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# **Optimising the extraction of plant milk from oats.**

A research report presented in partial fulfilment of the requirements for the  
degree of

Master of Food Technology

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# Abstract

In recent years, due to the various benefits of plant milk, it has become an ideal substitute for cow's milk, among which oat milk contains many beneficial substances for the human body such as  $\beta$ - Glucan, phytic acid, avenanthramides and phenolic compounds. However, the cost of oat milk production is relatively high. Therefore, this study aims to improve the yield of oat milk by determining the best enzymatic hydrolysis conditions of oat milk.

The study shows that the optimal production condition of oat milk is: the enzyme concentration is 16ul/500ml oat slurry, the incubation temperature is 76°C, and the slurry concentration is 8%, the hydrolysis time was 20 minutes and pH was not controlled.

Oat milk produced by the optimum process was UHT treated and tested for stability and rheology. Sedimentation occurred within 3 days at both 4°C and 25°C. The power law model explains the rheological behavior of oat milk at 25 ° C, 35 ° C, 45 ° C, 55 ° C, and 65 ° C.

Further recommendations to the process include addition of additives to improve stability and testing of sensory properties. The optimal process can be scaled for further testing.

Key words: oat milk, yield, rheology, stability

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# 1.Introduction

## 1.1 Background

Oats are one of the best complete nutrition foods in cereals, rich in protein and contain all 8 essential amino acids needed by the human body (Sterna et al., 2016). Most lipids in oats are unsaturated fatty acid (Rasane et al., 2015) which have many positive health impacts on the human body including lower blood cholesterol, reduce blood viscosity and improve blood microcirculation. In addition, oats and oat products provide high quality soluble dietary fiber such as beta glucan ( $\beta$ -glucan) (Tiwari & Cummins, 2012). Tiwari and Cummins (2011) found that  $\beta$ -glucan intake could regulate blood cholesterol and glucose levels.  $\beta$ -glucan also played a significant role in protecting skin against aging, preventing cancer, modulating the immune system and decreasing the risk of heart diseases (Daou & Zhang, 2012). Soluble  $\beta$ -glucan is the main functional component in oat products, among all oat products, oat milk which contains macronutrients from the oats is more convenient for consumers.

Cow's milk is considered as a good source of nutrients, but it cannot be digested and absorbed by everyone (Silva et al., 2020). Oat milk can be consumed as an alternative to cow's milk for those people who are suffering cow's milk protein allergy (CMPA) or lactose intolerance (LI). Onning et al. (1999) reported that the consumption of an oat beverage every day for 5 weeks can lower blood cholesterol and low-density lipoprotein (LDL) in people with hypercholesterolemia. Oat milk was widely used as a substitute for cow's milk. According to Research and Markets (Figure 1), the global market value of oat milk has grown rapidly in the past five years. Therefore, it is necessary to conduct research on how to maximize the processing yield of oat milk.

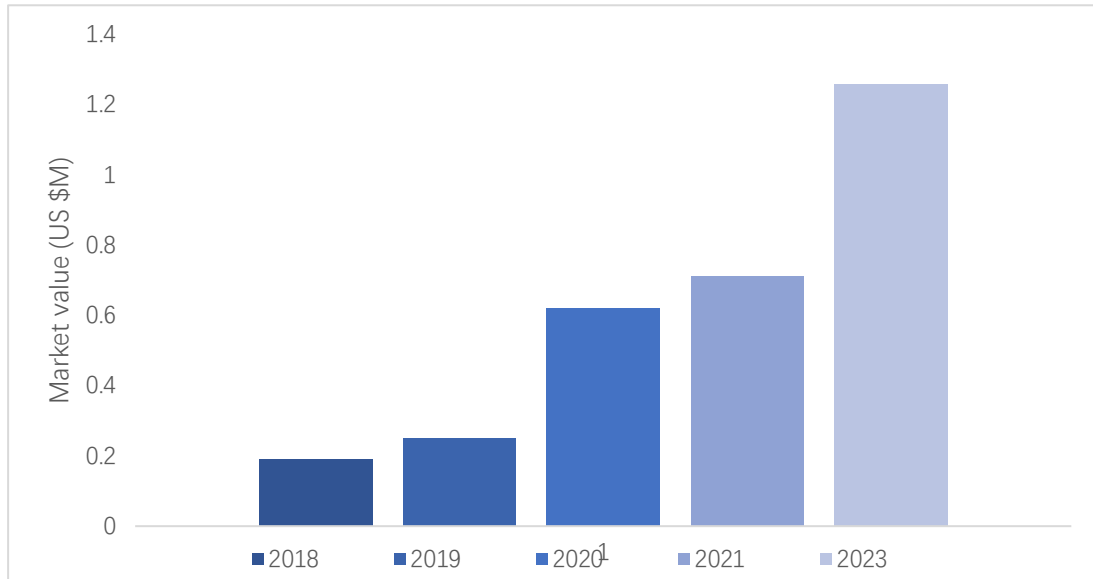


Figure 1 -The statistics of global oat milk market value from 2018 to 2023(Xin Lang Cai Jing,2023)

The production process of oat milk is the extraction of soluble nutrients of oats in water to make the product look like cow's milk (Mäkinen & Arendt, 2015). The typical process of oat milk involves oat kernel treatment, milling, enzyme reaction, separation, adding ingredients, heat treatment and homogenizing, then the final product is packaged by aseptic packaging. The main challenge in the production of oat milk is that during the heat processing the oat starch may be gelatinized (Tester & Karkalas, 1996). Using  $\alpha$ -amylase could convert the oat starch into maltodextrins to prevent gelatinization and facilitate the process of filtration and finally increase the yield of oat milk (Deswal et al., 2014b). Hence the proposal is for design an optimum process for the extraction of oat milk.

## 1.2 Purpose

- ✧ To design a process for extraction of plant-based milk from oats and maximize the processing yield and efficiency.

## 1.3 Objectives

- ✧ To determine the significant factors affecting the yield for the enzymatic extraction of oats in water.
- ✧ To determine optimum processing conditions for oat milk extraction using Response Surface Methodology
- ✧ To characterize the particle size, stability and rheology of oat milk using the optimal process.

## **2 Literature Review**

### **2.1 The prospect of Oat-based milk as an alternative to cow's milk**

The awareness of public health is constantly improving, the market for plant-based milk continues to grow. Consumers specially for those with lactose intolerance (LI) and cow's milk allergy (CMA) as well as vegetarians would like to choose plant-based milk as a substitute for cow's milk. Soy milk is an ideal choice as it is rich of protein, fiber, saponins and isoflavones (Bricarello et al., 2004). However, the protein in soy milk would still cause allergies because soy is one of the top 8 most common food allergens (Patel, 2015). Therefore, oat milk is an optional substitute as it is allergens free and rich in fibers the most well-known ingredient is beta glucan ( $\beta$ -glucan).

