented beverages are shown in Figure 5. The pH values of non-fermented beverages with different inulin concentrations were higher, suggesting a reduced amount of acidity. When compared to other inulin concentrations, the beverage with 1% inulin had the highest pH of all, measuring 6.913±0.006.

On the other hand, because a fermenting culture was present, the pH values of fermented beverages were lower, suggesting increased acidity. Comparable results were seen in fermented drinks containing 1% inulin, which had a comparatively higher pH of 4.55±0.02 than other fermented samples.

Figure 5: pH measurement of plain beverages



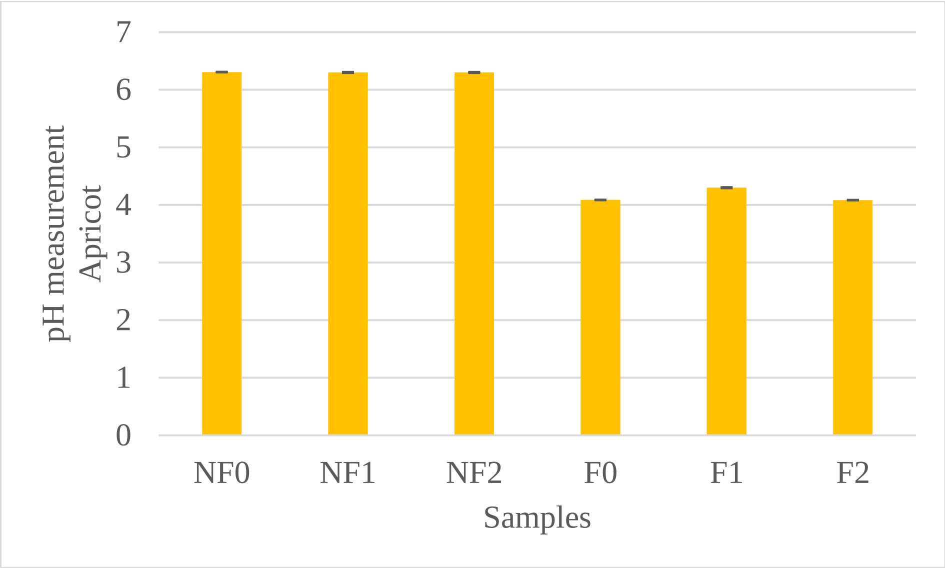
4.2.2) pH of apricot beverages

The pH trends in apricot beverages are depicted in Figure 6. Due to the presence of mesophilic cultures, the pH values of all fermented apricot beverage samples are lower than those of non-fermented beverages as noted it (**Lin et al. 2013).** The pH levels of non-fermented apricot beverages remained constant at varying inulin concentrations, exhibiting similar acidic properties.

The fermented apricot beverages showed a similar pattern, but the sample with 1% inulin had a little higher pH than the other fermented samples. Because of the apricot's inherent acidity, which accelerates fermentation and increases the production of organic acids, the pH values of these apricot beverages are lower than those found in samples of simple beverages

According to **(Granato et al., 2010).**, this combination results in apricot-flavored beverages having a lower pH than plain beverages, which is compatible with the flavor profile and intended sensory attributes of fruit-based products.

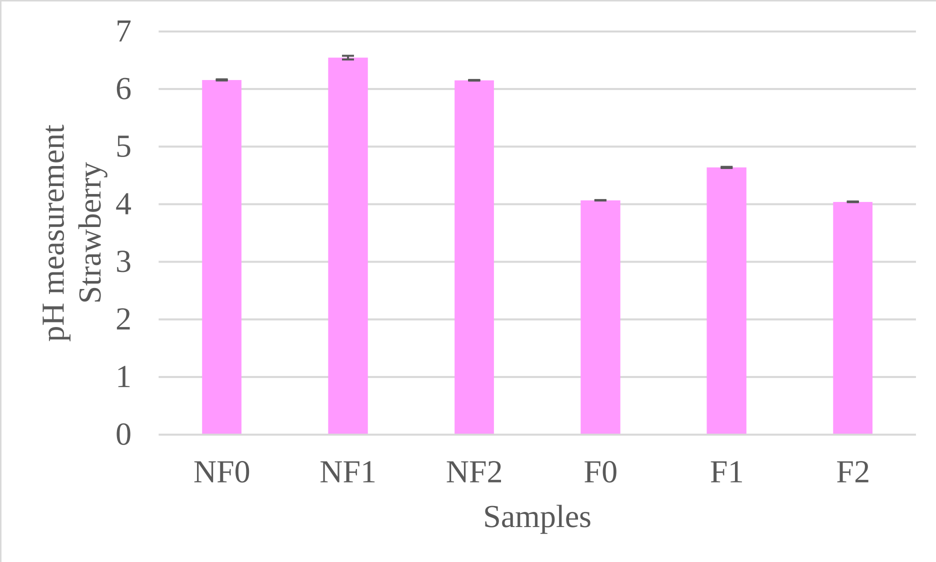
Figure 6: pH of apricot beverages



4.2.3) pH of strawberry beverages

The pH distribution of strawberry beverages is shown in Figure 7. The sample with 1% inulin had the highest pH of any non-fermented strawberry drink, measuring 6.547±0.032, which is similar to that of plain beverages and indicates an affinity for a more alkaline profile. Due to fermenting bacteria that primarily consume strawberry sugar, which may reduce acid production, the 1% inulin sample in the fermented strawberry beverages also displayed a slightly higher pH value of 4.64±0.01 when compared to other inulin concentrations **(Shah, 2000).**

