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Incorporation of sensitive bioactive compounds in functional dairy products

Master´s thesis

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Sustainable Agriculture and Food Security

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Disclaimer

This master's thesis is submitted as a requirement for the Master´s degree in Sustainable Agriculture and Food Security at the Czech University of Life Sciences Prague and the Master´s degree in Biosafety and Food Quality at the University of Pisa. The submission is made concurrently to both universities under the double degree program agreement.While the substantive content of this thesis remains consistent for both submissions, there are variations in the title page and the roles of the supervisor and co-supervisor, adhering to the respective guidelines of each university.The dual submission of this thesis is a reflection of the academic collaboration and educational innovation fostered by the double degree program, aiming to broaden the academic and cultural perspectives of its participants.

Declaration

I hereby declare that I have authored this master's thesis carrying the name **Incorporation of sensitive bioactive compounds in functional dairy products** independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

Prague 10.04.2024 _____

Summary

Abbreviations and Acronyms

AP: Apple Pomace

CVD: Cardiovascular Disease

DF: Dietary Fibre

AP-TMR: Apple Pomace - Total Mixed Ration

SNF: Solid Not Fat

TPA: Texture Profile Analysis

PS: Particle-size

PSD: Particle-size Distribution

TPC: Total Phenolic Content

ABSTRACT

In the study, a functional yogurt made by the addition of by-products from the food industry, namely apple pomace (AP) was prepared. Due to its abundance of components that are good for the body, such as ursolic acid, dietary fiber, antioxidants, and minerals, apple pomace may find application as a food ingredient, functional food, or dietary supplement. The essay aims to understand if there is the possibility of creating a fortified product with bioactive compounds (AP) and what effects they have on the main characteristics of the product, carrying out various analyses useful to understand the real possibility of completion.

The experiment was divided into two attempts, each lasting four weeks of storage, in which different types of yogurt, made from whole milk with the addition of AP from Idared variety apples, were prepared: control, 80g milk + 20g apple pomace, 90g milk + 10g apple pomace, 80g milk + 15g apple pomace and 5g sucrose ($C_{12}H_{22}O_{11}$), and lastly, freeze-dried apple slices, "packaged“ separately and mixed with yogurt before consumption. Weekly measurements were taken of texture, rheology, particle size, and pH. Afterward, a sensory analysis involving ten volunteers was conducted.

To determine any potential impacts on people, the phenol content of apple pomace was finally measured with the extraction *Folin Ciocalteu* method.

From the results obtained, it is clear that AP has a positive impact on the product, given that it emerged that the yogurts have appropriate hardness, a viscosity comparable to that of plain yogurts, and an average particle size distribution. The phenolic content is consistent with that of other studies, although this depends on the variety used. Therefore, the preparation of yogurts with AP, i.e., by-products from the food industry, is possible; furthermore, thanks to the substances contained in them, it is likely that there is also a beneficial effect on the body. The results obtained in this thesis can contribute to the nowelty sustainable approach in the food industry which is a key step for the effective utilization of by-product and sustainable food chain.

Chap. 1 - INTRODUCTION

Humanity is currently dealing with problems like a growing global population, food insecurity, pollution, and food waste. The last, among those listed, is a significant problem among them because of its abundance. Any nutritious food that is lost, deteriorated, or eaten by pests at any stage of the food supply chain is considered "*food waste*." It is estimated that due to inappropriate handling, technological problems, and consumer behavior, almost one-third of the world's food production ends up as food waste. Waste from processing fruits and vegetables contributes the most (about 50%). The majority of the time, these wastes are thrown away without being recycled or processed, which pollutes the environment. Nonetheless, numerous studies have demonstrated that they are also excellent sources of vitamins, minerals, fibre, and bioactive substances. (Asif *et al.,* 2024)

Therefore, it's essential to take advantage of these properties to produce functional foods or substances.

But what does "functional food" mean? Functional food is defined as follows by the "Concerted Action on Functional Food Science in Europe" (FUFOSE): *"a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. It is consumed as part of a normal food pattern. It is not a pill, a capsule, or any form of dietary supplement."* (*Food for Thought, Health, Repair... It's Functional Food*, n.d.).

Nowadays, in most industrialised countries, food is no longer seen only as an element of survival but rather as a "tool" capable of improving an individual's health. The search for substances capable of increasing the nutraceutical and flavor value of the traditionally proposed mixes is seen as an important product innovation and is of certain interest to the main brands operating in the sector. One of the main problems for the operation to be successful is the ability to produce sufficiently large quantities of final product and standardize production.

The production of functional yogurt with AP, therefore, may be a good idea for the future thanks to the combination of the intrinsic properties of AP and dairy products.

1.1-Apple pomace: A by-product with great potential

About 70%–75% of the juice is recovered overall in the industrial production process, producing 25%–30% apple pomace and 5%–11% sludge (liquid waste collected after clarifying and sedimentation with bentonite). (Bhushan *et al.*, 2008)

Produced at a rate of about 4 million metric tonnes annually worldwide, AP is one of the most common types of agri-food waste in many nations. Regretfully, AP has a very poor and inadequate recovery rate.

The most commonly applied disposal method for AP is to discard it directly into the soil in a landfill. Because AP is high in water (>70%), sugars, and organic acids—all of which are vulnerable to rapid microbial fermentation—it may result in significant soil and water pollution. The increase in microbial flora may impact the C/N ratio and reduce the amount of nitrogen that is accessible in the soil. (Gołębiewska *et al.*, 2022)

For these reasons, making effective use of these by-products is crucial. Exploitation and valorization of AP can reduce environmental impact and meet the requirements for sustainable development in the large-scale apple processing industry.

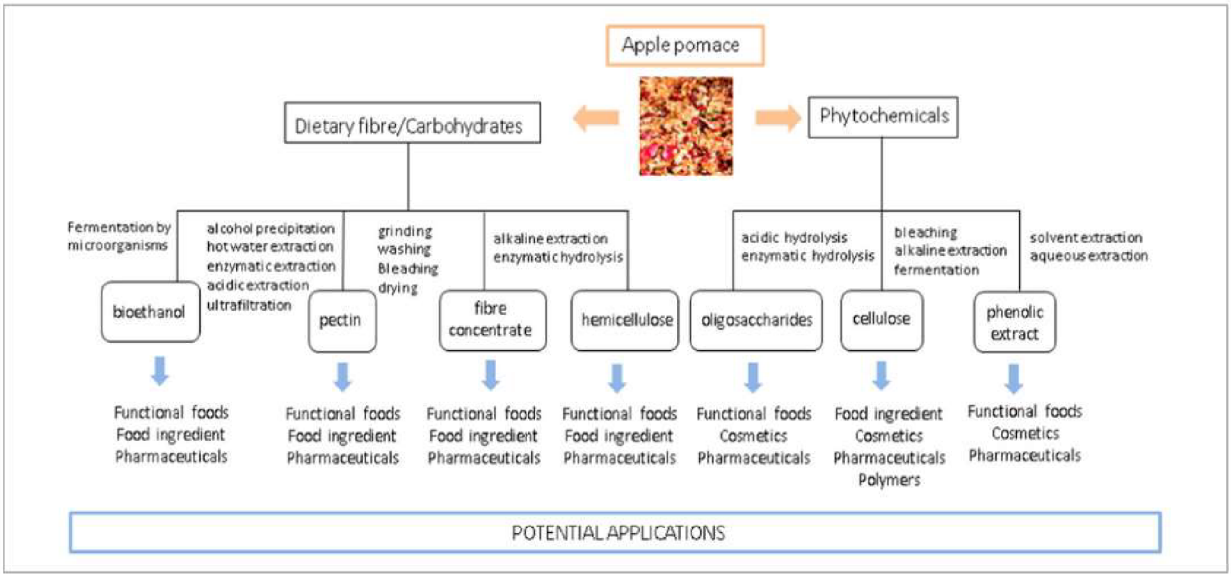


Figure 1 *– Potential applications of apple by-products.*

Source: (https://ift.onlinelibrary.wiley.com/doi/epdf/10.1111/1541-4337.12290?saml_referrer)

As you can see in the image above, the possibility of using AP is great. Several agricultural and food processing wastes can be partially converted into commercially viable products, such as nutrients, biofuels, and multipurpose ingredients.

AP is considered a good functional ingredient to be added to any kind of food product because of its high level of phenolic compounds, dietary fiber, and other nutrients. Nevertheless, it has been noted that adding AP can lower certain quality parameters of food products. AP is therefore added at a relatively low level as a functional element, and these amounts need to be closely watched.

An example is bakery products. Humans have been consuming and accepting these foods for hundreds of years, such as bread, cakes, and cookies. It is thought that adding AP to baked goods can increase their dietary fiber content and health benefits. Another example concerns snacks. It has been demonstrated that adding AP to extruded snack products increases their nutritional content without materially altering their physical characteristics. It can also serve as a substrate for the production of alcoholic beverages and edible mushrooms. Furthermore, it was discovered that the use of AP in dairy and confectionery items enhanced the qualitative attributes of the final product. (Lyu *et al.*, 2020)

AP was used in a study by Wang (Wang *et al.*, 2019) as a natural texturizer and stabilizer in set-type yogurt. AP at different concentrations (0.1%, 0.5%, and 1% w/w) was mixed with skim milk and fermented at 42 ◦C using a combination of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. The addition of 1% pomace resulted in a much higher onset pH and a shorter gelation period, according to the results. Furthermore, after being stored for 28 days, all of the enriched yogurts showed increased cohesion and consistency. Wang (Wang, n.d., 2018) recently assessed the possibility of freeze-dried AP powder as a dairy component.

According to this study, adding 1% AP powder raised the gelation pH and shortened the fermentation period during the yogurt-making process, resulting in the development of a harder, more uniform, and viscoelastic yogurt gel. In the same study, stirring AP into yogurt enabled the incorporation of an even higher amount of pomace (3%), and during the course of 28 days of cold storage, the matrix's viscosity, stiffness, and cohesiveness increased significantly while syneresis decreased.

This research highlights the potential of AP as a natural stabilizer, texturizer, and source of dietary fiber and polyphenols in dairy products such as yogurt. Even though AP contains pesticide residues and natural plant toxins that are thought to present health dangers, recent research indicates that consuming AP does not present a significant risk to human health. (Lyu *et al.*, 2020)

In a recent study from 2018, (Skinner *et al.*, 2018)- Nutrition Reviews, Volume 76, the composition of AP from a nutritional point of view was studied, as were the potential benefits it brings.

Because of its low-fat content, AP is not thought to be a rich source of fatty acids. AP, on the other hand, has been discovered to contain more protein than apples, probably because of the seeds. AP is high in fibre, and studies have shown that eating a diet rich in dietary fibers helps improve digestive health and lower the incidence of diverticular disease and several malignancies, including colorectal cancer. *(*Skinner *et al.*, 2018)

Values are the ranges reported for n = 6–38 samples. A single value indicates an n = 1 sample.

Constituents (fresh weight)	Whole apple	Apple pomace
Macronutrients, (%)		
Fat	0.16-0.18	1.1-3.6
Protein	0.24-0.28	2.7-5.3
Total Carbohydrate	13.81	44.5-57.4
Total Fibre	2.1-2.6	4.4-47.3
Major minerals, (mg/100 g)		
Sodium	0.9-1.1	185.3
Potassium	104.8-109.2	398.4-880.2
Calcium	5.7-6.3	55.6-92.7
Phosphorus	10.7-11.3	64.9-70.4
Magnesium	4.9-5.1	18.5-333.5

Table 1*: Comparison of the nutrient composition of whole apples versus apple pomace*

Source: (comprehensive analysis of the composition, health benefits, and safety of apple pomace | Nutrition Reviews | Oxford Academic (academic-oup-com.translate.goog)

1.1.1-Dietary Fiber

Dietary fiber (DF) had a hiatus for a large portion of the middle of the 20th century, but it began to gain traction again in the 1970s when Denis Parsons Burkitt published articles suggesting that dietary fiber could prevent obesity, diabetes mellitus, and colon cancer. Since then, there has been a rapid pace of study on the health advantages of dietary fiber, and over that time, our understanding of these advantages

has changed. The current guidelines for adult dietary fiber consumption in the US and most European nations are between 25 and 32 g for women and between 30 and 35 g for men per day. (Barber *et al.*, 2020)

Dietary fibers are the components that make up the majority of the weight of dried AP because they are indigestible to the human enzyme system. DF are separated into two categories: soluble and insoluble, based on their solubility in water. The insoluble group is made up of cellulose (*β-1,4-glycosidic linked glucoses*), hemicelluloses (*xyloglucan, galactomannan, and glucuronarabinoxylan*), and lignin (*polymerized coniferyl, sinapyl, and p-coumaryl alcohols*), which accounts for two-thirds of apple fibre. It has been found that there are 4.4 – 47.3 g of fibre per 100 g of AP. The variations in the reported fibre content of AP are probably caused by the use of various apple cultivars and techniques for measuring or removing dietary fiber.

Of the overall fibre composition in AP, lignin comprises 14.1%–18.9%, cellulose comprises 6.7%–40.4% and insoluble fibre comprises 33.8%–60.0%. Since the majority of soluble fibre in apples is contained in the skin, AP has a higher percentage of soluble fibre than apples. In particular, AP contains more pectin than apples. Because of its high fibre content, 100 g of AP can provide almost half of an individual's recommended daily fibre intake. (Barber *et al.*, 2020; Skinner *et al.*, 2018; Waldbauer *et al.*, 2017)

1.1.2-Phytochemicals in apple pomace

Through a variety of biological activities, free radicals are important chemicals that contribute to human diseases like cancer, heart disease, cerebrovascular disease, and ageing. It has been suggested that naturally occurring antioxidants are crucial in reducing the oxidative damage caused by free radicals. Natural foods and food-derived ingredients, like phenolic phytochemicals and antioxidant vitamins, have drawn a lot of interest recently because they are considered not harmful for health and do not qualify as "medicine." It is known that certain of these can protect against oxidative damage by acting as chemopreventive agents. One of the most common antioxidants found in fruits and vegetables is *vitamin C*, which has significant chemopreventive effects at a relatively high concentration without showing any signs of toxicity.

Nonetheless, it was found that *vitamin C* typically makes up less than 15% of the overall fruit activity. On the other hand, it has been proposed that phytochemicals have a significant role in the overall antioxidant capacity of fruits, vegetables, grains,

and tea. The potential health advantages of dietary phenolics, which have antioxidant properties stronger than those of vitamin C, have received a lot of attention lately. (Lee *et al.*, 2003)

As just said, therefore, the risk of inflammatory and oxidative stress-related illnesses is reduced by including phytochemicals in diets. Apples include five primary polyphenolic groups: *anthocyanins, hydroxycinnamates, flavonols, and dihydrochalcones*. Prior research has demonstrated that apples scavenge free radicals ex vivo and prevent lipid peroxidation. In an American study of commonly consumed fruits, apples were found to have the second-highest overall antioxidant activity, after cranberries, at about 100 μmol of *vitamin C* equivalents/g of fruit.

Because polyphenols are mostly found in the skin, the majority of them stay in the pomace. AP contains polyphenolic substances such as *ferulic acid, caffeic acid, p-Coumaric acid,* and *catechins*. AP has the potential to be a source of dietary antioxidants because it has been demonstrated to have greater scavenging capabilities than antioxidant *vitamins E and C. Quercetin* and its glucosides are the most abundant flavonoids. It is well known, thanks to many studies, that flavonoids have been connected to the prevention of cancer and cardiovascular disease (CVD). (Skinner *et al.*, 2018)

It should also be remembered that plants evolved a sophisticated chemical defence arsenal to combat pathogens, predators, and overgrowth by other plants. For this reason, studies have been done on the anti-pathogenic properties of apples. According to a study by Friedman *et al.* to test the bactericidal efficacy of apple skin extract against *non-MRSA-resistant Staphylococcus aureus, Lactobacillus monocytogenes RM2199, Salmonella enterica RM1309, and E. coli O157:H7,* a 4% stock solution was prepared and diluted. 50% of the aforementioned infections were killed by dilutions of the apple skin extract stock solution, which were >2.7%, 1.39%, 0.007%, and 0.002%, in that order. In *S. aureus* strains, the apple skin extract demonstrated remarkable growth suppression. *Procyanidins and phloridzin* were identified by the authors as the primary constituents in the extract. (Friedman *et al.,* 2013)

1.1.3-Storage and processing effects on apple phytochemicals

The phytochemical composition of apple fruit is not greatly impacted by storage.