Recently, there are several retail brands producing oat milk, for consumers to add to coffee, as the  $\beta$ -glucan in oat milk can provide coffee plenty of flavor. However, the price of oat milk is higher than cow's milk because of the high production cost (Aydar et al., 2020). Furthermore, compared with cow's milk, oat milk has a shorter shelf life as it tends to form precipitates, and the insoluble particles in plant-based milk led to a chalky mouthfeel. Improving the flavor of oat milk and optimizing the extraction efficiency can reduce production costs and make oat milk more acceptable to consumers.

#### **2.1.1 Functional Substance in oat**

Oats are rich in many kinds of nutrients, such as protein, fiber, vitamins and antioxidants. In addition, avenanthramides in oats are good for the immune system of body and the amount of protein in oats are higher than other grains, so oats have been recognized as one of the world's top 10 superfoods (Kim et al., 2021). The general composition of raw material of oat and oat milk (100 g) are shown in Table 1.

Table 1-General composition of oat and oat milk (100 g)

	Raw material	Oat milk
Protein(g)	12.5	2.3
Total lipids(g)	6.25	0.63
Carbohydrate(g)	67.5	21.3
Total fibers(g)	10	2
Total sugar(g)	2.5	2.09

Lysine is one of the essential amino acids for human beings. The human body cannot synthesize it by itself. It must be supplemented from food. The content of lysine in cereals is low and it is easily destroyed during processing. However, the content of lysine, in oats is higher than that in rice and wheat flour (Klose & Arendt, 2012).

Unsaturated fatty acids account for more than 95% of the total fatty acid content in oats, of which palmitic acid is 13%-28%, oleic acid 19%-53%, linoleic acid 24%-53%, linolenic acid 1%-5% (Martín-Diana et al., 2021). In addition, oats also contain lauric acid, palmitoleic acid, arachidonic acid, as well as trace amounts of lignoceric acid and nervonic acid (Capurso, 2021). Due to the high polyunsaturated fatty acid content of oats, consuming oats can lower cholesterol, regulate blood sugar and lower blood pressure. Oats are a source of dietary fiber, vitamins (Vitamin B, Vitamin E and Vitamin K) and minerals (Raguindin et al., 2021).

The dietary fiber in oats can be divided into water insoluble dietary fiber and polysaccharides. Oat polysaccharides mainly include soluble hemicellulose, natural pectin and beta-glucan, among which there are more studies on  $\beta$ -glucan (Figure 1). The biologically active ingredient with special physiological functions in oats is  $\beta$ -glucan, which has important biological functions such as lowering blood cholesterol, improving immunity, inhibiting lipid oxidation, and delaying aging.  $\beta$ -glucan is a linear polysaccharide formed by D-glucan linked by  $\beta(1\rightarrow4)$  and  $\beta(1\rightarrow3)$  glycosidic bonds.

The ratio of these two glycosidic bonds is about 7:3 (Yoo et al., 2020), but their ratios will vary due to different extraction conditions, extraction temperatures and analytical methods used in the study.

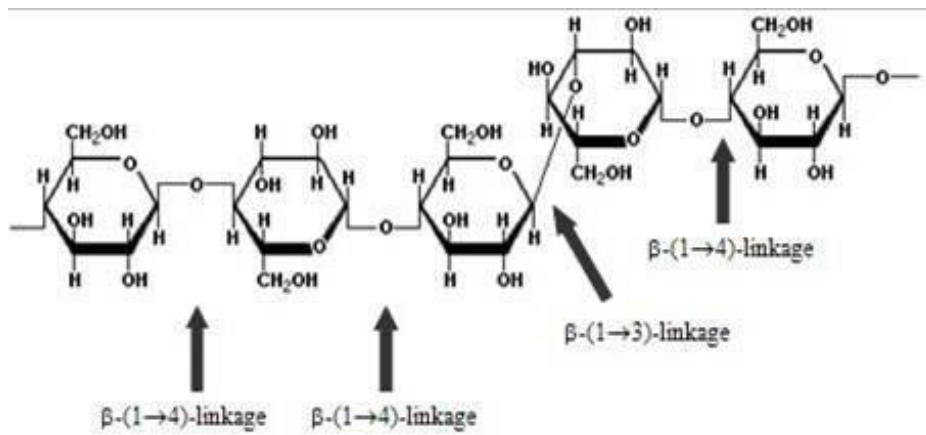


Figure 2-Molecular structure of  $\beta$ -glucan(Zhang et al., 2021)

### 2.1.2 The Application of Oats in Food

Currently, the most common oat product on the market is rolled oats, which is usually eaten for breakfast. Based on the nutritional effect of oats, extensive research has been carried out on its food development and utilization, the commercial oat products have been launched continuously. In recent years, the proportion of oat used in food has been increased significantly. Furthermore,  $\beta$ -glucan extracted from oats can be used to produce high-fiber beverages, pasta, cakes. It can also be mixed with starch in a certain proportion to process various puffed foods and used as ingredients for pet food and feed (Hu, 2005). In Japan an oat extract is used to develop and produce an oat EX food, which contains more than 5% soluble dietary fiber, this product can inhibit the absorption of saturated fatty acids and cholesterol, and has a positive effect on heart disease (You, 2003). Inglett and Newman (1994) have developed water-soluble dietary fiber products using oats as raw materials, which were added to meat products, dairy products and baked goods as a fat replacement. Because of the high viscosity of oat  $\beta$ -glucan, it has been used as a thickener in food, such as salad dressing, ice cream, jam and other foods (Charalampopoulos et al., 2002). At present,  $\beta$ -glucan is also widely

added to various types of foods such as frozen, ready-to-eat food, beverages, and fast food. The addition of  $\beta$ -glucan to these foods will not only affect the sensory properties of the food, but also improve the nutritional function of the food. In recent years, oat-based food has become more and more popular among consumers as they are perceived to have a positive impact on nutrition (Chalupa-Krebzdak et al., 2018). Compared with other oat foods, in the fast-paced modern life, oat milk is more convenient for consumers.

Önning (1999) reported that the consumption of oat beverage every day in 5 weeks can lower blood cholesterol and low-density lipoprotein (LDL) in people with hypercholesterolemia. Consuming oats can also lower after meal blood sugar and cholesterol in type 2 diabetic patients (Hou, 2015). Oat milk also contain flavonoids, unsaturated fatty acid and a variety of antioxidants that are beneficial to the human body. Studies have found that oat milk is low in protein but contains all 8 essential amino acids (Sterna, 2016). Unsaturated fatty acids and phytosterols in oat have many positive health impacts on the human body including lower blood cholesterol, protect skin against aging, prevent cancer, modulate the immune system and decrease the risk of heart diseases.