Figure 7: pH of strawberry beverages



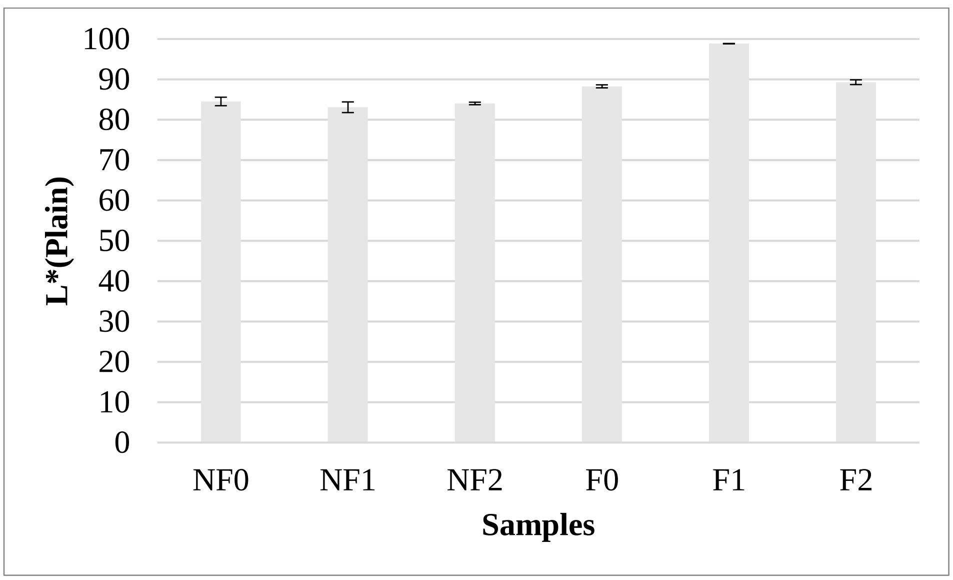
4.3) Color measurements of buttermilk beverages

4.3.1) Plain beverages

4.3.1.1) L* value

Figure 8 displays the L* values for simple beverages, which indicate sample lightness on a scale from 0 (black) to 100 (white). Every sample in the non-fermented plain beverages ranged between 83.00 – 84.50 in L* value, suggesting that they were all light in color. The sample with 1% inulin had the highest L* value for fermented plain beverages (98.87±0.035), indicating a significantly higher level of lightness than other fermented samples, based on previous findings (**Aksornsri et al. 2023).** This increase suggests that the purity and lightness of the beverage are enhanced when 1% inulin is added to a mesophilic culture. This quantity of inulin makes the beverage appear cleaner and brighter without significantly thickening it. According to research by **(Tamine and Robinson, 2007),** prebiotics like inulin can improve beverages' visual attractiveness by changing how they reflect light, giving them a more radiant and alluring appearance.

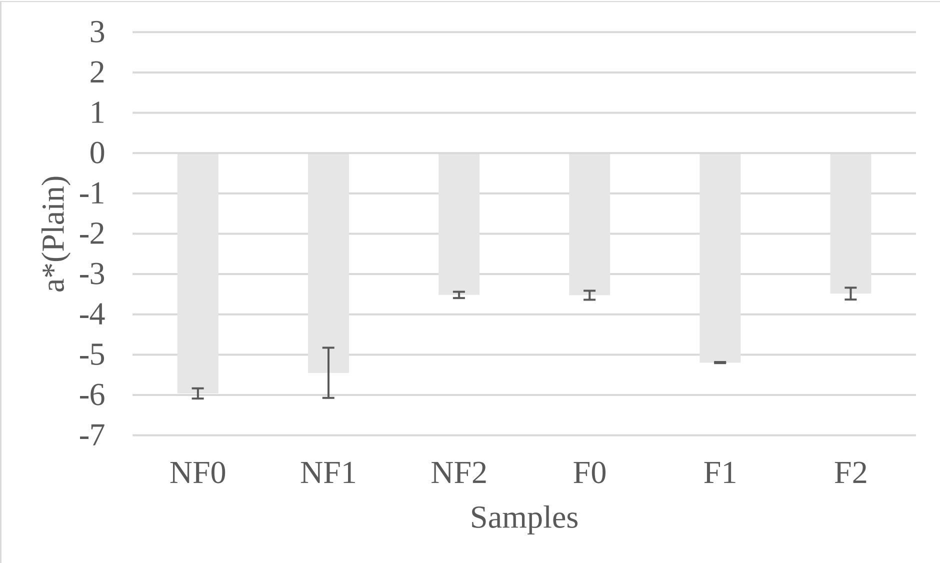
Figure 8: L*value of plain beverage



4.3.1.2) a* value

The a* values for plain beverages are displayed in Figure 9, where positive a* values suggest reddish hues and negative ones represent greenish tones. In this experiment, every sample of simple beverage had negative a* values, indicating that they were all greenish in hue. With the highest negative a* value of -5.96±0.126 among the non-fermented plain beverages, the inulin-free sample was more prominently green. The a* value of 5.20±0.013 for the 1% inulin sample for the fermented beverages was greater than that of other fermented samples, suggesting that a 1% inulin concentration would be optimal for the growth of the fermentation culture.

