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The Effect of Alpha-Amylase Types and Time of Enzyme Activation Towards the Sensory and Physicochemical Properties of Oat Milk

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HIGHLIGHTS

- Investigated effects of alpha-amylase type on oat milk's sensory attributes
- Explored enzyme activation timing on physicochemical oat milk properties
- Findings reveal enzyme type significantly influences oat milk viscosity
- Optimal enzyme timing enhances sweetness and consumer acceptability
- Study provides insights to improve oat milk production efficiency

ABSTRACT

Plant-based or non-dairy milk substitutes are becoming favored in the growing field of functional beverages driven by factors such as health benefits, allergies, lactose intolerance, and the rise in vegan diets. However, there are challenges to producing oat milk, such as achieving the desirable texture and taste. This study is aimed to evaluate the impact of alpha amylase and incubation time to the physicochemical and sensorial properties of oat milk. Oat milk making involves mixing of water, oat flour, and oil followed by filtration. Different types of amylase (1 and 2) are added with different incubation time (20 and 30 minutes). This research investigates the physicochemical characteristics and sensory qualities of enzymatic oat milk, encompassing pH, brix, stability, viscosity, 5-point hedonic test, and rank test. Moreover, it was found that there are significant differences in all of the attributes including pH, brix, viscosity, and stability with the p-value of less than 0.001. It was also found that the time of enzymatic activation had no impact towards the physicochemical and sensorial properties of oat milk. Lastly, sensorial properties of the oat milk prepared with amylase enzymes scored higher in all attributes compared to the negative control.

INTRODUCTION

In the field of newly developed food products for functional and specialty beverages, plant-based or non-dairy milk alternatives are expanding quickly globally. Plant-based milks now make about 16% of total milk sales. With a compound annual growth rate (CAGR) of 8.8%, the market for plant-based milks is currently valued at $13.24 billion globally and projected to grow to $30.79 billion by 2031. The expansion is driven by factors such as increasing self-diagnosis of lactose intolerance, which doubled from 2014 to 2016 in the US and Europe (8.7% CAGR); a growing trend towards veganism (9% global CAGR); and a growing demand for sustainable products (6% global CAGR) (Abrieux et al., 2022). Currently, customers prefer cow milk substitutes due to lactose intolerance, allergies to cow milk, hypercholesterolemia, calorie concerns, and a growing demand for vegan diets (Sethi et al., 2016).

Oat, a substitute to cow milk, is thought to provide several health benefits, including anti-cancer and hypocholesterolemic qualities. Moreover, oats have also been found to be a suitable addition to a celiac patient's diet (Rasane et al., 2015), as oat product consumption has been linked to lower blood cholesterol, a reduced risk of cardiovascular disease (CVD) and a decreased risk of diabetes, cancer, obesity, hypertension, and gastrointestinal issues. Oats have bioactive components such as β-glucan and other compounds that may have health benefits. Avenanthramides, or AVAs, are a special type of antioxidant found in oats that aid in preventing free radicals from causing harm to low-density lipoprotein (LDL) cholesterol (Alemayehu, 2023).The production and development of oat milk poses challenges relating to sensory and physiochemical qualities. Milk accepted by consumers must have an appealing flavor and texture similar to those of traditional dairy products. Starch, the primary ingredient in oats, affects the liquid’s viscosity and creates a sticky texture when heated through processing (Yu et al., 2023). The ability to maintain the intrinsic qualities of suspension, such as viscosity, appearance, consistency, color, and the ability to withstand destabilization processes such as flocculation, creaming, phase separation, and sedimentation, is sometimes referred to as physical stability. Research showed that additional enzymatic treatments utilizing amyloglucosidase, amylases, and other carbohydrate-degrading enzymes can enhance the stability and carbohydrate recovery of non-dairy milk substitutes (Dhankhar & Kundu, 2021). Numerous parameters, including β-glucan's solubility, concentration, and molecular weight, are known to impact oat-based drink’s viscosity (Patra et al., 2022).

Enzymes are essential to overcome the challenges in oat milk development and can be used to alter the physicochemical characteristics of oats by disintegrating unwanted substances and improving the milk's overall quality (Ren et al., 2023). Research done by Yu et al. (2023) utilizes amylase enzymes in the production of oat milk, which demonstrated that by using amylase, enzymatic hydrolysis may produce more glucose and maltose, which not only keeps high-viscosity colloids from forming but also enhances the taste of oat milk. Research on the characterization of α-amylase showed that the optimal temperature and pH for amylase activity were at 70°C and pH 7.5, respectively, with an incubation period ranging from 5 to 35 minutes (Cordeiro et al., 2002; Yadav & Prakash, 2011). Oat milk's viscosity and sliminess can be reduced with enzymatic treatment which improves the mouthfeel (Tan et al., 2023). Additionally, enzymes help to break down complex carbs and produce a smoother taste profile and mitigate off-flavor concerns (Emkani, 2022). During the production of oat milk, the use of enzymes to their full potential allows for the enhancement of its sensory qualities and helps create a delicious and superior substitute for conventional dairy milk. This study aimed to examine the effect of alpha amylase enzyme and time of enzymatic activation towards the physicochemical properties of oat milk, encompassing its pH, stability, brix, viscosity and sensorial properties using the 5-point hedonic scale.

MATERIALS AND METHODS

Research design

This research has 4 treatments utilizing 2 different kinds of amylase (amylase 1 and amylase 2 with different temperature activation and enzyme activity) along with 2 different times of enzymatic activation (20 minutes and 30 minutes) to modify physicochemical properties and sensory qualities. There are 4 different samples of oat milk that were assessed namely, positive control (benchmark), negative control (sample without amylase), treatment 1 (oat milk with alpha-amylase 1), and treatment 2 (oat milk with alpha-amylase 2). The samples, not including the positive control, went through 20 and 30 minutes of enzymatic time activation as another variable of the study. The physicochemical and sensorial analysis was done which includes pH, brix, stability (pH, sedimentation, creaming index, and brix), viscosity, 5-point hedonic scale, and rank test. The samples were then analyzed statistically to determine if there are significant differences throughout the treatments **(Figure 1)**.

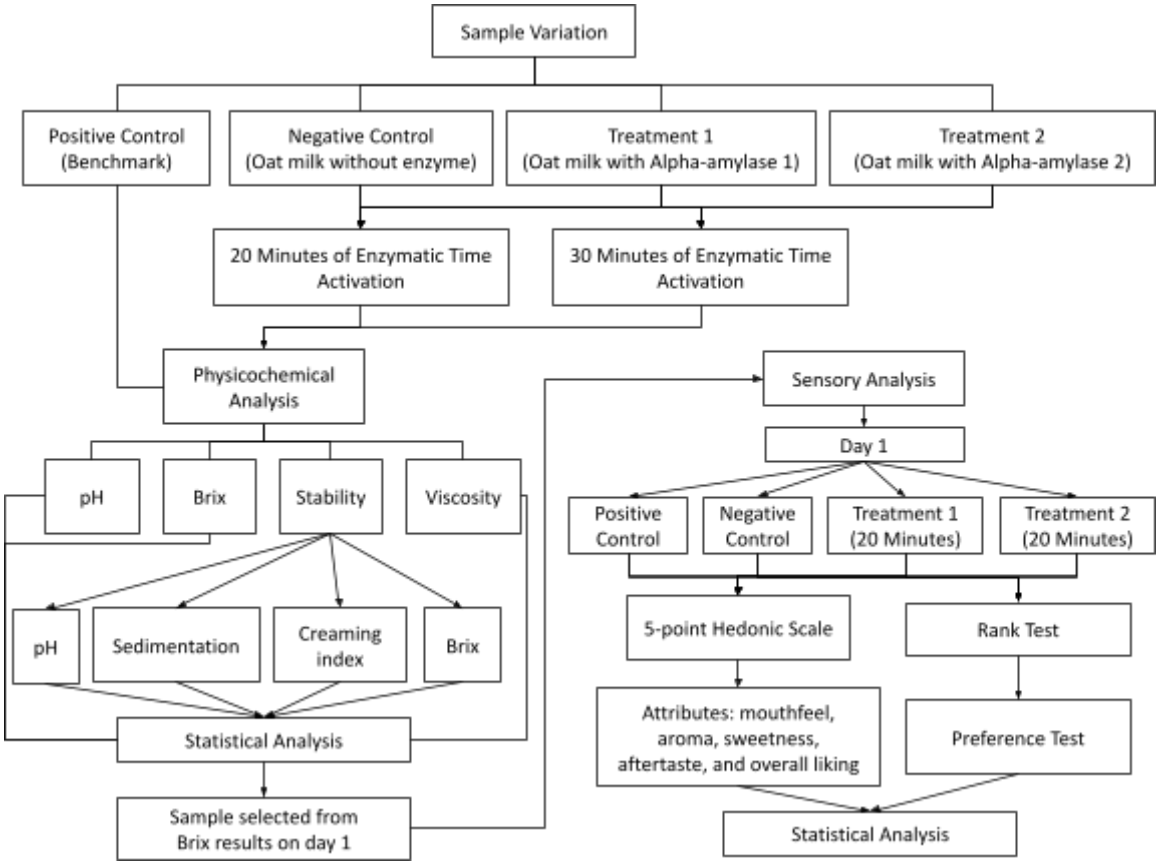

Figure 1. Study design of sample variations. Contains positive control, negative control, treatment 1 (oat milk with alpha-amylase 1) and treatment 2 (oat milk with alpha-amylase 2)