According to a Van der Sluis investigation, the phytochemical composition of apples did not significantly change while they were being preserved. Several types of apples showed no changes after 52 weeks of storage in a controlled atmosphere. The quantities of total catechins only slightly decreased in two kinds, while the contents of anthocyanins, phloridzin, and quercetin glycosides basically remained the same. After 25 weeks of storage, for all varieties, no decrease in chlorogenic acid was found. (van der Sluis *et al.*, 2001)

In another study, the concentration of total phenolics in the peel of *Golden Delicious* apples increased following a 60-day period of refrigeration. As expected, after 100 days of storage, the total phenolics started to decline; however, after 200 days, the total phenolics were still comparable to what they had been when the crop was first harvested. (Lattanzio *et al.*, 2001)

The phytochemical content is also influenced by the processing of apples. In the assay by Guyot *et al.*, it was found that 42% of the total phenolics were extracted in the juice, but more than half of the total phenolics were left in the AP. The largest extraction yields in juice were seen for *hydroxycinnamic* acids and *dihydrochalcones*, at 65% and 80%, respectively. Conversely, the *proanthocyanidins* had the lowest percentage. (32%). It has been discovered that apple phenols, especially *procyanidins*, attach to cell wall components, and this could result in reduced polyphenol concentrations in apple juice. (Guyot *et al.,* 2003)

1.2-Apple pomace production process and its different compositions

The by-products' compositions will vary depending on prior processing and the technologies employed. Pomace is the press residue left over following processing, which includes juice, cider, wine, distilled spirit, and vinegar production, as well as industrial processes like making jelly. Twenty to thirty-five percent of the apple fruit's fresh weight is made up of solid waste. A combination of peel, core, seed, calyx, stem, and pulp make up the residue. Up to 95.5% of the solid waste originates from the epi mesocarp, which makes up the majority of the waste.

Different companies may use different procedures when making juice and cider. While small businesses can employ a discontinuous vertical hydraulic press, commercial fruit juice uses the continuous press system most frequently.

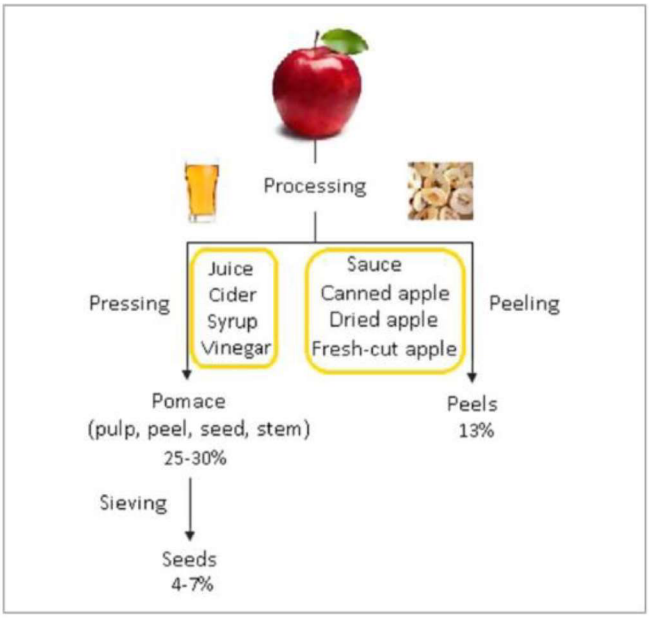


Figure 2 *- By-products of industrial apple processing*

Source: (https://ift.onlinelibrary.wiley.com/doi/epdf/10.1111/1541-4337.12290?saml_referrer)

Apples may be pressed, and skins, stalks, and pips removed before pressing. This will affect the pomace's composition. The final composition of the AP and pectin levels arepie also impacted by the inclusion of pectinolytic enzymes throughout the juice-making process. The cooking process involved in the production of the syrup and jelly, andmplies fibre extraction, particular scents, and sensory qualities from the pomace. The skins are blanched or chemically treated right away after peeling to stop enzymatic browning. AP is separated and sieved from apple seeds; the proportion of seeds it contains makes up about 4–7%. *Amygdalin*, a cyanogenic glycoside found in apple seeds, can be broken down by the *β-glucosidase* enzyme that is normally found in the human intestine to produce cyanide, which can be extremely dangerous to humans. A technique and apparatus for separating seeds from fruit pulp are proposed in a recent patent.

As mentioned earlier, different technologies are applied to apple fruits based on the final products, which affect the by-products' composition. For instance, during the syrup-making process, the cooking operation results in low levels of pectin and soluble

fibre in AP (2.9% and 0.0%, respectively).When apple peels undergo a blanching treatment, the amount of soluble fibre is concentrated to 32.1%, as opposed to 5.8% in untreated peels. Furthermore, the variation in cultivars, maturation stages, and quantification techniques may have an impact on the composition. (Rabetafika *et al.*, 2014)

1.3-Alternative uses of apple pomace

A substantial contribution to the energy budget can be made by using dried AP as fuel for processing plant steam generation. The economic viability of burning apple processing waste in-plant has been investigated in the past. It was hypothesised that by burning AP on-site, apple processors might cut their costs associated with disposing of garbage and using fossil fuels. To produce useful products including biogas, ethanol, butanol, citric acid, and pectinases, various microbial transformations of AP have been suggested. *Yeast* can be used to convert the fermentable carbohydrates, such as glucose, fructose, and sucrose, in AP into ethanol. It is thought that ethanol may be used as an alternative fuel to partially or entirely replace petroleum.

Traditionally, AP was used as cow feed. AP can be safely fed to animals, according to Narang and Lal's (Narang & Lal, 1985) evaluation of various agro-industrial wastes as feed for "*Jersy*" calves based on body weight gains, live body measurements, and metabolic trials.

A total mixed ration (AP-TMR) with 39% apple pomace was compared to conventional feeds (control) by Bae (Bae *et al.*, 1994). When compared to cows fed the control diet, they found that milk from cows fed AP-TMR had a higher protein content but a lower lactose concentration. For both diets, milk fat and solid-not-fat (SNF) were comparable. In addition, the body weight of cows fed AP-TMR was higher than that of cows fed control.

The use of AP is therefore very varied, ranging from the classic food use to that for fuel up to animal feed, but also as a filler, diluent, bulking agent, and substitute for microcrystalline cellulose. (Shalini & Gupta, 2010)

1.4-Use of fortified yogurts in recent years

Most regulatory bodies worldwide classify yogurt, often written *"yoghurt"* or *"yoghourt,"* as a fermented milk product that provides digested lactose and contains thermophilic lactic acid bacteria that have been specifically defined (in charge of fermenting milk) These strains are usually *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus.* It is a means of fortification and a source of various important nutrients, such as protein, calcium, potassium, phosphorus, and vitamins B2 and B12.

Sweeteners, fruits, and flavours can also be added to readily change the consistency and scent. Additionally, nuts, soy, and grains can be used to make yogurt as an alternative to the classic milk normally used.

The symbiosis of two bacterial strains (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) in a sterile setting at a certain temperature (36°C – 42°C) for 3–8 hours is what defines yogurt. In the finished product, both bacterial strains must continue to be active (with at least 10 million bacteria/g, according to CODEX 2003). Aroma is derived from the formation of lactic acid and various other compounds when milk lactose is used as the fermentation substrate.

Reduced pH has several benefits, including delaying the growth of unwanted microorganisms, converting milk's calcium and phosphorus into their soluble forms, and improving the digestibility and overall bioavailability of most proteins by allowing proteolytic enzymes to better break them down. (Fisberg & Machado, 2015)

There are two primary forms of yogurt: *stirred* and *set*. *Stirred* yogurts are made by breaking the intact gel and can be combined with fruit and flavours, resulting in a consistent semi-solid structure. On the other hand, *set-type* yogurts are fermented in a retail pot, producing a firm and compact gel structure. The network of aggregated casein micelles, which forms the foundation of yogurt gels, is susceptible to rupture from shearing, external forces, or spontaneous rearrangements during storage, leading to whey separation or syneresis. One approach to mitigate syneresis is the inclusion of stabilisers such as gelatin, pectin, carrageenan, and starch, which interact with casein micelles. However, while these stabilisers can prevent the attainment of 'natural functionality,' they may also introduce undesirable flavours and textures. (Wang, n.d. 2018)

There are several reports on adding fibre from various fruits and vegetables to yogurt, despite the paucity of research on AP enriched yogurt. Dello Staffolo, Bertola, Martino, and Bevilacqua conducted a sensory examination of yogurt that had been fortified with inulin, wheat, bamboo, and apple fibre. They discovered that the yogurt that had been fortified had high ratings for texture, colour, and flavour. The majority of fruit and vegetable fibre, in contrast to commercial stabilisers, may alter the hues of the fortified products, which might be a significant disadvantage of these preparations. (Staffolo *et al.*, 2004)

In a different investigation on yogurts supplemented with orange fibre, the increased water-holding capacity of the fibre was linked to decreased syneresis during cold storage, suggesting orange fibre as a substitute natural stabiliser *(*García-Pérez *et al.*, 2005).

While passionfruit by-products improved the cohesion, firmness, and consistency of fortified yogurts, they also markedly shortened the fermentation period. (do Espírito Santo *et al.*, 2012) Although the earlier studies enhanced the nutritional fibre content and enhanced the yogurt's texture, they used pure fruit or vegetable fibre, which may be more expensive and less available. Under these circumstances, entire fruits and vegetables—especially their byproducts—might be good candidates for yogurt fortification.

Since AP isn't often added to yogurts—as was already mentioned—this study will look at how it affects characteristics and storage.

With all the good characteristics present in apple pomace, the creation of a yogurt with added pieces can be an innovative and correct idea for a more eco-sustainable future, as well as being a potentially useful product for humans thanks to its contents.

Chap. 2 - MATERIALS AND METHODS

2.1-Initial test

The experiment commenced with a dress rehearsal, aiming to achieve several key objectives: determining the optimal amount of microbial culture necessary for yogurt production, identifying the ideal incubation period, and selecting the most suitable apple variety based on pulp characteristics and their impact on pH reduction. Initially, approximately 1 kg each of *Idared* and *Granny Smith* apples were procured, and the procedure was executed with simplicity.

The investigation began by assessing various weight ratios of apples to milk. Specifically, tests were conducted using 20 g of Idared apples with 200 mL of milk (1:10 ratio) and 40 g of Granny Smith apples with 200 mL of milk (1:5 ratio).

Subsequently, the quantity of microbial culture required for yogurt production was determined in accordance with the instructions provided on the packaging of Yo-Flex L812, which contains *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The packaging specifies a necessity of 11.8 g of culture for 250 L of milk. By applying a simple proportion, it was calculated that 0.0472 g of culture was needed per 1 L of milk.

$$11{,}8\ g : 250\ L = X : 1\ L$$

$$X = \frac{11{,}8\ g\ \times 1L}{250\ L} = 0{,}0472\ g\ of\ culture\ Yo - Flex\ L812$$

The test commenced by heating 800 mL of whole milk in a glass beaker until it reached a temperature of 50 degrees Celsius. Subsequently, the milk was allowed to cool for 15 minutes, reaching approximately 42 degrees Celsius, which is the optimal temperature for the growth of our microbial lactic culture (thermophilic). The previously determined quantities of the microbial culture were then added to the milk.

Meanwhile, two apples of each variety were carefully selected and sliced thinly, including the peel. These apple slices were then blended to create an apple "puree" **(Fig. 3)**. Subsequently, the puree was strained through a mesh to separate the excess juice.

Once the milk reached the desired temperature, it was poured into 100-milliliter plastic containers. The appropriate amounts of each apple variety were added to the milk: 10 grams of *Granny Smith* apples were added to 100 mL of milk, and 20 grams of

Idared apples were added to another 100 mL of milk. This resulted in a total of two containers, each containing 100 mL of milk and apple puree. This allocation was necessary to ensure that the desired ratios of apple to milk (20 g of *Granny Smith* in 200 mL and 40 g of *Idared* in 200 mL) were maintained, as previously described.

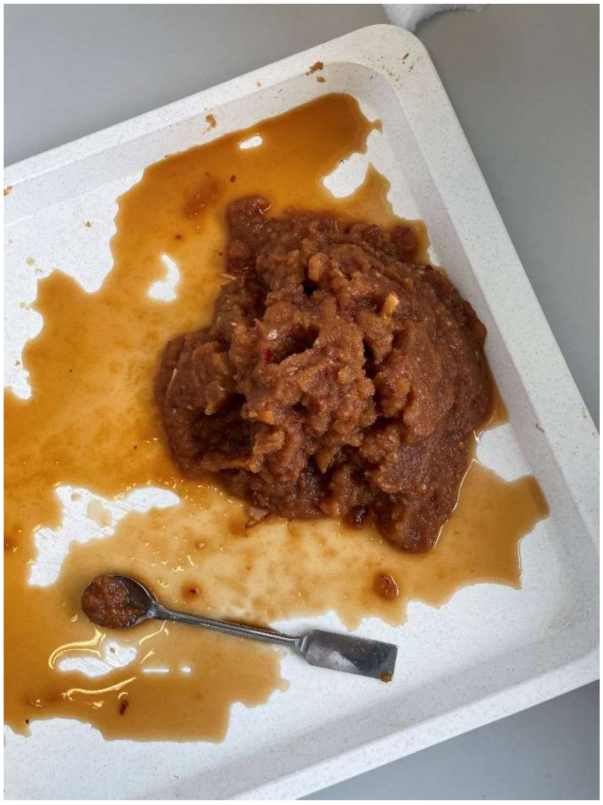


***Figure 3**: Apples of the Idared variety, after being blended with a minipimer*

Three containers, containing only milk, were also prepared as controls. Therefore, there were a total of 7 containers: 3 controls, 2 with *Idared*, and 2 with *Granny Smith.*

Following the completion of the preparation, the yogurt samples were left to incubate at 42°C in the oven for approximately 3 hours. The pH levels were tested before allowing the bacteria to ferment, and measurements were taken again 3 hours later to ensure adherence to the planned process. The table below **(Tab.2)** illustrates the discrepancies between the observed outcomes and the anticipated values, considering that the ideal pH for yogurt is <4.6.

Subsequently, corrective measures were implemented to address the error, including the addition of more microbial culture to enhance fermentation and extending the incubation period to 4 hours. Additionally, it was noted that the *Idared* variety

exhibited a higher pulp content compared to *Granny Smith* and demonstrated better efficacy in reducing the pH.

Ultimately, this preliminary experiment laid the groundwork for the development of a yogurt with the desired composition and values.

Type	*pH at preparation*	*pH after four hours*
Control 1	6.4	6.2
Control 2	6.5	6.2
Control 3	6.5	6.3
Granny Smith 1	6.0	6.0
Granny Smith 2	6.0	5.9
Idared 1	5.8	5.8
Idared 2	5.9	5.8

Table 2: pH of the yogurts for the first test.

2.2-Yogurt preparation

Once the optimal amount of microbial culture, the most suitable apple variety, and the correct incubation period were determined, the comprehensive testing phase commenced.