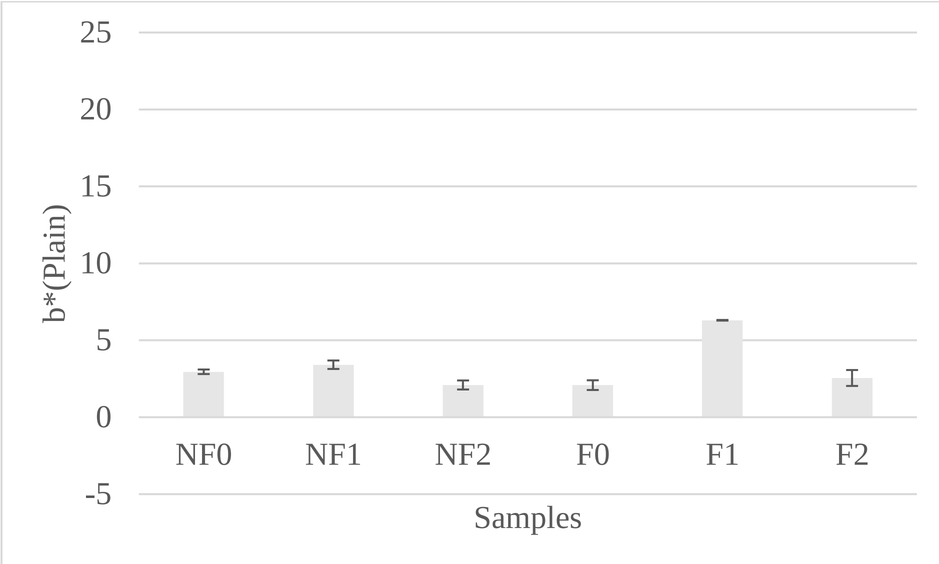
Figure 9: a* value of plain beverages



4.3.1.3) b* value

The b* values for plain samples are shown in Figure 10, where positive values denote yellowish hues, and negative values denote bluish tones. All of the simple samples had positive b* values and a yellowish tint. Among fermented and non-fermented samples, the samples with a 1% inulin concentration exhibited the greatest b* values, measuring 3.41±0.276 for the former and 6.30±0.022 for the latter. This suggests that the 1% inulin content adds to a more pronounced yellowish tint in the beverages compared to other samples because of its capacity to scatter light. This scattering effect increases the intensity of reflected light, making yellowish tones more pronounced and powerful, as seen in (**Granato et al., 2010).**

Figure 10: b* value of plain beverages

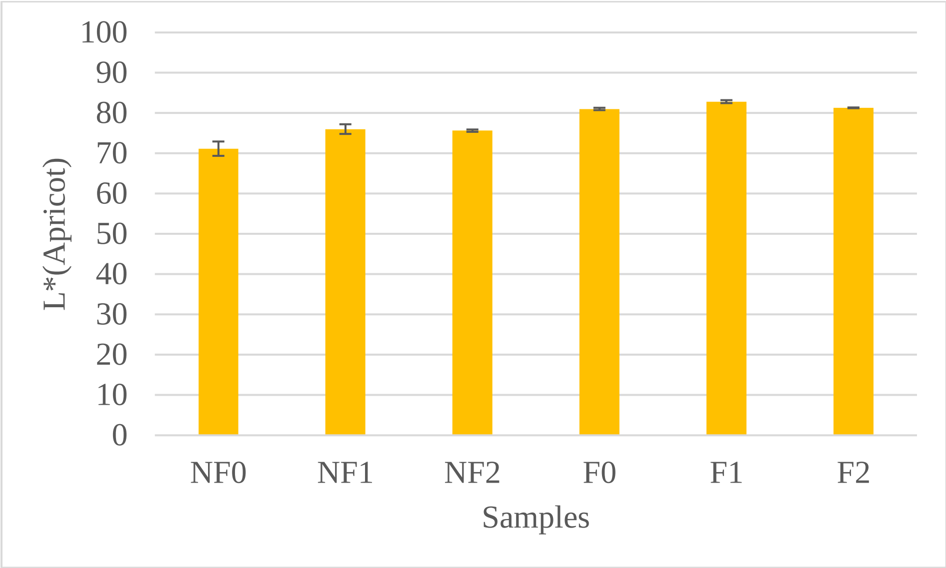


4.3.2) Apricot beverages

4.3.2.1) L* value

Figure 11 displays the L* values for apricot beverages. since of the apricot's natural hue, these beverages had lower L* values than plain samples since the apricot pigment lowers overall lightness. The samples with a 1% inulin concentration in both fermented and non-fermented apricot beverages had the highest L* values, 76.01±1.20 and 82.83±0.37. This suggests that, as noted in **(Aksornsri et al. 2023),** 1% inulin concentration is optimal for enhancing the purity and lightness of apricot beverages.