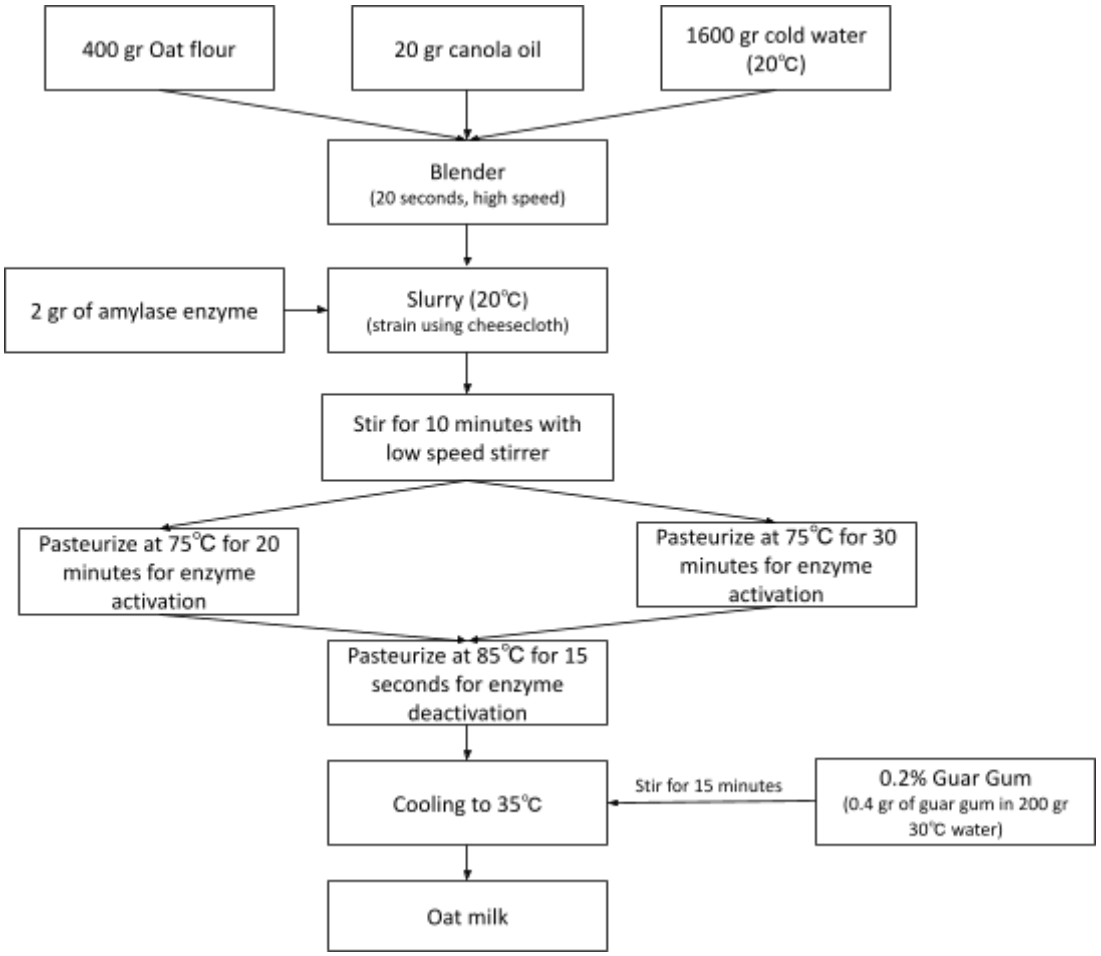

Figure 2. Oat milk preparation, which includes the processes that all the oat milk samples went through including 20 and 30 minutes of time variation

The oat milk production process entails utilizing 400 g of oat flour, 1800 g of cold water, 20 g of canola oil, 0.4 g of guar gum, and 2 g of amylase enzyme **(Figure 2)**. Equipment such as Tefal blender, Ika C-MAG HS 7 magnetic stirrer, cheesecloth, bowl, and Memmert water bath were used for a seamless procedure. Furthermore, instruments such as the Atago pocket refractometer, Brookfield manual viscometer, Hettich ROTINA 38 centrifuge, and Mettler Toledo pH meter were used for accurate physicochemical assessment. Every sample **(Figure 1)**, which includes samples from two different brands of amylase enzymes, a negative control, and a positive control which is oatside **(Table 1)**, was stored in small bottles and went through three manufacturing replications before receiving three technical replications in the physicochemical analysis.

Production of oat milk

In the experiment, 1600 g of water and 400 g of oat flour was combined. Following that, 20 g of oil was added to the oat flour and water mixture. The mixture underwent processing for 20 seconds to get a smooth consistency. The mixture was then filtered through a cheesecloth to remove any solid particles. To encourage an enzymatic reaction, the strained mixture was then treated with the addition of 2 g of amylase and mixed for ten minutes. Afterwards, the oat milk was placed in a water bath and pasteurized for 15 seconds at 85°C to guarantee that the enzyme becomes inactive. Lastly, before being served, 0.4 g of guar gum in 200 g of water was added and stirred for 15 minutes.

Table 1. Oat milk ingredients

Ingredients*	Amount (grams)		
	Negative Control	Treatment 1	Treatment 2
Oat Flour	400	400	400
Cold Water	1800	1800	1800
Canola Oil	20	20	20
Guar Gum	0.4	0.4	0.4
Alpha-amylase 1	-	2	-
Alpha-amylase 2	-	-	2

*The ingredients shown above state the ingredients for the production of oat milk for the samples.

Alpha-amylase 1 enzyme product specification

Amylase enzyme 1 has an enzymatic strength of 480 KNU-B/g and performs best in the pH range of 5.0 to 7.0 and the temperature range of 70°C to 80°C. It also shows excellent efficiency in the generation of sweetness and is suitable to use in food and beverage applications as it satisfies food-grade quality criteria.

Alpha-amylase 2 enzyme product specification

Amylase enzyme 2 has the enzymatic strength of 650 KNU-B/g and performs best at temperatures ranging from 70°C to 75°C, breaking down complex carbohydrates efficiently. The enzyme's optimal pH is reported at pH 5.5 to 6.0 which indicates its resilience to acidic conditions. These properties make amylase vital in a variety of industrial applications, notably food processing, where it plays an important role in starch hydrolysis and fermentation processes.

Benchmark product specification

The benchmark product has a pH of 7.38 determined by Mettler Toledo pH meter with a brix percentage of 11.77 quantified by Atago pocket refractometer. Moreover, it has a notable viscosity of 6.17 measured by the Brookfield manual viscometer. The benchmark itself has a noteworthy taste profile by being creamy, malty, and nutty.

Physicochemical analysis of oat milk

Oat milk's physical analysis involves determining several characteristics, including pH, brix, stability, and viscosity. The measurement of milk's pH provides information on the acidity or alkalinity of the liquid and the brix analysis reveals the sweetness of the liquid (Aydogdu et al., 2023; Jaywant et al., 2022). Evaluations of the milk's stability determine how long it can withstand sedimentation or separation, which is important for shelf-life estimations (Karlsson et al., 2019). Measurements of viscosity reveal the milk's thickness or flow properties, which might impact its mouthfeel and texture (Frøst et al., 2001). All of these investigations help to clarify the general quality and features of oat milk as a dairy substitute. Furthermore, the statistical analysis for the physicochemical properties of oat milk was the one-way ANOVA if the normality results are parametric and Kruskal-Wallis test if the results are non-parametric.

pH

The SevenEasy Mettler Toledo Benchtop pH meter was used to conduct pH analysis. To standardize the equipment, the pH meter was calibrated using pH buffers of 7, 4, and 10 (JoVE, 2024). The pH analysis was done with 30 mL of oat milk placed in shot cups and measured by a probe from the SevenEasy Mettler Toledo pH meter kit (National Institute of Standards and Technology, 2023). It is done in triplicates to ensure accuracy and reliability. This method of pH analysis makes it easier to draw strong conclusions about how acidic or alkaline the materials being studied are.