The test spanned over a period of 4 weeks, during which various parameters, including texture (hardness), rheology (viscosity), pH, and particle size, were measured. Assessments were conducted immediately post-preparation, after 7 days, 14 days, and at the conclusion of the 28-day period.

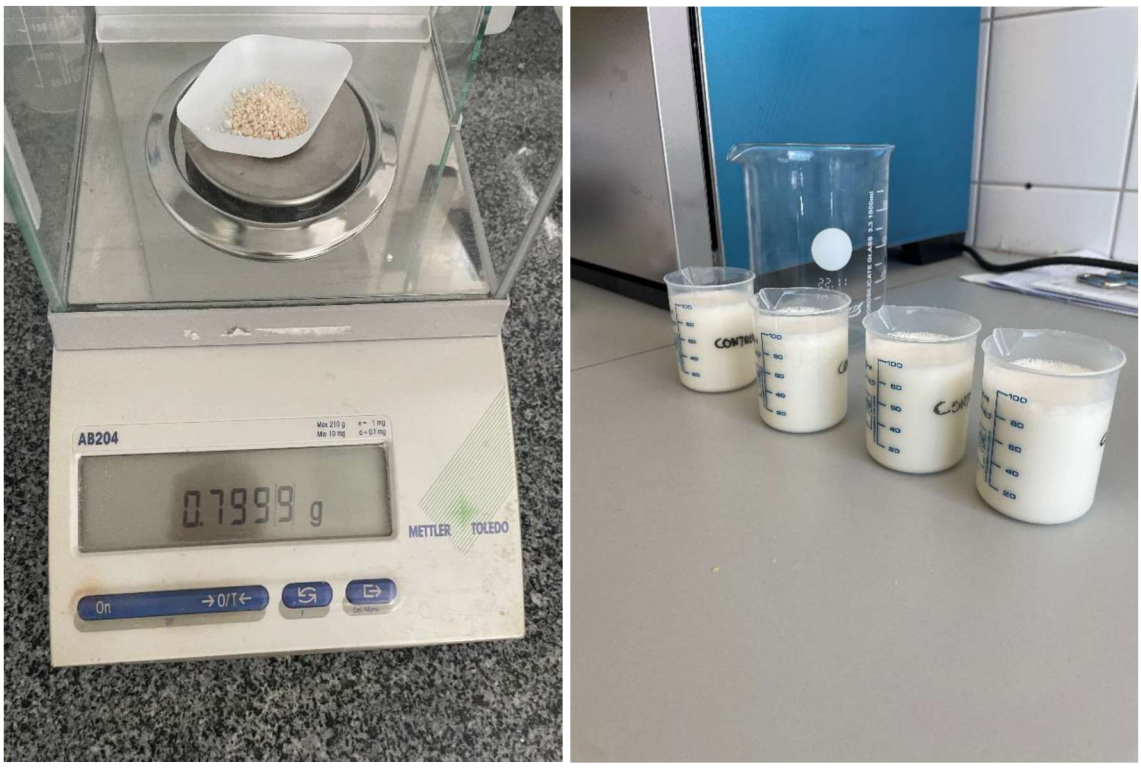
The experimental protocol mirrored that of the trial test, albeit with refined parameters. Utilizing 0.200 g of microbial culture per 1 L of milk, a total of 32 yogurt samples were prepared. These samples were divided into batches, with 8 samples designated for each week of storage, totaling 32 samples over the 4-week duration. The composition of each batch remained consistent, following the specified proportions.

It's important to note that this procedure was replicated in two separate attempts to ensure the reliability of the findings.

The necessary quantity was placed in a 100 mL plastic container

- Control 1
- Control 2
- 80 g milk + 20 g AP *(Idared)* – number 1
- 80 g milk + 20 g AP *(Idared)* – number 2
- 90 g milk + 10 g AP *(Idared)* – number 1
- 90 g milk + 10 g AP *(Idared)* – number 2
- 80 g milk + 15 g AP (Idared) + 5 g sucrose ($C_{12}H_{22}O_{11}$) – number 1
- 80 g milk + 15 g AP (Idared) + 5 g sucrose ($C_{12}H_{22}O_{11}$) – number 2

Based on a proportion, the amount of lactic bacteria is roughly 0.800 g, given the amount of milk used, which is around 4 L.



***Figures 4 and 5**: Weighing of 0.800 g of microbial culture and yogurt samples in preparation*

Next, 4 L of milk were heated to 50 degrees and then incubated with 0.800 g of culture for about 4-6 hours, to reach the required acidity (pH<4.6). Following the initial studies on 8 samples, the remaining samples were kept in the refrigerator at 4°C for subsequent analyses.

2.3-Texture Profile Analysis (TPA)

Texture plays a pivotal role in determining the acceptance of food products. In both quality control and product development processes, food industries commonly employ sensory panels to subjectively assess the texture of their offerings. However, such evaluations are inherently subjective and prone to variability.

Consequently, many companies complement sensory assessments with instrumental measurements to provide objective data.

The primary function of a texture analyzer is to quantitatively measure the deformation of a food item under specified conditions (Mermelstein, 2013). The Texture Profile Analysis (TPA) test, in particular, serves to simulate the chewing process and enables the evaluation of a wide array of textural attributes in both fresh and processed foods. These attributes include *'Springiness,'* which reflects a sample's ability to revert to its original shape after deformation; *'Hardness,'* denoting the maximum force required to compress the sample; *'Cohesiveness,'* indicating the extent to which a sample can be deformed before rupturing; *'Adhesiveness,'* representing the force required to resist surface adhesion*; 'Gumminess,'* quantifying the force necessary to disintegrate a semisolid sample into a swallowable state (calculated as *hardness* × *cohesiveness*); and *'Chewiness,'* measuring the effort required to masticate a solid sample to a swallowable state (computed as *springiness* × *gumminess*) (Chen & Opara, 2013).

2.3.1 – "Hardness" Measurement

The hardness of the yogurt samples was assessed using the *Shimadzu EZ-X Texture Analyzer* **(fig.6)**. A cylindrical probe with a diameter of 35 mm was employed, penetrating the samples at a constant rate of 1 mm/s to a depth of 15 mm, with a maximum force set at 50 N.

For each type of yogurt, two samples were tested in both the first and second attempts, and the results were averaged to ensure accuracy. Standard deviations were calculated to quantify variability and standardize the results.

Figure 6: *Texture Analyzer Shimadzu EZ-X*

2.4-Rheology

Rheology serves as a crucial tool for evaluating and describing the deformation and flow behavior of materials. It encompasses the study of how solids undergo deformation and how fluids flow at different rates.

The textural qualities experienced when consuming food, such as creaminess, juiciness, smoothness, brittleness, softness, and hardness, are often attributed to their rheological characteristics. Consequently, characterizing the rheological properties of food has become increasingly important for assessing the quality of raw materials, predicting material behavior during processing, and ensuring storage and stability requirements are met (*Basics of Rheology | Anton Paar Wiki*, n.d.).

2.4.1-"Viscosity" Measurement

Viscosity refers to the resistance of a fluid to flow. In any liquid, including dispersions containing larger particles, molecules and particles move past each other when in motion, experiencing internal friction that impedes flow. The presence of larger constituents contributes to higher viscosity levels in a fluid.

To measure viscosity, rotational testing with speed control or other techniques can be employed, yielding viscosity or flow curves as outcomes (*Basics of Rheology | Anton Paar Wiki*, n.d.).

For assessing yogurt's rheological behavior, a *Modular Compact Rheometer (MCR) series-MCR 72 (Anton Paar)* **(fig.7)** was utilized, employing a plate-plate geometry system with a 1 mm gap. Prior to analysis, yogurt samples were brought to room temperature and stirred with a glass rod in both clockwise and anticlockwise directions ten times each. Subsequently, approximately 5 mL of the sample were added to the dish for measurement. The shear rate was calculated in 1/s, and viscosity was reported in mPa·s.

$$\textit{Definition of viscosity: } \eta = \frac{\tau}{\gamma}$$

η = Viscosity

τ = Shear stress (N/m^2 = 1 Pa – Pascal)

γ = Shear rate ($1/s = 1\ s^{-1}$)

Figure 7: *Modular Compact Rheometer (MCR) series-MCR 72 (Anton Paar)*

2.5-“Particle Size” Measurement

Through specific analyses, it becomes feasible to discern the particle size (PS) and particle size distribution (PSD) of a given specimen. This versatile technique finds application across various materials such as solids, aerosols, emulsions, and suspensions. As an indispensable quality control tool across diverse sectors, the PS significantly influences the ultimate applications and outcomes of the product. In this study, the measurements were conducted utilizing a *Malvern Mastersizer 3000 instrument* **(fig.8)** equipped with an Hydro unit, sourced from *Malvern Instruments Ltd., Worcestershire, UK, 2013.*

Due to the dimensions of the AP particles, the analyses were exclusively performed on the control samples (in the absence of AP).

Figure 8: *Malvern Mastersizer 3000 equipped with Hydro unit*

2.6-“pH” Measurement

The pH, a fundamental parameter crucial for yogurt production, underwent evaluation using a laboratory-grade pH meter. This instrument was meticulously calibrated each day with pH 4.0, 7.0, and 9.0 buffer solutions to ensure accuracy and reliability in measurements. Each sample underwent initial pH analysis after 4 hours of incubation, followed by subsequent assessments at intervals of 7, 14, and 28 days.

The comprehensive and systematic approach enabled a thorough understanding of pH dynamics throughout the yogurt production process.

2.7-“ Total phenolic content” Measurement in AP

A portion of *Idared* apples was lyophilized at -55°C, 3 days **(fig.9)** utilizing the freeze-dryer available at the *Faculty of Agrobiology, Food, and Natural Resources of the Czech University of Life Sciences (CZU).*

The freeze-dried apples, including the peel, were subsequently utilized to determine the total phenolic content (TPC). Additionally, these freeze-dried apples were incorporated into the sensory analysis as a distinct component to be added to the yogurt prior to consumption.

***Figure 9**: freeze-dried apples of Idared variety*

First, approximately 72.5 g of apples were weighed and then homogenised using a mixer, following which the sample was transferred to a beaker with 70% aqueous methanol in a 1:1.3 (w/v) ratio, i.e., 72.5 g of homogenised apples and 100 mL of aqueous methanol. The apple homogenate with 70% methanol was then centrifuged and filtered with filter paper. The process was repeated in duplicate.

Total phenols were estimated by using the *Folin-Ciocalteu* reagent according to the method described by (Meneses *et al.*, 2013) with slight modifications. Briefly, 5 µL of the diluted sample was mixed with 200 µL of distilled water and 15µL of *Folin-Ciocalteau reagent* in a 96-well microplate **(fig.10)**, and allowed to stand for 5 min.

Then 60 µL of sodium carbonate solution (20% w/v) was added and incubated at room temperature for 60 min. The absorbance was measured at 725 nm with a spectrophotometric microplate reader (Synergy H1, BioTek). A standard curve was prepared using a standard solution of gallic acid (25–1000 µg/mL). The TPC was reported as milligram gallic acid equivalent per dry weight of material (mg GAE/g AP).

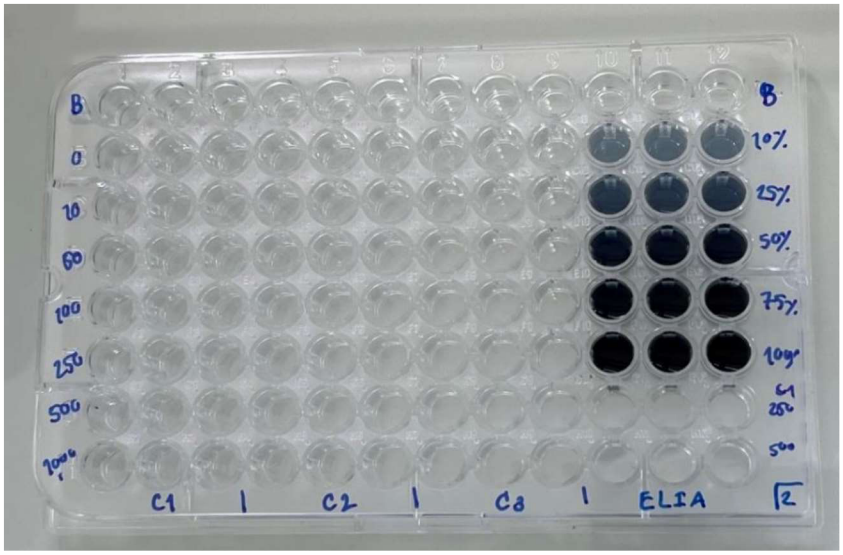


Figure 10: *Folin-Ciocalteau reagent in a 96-well microplate*

2.8- Sensory analysis test

Subsequently, a sensory analysis test was conducted employing a panel comprised of 10 individuals carefully selected from the student and doctoral cohorts within the *Faculty of Agrobiology, Food, and Natural Resources department*.

Each participant underwent tastings for every yogurt variant: 80 g milk + 20 g AP, 90 g milk + 10 AP, 80 g milk + 15 AP + 5 sugar, and a control yogurt, which was consumed alongside approximately 5 g of freeze-dried apple to mimic a yogurt packaged separately. Samples of 100 g each were presented to the panel in plastic containers labeled with randomized letters, approximately 1 hour and 30 minutes post-extrusion.

The parameters taken into consideration were:

1. *Color (from white to pink/yellow due to the apples)*
2. *Sour (smell)*
3. *Presence of syneresis on the top*
4. *Sweet (taste)*

5. *Density (consistency)*
6. *Sour (taste)*
7. *Smell of milk*
8. *Fruit Smell (apples)*
9. *Defects*
10. *Intensity of apple flavour*

The data were then subjected to comparison, acknowledging the inherent limitations stemming from the non-professional expertise of the participants and the relatively modest sample size. Nevertheless, overarching trends regarding yogurt preferences based on taste evaluations could be discerned.

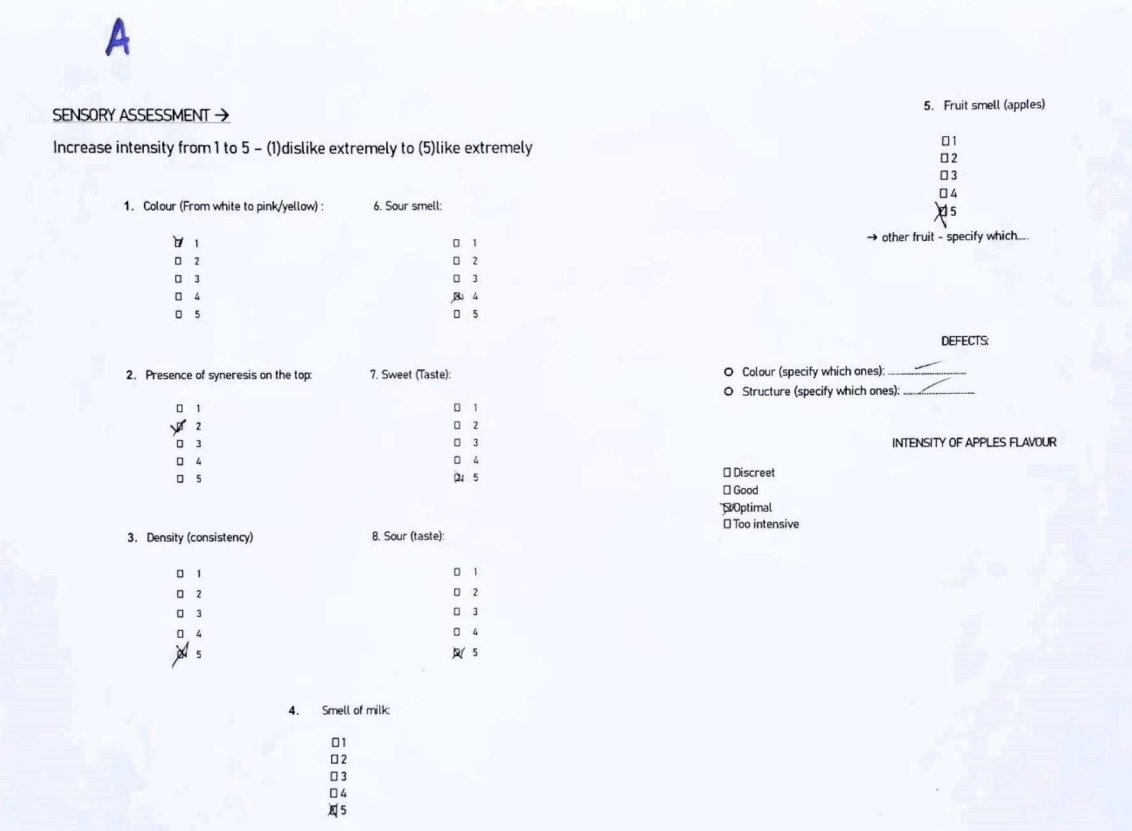


Figure 11: *Example of sensory analysis sheet used for the test*

Chap. 3 - RESULTS

3.1-"Hardness" Results

Hardness, expressed in Newtons (N), plays a critical role in Texture Profile Analysis (TPA). It is assessed both during preparation and in the subsequent weeks of storage, at intervals of 7, 21, and 28 days.