### **2.1.3 Market Outlook for Oat Milk**

Dairy has been an essential part of breakfast for many years. However, when considering ethical factors such as animal rights and environmental factors, many kinds of plant-based milks have appeared on the market in recent years. As oat milk contains dietary fiber ( $\beta$ -glucan) instead of cholesterol, and therefore regular consumption can contribute to a healthy diet. The global oat milk market continues to expand.

However, due to the complex production process of oat milk and the small production scale, the production cost of oat milk is high, there are many people that are not familiar with the oat milk. Compared with milk, the pretreatment of raw materials for plant milk is more complicated, and in order to obtain better taste, the production process also uses

enzymatic hydrolysis and emulsification process, which increases production cost. Therefore, using experimental methods to increase the yield and production efficiency of oat milk can provide guidance for industrial production of oat milk.

## 2.2 The Production Process of Oat Milk

The production process of oat milk is the extraction of soluble nutrients of oats in water to make the product look like cow's milk (Mäkinen et al., 2015). The general manufacturing process of oat milk is shown in Figure 3. The raw material is soaked and wet-milled to break the oats into a thick slurry and then soluble materials extracted usually with the aid of enzymes. Then oat residue is removed by filtration. The resulting filtered liquid is standardised and fortified usually with calcium, riboflavin, and vitamins A, D, B<sub>12</sub>. Following standardisation the milk is homogenised and sterilised by a heat treatment (Outi Elina Mäkinen & Arendt, 2016). Each step may affect the final flavor and quality of the product.

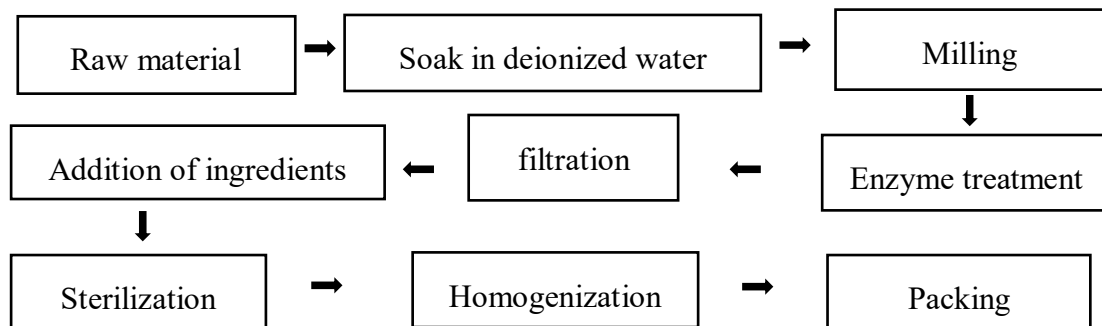


Figure 3-Manufacturing process of oat milk

The main component of oats is starch (50%-60%), so the main challenge in the production of oat milk is that during the enzyme treatment at elevated temperatures the oat starch is susceptible to gelatinisation (Tester & Karkalas, 1996). The gelatinisation phenomenon will greatly thicken the resulting solution and pose an adverse effect on the subsequent filtration. The addition of  $\alpha$ -amylase can convert starch

into dextrin and oligosaccharides resulting in a reduction in viscosity and a reduction in the potential of and increase in viscosity due to gelatinization and facilitating the filtration step (Silva, 2020). The yield of oat milk could be maximized by optimizing the enzymatic liquefaction process during production.

There are few articles about the production process of oat milk, some of the existing articles are about the nutritional aspects of plant milk. Deswal et al. (2014b) used central composite rotatable design (CCRD) to optimize the enzymatic conditions when preparing oat milk. The range of slurry concentration (w/w), enzyme concentration (w/w) were from 25 to 35 and 0.5 to 2.5 respectively, while the range of liquefaction time (min) was from 30 to 90. The results showed that the optimal conditions were: a slurry concentration of 27.1% w/w, an enzyme concentration of 2.1% w/w and a liquefaction time of 49 min. While in another article oat flour and water were mixed according to different material-to-liquid ratios (1:10, 1:15, 1:20, 1:25, 1:30) and gelatinized at 70°C for 10 minutes. Different amounts of enzymes (0.2, 0.4, 0.6, 0.8, 1, 2, 4, 8, 16, 32 u /g oats) were added to the gelatinized oat slurry to obtain enzymatic hydrolysis solutions at certain temperatures (45, 50, 55, 60, 65, 70, 75 °C) for different times (10, 20, 30, 40, 50, 60 minutes). Results showed when the hydrolysis time was 45 min, using a dosage of  $\alpha$ -amylase 6 U/g with a reaction temperature of 55°C, and the ratio of oat to water was 1 : 8 ( w:w) , the soluble solid content was 17.24 g /100 mL in oat milk (Cao et al., 2012). The effects of different degrees of hydrolysis on the viscosity, color and stability were also researched, these properties were stable and no longer changed when Dextrose Equivalent value achieved 50%. According to the characteristics of the enzyme reaction, the concentration of the substrate, the pH of the solution, the concentration of the enzyme, the reaction temperature and the reaction time may all affect the reaction results. Therefore, these five factors can be selected as independent variables to studied.

### **2.2.1 Alpha-Amylase**

Alpha-amylase also known as liquefaction amylase, liquefaction enzyme, or  $\alpha$ -1,4-dextrinase, can hydrolyze the alpha-1,4-glycosidic bond inside starch, and the hydrolyzed products are dextrin, oligosaccharides and monosaccharides. In the presence of an appropriate amount of calcium salt and table salt, when the pH value is 5.3~7.0, the  $\alpha$ -amylase can still maintain a high activity when the temperature is increased to 93~95°C. When producing oat milk, starch can be hydrolyzed by  $\alpha$ -amylase to increase the soluble solid content of oat base, prevent gel formation and improve the utilization rate of raw materials (Reyes-Jurado et al., 2021)

### **2.2.2 Production of Oat Base**

Hydrolysis of oats with amylase is an important step in oat milk production. Before wet milling, heat treatment may enhance aroma and flavor of the oat-milk. In order to study the effect of pre-treatment conditions on oat milk production, Tang xueyan (2014) baked oats at different temperatures and times, and soaked them for different times. They found optimal conditions were baking at 160 °C and 10 minutes, and then soaking the baked oats for 8 hours at room temperature. Furthermore, soaking for 8 hours before enzymatic treatment results in higher yield and titratable acidity than not soaking (Syed et al., 2020). While Kang Xu (2009) found that before milling soaking oat groats for 12 hours may produce more stable oat milk. Soaking time may vary depending on the oat to water ratio.