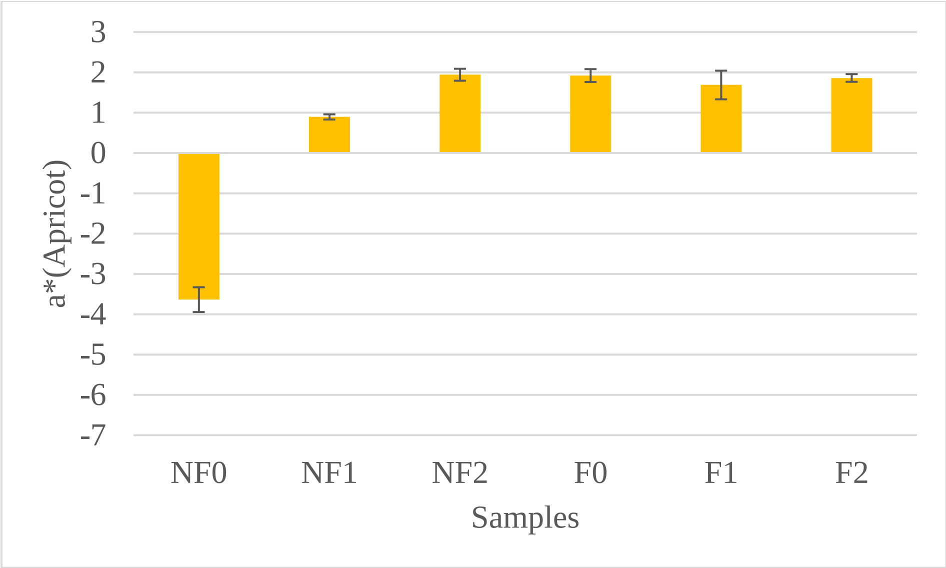
Figure 11: L* value of apricot beverages



4.3.2.2) a* value

Figure 12 displays the a* values for apricot beverages. Compared to plain beverages, only the non-fermented apricot beverage without inulin exhibited a negative a* value, indicating a greenish color. All other apricot samples displayed positive a* values, indicating a reddish tinge, due to the apricot's natural hue (**Palencia-Argel et al. 2022**).

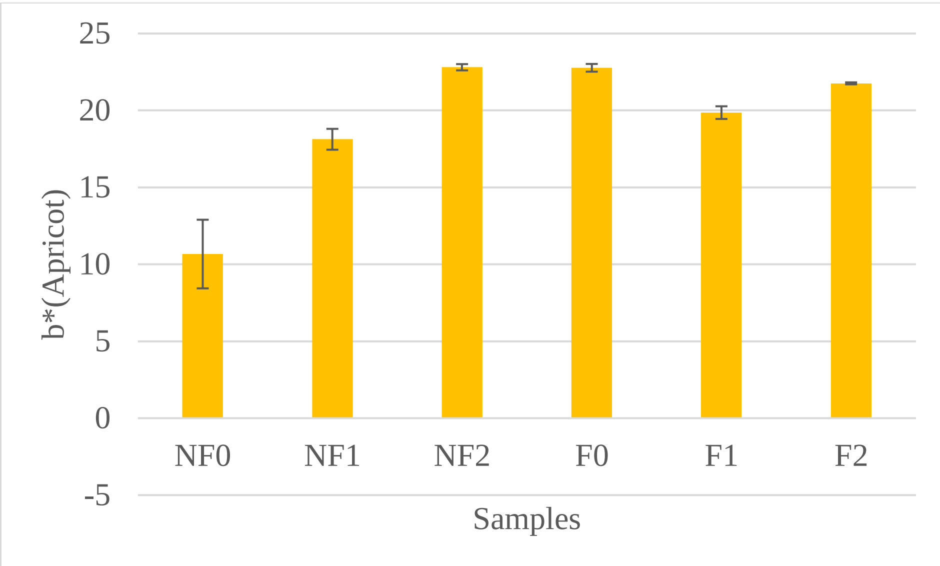
Figure 12: a* value of apricot beverages



4.3.2.3) b* value

In Figure 13, the b* values for apricot beverages are shown. In non-fermented apricot beverages, a higher inulin concentration resulted in a more pronounced yellowish color. Since the mesophilic culture could not grow sufficiently in the fermented apricot beverage without inulin, which displayed a higher yellow hue, the apricot's original yellowish color was preserved **(Palencia-Argel et al. 2022).**