LEGEND:

- CONTROL: Control plain yogurt
- 80+20: Yogurt with 80 g milk + 20 g AP
- 90+10: Yogurt with 90 g milk + 10 g AP
- 5SUGAR: Yogurt with 80 g milk + 15 g AP + 5 g Sucrose ($C_{12}H_{22}O_{11}$)

The values were calculated as the average with standard deviation for both attempts and each type of yogurt.

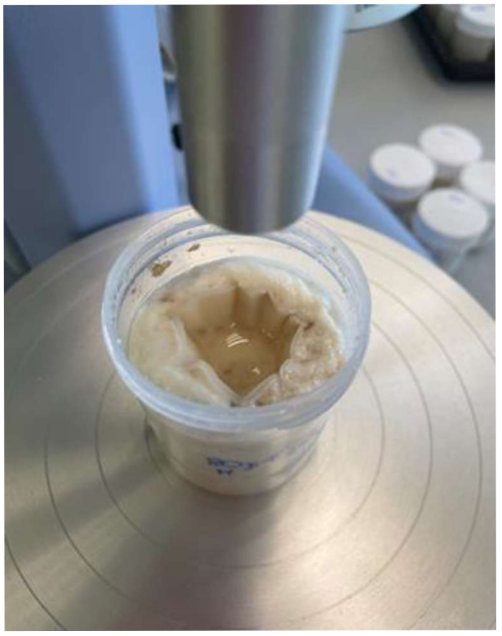


***Figure 12**: Suboptimal storage conditions evident in an 80+20 type sample.*

Expressed in "N"	*PREPARATION*	*7 DAYS*	*14 DAYS*	*28 DAYS*
CONTROL	0.381 ± 0,087	0,628 ± 0,038	0,593 ± 0,046	0,634 ± 0,051
80+20	0,364 ± 0,114	0,509 ± 0,047	0,535 ± 0,107	0,722 ± 0,342
90+10	0,392 ± 0,163	0,549 ± 0,019	0,561 ± 0,048	0,596 ± 0,066
5 SUGAR	0,362 ± 0,127	0,515 ± 0,056	0,546 ± 0,060	0,621 ± 0,109

***Tab 4**: hardness of each type of yogurt during the 4 weeks of storage*

According to the data presented in the table above, it is evident that none of the yogurt varieties containing AP have undergone a noteworthy increase in hardness.

The only variation was evident in the last week (28 days), when there was a slight increase in samples with 80+20 and 5SUGAR. This was due to improper storage, which led to mold formation and a subsequent hardness increase in some samples of the second attempt. **(Fig.12)**

However, a study conducted by (Wang *et al.,* 2019), revealed that over a 28-day period of cold storage, the addition of AP to stirred yogurt significantly decreased syneresis while simultaneously augmenting the viscosity, firmness, and cohesiveness of the matrix.

3.2-“Viscosity” Results

As apparent viscosity significantly influences both the overall characteristics of the gel and the stability of dairy products, understanding rheological behavior becomes pivotal. The subsequent graphs present a comparative analysis of average viscosities (mPa·s) over the four-week period for all three yogurt types (80g milk + 20g AP; 90g milk + 10g AP; 80g milk + 15g AP + 5g sucrose) across both attempts **(Fig. 13)**. Additionally, individual trends in viscosity over the weeks of storage were examined for each type **(Fig. 14a-14d).**

In all graphs, viscosity was calculated within a range of 0.526 to 12.6 shear rates (1/s). Lower and higher values were omitted as they resulted in overlapping curves, making interpretation challenging. The first graph illustrates a clear trend wherein increased AP content correlates with higher viscosity. This observation aligns with findings from various studies, such as that of Wang.

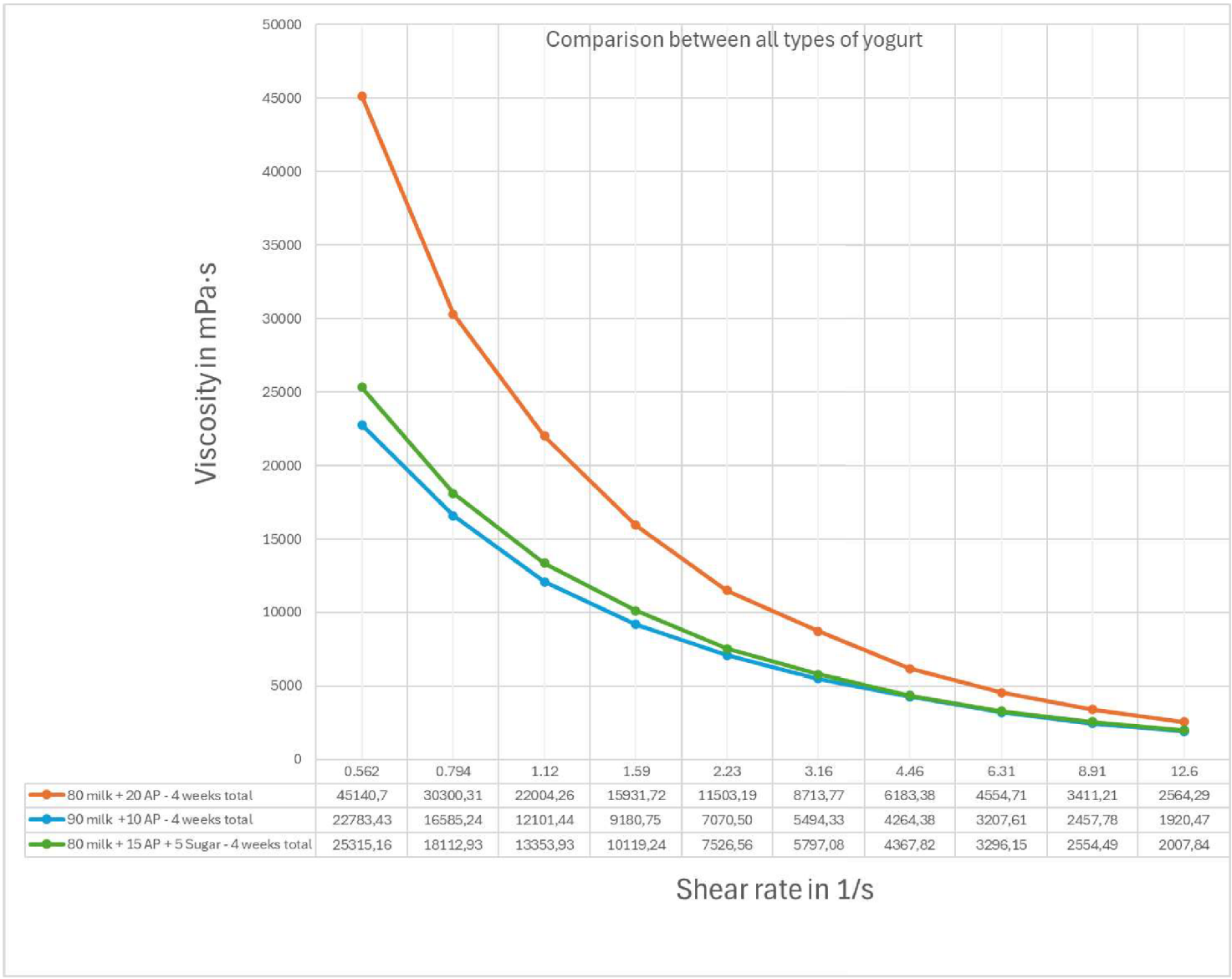
The addition of apple pomace altered the rheological properties of yogurt gel, resulting in increased viscosity and a gel with more liquid-like characteristics. AP, containing both soluble and insoluble fibre, has been shown to possess a significant water-holding capacity as well as the ability to modify viscosity and texture. These properties potentially account for the observed increase in yogurt viscosity in the presence of apple pomace (Wang *et al.*, 2019). Throughout all storage periods, the viscosity index of yogurt samples was influenced by the addition of apple pomace. Moreover, as the dosage of apple pomace increased, this parameter exhibited a corresponding rise.

Each sample exhibited shear-thinning fluid behavior as the shear rate increased, as evidenced by a decrease in apparent viscosity. Across the entire shear rate range under investigation, the viscosity of the control sample decreased continuously with shear rate, indicating a pseudoplastic tendency. Conversely, when AP was added, the viscosity curves tended to change shape.

Notably, in the viscosity curves of yoghurts supplemented with 20 g of apple pomace **(fig. 14b),** compared to the trend observed in the control sample, a decrease in initial shear rate was followed by a higher viscosity value at shear rates exceeding 0.562 1/s. This trend gradually diminished over time, nearly disappearing by the fourth week (Wang *et al.,* 2019). Furthermore, it is pertinent to recall that pectins, present in AP, possess the capability to stabilize casein aggregates through steric and electrostatic mechanisms. This may result in the formation of casein-pectin complexes, contributing to the stabilization of the yogurt gel network (Liu *et al.,* 2006).

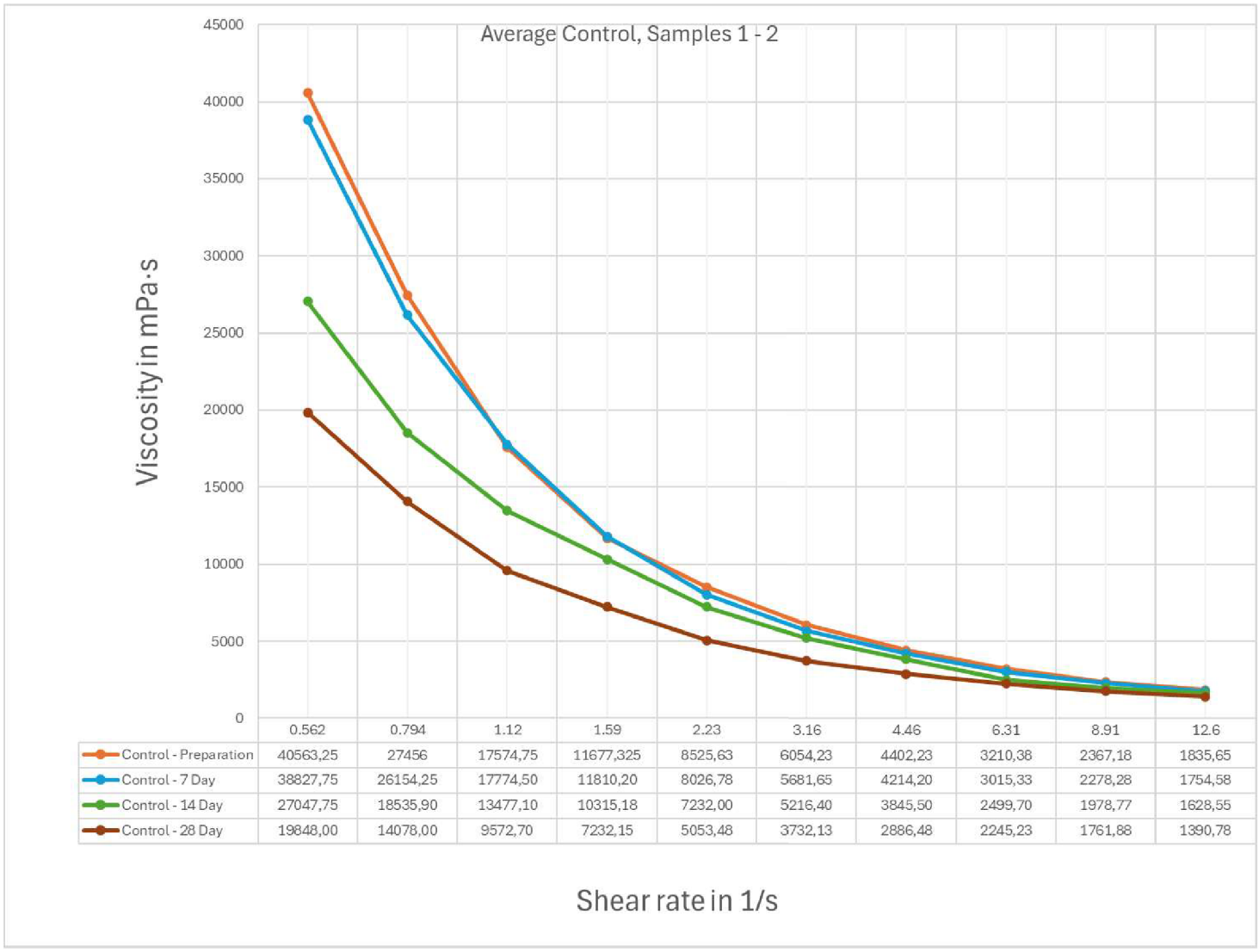
COMPARISON BETWEEN ALL TYPES OF YOGURT

Fig.13: *Comparison of the three types of yogurt over a four-week period.* ***(1st & 2nd ATTEMPT).***