Brix

The Atago Pocket Refractometer, an instrument for determining a solution's sugar concentration, was used to perform brix analysis (Global Instruments, 2024). To achieve precise readings, the refractometer was calibrated using an aquadest solution before measurements. The purpose of the calibration phase was to reduce possible errors and standardize the equipment. Brix analysis was measured using 5 mL of oat milk pipetted to the refractometer reader and the start button was pressed to get the analytical readings. Measurements were carried out in triplicates to ensure the accuracy of the data and provide a thorough assessment of the sugar concentration in the samples.

Stability

Determining the milk's capacity to withstand phase separation over time requires stability testing. The oat milk sample's proportion of creaming or sedimentation is measured using the creaming index formula. The creaming index formula is $Cl\% = (Hs/Ht)\, x\, 100\%$, where the overall height of the emulsions is equal to the height of the serum layer, which is the total of the transparent or turbid layers at the container's bases. With this procedure, the height of the cream layer that forms at the top of the sample container is usually measured and expressed as a percentage of the sample height (Zhang et al., 2020). Oat milk stability testing included storing 50 mL of oat milk per sample in the fridge at 18°C-22°C for 5 days in small bottles. The creaming index and sedimentation data of the oat milk was centrifuged and calculated on day 1, day 2, and day 5 along with the pH and brix. Stability testing is done in triplicates to ensure the dependability of the results. Oat milk manufacturers can assess the stability by using this method to guarantee quality and extending shelf life (Lal et al., 2006).

Viscosity

The Brookfield Manual Viscometer is utilized in the physicochemical analysis. An understanding of the fluid's flow characteristics, texture, and mouthfeel is important while developing oat milk. The viscosity of the oat milk is measured manually using a viscometer to ensure product consistency and optimize processing conditions (Yang & Lin, 2021). Then, to guarantee accuracy and to facilitate statistical analysis of the data, 300 mL of oat milk is measured in triplicates using spindle 1 and at a speed of 60 from the Brookfield Manual Viscometer kit. Oat milk producers can enhance consumer satisfaction and product appeal by customizing processing parameters to obtain desired texture and quality features by methodically evaluating viscosity.

Sensorial analysis of oat milk

Oat milk is subjected to sensory evaluation, which includes assessing its flavor, aroma, texture, and appearance. To obtain subjective input from panelists, techniques such as ranking tests and the 5-point hedonic scale are used in this assessment. Panelists can score several aspects of the oat milk on a 5-point

hedonic scale that ranges from "dislike very much" to "like very much," making it possible to quantify the total acceptance (Alsado et al., 2023).

Furthermore, panelists in ranking tests, which are usually examined using the Friedman test, arrange samples of oat milk according to characteristics like flavor or texture. This helps reveal relative preferences and pinpoint ideal formulas (Ramachandran & Tsokos, 2021). Oat milk manufacturers can understand consumer preferences, improve product formulations, and increase overall product acceptance in the market by using these sensory analysis methods.

Hedonic test

A sensory evaluation method named the hedonic test determines how consumers perceive oat milk samples and what they prefer. Thirty untrained panelists, aged 25 to 45, participated in the hedonic test and rank test, as the age group represents the target market for oat milk. To minimize sample order bias, panelists are given four oat milk samples separately throughout the test, also known as a monadic presentation, with water as the palate cleanser. Each sample is evaluated by the panelists according to five criteria which are mouthfeel, aroma, aftertaste, sweetness, and overall liking (Su et al., 2022). Panelists are obliged to justify their assessment for the overall liking attribute, which allows for a more thorough understanding of their preferences. Statistical methods using JASP such as the Kruskal-Wallis test for non-parametric results and ANOVA test for the parametric results are used to evaluate the hedonic test data.

Rank test

A rank test is used after the hedonic test, in which participants assess samples according to preference. The samples are ranked by participants from 1 (most preferred) to 4 (least preferred). To ascertain whether there are statistically significant variations in preference between the samples, the Friedman test and Conover’s test using JASP software evaluates the data. The Friedman test is applied when the dependent variable being measured is ordinal to test for differences across groups (Laerd Statistics, 2018). This approach takes into consideration the ordinal character of the data and assists in determining whether preferences are constant amongst the samples (Carabante, 2017).

Statistical analysis

The study uses JASP software to conduct the statistical analysis. Initial normality assessment for sensory and physicochemical analysis, such as pH, brix, and viscosity, were performed with Shapiro-Wilk tests. Based on normality results, Kruskal-Wallis (non-parametric) or one-way ANOVA (parametric) to determine the significance followed by Dunn's or Tukey's test for post-hoc. Stability tests among samples work similarly, starting with the same sample comparison procedures. Additional analyses are performed to look for variations between days. Shapiro-Wilk, Friedman Test (parametric), or Repeated Measures ANOVA (non-parametric) are used. The Conover’s or Bonferroni tests are used for post-hoc evaluation, respectively.

RESULTS

Physicochemical analysis

As part of the physicochemical analysis, measurements of the pH and brix levels as well as assessments of viscosity and stability, including creaming and sedimentation tests, were conducted.

Table 2. Physicochemical analysis

Samples	Physiochemical Analysis*		
	pH	Brix	Viscosity
Positive Control (benchmark)	7.47 ± 0.075[a]	13.91 ± 1.911[a]	6.17 ± 0.167[b]
Negative Control, 20 Minutes	6.19 ± 0.213[b]	2.94 ± 0.76[b]	42277.78 ± 26731.35[a]
Negative Control, 30 Minutes	6.31 ± 0.065[b]	1.46 ± 0.601[b]	12944.44 ± 6214.53[a]
Amylase 1, 20 Minutes	6.44 ± 0.0925[a]	12.33 ± 0.751[a]	6.89 ± 0.509[b]
Amylase 1, 30 Minutes	6.44 ± 0.049[a]	12.1 ± 0.12[a]	6.72 ± 0.096[b]
Amylase 2, 20 Minutes	6.36 ± 0.055[b]	12.57 ± 0.115[a]	6.89 ± 0.192[b]
Amylase 2, 30 Minutes	6.32 ± 0.022[b]	12.3 ± 0.581[a]	6.78 ± 0.255[b]
p-value	<.001	<.001	<.001

*Physicochemical properties (pH, total soluble solids in °brix, and viscosity) of control (positive and negative) and enzyme-treated samples measured after 20 and 30 minutes of incubation. Values represent mean ± standard deviation from three independent batches with technical replicates. Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to the Kruskal–Wallis test followed by Dunn's post-hoc analysis.

Based on the results, it was observed that the positive control (benchmark) had the highest pH value with the score of 7.47 ± 0.075 and negative control, 20 minutes had the lowest pH value with the score of 6.19 ± 0.213. Moreover, it was discovered that there are no significant differences ($p>0.05$) found between the positive control (7.47 ± 0.075); amylase 1, 20 minutes (6.44 ± 0.0925); and amylase 1, 30 minutes (6.44 ± 0.049). The samples that were observed to have significant differences ($p<0.05$) with the positive control are amylase 2, 20 minutes (6.36 ± 0.055); amylase 2, 30 minutes (6.32 ± 0.022); negative control, 30 minutes (6.31 ± 0.065); and negative control, 20 minutes (6.19 ± 0.213).

The brix levels were also analyzed and concluded in **Table 2** as a part of the physicochemical testing. From the results, it was concluded that there are no significant differences ($p>0.05$) between the positive control (13.91 ± 1.911); amylase 2, 20 minutes (12.57 ± 0.115); amylase 1, 20 minutes (12.33 ± 0.751); amylase 2, 30 minutes (12.3 ± 0.581); and amylase 1, 30 minutes (12.1 ± 0.12). On the contrary, the negative control, 20 minutes (2.94 ± 0.76); and negative control, 30 minutes (1.46 ± 0.601) have been found to have significant differences ($p<0.05$) with all the other samples besides one another.