During milling the oat kernels are mixed together with water. After milling is the enzymatic process. In this step natural enzymes are added to break the oat starch into maltose which will provide the oat base natural sweetness. The hydrolysis of starch may prevent gel formation therefore facilitate filtration. It is very important to study the optimum conditions for the enzymatic reaction in this process. Hydrolysis time, dosage of amylase, temperature and the ratio of oat to water are key factors affecting the

enzymatic hydrolysis. Deswal et al. (2014b) studied the effect of slurry concentration, enzyme concentration and liquefaction time on oat milk used response surface methodology and found that when increasing the enzyme concentration, the water-soluble extract of the product will also increase. The research showed oat milk is a pseudoplastic fluid. The optimal conditions were found when the slurry concentration was 27.1 % w/w the enzyme concentration was 2.1% w/w and the reaction time was 49 minutes. It is worth noting that pH may also affect the yield of the process. The isoelectric point which translating to the lowest solubility of most cereal proteins is under 5 (Wolf, 1970), so the extraction step may be improved by slightly increasing the pH of the slurry. Higher extraction temperatures can increase the rate of fat extraction but may cause protein denaturation and reducing protein solubility and protein yield (Rustom et al., 1991). Papain extracted from papaya can hydrolyze large proteins into small peptides or amino acids. The use of papain increased the protein yield of soy milk (Rustom et al., 1993) but the use of papain for oat milk has not been found in the literature. As oats contain less protein than soy, the use of papain which acts on protein is likely not as effective for oat milk than soy milk.

### **2.2.3 Filtration and the addition of ingredients**

Filtration separates large particles insoluble fibers that remain after extraction and hydrolysis. However, oat milk does contain small insoluble solids that are ideally suspended in an aqueous phase referred to as the continuous phase. These suspended solids affect the consistency and viscosity of oat milk and play an important role in the acceptance by consumers.

In order to achieve a stable suspension of small insoluble particles the surfactant, sodium stearyl-2 lactylate (SSL) can be added specifically to partially hydrolyzed oat proteins (Bricarello et al., 2004). Hydrocolloids and emulsifiers have been proved to increase the viscosity of continuous phase (Rustom et al., 1995) and to enhance physical stability. Commonly used stabilizers include sodium carboxymethyl cellulose (CMC-

Na), xanthan gum, sucrose esters glycerol monostearate and guar gum. An orthogonal design experiment was carried out by Tang xueyan (2014) on the effect of compound stabilizers on oat milk. They found the best composite stabilizer combination is: 0.15% of sodium carboxymethyl cellulose, 0.07% of xanthan gum, 0.05% of glycerol monostearate and 0.07% of the sucrose esters.

Compared with cow's milk, oats lack calcium and other minerals, after removal of coarse plant material other ingredients including vitamins, minerals, sweeteners (Mäkinen Wanhalinna et al., 2015). Oat milk is usually fortified with calcium to achieve a level of calcium similar to cow's milk. Because of the higher bioavailability, calcium carbonate is more commonly used than tricalcium phosphate nowadays (Zhang et al., 2007). Furthermore, oats products contain antinutritional components such as phytic acid which prevents the human body absorption of calcium, zinc and iron (Sethi et al., 2016). Addition of phytase for oat milk can reduce the inhibition of phytic acid on mineral absorption. Flavorings such as salt, vegetable oils and citric acid are used to increase the flavor and taste of oat milk.

#### **2.2.4 Sterilization and homogenization**

Sterilization and homogenization are final stage of oat milk processing. Traditional pasteurization and Ultra High Temperature (UHT) are used to inactive harmful bacterial and extend the shelf life of oat milk. After sterilization commercial oat milk is usually aseptically packaged. High temperature also can deactivate the lipase which cause rancidity and short storage life for oat products (Lehtinen et al., 2003). Oat milk contains insoluble substances which may affect mouthfeel.

The homogenization process can be used to decrease the particle size distribution by breaking down aggregates and lipid droplets (Maghsoudlou et al., 2016) in order to make sure all the lipids mix properly and improve the taste and stability of oat milk. When studying the effect of temperature on the viscosity on oat beverages, Yuzhu Liu (2017) found that the viscosity of oat beverage decreases with increasing temperature.

The research was of great significance for studying the influence of temperature on the stability of oat beverages and the sensory quality of the products. Cruz et al. (2007) found that ultra-high-pressure homogenization (UHPH) reduces microbial counts and can be used for preservation. The synergy of thermal technologies and homogenization methods may achieve better quality of the final product. However, the optimal homogenization process of oat milk has not been studied.

### **2.3 Response Surface Methodology**

Design of Experiment (DOE) is a scientific research method that reduces experimental errors, production costs, and experimental workload by rationally designing experiments and scientifically analyzing experimental results. It is an experimental method to determine the optimal production process. A complete experimental design includes four stages: experimental planning, experimental implementation, experimental result analysis and experimental conclusion. The implementation process of DOE is shown in Figure 4.

#### **2.3.1 The concept of Response Surface Methodology**

In actual production, it is necessary to study how the response variables depend on the independent variables in order to obtain the optimal settings of the independent variable. When the number of independent variables is less than 3, response surface methodology is one of the best methods.

#### **2.3.2 Application of Response Surface Methodology**

When response surface methodology is used to design an experiment, a two-level factor experiment is usually used first to fit a linear regression equation. While the model is curved, a quadratic regression equation is fitted by the response surface methodology. The Central Composite Design is the most common method because it is sequential. The steps of the response surface experiment are shown in Figure 5.