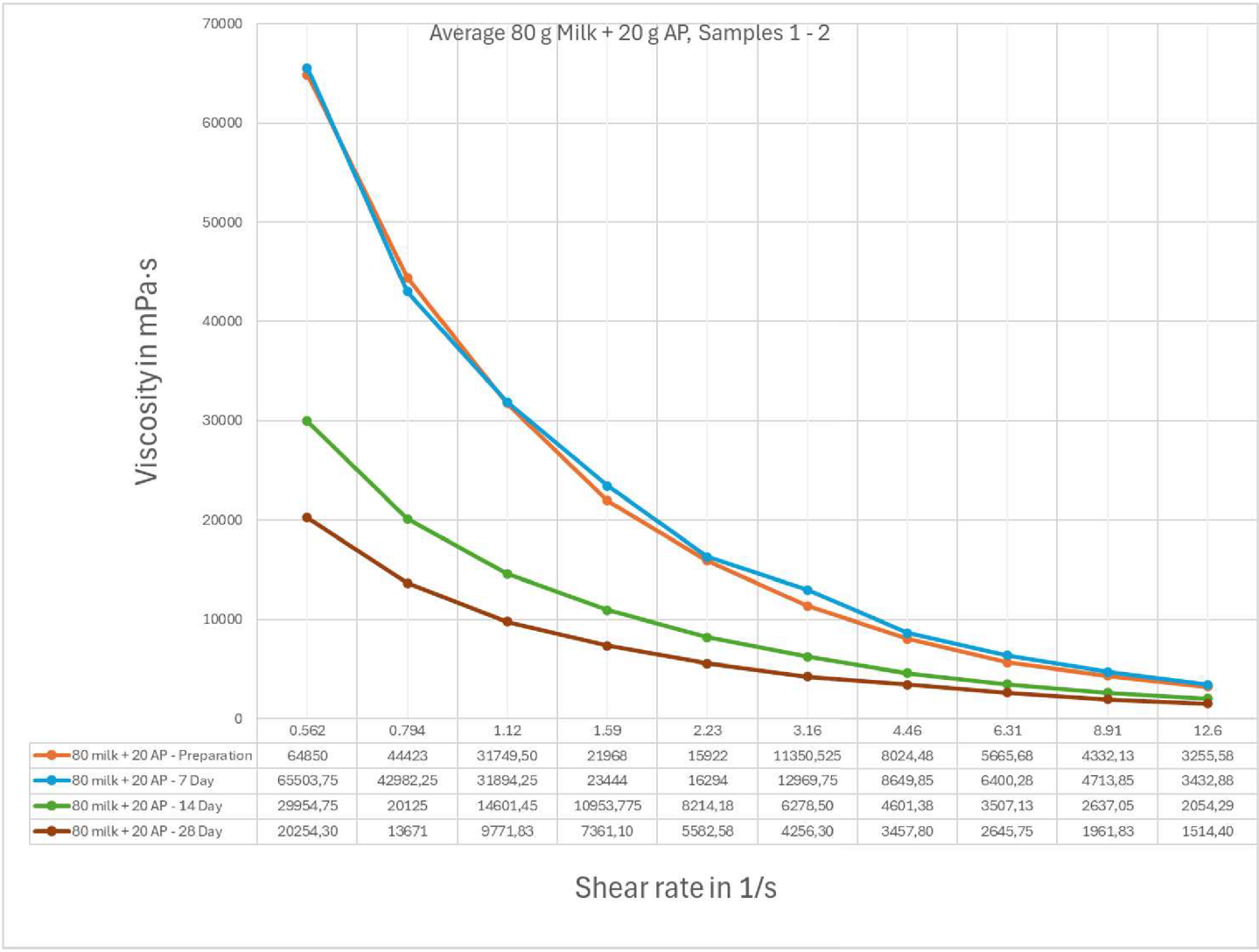
	0.562	0.794	1.12	1.59	2.23	3.16	4.46	6.31	8.91	12.6
80 milk + 20 AP - 4 weeks total	45140,7	30300,31	22004,26	15931,72	11503,19	8713,77	6183,38	4554,71	3411,21	2564,29
90 milk +10 AP - 4 weeks total	22783,43	16585,24	12101,44	9180,75	7070,50	5494,33	4264,38	3207,61	2457,78	1920,47
80 milk + 15 AP + 5 Sugar - 4 weeks total	25315,16	18112,93	13353,93	10119,24	7526,56	5797,08	4367,82	3296,15	2554,49	2007,84

Fig. 14a**: Progression of yogurt control over the course of four weeks **(1st & 2nd ATTEMPT).



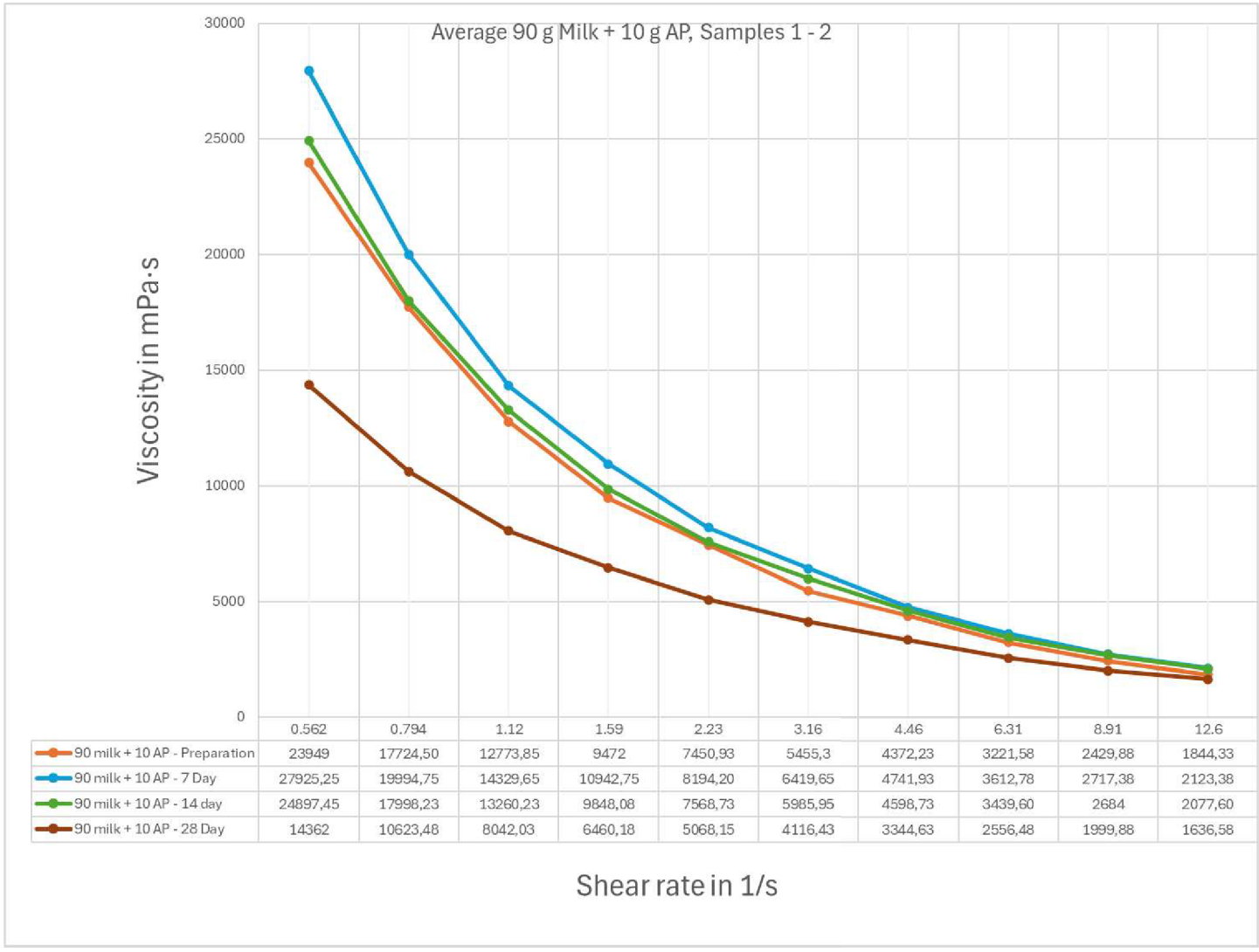
	0.562	0.794	1.12	1.59	2.23	3.16	4.46	6.31	8.91	12.6
Control - Preparation	40563,25	27456	17574,75	11677,325	8525,63	6054,23	4402,23	3210,38	2367,18	1835,65
Control - 7 Day	38827,75	26154,25	17774,50	11810,20	8026,78	5681,65	4214,20	3015,33	2278,28	1754,58
Control - 14 Day	27047,75	18535,90	13477,10	10315,18	7232,00	5216,40	3845,50	2499,70	1978,77	1628,55
Control - 28 Day	19848,00	14078,00	9572,70	7232,15	5053,48	3732,13	2886,48	2245,23	1761,88	1390,78

Fig. 14b**: Progression of yogurt 80g milk +20g AP over the course of four weeks **(1st & 2nd ATTEMPT).



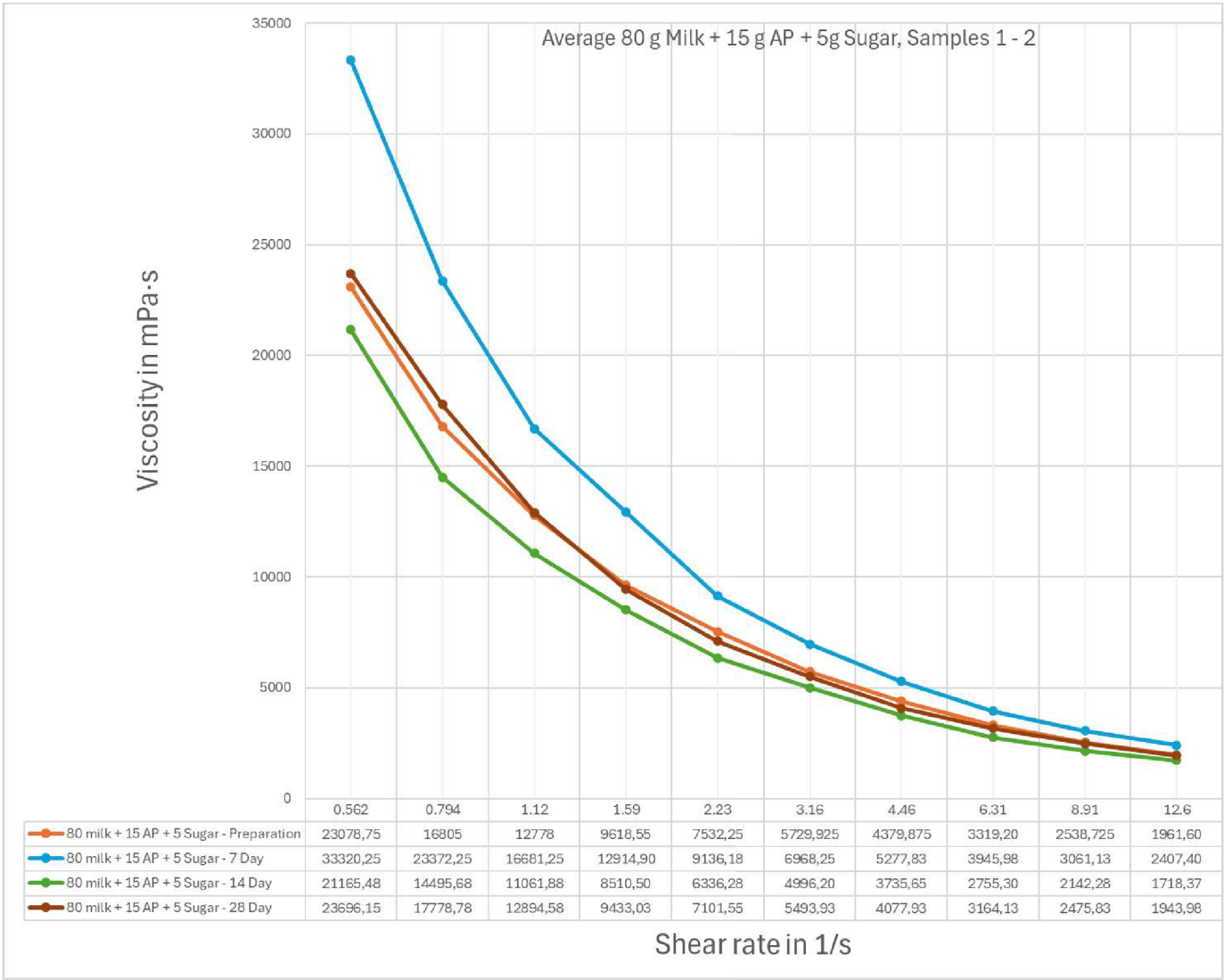
	0.562	0.794	1.12	1.59	2.23	3.16	4.46	6.31	8.91	12.6
80 milk + 20 AP - Preparation	64850	44423	31749,50	21968	15922	11350,525	8024,48	5665,68	4332,13	3255,58
80 milk + 20 AP - 7 Day	65503,75	42982,25	31894,25	23444	16294	12969,75	8649,85	6400,28	4713,85	3432,88
80 milk + 20 AP - 14 Day	29954,75	20125	14601,45	10953,775	8214,18	6278,50	4601,38	3507,13	2637,05	2054,29
80 milk + 20 AP - 28 Day	20254,30	13671	9771,83	7361,10	5582,58	4256,30	3457,80	2645,75	1961,83	1514,40

Fig. 14c**: Progression of yogurt 90g milk +10g AP over the course of four weeks **(1st & 2nd ATTEMPT).



	0.562	0.794	1.12	1.59	2.23	3.16	4.46	6.31	8.91	12.6
90 milk + 10 AP - Preparation	23949	17724,50	12773,85	9472	7450,93	5455,3	4372,23	3221,58	2429,88	1844,33
90 milk + 10 AP - 7 Day	27925,25	19994,75	14329,65	10942,75	8194,20	6419,65	4741,93	3612,78	2717,38	2123,38
90 milk + 10 AP - 14 day	24897,45	17998,23	13260,23	9848,08	7568,73	5985,95	4598,73	3439,60	2684	2077,60
90 milk + 10 AP - 28 Day	14362	10623,48	8042,03	6460,18	5068,15	4116,43	3344,63	2556,48	1999,88	1636,58

Fig. 14d**: Progression of yogurt 80g milk + 15g AP + 5g Sugar over the course of four weeks **(1st & 2nd ATTEMPT).



	0.562	0.794	1.12	1.59	2.23	3.16	4.46	6.31	8.91	12.6
80 milk + 15 AP + 5 Sugar - Preparation	23078,75	16805	12778	9618,55	7532,25	5729,925	4379,875	3319,20	2538,725	1961,60
80 milk + 15 AP + 5 Sugar - 7 Day	33320,25	23372,25	16681,25	12914,90	9136,18	6968,25	5277,83	3945,98	3061,13	2407,40
80 milk + 15 AP + 5 Sugar - 14 Day	21165,48	14495,68	11061,88	8510,50	6336,28	4996,20	3735,65	2755,30	2142,28	1718,37
80 milk + 15 AP + 5 Sugar - 28 Day	23696,15	17778,78	12894,58	9433,03	7101,55	5493,93	4077,93	3164,13	2475,83	1943,98

3.3-“Particle Size” Results

Considering the dimensions of the AP particles, the analysis of particle size distribution was exclusively conducted on the two control samples over the span of four weeks for both attempts **(Fig.15a-15d; Fig.16a-16d).**

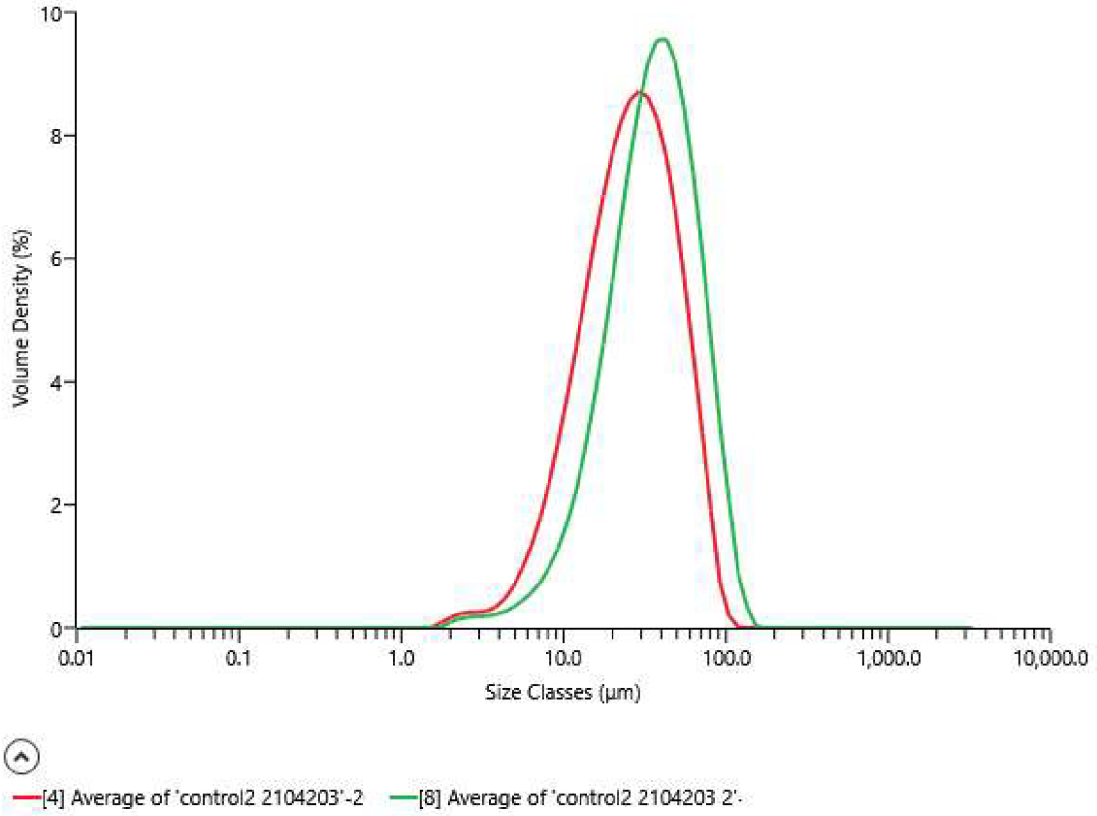
The size of the particles plays a crucial role in yogurt stability. However, consistent with the characteristics of standard yogurt, all measurements exhibited a monomodal trend.