The viscosity test was then analyzed and gathered in **Table 2** as a part of the physicochemical analysis. Following the discovery that the normality test was not normally distributed within the samples, a statistical analysis was carried out using the post-hoc Kruskal-Wallis and Dunn tests. The results stated that there are no significant differences ($p>0.05$) between the positive control (6.17 ± 0.167); amylase 2, 20 minutes (6.89 ± 0.192); amylase 1, 20 minutes (6.89 ± 0.509); amylase 2, 30 minutes (6.78 ± 0.255); and amylase 1, 30 minutes (6.72 ± 0.096). On the other hand, the negative control, 20 minutes (42277.78 ±

26731.35); and negative control, 30 minutes (12944.44 ± 6214.53) have been found to have significant differences ($p<0.05$) with all the other samples apart from one another.

Stability analysis

In order to do the stability analysis, a number of variables were looked at, including pH values, brix levels, creaming index, and sedimentation behaviors. Collectively, these factors offered information about the sample's potential to hold onto its structure and composition throughout time, guaranteeing its overall performance and quality remain steady.

pH stability analysis

The pH stability analysis comprised of samples positive control, negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes).

Table 3. pH stability analysis

Samples***	pH Stability Analysis*		
	Day 1**	**Day 2****	**Day 3****
Positive Control (benchmark)	7.47 ± 0.075^{bx}	7.23 ± 0.61^{bx}	7.58 ± 0.045^{ax}
Negative Control, 20 Minutes	6.19 ± 0.213^{by}	6.46 ± 0.026^{ay}	6.49 ± 0.035^{ay}
Negative Control, 30 Minutes	6.31 ± 0.065^{by}	6.46 ± 0.037^{ay}	6.48 ± 0.054^{ay}
Amylase 1, 20 Minutes	6.44 ± 0.0925^{bx}	6.55 ± 0.003^{ax}	6.53 ± 0.091^{ax}
Amylase 1, 30 Minutes	6.44 ± 0.049^{bx}	6.59 ± 0.056^{ax}	6.66 ± 0.113^{ax}
Amylase 2, 20 Minutes	6.36 ± 0.055^{by}	6.45 ± 0.022^{ay}	6.45 ± 0.037^{ay}
Amylase 2, 30 Minutes	6.32 ± 0.022^{by}	6.45 ± 0.031^{ay}	6.44 ± 0.023^{ay}

*The results shown above is the mean ± standard deviation of pH taken from technical replicates of 3 batches with different letters a, b, c in the same row, and x, y, z in the same column to denote statistical significance ($p<0.05$).

**Samples comparison on day 1, 2, and 5 (column) uses Shapiro-Wilk as a normality test followed by Kruskal-Wallis test and Dunn test as a post-hoc test.

***Positive control, negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), and amylase 2 (30 minutes) uses Shapiro-Wilk as a normality test followed by Friedman test and Conover test as a post-hoc test (row). Amylase 1 (30 minutes) and amylase 2 (20 minutes) uses Shapiro-Wilk as a normality test, followed by repeated measures ANOVA, and Bonferroni test as a post-hoc test.

Based on the results, it was found there was a slight increase on samples amylase 1 (20 minutes), amylase 2 (20 minutes), negative control (20 minutes), positive control, amylase 1 (30 minutes), amylase 2 (30 minutes), negative control (30 minutes) in the pH stability analysis. Following statistical analysis done **(Table 3)**, it can be concluded that there was an instability or significant differences ($p<0.05$) in the pH of all the samples mainly on day 1 towards both day 2 and day 5.

On day 1, the samples that did not have significant differences ($p>0.05$) with the positive control (7.47 ± 0.075) were amylase 1, 20 minutes (6.44 ± 0.0925) and amylase 1, 30 minutes (6.44 ± 0.049). Moreover, the samples that had significant differences ($p<0.05$) with the positive control (7.47 ± 0.075) were negative control, 20 minutes (6.19 ± 0.213); negative control, 30 minutes (6.31 ± 0.065); amylase 2, 20 minutes (6.36 ± 0.055); and amylase 2, 30 minutes (6.32 ± 0.022).

On day 2, the samples that did not have significant differences ($p>0.05$) with the positive control (7.23 ± 0.61) were amylase 1, 20 minutes (6.55 ± 0.003) and amylase 1, 30 minutes (6.59 ± 0.056). The samples that had significant differences ($p<0.05$) with the positive control (7.23 ± 0.61) were negative control, 20 minutes (6.46 ± 0.026); negative control, 30 minutes (6.46 ± 0.037); amylase 2, 20 minutes (6.45 ± 0.022); and amylase 2, 30 minutes (6.45 ± 0.031).

On day 5, the samples that did not have significant differences ($p>0.05$) with the positive control (7.58 ± 0.045) were amylase 1, 20 minutes (6.53 ± 0.091) and amylase 1, 30 minutes (6.66 ± 0.113). Lastly, the samples that had significant differences ($p<0.05$) with the positive control (7.58 ± 0.045) were negative control, 20 minutes (6.49 ± 0.035); negative control, 30 minutes (6.48 ± 0.054); amylase 2, 20 minutes (6.45 ± 0.037); and amylase 2, 30 minutes (6.44 ± 0.023).

Brix stability analysis

The brix stability analysis includes positive control, negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes).

Table 4. Stability analysis brix

Samples***	Brix Stability Analysis*		
	Day 1**	Day 2**	Day 3**
Positive Control (benchmark)	13.91 ± 1.911^{ax}	13.43 ± 1.48^{ax}	13.11 ± 0.54^{ax}
Negative Control, 20 Minutes	2.94 ± 0.76^{ay}	3.67 ± 2.49^{ay}	1.67 ± 0.55^{ay}
Negative Control, 30 Minutes	1.46 ± 0.601^{by}	1.99 ± 1.31^{by}	2.52 ± 1.35^{ay}
Amylase 1, 20 Minutes	12.33 ± 0.751^{ax}	12.42 ± 0.501^{ax}	12.42 ± 0.7^{ax}
Amylase 1, 30 Minutes	12.1 ± 0.12^{bx}	12.69 ± 0.038^{ax}	12.46 ± 0.37^{ax}
Amylase 2, 20 Minutes	12.57 ± 0.115^{bx}	12.97 ± 0.233^{ax}	12.78 ± 0.157^{ax}
Amylase 2, 30 Minutes	12.3 ± 0.581^{bx}	12.5 ± 0.61^{ax}	12.78 ± 0.157^{abx}

*The results shown above is the mean ± standard deviation of brix taken from technical replicates of three batches with different letters a, b, c in the same row, and x, y, z in the same column to denote statistical significance ($p<0.05$).

**Samples comparison on day 1, 2, and 5 (column) uses Shapiro-Wilk as a normality test followed by Kruskal-Wallis test and Dunn test as a post-hoc test.

***Negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes) uses Shapiro-Wilk as a normality test

followed by Friedman test and Conover test as a post-hoc test (row). Positive control uses Shapiro-Wilk as a normality test, followed by repeated measures ANOVA, and Bonferroni test as a post-hoc test.

From the results **(Table 4)**, it was discovered that there are several samples that had instability in brix which had significant differences throughout the days mainly on day 1 with day 2 and 5. The samples that had instability were negative control (30 minutes), amylase 1 30 (minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes).

On day 1, the samples that did not have significant differences ($p>0.05$) with the positive control (13.91 ± 1.911) were amylase 1, 20 minutes (12.33 ± 0.751); amylase 1, 30 minutes (12.1 ± 0.12); amylase 2, 20 minutes (12.57 ± 0.115); and amylase 2, 30 minutes (12.3 ± 0.581). On the other hand, the samples that had significant differences ($p<0.05$) with the positive control (13.91 ± 1.911) were negative control, 20 minutes (2.94 ± 0.76) and negative control, 30 minutes (1.46 ± 0.601).

On day 2, the samples that did not have significant differences ($p>0.05$) with the positive control (13.43 ± 1.48) were amylase 1, 20 minutes (12.42 ± 0.501); amylase 1, 30 minutes (12.69 ± 0.038); amylase 2, 20 minutes (12.97 ± 0.233); and amylase 2, 30 minutes (12.5 ± 0.61). Moreover, the samples that had significant differences ($p<0.05$) with the positive control (13.43 ± 1.48) were negative control, 20 minutes (3.67 ± 2.49) and negative control, 30 minutes (1.99 ± 1.31).