Figure 13: b* value of apricot beverages

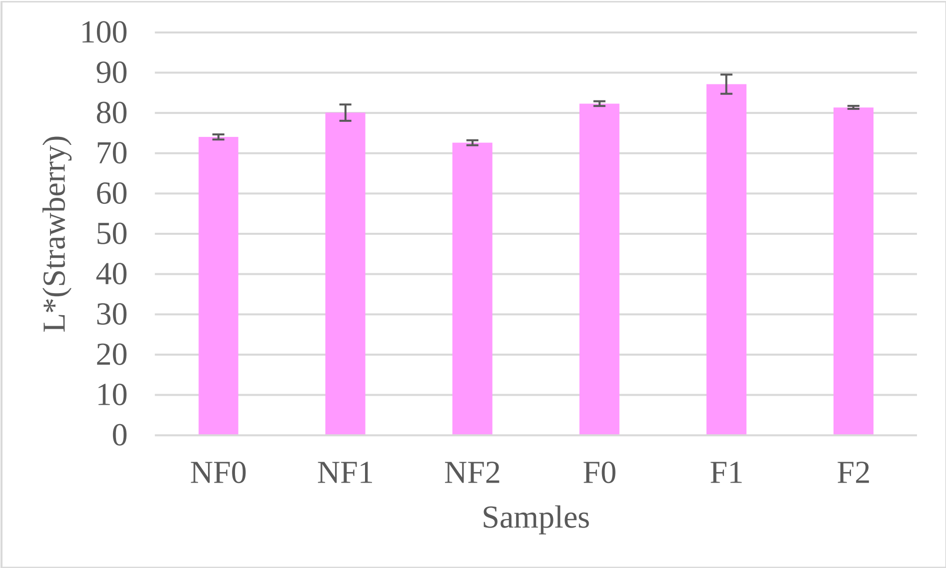


4.3.3) Strawberry beverages

4.3.3.1) L* value

The L* values for strawberry beverages are shown in Figure 14. When comparing the L* values of strawberry beverages to plain samples, it was found that fermented samples tended to be lighter due to fermentation **(Kusnadi and Setiawati, 2021).** The highest L* values in both fermented and non-fermented strawberry beverages were found in samples with a 1% inulin concentration of 87.17±2.38 and 80.10±2.02, respectively. Accordingly, 1% inulin might be the best amount to use in strawberry drinks to make them lighter.

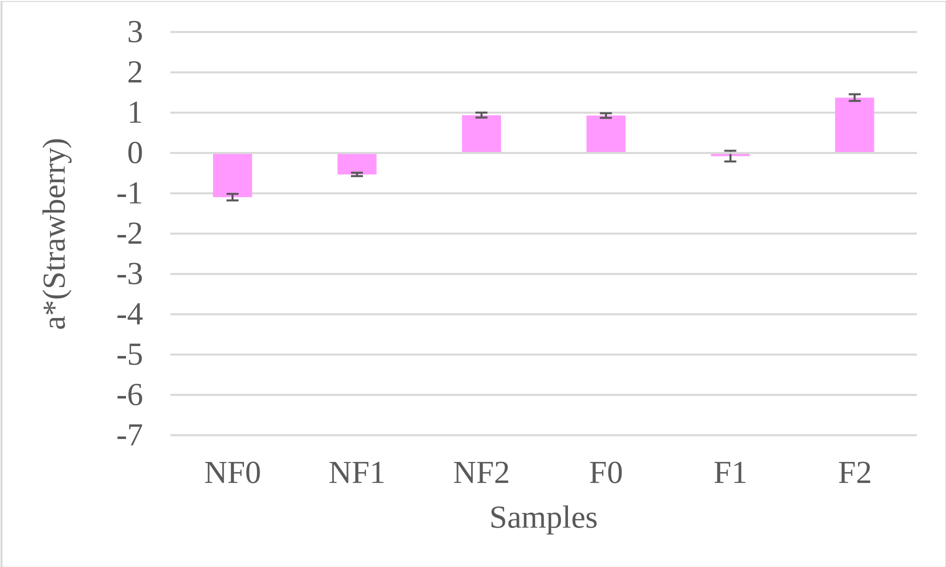
Figure 14: L* value of strawberry beverages



4.3.3.2) a* value

Figure 15 displays the a* values for strawberry beverages. Negative a* values indicated a greenish hue in the non-fermented samples containing 0% and 1% inulin. Among the fermented samples, only the 1% inulin sample showed a negative a* value and a greenish tint. The remaining samples has positive a* values suggested an affinity for a reddish hue.