In a study by Cayot *et al.* in 2008, it was elucidated that the perception of creaminess seemed to diminish when particle size exceeded 150 µm. Nevertheless, the correlation between gel firmness and particle size does not provide a straightforward explanation for the sensation of creaminess, as evidenced by the findings of two commercial products containing fat and starter (Cayot *et al.*, 2008).

On average, across all samples, it was observed that approximately 90% of the particles had a diameter of about 50 µm, aligning with the aforementioned study's results. The sole deviation was noted on the day of preparation during the second attempt **(Fig. 16a).** This bimodal distribution does not appear significant, as it is possible that errors occurred during the analysis procedure, resulting in the presence of larger particles, as depicted in the secondary curve. (Bimodality is often characterized by the presence of larger particle modes exceeding several hundred microns and smaller particle modes with sub-100-µm sizes.)

FIRST ATTEMPT

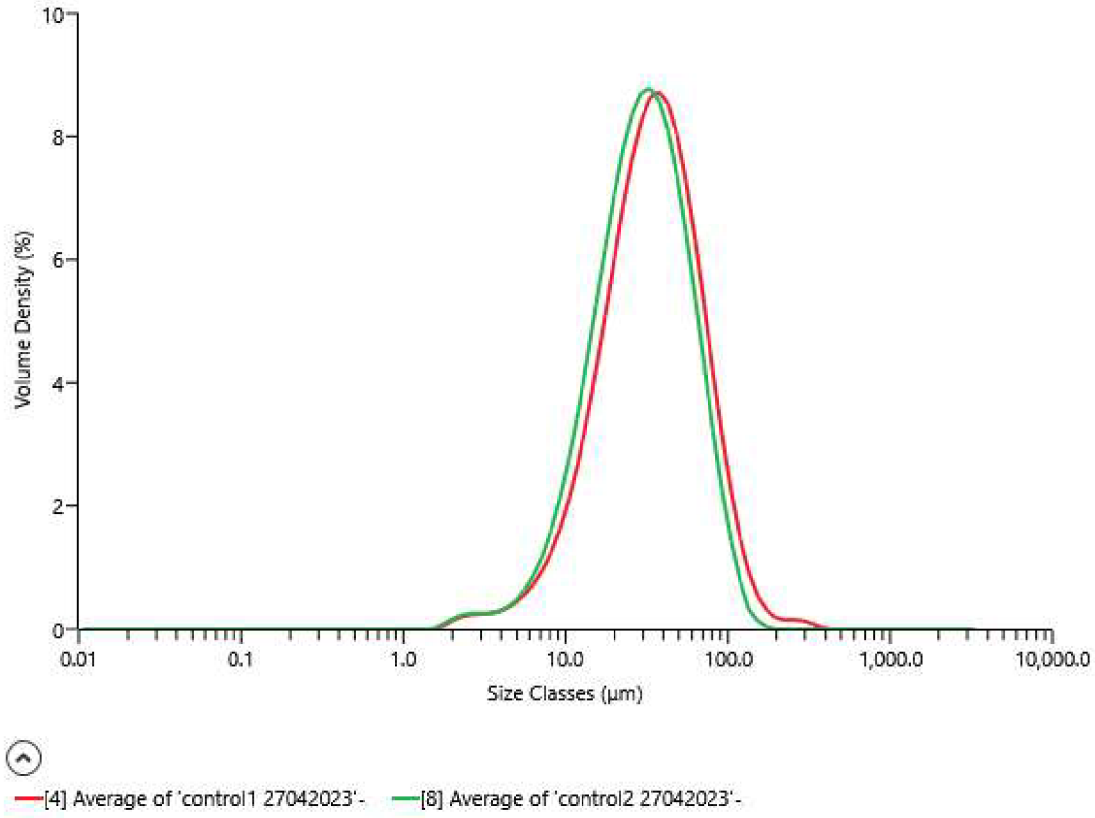
Fig.15a: *Particle-Size Distribution (PSD), at the time of preparation.* ***(1 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control2 2104203	10.2	28.0	63.0
	2	control2 2104203	9.75	25.7	55.1
	3	control2 2104203	9.45	24.4	52.1
	4	Average of 'control2 2104203'	9.80	25.9	56.8
	5	control2 2104203 2	15.2	39.9	84.3
	6	control2 2104203 2	14.1	35.8	72.8
	7	control2 2104203 2	13.4	33.2	66.7
	8	Average of 'control2 2104203 2'	14.2	36.1	74.7
Mean			**12.0**	**31.1**	**65.7**
1xStd Dev			**2.42**	**5.86**	**11.1**
1xRSD (%)			**20.1**	**18.8**	**16.9**

Table 5a*: Mastersizer parameters at preparation.*

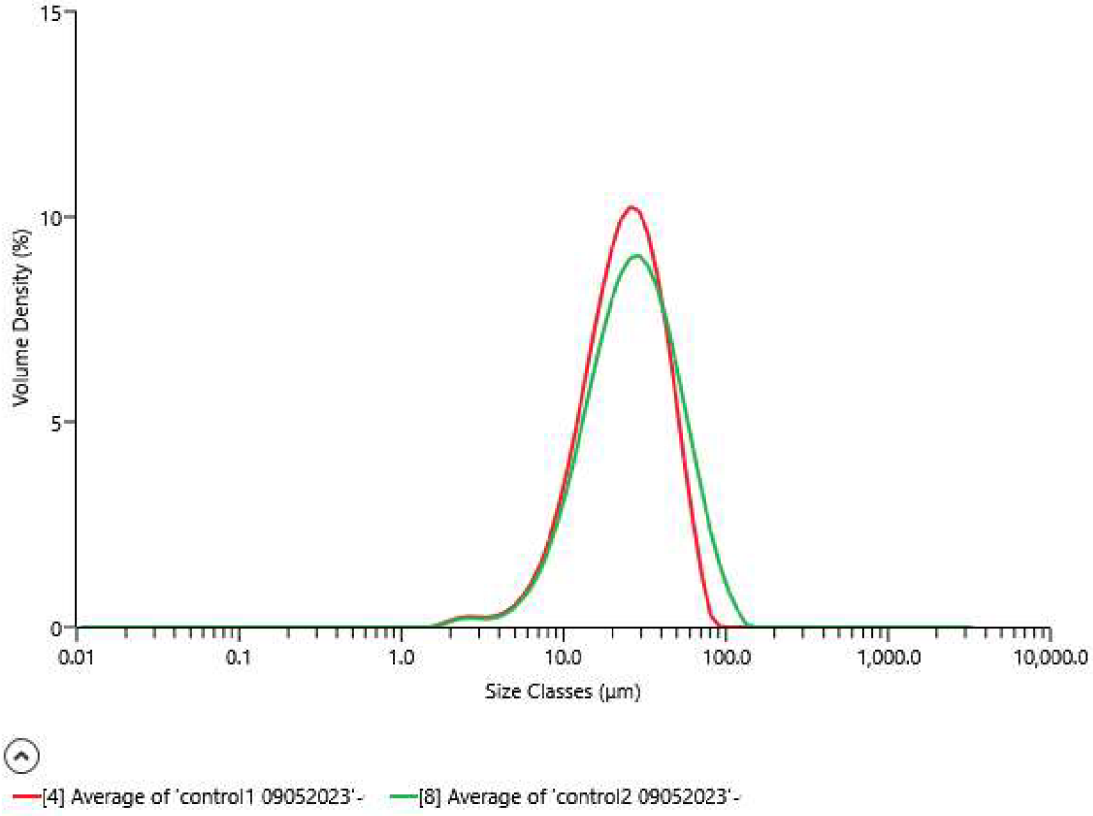
Fig.15b: *Particle-Size Distribution (PSD), after 7 days.* ***(1 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control1 27042023	13.5	38.1	89.9
	2	control1 27042023	12.6	33.9	76.7
	3	control1 27042023	12.1	31.3	70.2
	4	Average of 'control1 27042023'	12.7	34.2	79.5
	5	control2 27042023	12.1	33.0	77.6
	6	control2 27042023	11.4	29.7	66.0
	7	control2 27042023	10.9	27.4	59.4
	8	Average of 'control2 27042023'	11.4	29.8	67.7
Mean			**12.1**	**32.2**	**73.4**
1xStd Dev			**0.833**	**3.33**	**9.50**
1xRSD (%)			**6.89**	**10.4**	**12.9**

Table 5b*: Mastersizer parameters after 7 days.*

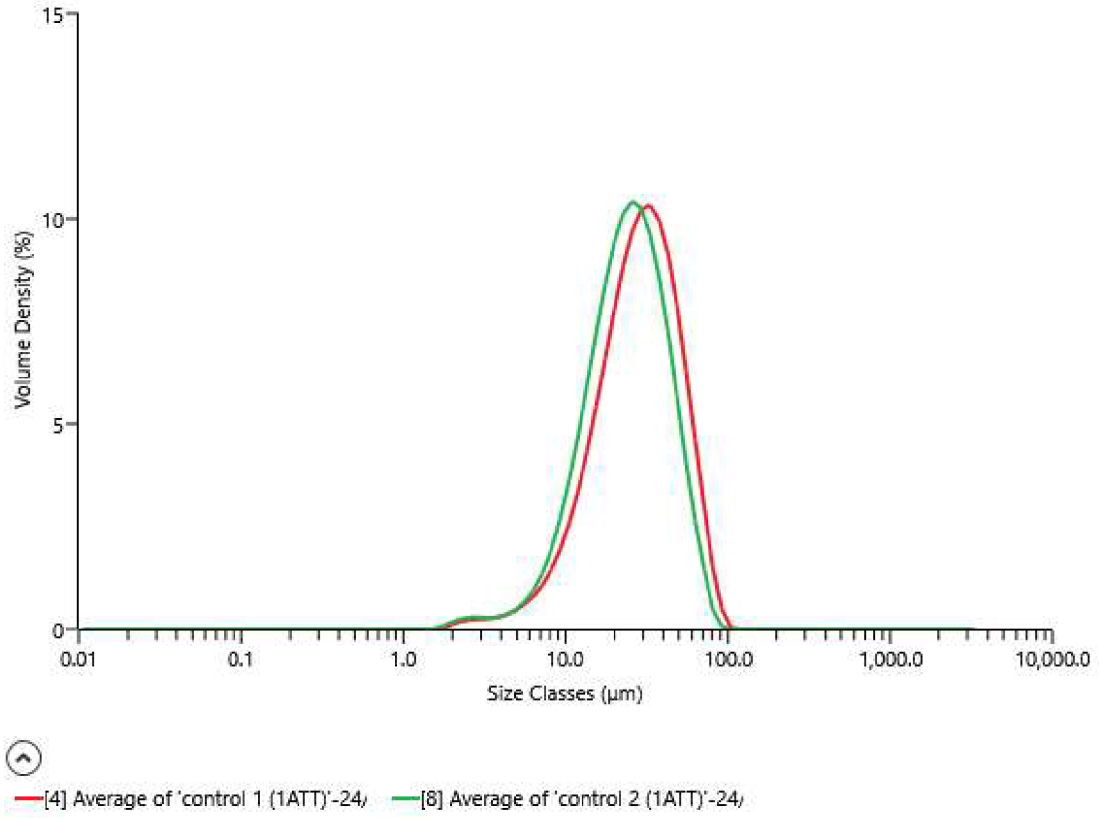
Fig.15c: *Particle-Size Distribution (PSD), after 14 days.* ***(1 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control1 09052023	10.7	25.5	50.9
	2	control1 09052023	10.3	24.0	47.2
	3	control1 09052023	10.0	22.8	44.1
	4	Average of 'control1 09052023'	10.3	24.1	47.5
	5	control2 09052023	11.4	28.9	66.3
	6	control2 09052023	10.8	26.7	59.1
	7	control2 09052023	10.4	25.0	54.2
	8	Average of 'control2 09052023'	10.8	26.8	60.0
Mean			**10.6**	**25.5**	**53.7**
1xStd Dev			**0.425**	**1.93**	**7.64**
1xRSD (%)			**4.00**	**7.57**	**14.2**

Table 5c*: Mastersizer parameters after 14 days*

Fig.15d: *Particle-Size Distribution (PSD), after 28 days.* ***(1 ATTEMPT)***

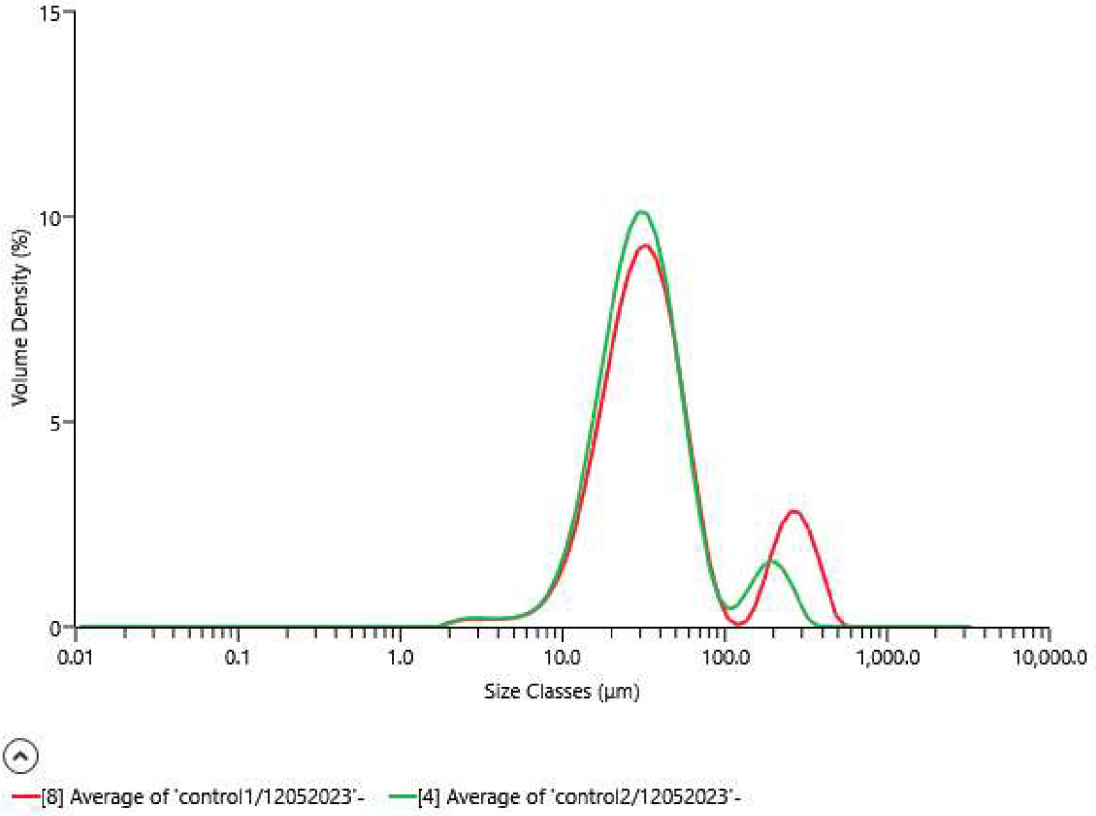


	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control 1 (1ATT)	12.5	30.5	60.7
	2	control 1 (1ATT)	11.8	28.5	55.4
	3	control 1 (1ATT)	11.4	26.9	51.3
	4	Average of 'control 1 (1ATT)'	11.9	28.6	55.9
	5	control 2 (1ATT)	11.1	25.9	51.8
	6	control 2 (1ATT)	10.6	24.2	47.3
	7	control 2 (1ATT)	10.2	22.9	44.4
	8	Average of 'control 2 (1ATT)'	10.6	24.2	48.0
Mean			**11.3**	**26.5**	**51.8**
1xStd Dev			**0.778**	**2.64**	**5.33**
1xRSD (%)			**6.90**	**9.99**	**10.3**