On day 5, the samples that did not have significant differences ($p>0.05$) with the positive control (13.11 ± 0.54) were amylase 1, 20 minutes (12.42 ± 0.7); amylase 1, 30 minutes (12.46 ± 0.37); amylase 2, 20 minutes (12.78 ± 0.157); and amylase 2, 30 minutes (12.78 ± 0.157). Lastly, the samples that had significant differences ($p<0.05$) with the positive control (13.11 ± 0.54) were negative control, 20 minutes (1.67 ± 0.55) and negative control, 30 minutes (2.52 ± 1.35).

Creaming stability analysis

The creaming stability analysis encompasses positive control, negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes).

Table 5. Stability analysis creaming index

Samples***	Creaming Index Stability Analysis*		
	Day 1**	Day 2**	Day 5**
Positive Control (benchmark)	0.81 ± 0.09^{bx}	6.36 ± 4.68^{abx}	11.45 ± 4.43^{ax}
Negative Control, 20 Minutes	$_{0}ax$	$_{0}ay$	$_{0}ay$
Negative Control, 30 Minutes	$_{0}ax$	$_{0}ay$	$_{0}ay$
Amylase 1, 20 Minutes	0.93 ± 0.107^{ax}	1.51 ± 0.822^{ax}	3.63 ± 2.72^{ay}
Amylase 1, 30 Minutes	0.81 ± 0.107^{ax}	1.55 ± 0.704^{ax}	2.16 ± 0.55^{ay}
Amylase 2, 20 Minutes	1.23 ± 0.157^{bx}	1.47 ± 0.798^{abxy}	2.12 ± 0.864^{ay}
Amylase 2, 30 Minutes	1.27 ± 0.25^{bx}	1.6 ± 1.06^{abx}	2.14 ± 0.59^{ay}

*The results shown above is the mean ± standard deviation of the creaming Index taken from technical replicates of 3 batches with different letters a, b, c in the same row, and x, y, z in the same column to denote statistical significance ($p<0.05$).
**Samples comparison on day 1, 2, and 5 (column) uses Shapiro-Wilk as a normality test followed by One-way ANOVA test and Tukey's test as a post-hoc.
***Positive control, amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes) uses Shapiro-Wilk as a normality test, followed by repeated measures ANOVA, and Bonferroni test as a post-hoc (row).

According to results **(Table 5)**, there was an increase in the creaming percentage in samples amylase 1 (20 minutes), amylase 2 (20 minutes), positive control, amylase 1 (30 minutes), and amylase 2 (30 minutes). However, in negative control (20 & 30 minutes) no creaming was found. Based on the statistical analysis done, the samples are known to be relatively unstable in terms of creaming since there are several significant differences ($p<0.05$) found between the days in several samples. For instance, the positive control sample, amylase 2 (20 minutes) sample, and amylase 2 (30 minutes) sample had significant differences ($p<0.05$) throughout the days.

On day 1, all the samples had no significant differences ($p>0.05$) towards the positive control. However, on day 2, the samples that had significant differences than the positive control samples were the negative control (20 & 30 minutes) samples since they had no creaming index. Lastly, on day 5, the samples that were found to have significant differences ($p<0.05$) to the positive control (11.45 ± 4.43) were negative control, 20 minutes (0); negative control, 30 minutes (0); amylase 1, 20 minutes (3.63 ± 2.72); amylase 1, 30 minutes (2.16 ± 0.55); amylase 2, 20 minutes (2.12 ± 0.864); and amylase 2, 30 minutes (2.14 ± 0.59).

Sedimentation stability analysis

The sedimentation stability analysis incorporates positive control, negative control (20 minutes), negative control (30 minutes), amylase 1 (20 minutes), amylase 1 (30 minutes), amylase 2 (20 minutes), and amylase 2 (30 minutes).

Table 6. Stability analysis sedimentation

Samples***	Sedimentation Stability Analysis*		
	Day 1**	Day 2**	Day 5**
Positive Control (benchmark)	2.36 ± 0.24^{ax}	4.88 ± 1.14^{ay}	6.99 ± 1.45^{ay}
Negative Control, 20 Minutes	96.53 ± 1.8^{ax}	93.81 ± 6.12^{ax}	83.83 ± 12.66^{ax}
Negative Control, 30 Minutes	97.09 ± 0.027^{ax}	96.65 ± 2.9^{ax}	83.26 ± 10.66^{ax}
Amylase 1, 20 Minutes	3.56 ± 0.257^{bx}	5.79 ± 3.37^{aby}	8.15 ± 1.31^{ay}
Amylase 1, 30 Minutes	3.14 ± 0.32^{bx}	5.28 ± 3.02^{aby}	9.07 ± 0.93^{ay}
Amylase 2, 20 Minutes	8.45 ± 1.39^{ax}	9.79 ± 0.36^{axy}	10.93 ± 1.2^{ax}
Amylase 2, 30 Minutes	3.8 ± 0.06^{ax}	9.88 ± 0.27^{ax}	10.55 ± 0.39^{axy}

*The results shown above is the mean ± standard deviation of sedimentation taken from technical replicates of 3 batches with different letters a, b, c in the same row, and x, y, z in the same column to denote statistical significance ($p<0.05$).
**Samples comparison on day 1, 2, and 5 (Column) uses Shapiro-Wilk as a normality test followed by One-way ANOVA test and Tukey's test as a post-hoc test.
***Amylase 2 (30 minutes), negative (20 minutes), and negative (30 minutes) uses Shapiro-Wilk as a normality test followed by Friedman test and Conover test as a post-hoc test (row). Positive control, amylase 1 (20 minutes), amylase 1 (30 minutes), and amylase 2 (20 minutes) uses Shapiro-Wilk as a normality test, followed by repeated measures ANOVA, and Bonferroni test as a post-hoc test.

Table 6 stated that there was some sedimentation instability and significant differences on samples of amylase 1 (20 minutes) and amylase 1, (30 minutes) on days 1, 2, and 5. Moreover, on day 1, it was discovered that none of the samples had any significant differences ($p>0.05$) towards the positive control. On day 2, the samples that had no significant differences ($p>0.05$) with the positive control (4.88 ± 1.14) were amylase 1, 20 minutes (5.79 ± 3.37); amylase 1, 30 minutes (5.28 ± 3.02); and amylase 2, 20 minutes (9.79 ± 0.36). On the other hand, the samples that had significant differences ($p<0.05$) towards the positive control (4.88 ± 1.14) were negative control, 20 minutes (93.81 ± 6.12); negative control, 30 minutes (96.65 ± 2.9); and amylase 2, 30 minutes (9.88 ± 0.27).

On day 5, the samples that had no significant differences ($p>0.05$) with the positive control (6.99 ± 1.45) were amylase 1, 20 minutes (8.15 ± 1.31); amylase 1, 30 minutes (9.07 ± 0.93); amylase 2, 20 minutes (10.93 ± 1.2); and amylase 2, 30 minutes (10.55 ± 0.39). Lastly, the samples that had significant differences ($p<0.05$) towards the positive control (6.99 ± 1.45) were negative control, 20 minutes (83.83 ± 12.66) and negative control, 30 minutes (83.26 ± 10.66).

Sensorial analysis

The sensory analysis was performed specifically on samples that showed statistical significance and had the closest mean in relation to the positive samples (benchmark), specifically amylase 1 and amylase 2 made with 20 minutes of enzymatic activation **(Table 2)**. The sensory analysis utilized both a 5-point hedonic test and a rank test, as well as visual representations such as a spider web graph and a bar chart. These methods gave a full assessment of the sample's sensory characteristics, allowing for a more detailed understanding of the oat milk's aroma, mouthfeel, sweetness, aftertaste, and overall liking.