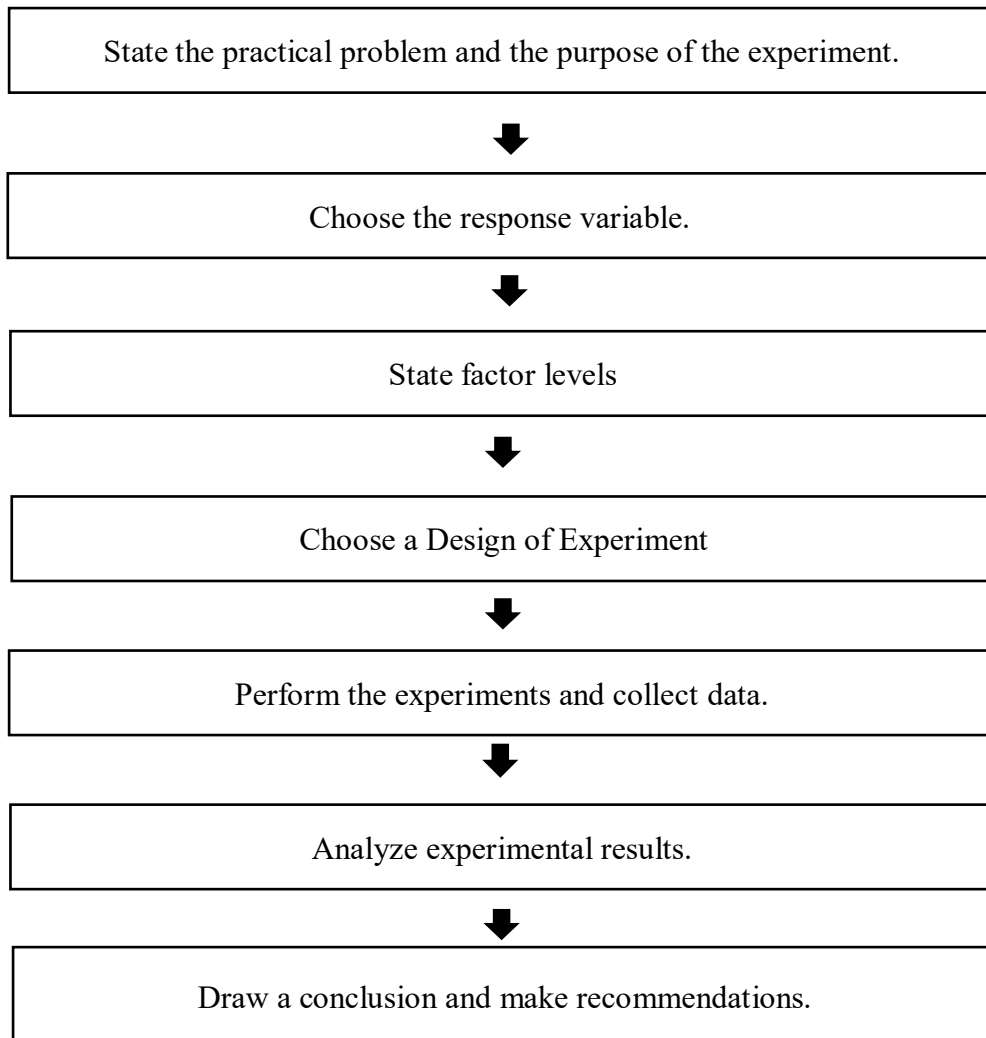


Figure 4-Flow chart of DOE

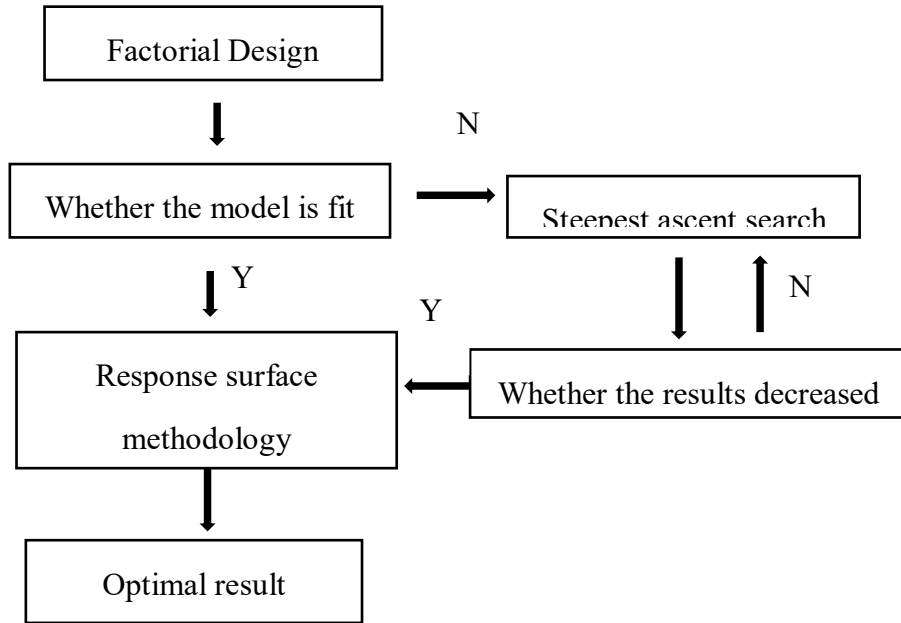


Figure 5-Steps of response surface methodology

## 2.4 Physical and chemical quality of Oat Milk

Rheological properties are one of the main parameters related to the taste and stability of oat milk, as well as the design and selection of process equipment. Studies by (Deswal et al., 2014a) have shown that oat milk is a pseudoplastic fluid whose viscosity decreases with increasing shear rate. It is believed that the effect of temperature on the viscosity of a beverage can be described by Arrhenius equation (Faccin et al., 2009). The Arrhenius equation relating  $k$  at any given temperature and  $k_{ref}$  at the reference temperature is:

$$k = k_{ref} e^{\frac{E_A}{RT} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)} \quad (1)$$

With the increase of temperature, the viscosity of the beverage gradually decreased, and the rate of decrease was related to the temperature, while the rate of decrease of the

viscosity slowed down with the increase of temperature.

The acidity of a developed drink affects the taste and stability of a beverage. In order to increase the stability of oat milk and improve the flavor of beverages, an appropriate amount of salt, vitamin C, and sodium tripolyphosphate can be added to the product (Tang xueyan, 2014). The addition of an appropriate amount of salt will reduce the intrinsic viscosity of xanthan gum and increase the sweetness of beverages appropriately (Viturawong et al., 2008). Tripolyphosphate is a common additive in beverages, which has a certain auxiliary effect on preventing protein denaturation and precipitation in beverages (Baoping, 1996).

Overall, the  $\beta$ -glucan, unsaturated fatty acids in oats as an important source of soluble dietary fiber. The oat milk market is growing internationally. But oat milk is sold at a higher price compared with cows milk because of the production cost is expensive and compared to dairy its taste is not widely accepted. Therefore, it is important to improve the production efficiency and yield of oat milk. Optimizing the concentration of enzymes and reaction time in the extraction process is important. In order to improve the stability and taste of oat milk, the application of suitable emulsifiers and additives in oat milk deserves further study.

# 3. Materials & Methods

## 3.1 Research plan

Converting oats into traditional liquid food make the nutrition in oats easier to digest and it is more convenient for people to consume oats. However, the production cost of oat milk is much higher than that of traditional milk.

In order to increase the processing yield and efficiency of oat milk experiments should be set up.

The project was focused on optimizing the extraction conditions of oat milk. The research could be divided into two parts, (1) generating an empirical model for the extraction process, and (2) evaluating the quality of the final product.

## 3.2 Materials

Instant oats (Harraways Oats Rolled) that have been rolled into flat flakes but contain all parts of oats grain were purchased from Countdown, New Zealand. Quick Oats are like rolled oats, but they're steamed longer and rolled thinner. This makes them cook faster, with a smooth creamy texture. The quick oats could absorb water easier therefore shorten experimental time compared to traditional rolled oats.

Food grade BAN480L alpha-amylase (Table 2) from Novozymes used for the study was a liquid form.

Table 2-Information of  $\alpha$ -amylase

Characteristic	Unit	Value
Alpha-amylase unit KNU-B	/g	530
pH at 25°C		6.10
Density	g/ml	1.218
Total viable count	/g	100
Coliform bacteria	/g	< 10
Molds	/g	< 10
Yeast	/g	< 10

### 3.3 Preparation of Oat milk

The experimental process is illustrated in Figure 6, after enzymatic hydrolysis the oat base was heated to 100°C to inactive the amylase. After sterilization, the oat milk was allowed to cool at room temperature. Once it returned to ambient temperature the milk was filled into 6 bottles and separated the sample into two groups of three bottles each. One group was stored at 4°C and the other group was placed at the cabinet in the PD Lab at ambient temperature for shelf life stability testing.