Table 5d*: Mastersizer parameters after 28 days.*

SECOND ATTEMPT

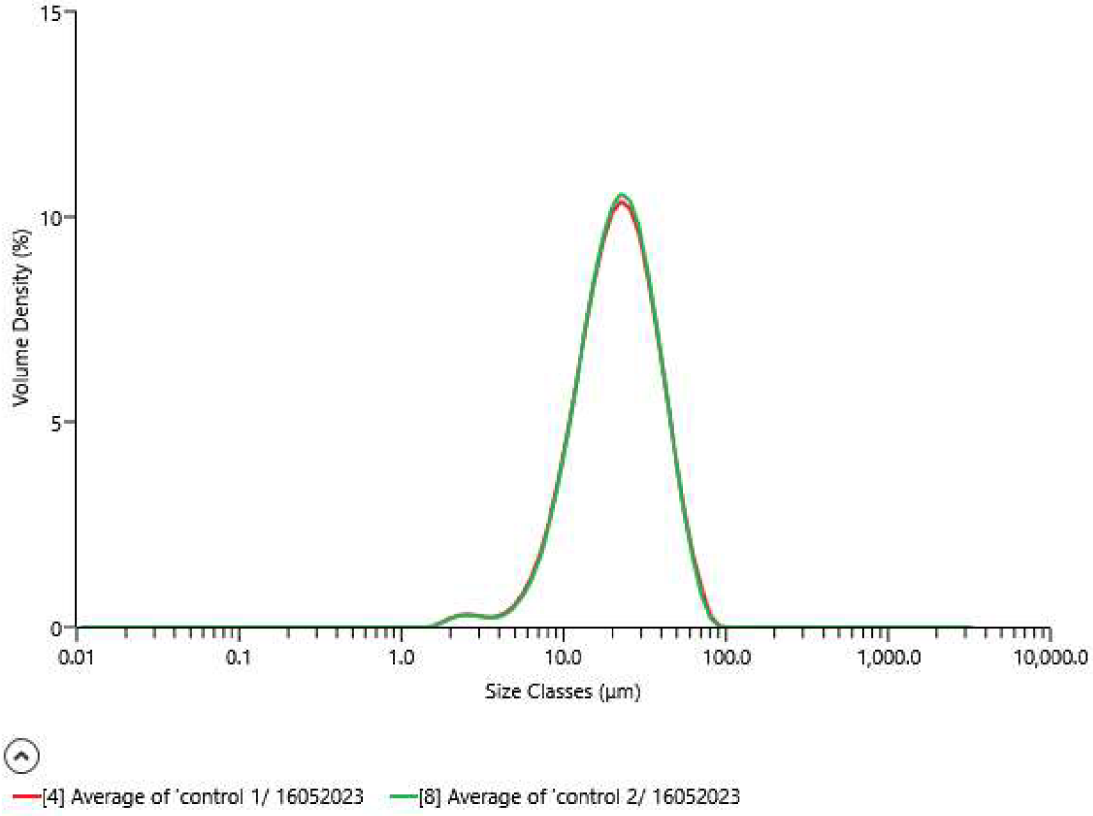
Fig.16a: *Particle-Size Distribution (PSD), at the time of preparation.* ***(2 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control2/12052023	13.7	31.4	72.4
	2	control2/12052023	13.8	30.4	71.1
	3	control2/12052023	14.1	30.2	121
	4	Average of 'control2/12052023'	13.9	30.7	73.8
	5	control1/12052023	14.6	34.9	183
	6	control1/12052023	14.5	33.5	233
	7	control1/12052023	14.6	32.8	256
	8	Average of 'control1/12052023'	14.6	33.7	228
Mean			**14.2**	**32.2**	**155**
1xStd Dev			**0.396**	**1.76**	**79.2**
1xRSD (%)			**2.78**	**5.48**	**51.2**

Table 6a*: Mastersizer parameters at preparation.*

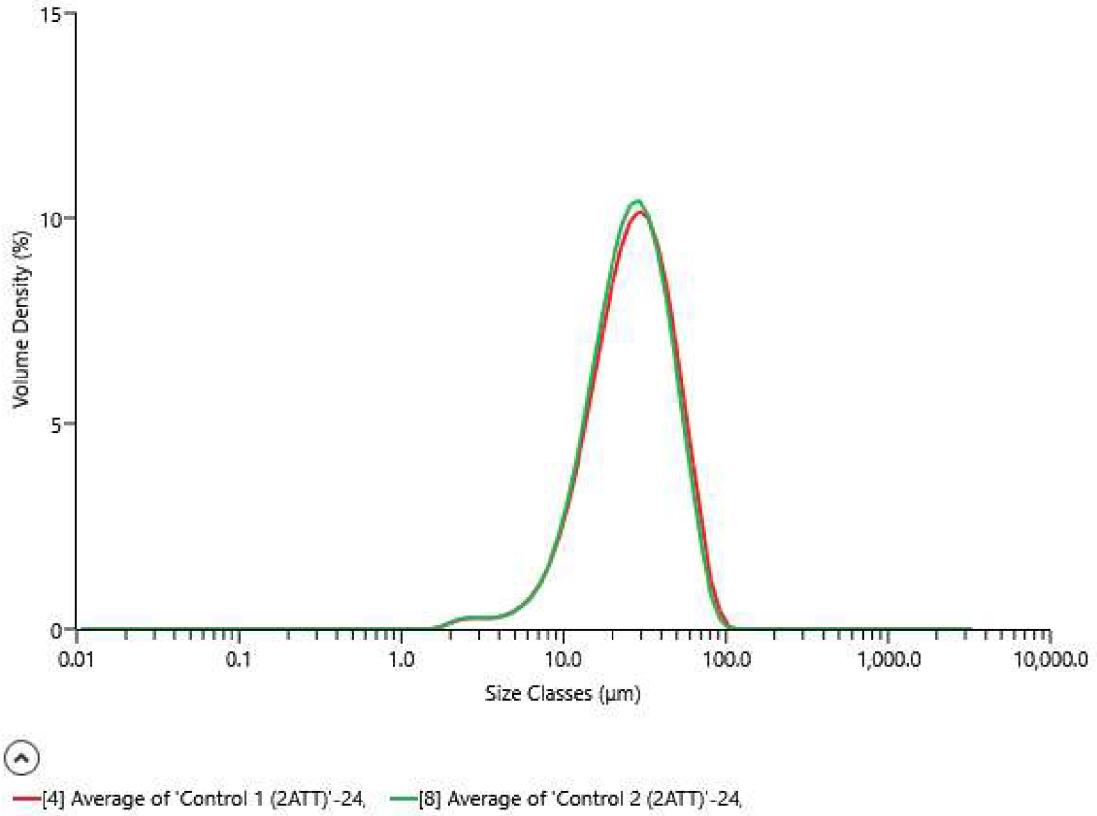
Fig.16b: *Particle-Size Distribution (PSD), aafter 7 days.* ***(2 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	control 1/ 16052023	9.89	22.4	45.4
	2	control 1/ 16052023	9.72	21.6	43.5
	3	control 1/ 16052023	9.54	21.0	41.6
	4	Average of 'control 1/ 16052023'	9.71	21.7	43.6
	5	control 2/ 16052023	10.1	22.6	45.8
	6	control 2/ 16052023	9.90	21.7	43.0
	7	control 2/ 16052023	9.66	20.9	40.3
	8	Average of 'control 2/ 16052023'	9.90	21.7	43.1
Mean			**9.81**	**21.7**	**43.3**
1xStd Dev			**0.179**	**0.615**	**1.81**
1xRSD (%)			**1.83**	**2.83**	**4.18**

Table 6b*: Mastersizer parameters after 7 days.*

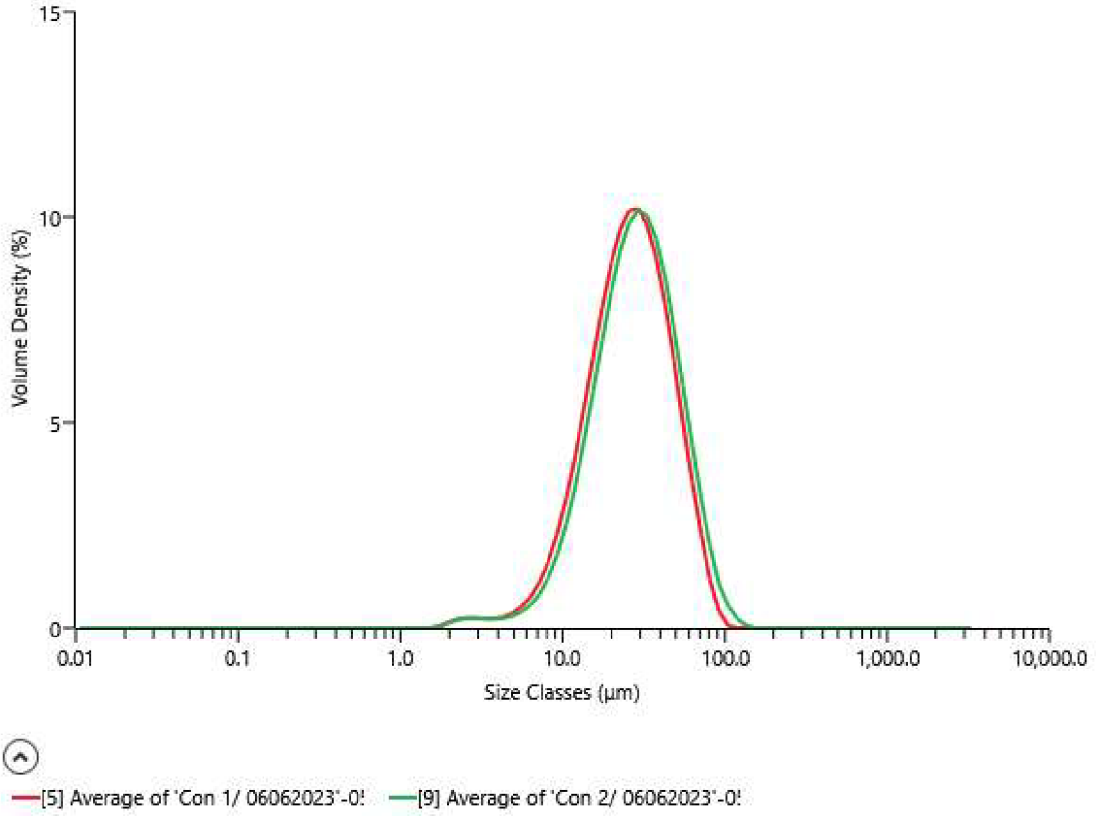
Fig.16c: *Particle-Size Distribution (PSD), aafter 14 days.* ***(2 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	Control 1 (2ATT)	12.2	29.7	60.2
	2	Control 1 (2ATT)	11.5	26.8	52.6
	3	Control 1 (2ATT)	10.9	24.9	48.3
	4	Average of 'Control 1 (2ATT)'	11.5	27.0	53.9
	5	Control 2 (2ATT)	12.1	28.4	56.9
	6	Control 2 (2ATT)	11.4	25.9	50.3
	7	Control 2 (2ATT)	10.8	24.1	45.8
	8	Average of 'Control 2 (2ATT)'	11.4	26.0	51.1
Mean			**11.5**	**26.6**	**52.4**
1xStd Dev			**0.496**	**1.82**	**4.62**
1xRSD (%)			**4.33**	**6.86**	**8.82**

Table 6c*: Mastersizer parameters after 14 days.*

Fig.16d: *Particle-Size Distribution (PSD), after 28 days.* ***(2 ATTEMPT)***



	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	2	Con 1/ 06062023	12.1	27.9	57.1
	3	Con 1/ 06062023	11.4	25.8	51.5
	4	Con 1/ 06062023	10.9	24.5	48.4
	5	Average of 'Con 1/ 06062023'	11.4	26.0	52.3
	6	Con 2/ 06062023	13.2	31.0	65.6
	7	Con 2/ 06062023	12.5	28.3	57.5
	8	Con 2/ 06062023	11.9	26.3	51.5
	9	Average of 'Con 2/ 06062023'	12.5	28.4	58.1
Mean			**12.0**	**27.3**	**55.3**
1xStd Dev			**0.750**	**2.04**	**5.44**
1xRSD (%)			**6.25**	**7.46**	**9.84**

Table 6d*: Mastersizer parameters after 28 days.*

3.4-“pH” Results

The graphs presented below illustrate that, in comparison to the control, there is a more rapid decline in pH following the addition of AP, particularly within the initial 4 hours. Furthermore, a higher concentration of AP correlates with a more pronounced pH decrease.

Among the acids identified in AP are ferulic, p-coumaroylquinic, caffeic, betulinic, ursolic, and oleanolic acids. Consequently, the observed pH reduction may be attributed to the acidic nature of AP, attributable to the presence of these natural acids (Jovanović *et al.*, 2020).

Lactic acidification, resulting from lactose fermentation by the cooperative action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* leads to a progressive reduction in pH and the acquisition of increased acidity in yogurt during storage.