Hedonic test

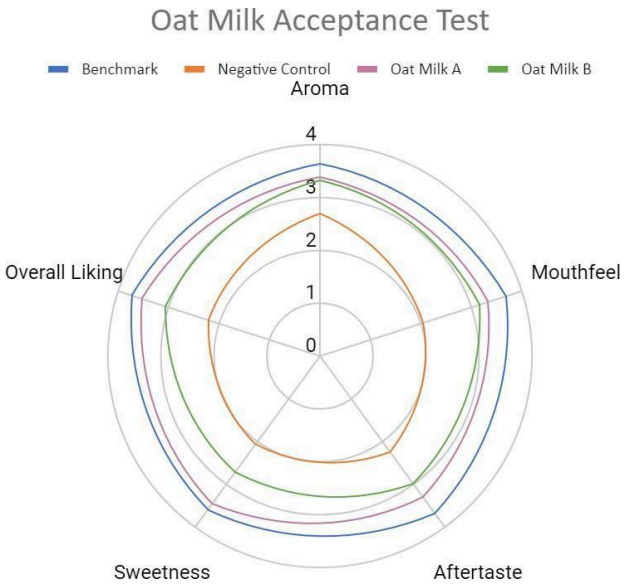

Figure 4. Spider web graph illustrating the sensory acceptance test of oat milk samples. The evaluation compared the benchmark, negative control, oat milk A (amylase 1, 20 minutes), and oat milk B (amylase 2, 20 minutes) across five attributes: aroma, mouthfeel, aftertaste, sweetness, and overall liking. Scores were obtained using a 5-point hedonic scale, with higher values indicating greater consumer preference

Based on **Figure 4**, the acceptance test was the highest in the benchmark sample followed by oat milk A, oat milk B, and the negative control in all attributes including aroma, mouthfeel, aftertaste, sweetness, and overall liking.

Table 7. Results from 5-point hedonic test

Sample code	5-Point Hedonic Test*				
	Aroma**	**Mouthfeel****	**Aftertaste****	**Sweetness****	**Overall Liking****
Positive Control (benchmark)	3.63 ± 0.67^{a}	3.7 ± 0.75^{a}	3.7 ± 0.75^{a}	3.6 ± 0.81^{a}	3.73 ± 0.74^{a}
Negative Control, 20 Minutes	2.7 ± 0.99^{b}	2.03 ± 0.96^{c}	2.23 ± 0.94^{c}	2.07 ± 0.94^{c}	2.2 ± 0.89^{c}
Oat milk A	3.4 ± 0.86^{a}	3.33 ± 0.88^{ab}	3.3 ± 0.95^{ab}	3.47 ± 0.97^{a}	3.53 ± 0.89^{ab}
Oat milk B	3.33 ± 0.88^{a}	3.17 ± 0.87^{b}	2.97 ± 1.03^{b}	2.7 ± 0.95^{b}	3.07 ± 0.94^{b}

*The results shown above is the mean ± standard deviation of aroma, mouthfeel, aftertaste, sweetness, and overall liking taken from the 5-point hedonic test with different letters a, b, c in the same column to denote statistical significance ($p<0.05$) based on Kruskal-Wallis and Dunn as a post-hoc analysis.
**1= Dislike Very Much, 3= Neither Like or Dislike, 5= Like Very Much

The 5-point hedonic test was done to ensure the acceptability of the oat milk in this study. As shown from **Table 7** above, it was seen that the benchmark had the highest score for all attributes which are aroma (3.63 ± 0.67), mouthfeel (3.7 ± 0.75), aftertaste (3.7 ± 0.75), sweetness (3.6 ± 0.81), and overall

liking (3.73 ± 0.74). The negative control, on the other hand, had the lowest score for all attributes including aroma (2.7 ± 0.99), mouthfeel (2.03 ± 0.96), aftertaste (2.23 ± 0.94), sweetness (2.07 ± 0.94), and overall liking (2.2 ± 0.89). Moreover, the negative control also had significant differences with all of the samples in all attributes provided. Based on Kruskal-Wallis and Dunn as a post-hoc analysis, it was found that oat milk A had no significant differences with the benchmark in all of the attributes provided. However, significant differences were found between oat milk B and the benchmark in several attributes including mouthfeel, aftertaste, sweetness, and overall liking.

Rank test

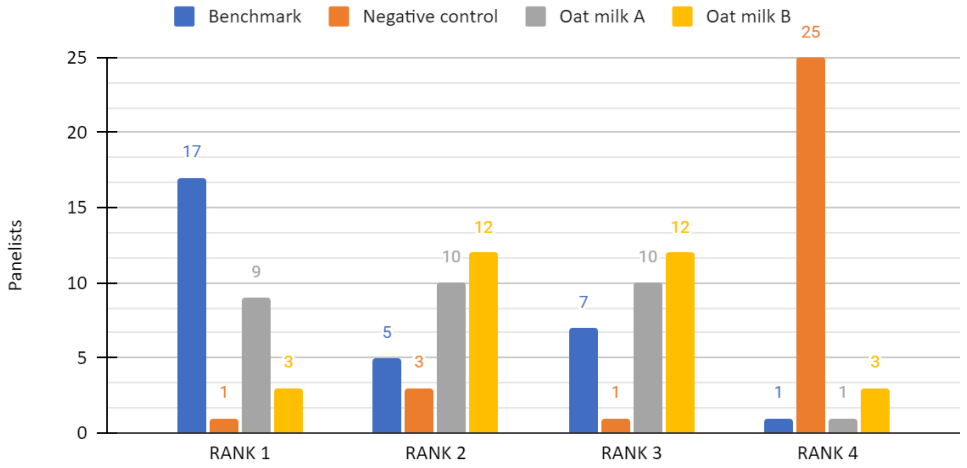

Figure 5. The evaluation compared the benchmark, negative control, oat milk A (amylase 1, 20 minutes), and B (amylase 2, 20 minutes). Panelists ranked the four samples from rank 1 (most preferred) to rank 4 (least preferred). The distribution of rankings is shown as the number of panelists per category, illustrating differences in overall preference among treatments

Based on **Figure 5**, the benchmark was found to be the most preferred with 17 panelists selecting it as rank 1. Oat milk B was ranked highest in both rank 2 and 3 with 12 panelists selecting it in both categories, respectively. While oat milk A was selected by 10 panelists on both rank 2 and 3. Lastly, the negative control was observed to be the least preferred with 25 panelists selecting it as rank 4 from all the samples.

Table 8. Results of rank test

Sample code	Overall Liking*
Benchmark	1.73 ± 0.94^{c}**
Negative Control	3.67 ± 0.8^{a}**
Oat Milk A	2.1 ± 0.88^{bc}**
Oat milk B	2.5 ± 0.82^{b}**

*The results shown above is the mean ± standard deviation of overall liking taken from the rank test with different letters a, b, and c in the same column to denote statistical significance ($p<0.05$) based on Friedman test and Conover's test as a post-hoc analysis.
**1=Most preferred, 3=Least preferred.

The rank test was done as a preference testing as a part of the sensory analysis. The results from **Table 8** were based on the Friedman test and Conover's test as a post-hoc test. It was then found that the benchmark was the most preferred followed by oat milk A, oat milk B, and negative control as the least preferred. Moreover, there were significant differences found in all the samples except samples oat milk A and oat milk B.

DISCUSSION

Physicochemical analysis

The physicochemical analysis examined a variety of parameters, including pH, brix, viscosity, and stability which includes pH, brix, creaming index, sedimentation. These tests were performed to completely assess the sample's qualities. Assessing these parameters is critical for establishing suitability for various uses and guaranteeing product quality.

pH

The samples consist of oat milk made from two types of amylase (amylase 1 and amylase 2), along with a positive (benchmark) and negative control (no alpha amylase treatment). Enzyme activation was tested at 20 and 30 minutes. The initial p-value of less than 0.001 suggests statistical significant differences ($p<0.05$) in the observed variations between the samples (Andrade, 2019). The highest pH recorded was the positive control (7.47), demonstrating that it is the most alkaline pH under the studies conditions. The lowest pH value was recorded by the negative control at 20 minutes (6.19), which did not differ significantly from the negative control at 30 minutes (6.31). In comparison, Guo (2009) stated that the pH of unfermented oat slurry was 6.3, making it a slightly acidic beverage.

Based on results on **Table 2**, it was found that oat milk made with amylase 2 (20 and 30 minutes) and negative control (20 and 30 minutes) had no significant difference in pH. The difference in properties of the alpha-amylase used may indirectly affect the final pH as oat milk made with alpha-amylase 1 and alpha-amylase 2 has significant differences ($p<0.05$). Enzymes usually function optimally in a specific range of temperatures (Mazumder et al., 2018). The rate of enzymatic processes may vary if two alpha-amylases, which have different optimal temperature ranges, are utilized in environments where the temperature differs from each of their individual optimal conditions (Sharif et al., 2023). For instance, the oat milk was made with 75°C of enzymatic activation whereas alpha-amylase 1 had an optimum temperature range of 70°C to 80°C and alpha-amylase 2 with an optimum temperature range of 70°C to 75°C. This could lead to difference in results since the temperature was more optimum for alpha-amylase 1 to react. Deviance from ideal circumstances, including changes in temperature, pH, or incubation period, led to a decrease in alpha-amylase activity, which was probably caused by denaturation, breakdown, or inhibition of the enzyme (Zar et al., 2013). Moreover, according to research done by Aydogdu et al. (2022), the measurement of pH at high temperature in milk protein solutions showed a reduction in pH as temperature increases. Upon this discovery, it can be assumed that heating during the oat milk process affects the pH of the end result.