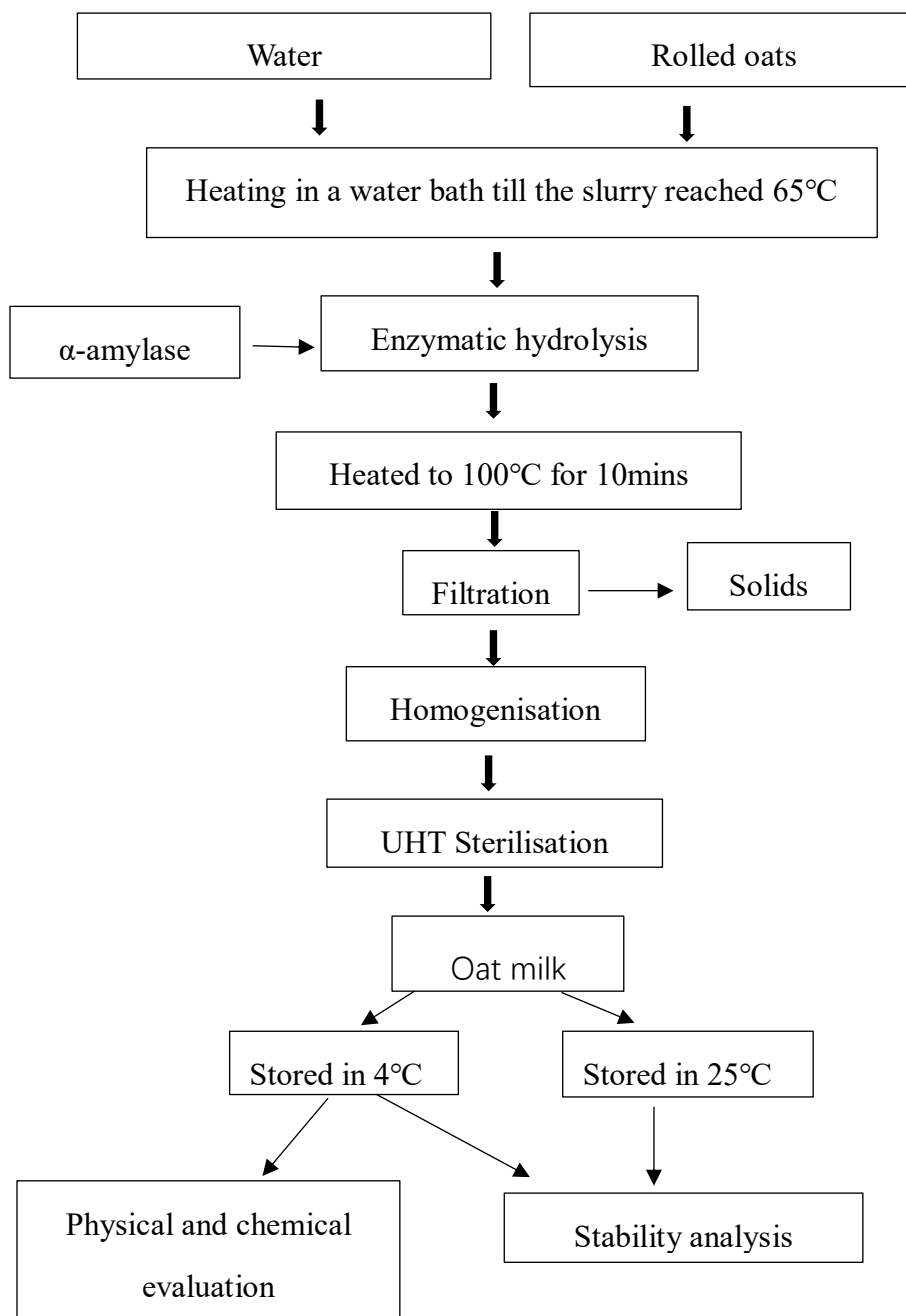


Figure 6-Experimental process

### 3.3.1 Gelatinization of aqueous oat

Oats and distilled water in a certain ratio for each treatment were added to a steel vessel and placed in a water bath at the incubation temperature. The slurry was allowed to heat in the water bath until it reached temperature of the water bath.

### **3.3.2 Enzymatic Hydrolysis**

Once the slurry had reached the incubation temperature alpha-amylase was added at the required dosage and allowed to hydrolyse the slurry for the hydrolysis time of the experimental treatment. During the enzymatic hydrolysis process, because the oat slurry formed gel, stirring is required immediately when adding amylase so that the starch could be fully hydrolyzed and prevent gelatinization. Before filtration the oat base was transferred to a pot of boiling water on the stove top for 10 minutes to inactivate the enzyme.

### **3.3.3 Filtration**

After inactivating the enzyme, 100ml of water is added to the liquefied oat to reduce the viscosity of the oat aqueous solution, thereby promoting filtration efficiency. The diluted liquefied oats were filtrated through cheese cloth to obtain oat milk. The oat milk was the material that passed through the cheese cloth.

### **3.3.4 Homogenization**

After the original oat milk cooling to 60 °C, a homogenizer (FBF ITALIA, PARMA-ITALY) was used to homogenize the oat base, and the operate condition was 180 bar.

### **3.3.5 Sterilization**

Approximately 1.5 L oat milk sample produced by enzymatic hydrolysis was sterilized by Ultra High Temperature (UHT) in the pilot plant, the operating conditions were 135 °C, 6 s. The sample was allowed to cool to room temperature and then divided into two groups of three plastic bottles. One group of 3 bottles was stored at 4 °C and one group of 3 bottles was stored at ambient temperature (about 22 °C) and these were used for physical and chemical evaluation. Both groups were used for stability analysis and the data were compared.