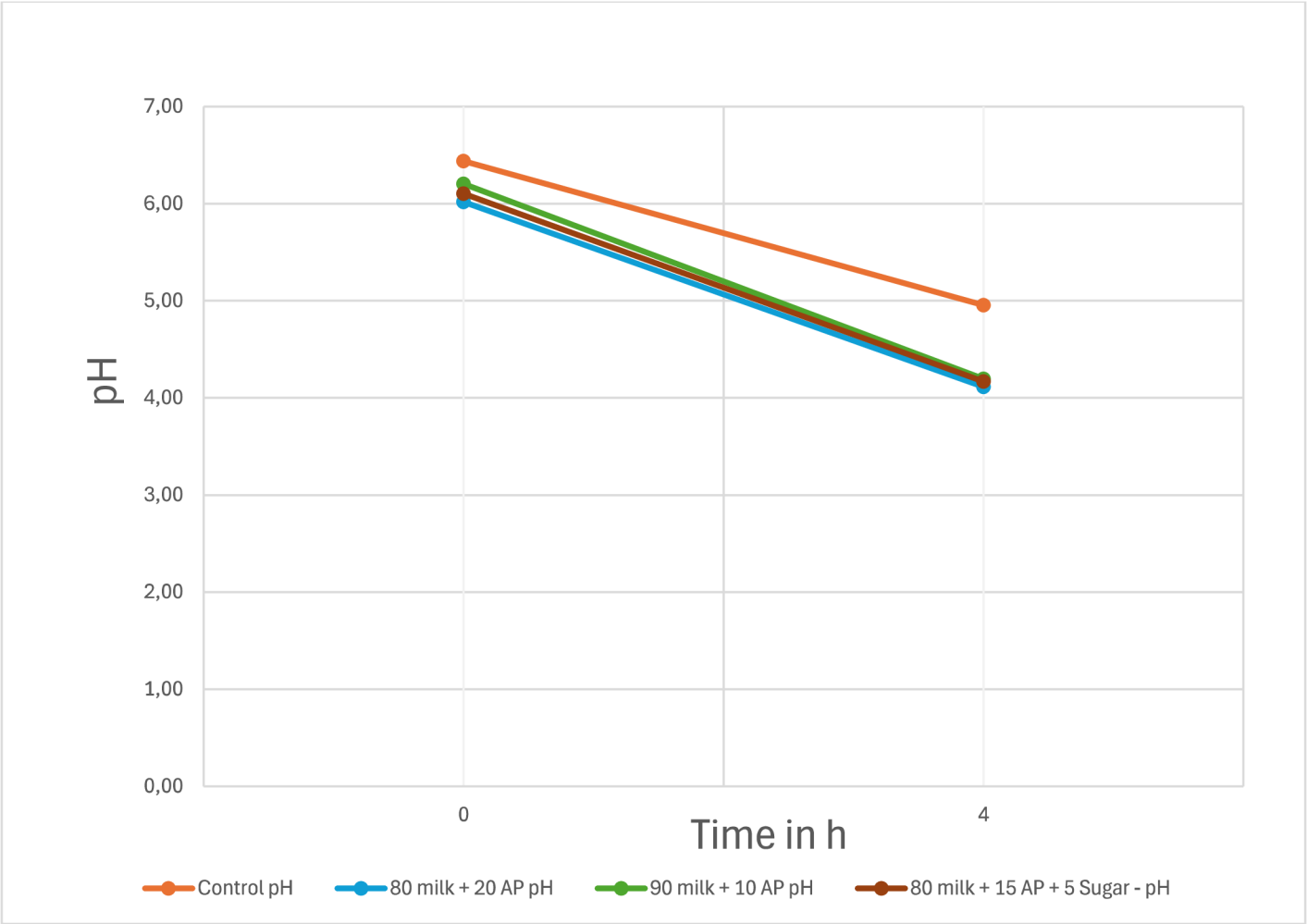
Despite these dynamics, the process proved to be successful, as evidenced by the maintenance of a consistent pH value in line with the average values observed in standard yogurt (<4.6).

FIRST ATTEMPT

Table** 7: pH Measurement **(1 ATTEMPT)

Number of samples		Freshly created Yogurts				Beginning Point
1	Control	6,45	6,27	6,14	6,04	
2	Control	6,43	6,25	6,18	6,12	
1	80g milk + 20g AP	6,02	5,91	5,94	5,87	
2	80g milk + 20g AP	6,02	5,97	5,90	5,81	
1	90g milk + 10g AP	6,19	6,2	6,10	5,94	
2	90g milk + 10g AP	6,22	6,14	6,05	5,79	
1	80g milk + 15g AP + 5g Sugar	6,11	6,05	6,07	5,90	
2	80g milk + 15g AP + 5g Sugar	6,10	6,08	5,98	5,95	
		↓	↓	↓	↓	
Number of samples		**AFTER 4 h - PREPARATION**	**AFTER 7 DAY**	**AFTER 14 DAY**	**AFTER 28 DAY**	**After storage**
1	Control	4,23	4,32	4,33	4,29	
2	Control	4,20	4,30	4,34	4,30	
1	80g milk + 20g AP	4,12	4,18	4,16	4,14	
2	80g milk + 20g AP	4,11	4,18	4,16	4,18	
1	90g milk + 10g AP	4,18	4,25	4,25	4,27	
2	90g milk + 10g AP	4,21	4,25	4,23	4,25	
1	80g milk + 15g AP + 5g Sugar	4,13	4,22	4,20	4,21	
2	80g milk + 15g AP + 5g Sugar	4,21	4,23	4,33	4,22	

Fig.17: *Average pH for each type of yogurt freshly prepared and after 4 hours* ***(1 ATTEMPT)***

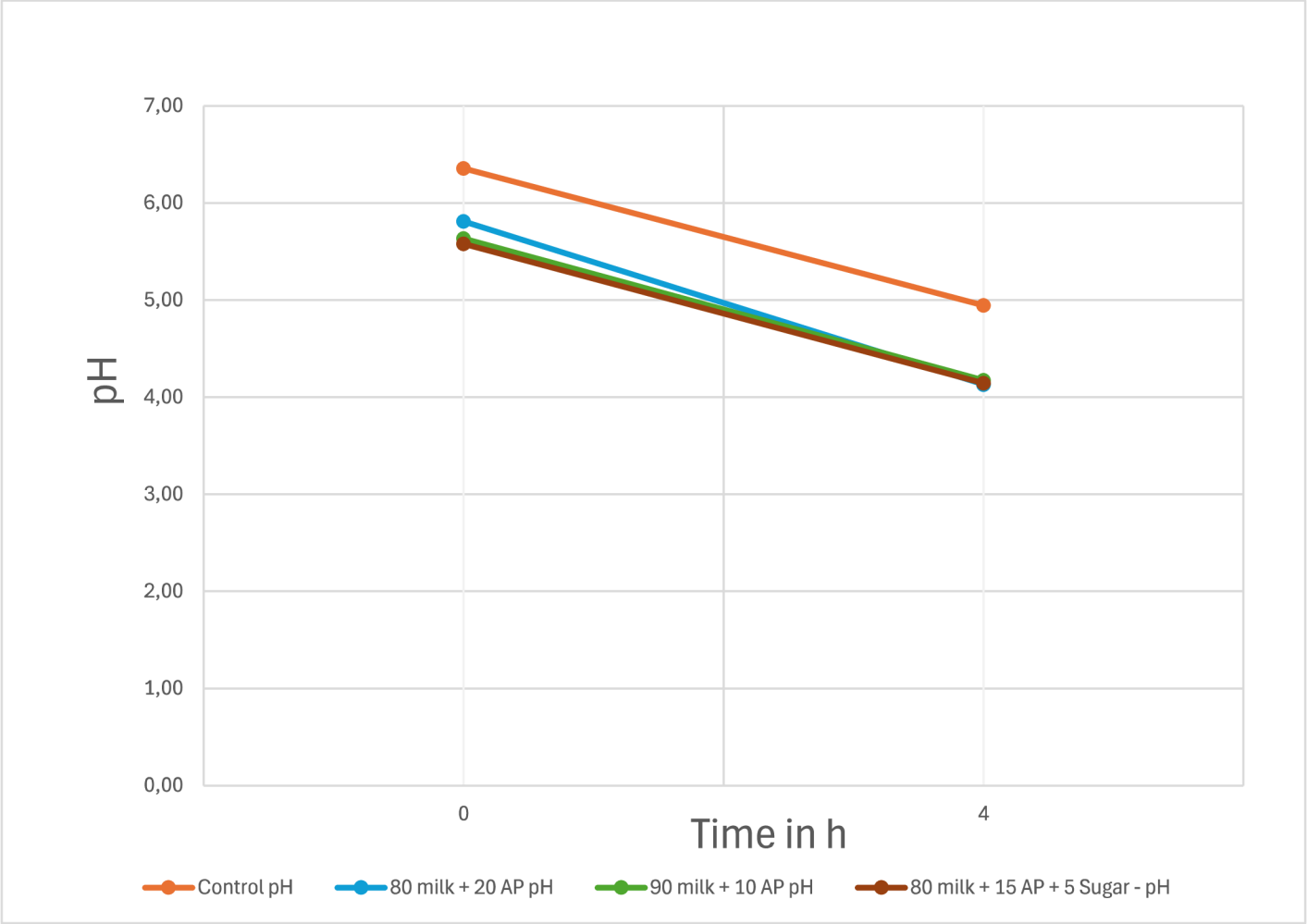


SECOND ATTEMPT

Table 8**: pH Measurement **(2 ATTEMPT)

Number of samples		Freshly created Yogurts				Beginning Point
1	Control	6,43	6,19	6,04	6,06	
2	Control	6,28	6,25	6,16	6,15	
1	80g milk + 20g AP	5,74	5,68	5,75	5,81	
2	80g milk + 20g AP	5,88	5,66	5,76	5,75	
1	90g milk + 10g AP	5,63	5,65	5,61	5,59	
2	90g milk + 10g AP	5,64	5,66	5,62	5,58	
1	80g milk + 15g AP + 5g Sugar	5,60	5,44	5,36	5,47	
2	80g milk + 15g AP + 5g Sugar	5,56	5,49	5,47	5,30	
		↓	↓	↓	↓	
Number of samples		**AFTER 4 h - PREPARATION**	**AFTER 7 DAY**	**AFTER 14 DAY**	**AFTER 28 DAY**	**After storage**
1	Control	4,29	4,26	4,30	4,24	
2	Control	4,19	4,28	4,29	4,27	
1	80g milk + 20g AP	4,11	4,12	4,19	4,15	
2	80g milk + 20g AP	4,15	4,14	4,16	4,16	
1	90g milk + 10g AP	4,17	4,19	4,21	4,21	
2	90g milk + 10g AP	4,18	4,20	4,21	4,20	
1	80g milk + 15g AP + 5g Sugar	4,13	4,15	4,16	4,17	
2	80g milk + 15g AP + 5g Sugar	4,16	4,15	4,17	4,16	

Fig.18: *Average pH for each type of yogurt freshly prepared and after 4 hours* ***(2 ATTEMPT)***



3.5-“Total Phenolic Content” Results

Following the measurement of the Total Phenolic Content (TPC), it was determined to be 1911,86 µg GAE/g sample, equivalent to 1,912 mg GAE/g sample (freeze-dried apple pomace).

The quantity of detectable phenols largely depends on the extraction method, such as: Ultrasound-Assisted Extraction (UAE), Ultraturrax Extraction (UTE), Microwave-assisted extraction (MAE) and others, as well as the variety utilized, which undoubtedly influences the final amount.

In the scope of our experimental methodology, an organic alcohol solvent, specifically methanol, was utilized for the extraction process.

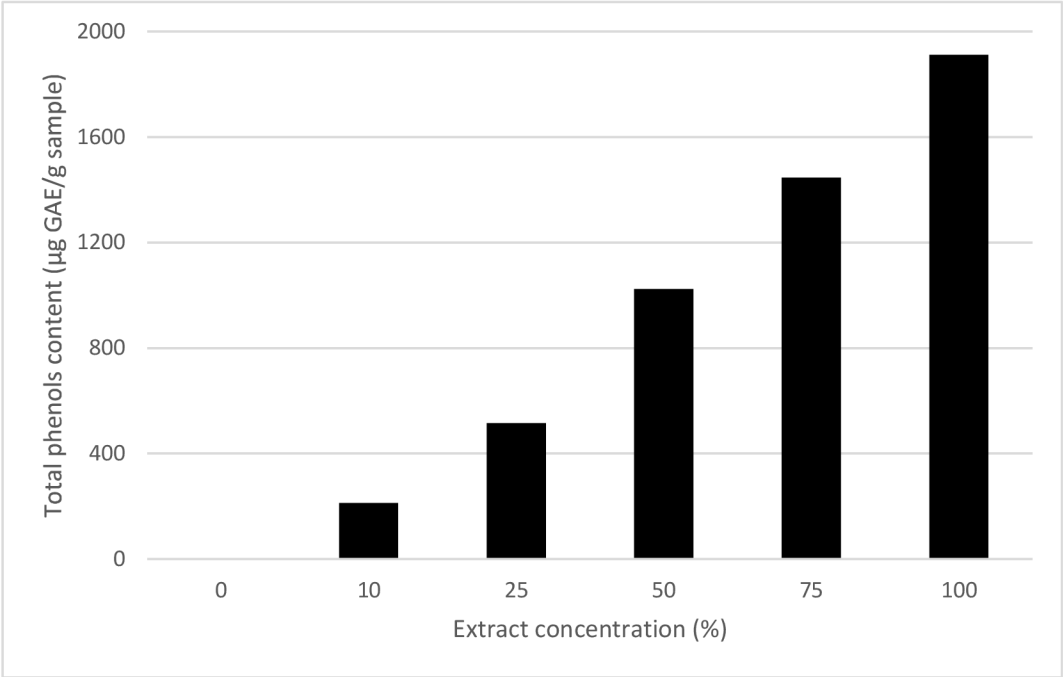


Fig.19: *Phenol quantity based on extraction percentage (µg/kg).*

3.6- Feedbacks derived from sensory analysis

The initial sample subjected to sensory analysis comprised the control, augmented with freeze-dried apples. Generally, this iteration exhibited minimal syneresis and bore the appearance of typical yogurt, garnering moderate ratings from the panel. The aroma of milk was pronounced, as expected, albeit well-received. Notably, the addition of freeze-dried apple pieces was positively acknowledged by the panel.

Yogurt formulations containing 80 g of milk with 20 g of AP, and 90 g of milk with 10 g of AP, were met with overall approval. However, one sample with 80 g of milk and 20 g of AP exhibited pronounced liquidity and substantial syneresis, indicative of potential storage or preparation irregularities.

In contrast to the aforementioned, where the apple flavour intensity was deemed optimal, formulations with 90 g of milk and 10 g of AP fell short in perceived apple flavour. Lastly, evaluations were conducted for the samples to which 5 g of sugar had been added.This addition was noted for imparting a notably sweet taste profile, further enhancing the aromatic presence of the apples. The intensity of both the sweetness and the apple aroma was deemed exceptional.

Overall, the yogurt samples exhibited commendable consistency and minimal syneresis, aligning with expectations following the incorporation of AP. Among the variants, the formulation comprising 80 g of milk with 15 g of AP and 5 g of sugar emerged as the preferred option, owing to its heightened sweetness profile, which elicited greater appreciation.

However, it is essential to underscore that the sensory evaluation was conducted by a non-specialized panel, thus precluding the data's applicability for formal statistical analysis. Nonetheless, these observations provide valuable insights into the preferences of the general populace, offering a foundational understanding of predominant taste preferences.

Chap. 4 – CONCLUSIONS

Apple pomace, a significant by-product generated in large quantities by the food industry, represents an untapped resource with diverse potential applications. Aside from its conventional use as a biofuel feedstock, it offers promising prospects as a functional ingredient within the food industry. Extensive scientific research has elucidated the beneficial effects of the phenolic compounds inherent in apple pomace on human health. When incorporated into yogurt formulations, these compounds synergistically augment the already commendable attributes of yogurt, presenting an enticing avenue for product innovation.

Thanks to the presence of phenolic compounds beneficial to the human body, as well as the fact that apple pomace enhances yogurt viscosity while maintaining comparable hardness to that of conventional yogurt, we can conclude, based on our results, that the development of a yogurt incorporating apple pomace is a feasible endeavor. However, the transition to large-scale production necessitates attention to process standardization. This may entail the incorporation of preservatives or stabilizers to mitigate the risk of microbial contamination, given the high sugar content of apples, which can serve as a substrate for microbial growth;

However, it is important to note that the natural acidic properties of apples, coupled with the fermentation of lactose by lactic acid bacteria, create an environment unfavorable to the proliferation of certain pathogens ($pH<4.6$), although their effectiveness against molds may be limited. Furthermore, sensory evaluation studies, albeit conducted on a modest scale, have demonstrated that the addition of apple pomace enhances the overall palatability of yogurt, suggesting the potential for market competitiveness with minimal sugar supplementation.

In light of these findings, the prospect of industrial-scale promotion of apple pomace-enriched yogurt is promising. However, realizing this potential requires further research efforts and meticulous formulation optimization to ensure product quality and consumer acceptance on a broader scale. Thus, while our study lays the groundwork for future exploration, continued investigation and rigorous evaluation are imperative for the successful development and commercialization of this innovative product.

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