Time of enzymatic activation was also found to have no significant effect towards pH, as the pH of oat milk remained mostly unaffected. Instead, pH was influenced more by the processing conditions. According to Zar et al. (2013), when the incubation period was increased from 5 to 10 minutes, alpha-amylase activity showed a significant spike. From 10 to 30 minutes, the activity increased gradually. The research indicates that there is little difference in the rate of enzymatic activity between 20 and 30 minutes of enzyme activation. This lack of effect could be due to the study's comparatively short time

frame. Therefore, it can be concluded that amylase 2 (20 and 30 minutes) does not impact the pH of oat milk which accepts the null hypothesis.

Brix

The positive control was also seen to have the highest brix content with the value of 13.91% **(Table 2)**. According to Considine & Frankish (2023), brix is the result of calibrating a solution's refractive index using dissolved sucrose. Therefore, it can be concluded that having a higher brix concentration indicates that the solution may be sweeter. On the other hand, the negative control with 30 minutes of enzymatic time activation had the lowest brix content with the value of 1.46. Based on research done by Magwaza & Opara (2015), brix is the proportion of total soluble solids in a pure aqueous sucrose solution expressed as a mass percentage which may conclude higher sweetness is correlated with higher brix readings. Due to this, it can be assumed that the negative control (both 20 and 30 minutes) may have significantly lower sweetness than other samples. The α-amylase is a member of the endo-amylase family, which is responsible for catalyzing the first phase of starch hydrolysis into shorter oligosaccharides by cleaving the glycosidic linkages. The final products of α-amylase activity are branching oligosaccharides comprising glucose units, maltose, and maltotriose, and oligosaccharides of variable length with dextrins (de Souza et al., 2010). Moreover, according to Babolanimogadam et al. (2022), α-amylases samples displayed the lowest starch and highest reducing sugar content. The use of amylase in enzymatic hydrolysis technology allows for increased production of glucose and maltose, enhancing the sweetness of oat milk and preventing the creation of highly viscous colloids (Yu et al., 2023). Zar et al. (2013) reported that there was a significant rise in alpha-amylase activity when the incubation period was increased from 5 to 10 minutes. After 10 to 30 minutes, there was a gradual rise in activity. Due to its short duration, the study indicates that there is little difference in enzymatic activity between 20 and 30 minutes of enzyme activation. From the results in **Table 2**, it was observed that all the samples except negative control (20 and 30 minutes) had no significant difference with the positive control. Therefore, the null hypothesis has been rejected.

Viscosity

From the results in **Table 2**, it was found that the negative control that had a 20 minute enzymatic activation time had the highest viscosity with the value of 42277.78 centipoise. On the contrary, the positive control had the lowest viscosity with the readings of 6.17 centipoise. Rosida et al. (2020) stated that the enzyme α-amylase functions in two primary phases. Initially, it rapidly breaks down amylose into maltose, causing the viscosity to quickly decrease. Alpha-1,4-glycosidic polysaccharides are hydrolyzed more easily by amylase, which results in the production of D-glucose, oligosaccharides, maltose, and dextrin. The data also concluded that there are no significant differences towards the amylase treatment samples (both amylase 1 and 2 treatments with both 20- and 30-minutes enzymatic activation) with the positive sample which indicates that the samples with amylase treatment had lower viscosity than the samples that had not undergone amylase treatment. According to Koyama et al. (2013), the viscosity of the mixture is influenced by the time of amylase-induced starch breakdown.

Stability testing

Stability testing is a complete evaluation that includes pH levels, brix readings, creaming index evaluation, and sedimentation analyses. Altogether, these criteria reveal an understanding of the product's stability and quality, as well as an overview of its qualities as time progresses.

pH

The pH of the samples showed instability, particularly between day 1 and the later storage days. For example, the negative control (20 minutes) increased from 6.19 ± 0.213 on day 1 to 6.46 ± 0.026 on day 2 and 6.49 ± 0.035 on day 5, while the negative Control (30 minutes) rose from 6.31 ± 0.065 on day 1 to 6.46 ± 0.037 on day 2 and 6.48 ± 0.054 on day 5. In contrast, amylase 1 (20 and 30 minutes), amylase 2 (20 minutes), and the positive control remained relatively stable, showing only minor fluctuations without significant differences across storage days.

According to Deziderio et al. (2023), immediately upon processing, the pH and brix levels of oat milk rapidly alter. Remaining enzymatic activity is often the source of this initial phase of adjustment. The release of sugars can therefore result in notable fluctuations in pH and brix levels during the course of the first day following processing. Moreover, based on research done by Kumar et al. (2014),enzymes included in oat milk, such as those added during processing, still catalyze processes after the initial adjustment period, but at a slower rate. This continuous enzymatic activity leads to the long-term alterations in pH and brix levels noticed in the subsequent days. However, the amount of these shifts decreases as amylase in oat milk reaches an improved state of equilibrium gradually.

Brix

The brix levels, on the other hand, had no statistically significant differences found between days 1, 2, and 5 in Positive control samples and amylase 1 (20 minutes). Significant variations were noted in other samples for brix levels, usually from day 1 to day 2 and day 5. According to research by Deziderio et al. (2023), oat milk's pH and Brix levels change rapidly right after processing. This first period of adjustment is frequently caused by remaining enzymatic activity. As a result, the release of sugars can cause significant variations in pH and brix levels throughout the first day after processing. The enzyme amylase is essential for converting starches into less complex carbohydrates like glucose and maltose (de Souza et al., 2010). Amylase may be added during the manufacturing of oat milk to aid in this breakdown process, which may have an effect on the final brix levels, a measurement of all soluble solids, including sugars of the oat milk (Marwati et al., 2018).

The difference in amylase enzymes used may also impact the brix levels in oat milk as their properties differ. The enzymatic strength of amylase Enzyme 1 is 480 KNU-B/g, whereas the enzymatic strength of amylase Enzyme 2 is higher at 650 KNU-B/g. Increased enzymatic activity of amylase would result in more effective starch breakdown, which would raise the sugar content and brix levels of the oat milk. Decreased amylase activity, on the other hand, may result in decreased starch conversion and sugar content, which would decrease brix levels (Streimikyte et al., 2022). Because the amylase enzyme breaks down starches into sugars throughout the production process, its strength can therefore directly affect the brix levels in oat milk.

Creaming index

A variety of samples showed noticeable increases in the proportion of creaming, with the negative Control samples (20 & 30 minutes) being the exception as creaming was not found in it. This condition may be explained by the absence of amylase in the negative control which results in more gelatinized starch. Based on research done by Olson (2021), swollen or gelatinized starch granules can adhere to the surface of fat globules and form a shield that keeps them from coming together to form bigger fat clusters and cause creaming. According to Yulianingsih & Gohtani (2019), when oat milk is heated or processed, starch gelation may take place, adding to its stability. The network structure created by gelatinized starch holds fat and

water globules, stopping them from moving and resulting in creaming. The viscosity is improved and higher by this starch gel matrix.

Significant variations in creaming across samples on different days were identified by statistical analysis; these variations were most apparent in the Positive Control and amylase 2 samples. Amylase 1 samples showed no significant difference towards creaming index in days 1, 2, and 5. This may be due to having different properties in the amylase enzymes used. The enzymatic strength of amylase Enzyme 1 is 480 KNU-B/g, while the enzymatic strength of amylase Enzyme 2 is 650 KNU-B/g. amylase 2's higher amylase activity promotes more effective starch transformation, which raises the sugar level while decreased amylase activity can lead to a decrease in sugar content and starch conversion (Streimikyte et al., 2022). This condition may be explained by the oat milk's sugar content rising as a result of the enzymatic hydrolysis, raising the amount of solids that are dissolved (Fredrick, 2011). Additionally, as seen from the results, all samples that were made with the addition of enzymes had creaming in them. Products that resemble thin beverages would gradually separate; this separation could take the form of slight creaming, phase separation, or even complex formation. Due to physical barriers like viscosity, which can slow down the separation process, amylase-treated oat milk separates more at both times of activation than the negative control samples because it is less viscous (Patra et al., 2022). The rheological characteristics of oat milk can also be impacted by amylase's enzymatic degradation of starch (Ren et al., 2023). Enzymatic action may cause changes in viscosity and fluid behavior, which could affect the emulsion's stability. These changes in rheology may affect oat milk's capacity to suspend fat globules and other particles, which may cause creaming (Jiang et al., 2018).