## 3.4 Design of experiment

The total solids of the oat milk were estimated by Air-Oven Method. After enzymatic hydrolysis, approximately 2 g of oat milk was weighed into aluminum dishes, the dishes and the lid under the dish were placed in the 108 °C air oven for 3 hours. After three hours the lid was placed on the dish and the dish was rapidly transferred to the desiccator for half an hour to cool. The dish was then weighed. Total Solids (T.S.) Content was calculated as Eq (2), the extraction yield was calculated as the weight of oat milk extracted and Solids Yield was calculated as Eq (3).

$$\%T.S. = \frac{w_2 - w_3}{w_1} \times 100 \quad (2)$$

where % T.S. = % total solids

w1 = weight in grams of sample

w2 = weight (g) of moisture dish + lid + sample (before drying)

w3 = weight (g) of moisture dish + lid + sample (after drying)

$$\%S.Y. = \frac{W \cdot TS}{M} \times 100 \quad (3)$$

Where % S.Y. = % solids yield

W = weigh of oat milk

T.S.= total solids

M = weigh of oats

### 3.4.1 Change of average soluble solid content during hydrolysis

Before preliminary experiment, the change of soluble solids of original oat liquid over time during the enzymatic hydrolysis could be studied to select the appropriate hydrolysis time. Preparation of original oat liquid is based on gelatinization of rolled oat. 100 g rolled oat and 450 g water were mixed to get 10% oat slurry, and the slurry was heated to 60°C by a water bath, then 30 ul  $\alpha$ -amylase was added to the slurry. 10 mL sample was taken out every 2 minutes for the first 20 minutes and every 5 minutes

for the last 25 minutes. At the end of the time the vessel with the liquified oats was removed from the water bath and placed into boiling water for 10 minutes to inactivate the enzyme. The vessel was removed and allowed to cool to ambient temperature. The solution was then filtered with a cheese cloth and the soluble solids (brix) was measured by a refractometer.

### 3.4.2 Preliminary Experiment

In order to enhance the process efficiency, optimization of  $\alpha$ -amylase hydrolysis is the key step during the production of oat milk. Many factors would affect the enzymatic hydrolysis, in the preliminary experiment the factors considered were, volume of enzyme, slurry concentration, hydrolysis time, incubation temperature and pH (Table 3) and the soluble solid of the oat milk was response in the model. A 1/2 fraction design was created with Minitab20.

Table 3-Values of factors for the preliminary experiment

Independent variables	coded symbol	range of levels	
		-1	1
volume of enzyme (ul)/500ml slurry	A	5	30
slurry concentration (%)	B	5	15
hydrolysis time (mins)	C	20	30
incubation temperature (°C)	D	50	80
pH	E	4.8	7

The regression model was used to fit the first-order regression model as follows:

$$Y = \beta_0 + \beta_1 X_A + \beta_2 X_B + \beta_4 X_D + \beta_{14} X_A X_D + \dots + e \quad (4)$$

Where Y is the response,  $\beta_0$  is the intercept and  $\beta_i$ ,  $\beta_{ij}$  are the regression coefficients for the model.  $X_A$ ,  $X_B$  and  $X_D$  are the factors,  $e$  is the error.

In the analysis of variance (ANOVA) table, a factor with a p-value less than 0.05 is a significant factor, while a factor with a p-value more than 0.05 is a non-significant

factor.

### 3.4.3 Application of Response Surface Methodology

Response surface methodology (RSM) could be used to obtain a high yield of the product with fewer trails. According to preliminary experiment, Enzyme volume, slurry concentration and incubation temperature are the three independent variables of the central composite rotatable design, while solids yield is the dependent variable. The method of steepest ascent was used to determine the range of independent variables. In the equation (3)  $\beta_1, \beta_2, \beta_4$  are the growing vectors,  $r = \sqrt{\beta_1^2 + \beta_2^2 + \beta_4^2}$  is the length of the vector. Therefore, the growth direction of  $X_A, X_B, X_D$  were  $\varphi = \frac{\beta_1}{r}, \varphi = \frac{\beta_2}{r}, \varphi = \frac{\beta_4}{r}$ , respectively. Table 4 was the design of steepest ascent trial.

Table 4-Design of steepest ascent

	E1	E2	E3	E4	E5
Enzyme volume(ul)/500ml slurry	8	12	16	20	24
Incubation temperature	54	60	66	72	78
Slurry concentration	8	10	12	14	16

When the response variable declined, the experimental range of the RSM can be determined. The factors and levels of CCD were shown in Table 5, pH was not controlled and the incubation time for all treatments was 30 minutes for all treatments. Total solids (Y1), Extraction Yield (Y2) and Solids Yield (Y3) were responses of the model. The Response Surface Design was created by Minitab20. The results were analyzed by Minitab20 as well. Optimal enzymatic hydrolysis was determined by

model analysis.

Table 5-Factors and levels of the experiment design

	-1.68179	-1	0	1	1.68179
V-amylase(ul)	7	10	15	20	23
Concentration	7.9%	10%	13%	16%	18.1%
Temperature	53	56	65	74	77

### **3.5 Validation of the model**

The enzymatic hydrolysis was performed according to the method shown in Figure 5, and operating conditions were the ones generated by the model. Total solids of oat milk was calculated by Eq (2) and the extraction yield was calculated by Eq (3). The model is correct when the responses were within the 95% confidence interval of the model.

### **3.6 Physical and Chemical evaluation**

The quality evaluation was carried out by the sample produced under the optimal enzymatic assisted aqueous extraction conditions. A fresh batch of oat milk was made using the optimal conditions and then homogenised and sterilised and placed into bottles as described previously. All measurements were carried out in triplicate and the average value was calculated.

The particle size distribution was obtained by integrated laser light scattering technique (Mastersizer2000). The measurement of soluble solid content is the same as previously mentioned.

Amino acids which contain nitrogen are the basic unit of protein, and it is generally

believed that the nitrogen content in proteins is about 16%. The protein content was measured using Kjeltac 2100 System (Tecator, Sweden), after digestion and distillation, the sample was titrated with 0.01M HCl to a grey-mauve end point. The calculation of protein content in oat milk was shown in Eq (5).

$$\%protein = \frac{0.01 \times A \times 14 \times 100}{1000 \times B} \times 5.83 \quad (5)$$

Where, A= mL HCl used

B= weight (g) of sample.

Rheological properties of oat milk were measured by a MCR301 rheometer. Due to the low viscosity and large particle of oat milk, cup and bob geometry was used test temperature was controlled by Peltier system. Temperature equilibrium was achieved by holding the sample in the geometry with no shearing for 10 minutes. Following equilibration, the samples were measured using a shear sweep up from of 0.1 s<sup>-1</sup> to 100 s<sup>-1</sup> at 25 °C, 35 °C, 45 °C, 55 °C and 65 °C. All the measurements were carried out in triplicate. Power law equation was used to fit the rheological behavior of oat milk.

### 3.7 Stability Analysis

Oat milk is stored at 4 °C and 25 °C for 30 days. Samples were taken from the three bottles for each temperature to measure sedimentation, pH and soluble solids every three days. Pictures were taken on the first and the 30<sup>th</sup> day.

The soluble solids were estimated by a handheld refractometer (Atago, Japan) and were expressed as °Brix. The pH of oat milk was measured by pH meter (Massachusetts, U.S.A). A centrifuge method was used to measure the sedimentation amount of oat milk, oat milk stored at 4 °C and 25 °C were shaken well before measurement, 100ml of oat milk was taken into centrifuge tube, the tubes was centrifuged at 5000 rpm for 5 minutes using a centrifuge (Massachusetts, U.S.A). The weight the sedimentation after drying at 108 °C air oven for 20 minutes was determined.