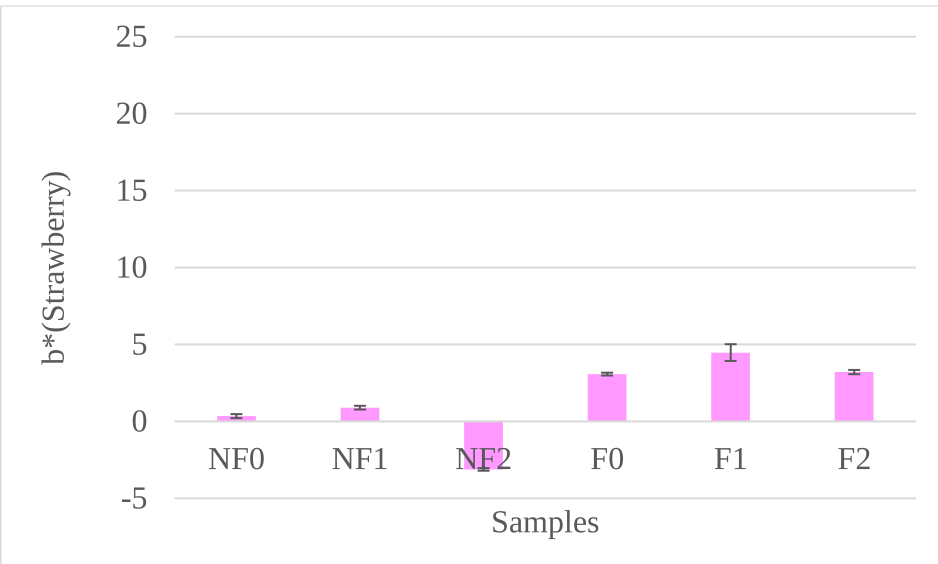
Figure 15: a* value of strawberry beverages



4.3.3.3) b* value

Strawberry beverage b* values are shown in Figure 16. While the other samples displayed positive b* values, indicating a shift toward a yellowish tone, the non-fermented samples with a 2% inulin concentration displayed a negative b* value, indicating a bluish hue. At 4.47±0.55, the fermented sample with 1% inulin had the greatest b* value when comparing the b* values of strawberry beverages with plain beverages. This value nearly matched the higher values found in plain samples.

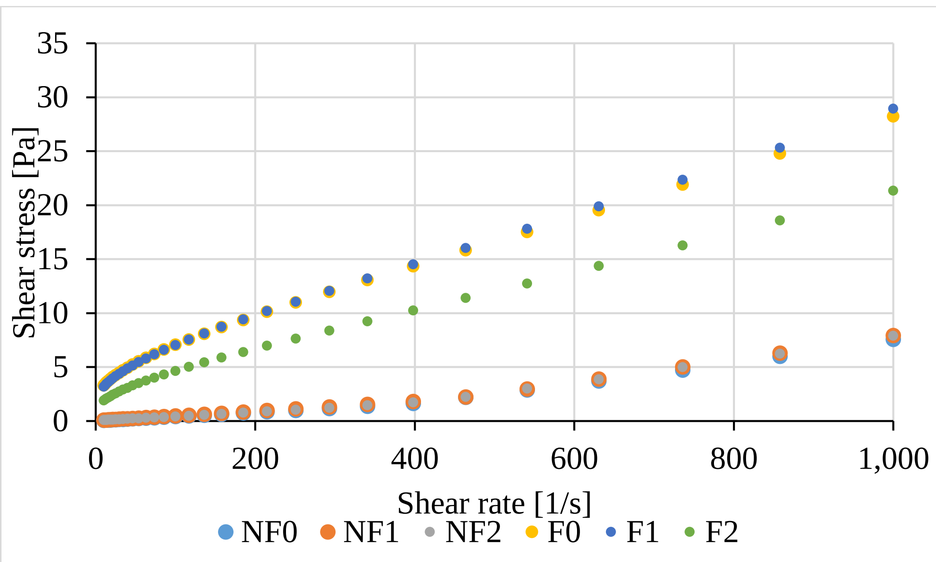
Figure 16: b* value of strawberry beverages



4.4) Rheology measurement of buttermilk beverages

Figure 17 illustrates the shear-thinning flow behavior of apricot drinks at 20 °C, where shear stress rises nonlinearly with increasing shear rates. All the samples showed minimal resistance at low shear rates, which gradually increased as shear rates increased. The viscosity profiles of fermented beverages containing 1% and 2% inulin were similar, and both reached a peak shear stress of 28.90 Pa at 1000 [1/s], indicating that inulin concentrations above 1% only add a small amount of extra thickness. The viscosity-enhancing effects of fermentation, because of the formation of exopolysaccharides, were highlighted by the consistently lower shear stress seen in non-fermented samples. Overall, improving the thickness and consistency of apricot beverages requires both fermentation and inulin content **(Janhøj et al. 2007).** Other samples followed the same trend.