The significant increase in the positive control may be caused by several factors such as different types of stabilizers used as the other samples. The potential of an emulsion to withstand changes in its qualities is referred to as "emulsion stability" which means the more stable an emulsion is, the slower alterations in its properties will take place. Physical processes that alter the component, such as creaming, sedimentation, flocculation, coalescence, Ostwald ripening, and phase inversion, may result in changes in the properties of the emulsion (McClements., 2009). The droplets will cream as long as there is a density differential between the water and the oil. Consequently, using stabilizing agents to increase fluidity can greatly reduce the rate of creaming (Wilde, 2019). Based on the research done by Song et al., (2023), different types of stabilizing agents have different effects on the emulsion stability in the sample. Wilde (2019) also mentioned another important consideration is the emulsifier's solubility. Rapid adsorption to the interface is made possible by the high free monomer concentration of a highly soluble emulsifier. The adsorption rate will be slowed by poorly soluble emulsifiers because they will form aggregates, micelles, or vesicles and have a reduced concentration of free monomers. In conclusion, the selection of the emulsifying agent presents the most difficulty in the stabilization and emulsification of food emulsions.

Sedimentation

Moreover, sedimentation instability was found in some samples, especially amylase 1 samples, according to statistical analysis. Notably, no significant variations were seen on amylase 2 samples, Positive control and negative control. Creaming is the result of the fat droplets gravitationally separating from one another, whereas sedimentation issues lead to the formation of clumps or aggregates within the system (Olson, 2021). Oat proteins, fibers, and starch granules are among the suspended particles found in oat milk, which is a colloidal dispersion. Sedimentation may be significantly exacerbated by oat milk's irregular particle size distribution as it builds up at the bottom of the container as a result of larger particles settling faster than smaller ones (Dhankhar & Kundu, 2021). Sedimentation may occur from the gelation of certain

components in oat milk, especially starch. Water and other substances are trapped by gel networks, which causes them to split from the liquid phase and sink to the bottom of the container (Zhou et al., 2023).

Since it was found that there are varying properties from both the enzymes, there may be several possibilities as to why amylase 1 samples differ from amylase 2 samples. As said, the enzymatic strength of amylase Enzyme 1 is 480 KNU-B/g, whereas the enzymatic strength of amylase Enzyme 2 is higher at 650 KNU-B/g. Particle size and composition suspended in oat milk can be influenced by the rate and degree of starch hydrolysis, which is determined by the enzymatic strength. The key parameters that affected the system's stability were the particle diameter, gravity, the densities of the dispersed and continuous phases, and the viscosity of the continuous phase. The hydrolysis of starch affected the stability of the oat milk and caused the system's viscosity to decline (Zhou et al., 2023). According to Zhang et al. (2024), since enzyme activity alters the size as well as distribution of suspended particles, it can change the viscosity and texture of oat milk. Differences in viscosity and texture can result from variations in enzyme strength, and these variations may then affect the behavior of sedimentation.

Sensory analysis

Sample oat milk A was developed by activating amylase 1 for 20 minutes, whereas sample oat milk B was created by activating amylase 2 for the same amount of time. Sensory analysis was performed utilizing both a 5-point hedonic test and a rank test to measure the samples' sensory qualities. The sensorial analysis was done based on the brix results that has a closer mean to the positive control. Based on research done by Arilla et al. (2023), brix played a significant role in the testing of sensorial analysis. Brix was also found to be related to the sweetness of the product that was the leading attribute in the 5-point hedonic test.

5-point hedonic scale

The sensory testing was done with a 5-point hedonic scale and rank test. The Benchmark samples were found to be the most favorable in all attributes including aroma, mouthfeel, aftertaste, sweetness, and overall liking **(Table 7)**. It was also found that there were no significant differences between oat milk A, oat milk B, and the Benchmark samples in the aroma, mouthfeel, aftertaste, and overall liking attribute which indicates a positive response from the panelists towards the oat milk with alpha-amylase treatments.

The sweetness attribute, however, appeared to have significant differences between the Benchmark, oat milk B, and the negative control. Additionally, the negative control was discovered to have significant differences in all attributes with all the other samples which indicates that amylase treatment may have an effect on the oat milk samples. According to research by Zhang et al. (2024), amylase treatment improves the sensorial attributes of aroma, color, taste, and mouthfeel of oat milk. The study itself presented a novel approach to improve the acceptability and quality of oat milk by utilizing oat core flour as a basic ingredient as the viscosity of oat core flour might be effectively decreased by deep liquefaction and saccharification using amylase enzymes. The sweetness and aftertaste attribute, on the other hand, may be explained by the increase of sugar levels after amylase enzyme treatment done on the oat milk. Based on the study done by Babolanimogadam et al. (2022), in comparison to the other treatments, the α-amylase exhibited the highest reducing sugar content and the lowest starch concentration. Amylases' main function is to hydrolyze the glycosidic bonds that hold starch molecules together, converting complex carbohydrates into simple sugars which in turn makes the oat milk sweeter when enzymatic treatment is done (Akinfemiwa, 2023).

The mouthfeel aspect may be heavily related towards the viscosity of the oat milk. A thick and creamy mouthfeel had a positive correlation with apparent viscosity and steady shear rates (Greis et al., 2022). Oat milk that was made using amylase enzymes were seen to have no significant differences towards the positive control. By making the filtration process easier, the enzymatic liquefaction of oat starch lowers viscosity and boosts the output of oat milk (Deswal et al., 2013). Other than that, the aroma of oat milk may also be influenced by the addition of amylase enzymes in the oat milk signified by the results on **Table 7**. According to research done by Villas-Boas et al. (2019), the Maillard reaction, a reaction involving amino acids and reducing sugars, can be aided by the alpha-amylase enzyme's enzymatic breakdown of starches. This process produces a variety of delectable compounds, such as those that give oat milk its caramelized, roasted, and nutty scents (Sun et al., 2023). In conclusion, the null hypothesis has been rejected since amylase enzymes are seen to have significantly affected the sensorial properties of oat milk.

Rank test

The rank test obtained a positive correlation to the 5-point hedonic test **(Table 8)**. It can be concluded that the Benchmark had the highest preference followed by oat milk A, oat milk B, and negative control with significant differences between the samples. However, oat milk A and oat milk B samples are seen to have no significant differences between the 2. This rank test may be explained by the addition of amylase enzymes which in turn hydrolyze the starch into simple sugars. These sugars add to sweetness, which is frequently linked to better sensory evaluations. As a result, samples that were treated with amylase enzymes might have been rated more favorably because of their enhanced perceived sweetness (Alsado et al., 2023). Oat milk's viscosity and creaminess can be changed by enzymatic activity, which can also affect the milk's mouthfeel and texture. Higher sensory ratings may have resulted from samples containing amylase enzymes showing better texture qualities, such as creamier mouthfeel and smoother consistency (Yu et al., 2023). It's possible that samples with amylase enzymes were preferred more highly overall than samples without enzymes due to their increased flavor and improved texture. Lastly, oat milk A samples and the benchmark were also seen to have no significant differences which rejects the null hypothesis of the research.

CONCLUSION

The findings of this study demonstrate that activation time had minimal influence on oat milk quality, whereas enzymatic treatment with amylase significantly affected key physicochemical parameters, including °brix, viscosity, creaming index, and sedimentation rates. Enzyme-treated samples displayed improved stability compared to the negative controls, with amylase 1 treatments showing the closest resemblance to the benchmark. Sensory evaluation further revealed that enzyme-treated samples achieved acceptability levels similar to the positive controls, suggesting that enzymatic modification can enhance the overall quality and consumer appeal of oat milk. These outcomes confirm the research objective by showing that enzymatic treatment, rather than activation time, plays a more decisive role in improving both the stability and quality of oat milk.

Further research can be explored through variations of time activation in combination with different types of amylase such as beta amylase.

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