Figure 17: Rheology measurements

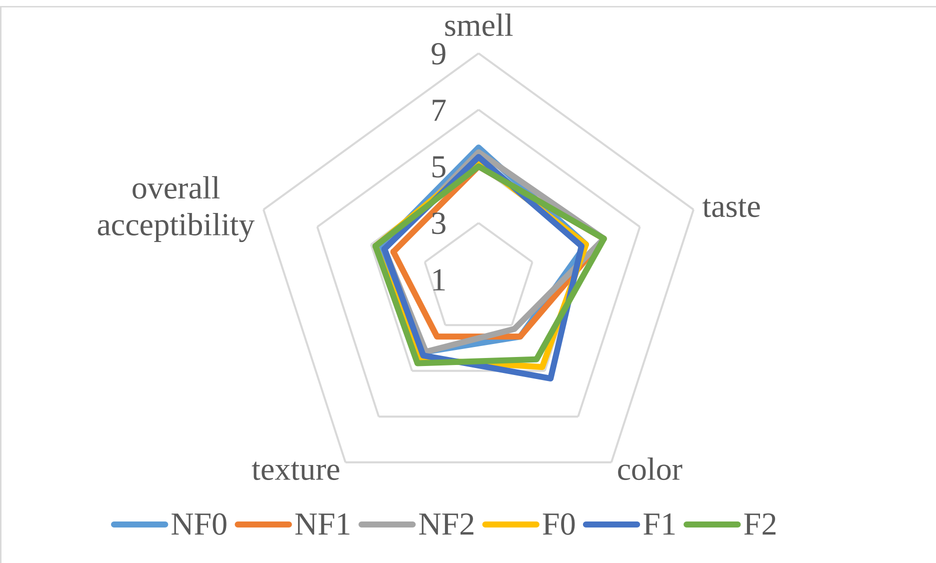


4.5) Sensory analysis of buttermilk beverages

4.3.5.1) Plain beverages

The smell, taste, color, and texture of plain, apricot, and strawberry beverages varied significantly when fermentation and inulin concentrations were considered. Figure 18 illustrates the sensory view about plain beverages. Fermentation enhanced texture and slightly deepened color in plain apricot beverages, with fermented samples with 2% inulin receiving the highest texture rating. Color measurements showed that the sample fermented with 1% inulin, which had the highest brightness and yellow hue, looked more vibrant and improved overall acceptance. Smell and taste remained consistent between fermented and non-fermented sample

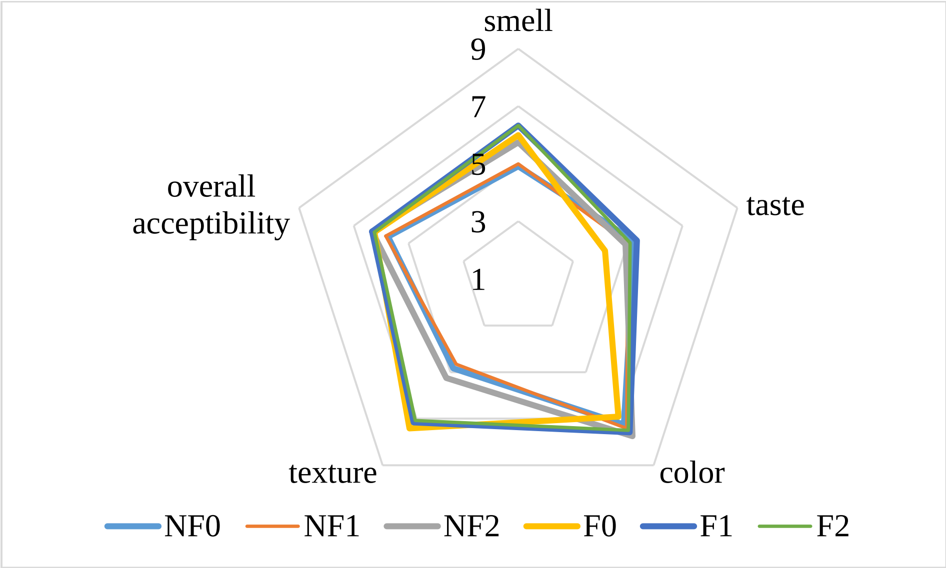
Figure 18: Sensory analysis of plain beverages



4.3.5.2) Apricot beverages

Figure 19, shown the sensory analysis of apricot beverages. The sensory qualities of apricot beverages were improved in all respects, especially in color and smell. In fermented samples, the color characteristic scored well, as seen by the b* values showing a more vivid yellow hue. Both fermentation and inulin appear to have a favorable effect on beverage thickness and color intensity, as seen by the highest scent and color ratings for fermented samples with inulin additions and an increase in texture ratings.

Figure 19: Sensory analysis of apricot beverages



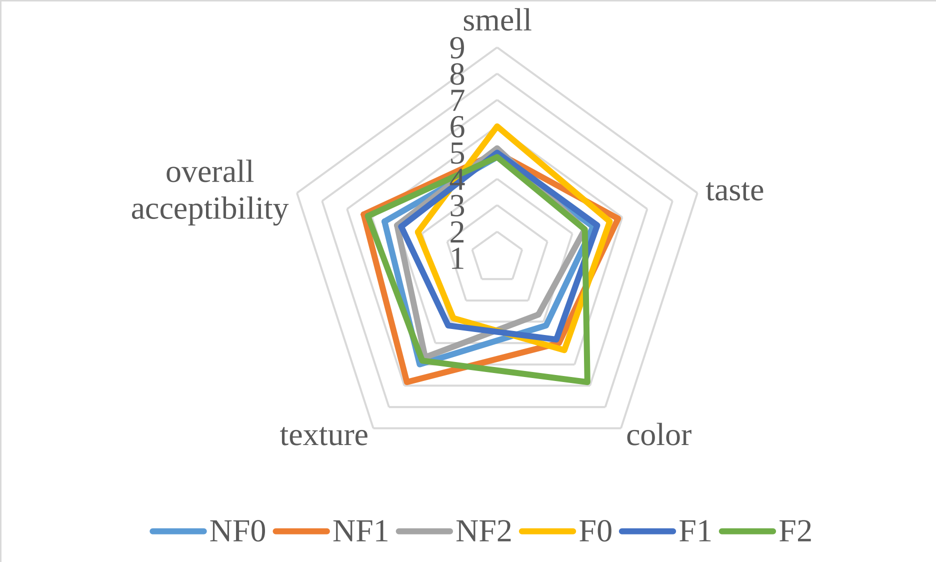
4.3.5.3) Strawberry beverages

Figure 20 has demonstrated the sensory analysis of strawberry beverages. The addition of inulin to strawberry-apricot beverages enhanced the smell in non-fermented samples, while taste ratings varied among all samples, reaching their highest in non-fermented samples containing 1% inulin. The greatest color value was found in fermented samples, indicating that fermentation greatly increased color intensity. Inulin-containing non-fermented samples had a noticeably thick texture, whereas both fermented and non-fermented samples received good texture ratings.

According to the investigation, inulin content and fermentation significantly improve color, texture, and occasionally taste and fragrance, especially in formulations made with apricots.

We concluded our results based on previous findings. **(Hidas et al., 2023)**

Figure 20: Sensory analysis of strawberry beverages



5) Conclusion and Suggestions

This thesis concludes that buttermilk is a useful ingredient in producing nutritious, functional drinks. The physical and sensory characteristics of the beverages were significantly influenced by the addition of inulin and the fermentation process; 1% inulin was shown to be the most effective in improving texture and color in plain, apricot, and strawberry flavors. While the addition of inulin raised the dry matter content, favoring greater density and better mouthfeel, fermentation successfully reduced pH levels, resulting in a balanced acidity profile that improves taste and shelf life. The results of the study are consistent with research showing how dairy by-products can be used to make sustainable, high-value goods that satisfy consumer desire for eco-friendly and health-conscious solutions. According to rheological evaluations, fermentation and increased inulin concentrations improved viscosity, especially in apricot beverages, facilitating the production of drinks with a suitable consistency. This study highlights buttermilk's potential as a sustainable foundation for functional drinks, providing an environmentally responsible way to use dairy byproducts while satisfying modern consumer demands for enticing and wholesome drinks. To properly identify the ideal concentration for balancing sensory and textural qualities, it is advised that future research refine inulin concentrations by evaluating intermediate levels, such as 1.5%. Additionally, experimenting with different fermentation times may help improve the texture consistency, flavor complexity, and acidity profile. Further enhancements in nutritional and sensory aspects might be possible by looking at different prebiotic fibers or advantageous plant extracts. The beverage's commercial relevance and appeal will be increased by extending consumer sensory panels to collect more comprehensive demographic information, carrying out stability experiments under various storage settings to determine long-term quality, and experimenting with novel flavor profiles. All of these suggestions work together to improve and expand the selection of buttermilk-based beverages, establishing them as a premium, useful choice in the sustainable food and beverage industry.

6) Summary

The purpose of this thesis was to examine the potential for using buttermilk, a nutrient-dense dairy industry by-product, as a base for functional drink production. With a focus on plain, apricot, and strawberry flavors, different formulations with varying inulin concentrations (0%, 1%, and 2%) were examined using both fermentation and non-fermentation procedures. Important investigations, including evaluations of dry matter content, pH, color characteristics, rheology, and sensory attributes, were conducted to optimize texture, viscosity, and overall consumer appeal.

The findings showed that adding inulin often raised the dry matter content, with 1% inulin offering the best texture and mouthfeel balance. In all samples, fermentation increased acidity and decreased pH, resulting in a well-balanced flavor quality that satisfies customer preferences. L*, a*, and b* color measurements revealed that 1% inulin improved color vibrancy and brightness, especially in strawberry and apricot flavors. This change in color profile satisfied customer demands for items that look lively and natural while also enhancing the visual appeal. These results are consistent with the results of sensory research, which showed that fermented foods containing inulin were highly rated for texture, color, and general appeal. Rheological investigations also verified that fermentation and increased inulin concentrations significantly raised viscosity, especially in apricot beverages, assuring a desired consistency. This increase in viscosity helped create a richer, more substantial consistency, which is a desirable quality for functional drinks, while preserving the nutritional advantages of the inulin and buttermilk.

Buttermilk's potential as a sustainable and useful ingredient in the beverage industry is demonstrated by this study. Through the combined impacts of inulin enrichment and fermentation, the study effectively illustrated how dairy by-products may be reused to produce enticing, health-conscious goods that appeal to consumers who are concerned about the environment.

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8) List of figures and Tables

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