# 4 Results & Discussion

## 4.1 Determination of extraction conditions of oat milk

### 4.1.1 The effect of enzymatic hydrolysis time on soluble solids

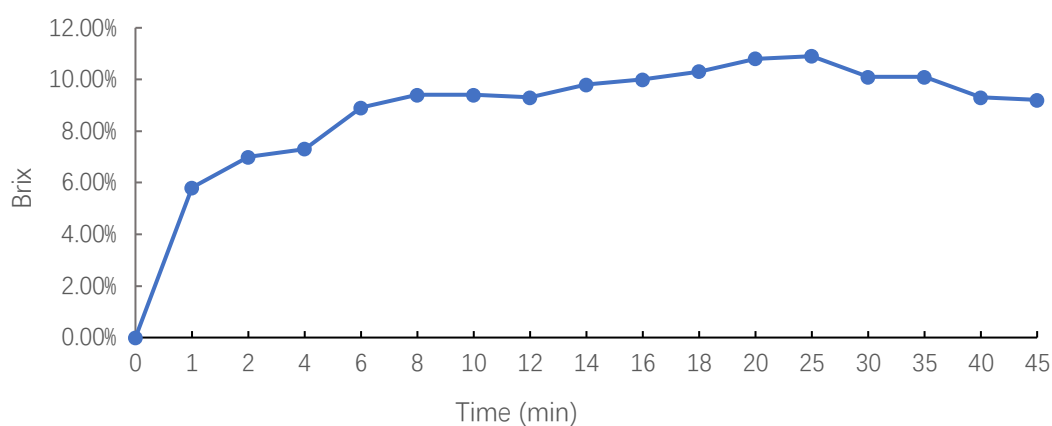


Figure 7-Curve of average soluble solid content during hydrolysis

It can be seen from Figure 7 that a soluble solids level of 6% occurs within the first minute and increases to a peak at 25 minutes of 10% solids. When the enzymatic hydrolysis time was longer than 25 minutes, the content of soluble solids decreased slightly.

When  $\alpha$ -amylase acts on starch, it will randomly cut  $\alpha$ -1,4-glucosidic bonds from the inside of the molecule, hydrolyze the starch to generate dextrin and some reducing sugars, and quickly reduce the viscosity of the starch solution in a short time. During the production of oat milk, the increase of soluble solid content is partly due to the hydrolysis of macromolecules of insoluble starch into soluble dextrin and reducing

sugar, and the dissolution of proteins and other substances. With the increase of liquefaction time, the substrate gradually decreases, the enzymatic hydrolysis speed would slow down, and the accumulation of enzymatic hydrolysis products would also inhibit the enzymatic hydrolysis. Therefore, the soluble solids would no longer increase.

Tang (2017) found that when the enzymatic hydrolysis time of rice was more than 20 minutes, the Dextrose Equivalent (DE) of rice milk beverage would no longer increase. That means the soluble dextrin and reducing sugar would stop increasing. However, as the enzymatic hydrolysis time prolongs, the already dissolved proteins are treated for a long time under high temperature conditions, causing protein aggregation to precipitate, resulting in protein loss and ultimately reducing the total soluble solids. Therefore, the enzymatic hydrolysis time in the experiment should not exceed 30 minutes.

#### **4.1.2 Screening of significant factors in the model**

Table 6 relates to the design matrix and results of preliminary experiment.

Table 6-Data of preliminary experiment

Trial number	Volume of enzyme	Slurry concentration	Hydrolysis time	Incubation temperature	pH	Total Solids
1	5ul	15%	30minutes	50°C	7	11.77%
2	30ul	5%	20 minutes	80°C	7	18.25%
3	5ul	15%	20 minutes	80°C	7	14.99%
4	5ul	5%	30 minutes	80°C	7	12.92%
5	5ul	15%	20 minutes	50°C	4.8	12.83%
6	5ul	5%	20 minutes	50°C	7	12.26%
7	30ul	5%	30 minutes	80°C	4.8	16.85%
8	30ul	15%	20 minutes	50°C	7	17.44%
9	30ul	15%	20 minutes	80°C	4.8	18.92%
10	30ul	5%	30 minutes	50°C	7	15.33%
11	30ul	5%	20 minutes	50°C	4.8	14.25%
12	5ul	15%	30 minutes	80°C	4.8	14.01%
13	5ul	5%	20 minutes	80°C	4.8	12.69%
14	5ul	5%	30 minutes	50°C	4.8	13.39%
15	30ul	15%	30 minutes	80°C	7	18.91%
16	30ul	15%	30 minutes	50°C	4.8	15.86%

The experimental results were analyzed by Minitab20 and the Analysis of Variance is shown in Table 7

Table7-Analysis of Variance

<b>Source</b>	<b>DF</b>	<b>Adj SS</b>	<b>Adj MS</b>	<b>F-Value</b>	<b>P-Value</b>
Model	4	0.007970	0.001992	26.86	0.000
Linear	3	0.007768	0.002589	34.90	0.000
A	1	0.005987	0.005987	80.70	0.000
B	1	0.000483	0.000483	6.51	0.027
D	1	0.001298	0.001298	17.49	0.002
2-Way Interactions	1	0.000202	0.000202	2.73	0.127
A*D	1	0.000202	0.000202	2.73	0.127
Error	11	0.000816	0.000074		
Total	15	0.008786			

According to the analysis of variance of the preliminary experiment, the model F-value of 26.86 with P-value 0.000 implies the model is significant. It can be seen that slurry concentration, volume of enzyme, and incubation temperature have a significant impact on the model. When factors A, B, and D (enzyme volume, slurry concentration and temperature are at the high levels the soluble solids of oat milk are the highest with 18.9% soluble solids. The other factors pH and hydrolysis time did not have a significant effect. The Regression Equation of preliminary experiment in uncoded units of model is

$$Y = 0.15042 + 0.01934 \text{ volume of enzyme} + 0.00549 \text{ slurry concentration} + 0.00901 \text{ incubation temperature} \quad (6)$$

It can be deduced from Equation (6) that with the increase of each factor, the solids yield would increase as well. However, it is expected that a temperature higher than 80 °C the enzyme will be inactivated, and degree of hydrolysis will be lower. When

the temperature exceeds 90°C, it would be deactivated (D'Aoust et al., 1988).

## 4.2 Fitting of Response Surface Method

The Regression Equations of Total Solids(Y1), Extracted Yield (Y2) and Solids Yield (Y3) were shown in Eq (6), Eq (7) and Eq (8)

$$Y1 = 0.16202 + 0.01095 A + 0.01769 B + 0.00473 C - 0.01007 A*A - 0.01005 B*B + 0.01195 A*B \quad (6)$$

$$Y2 = 388.44 - 44.85 B - 18.95 B*B \quad (7)$$

$$Y3 = 0.5075 + 0.02610 A - 0.09380 B + 0.02081 C - 0.05748 A*A - 0.03890 B*B \quad (8)$$

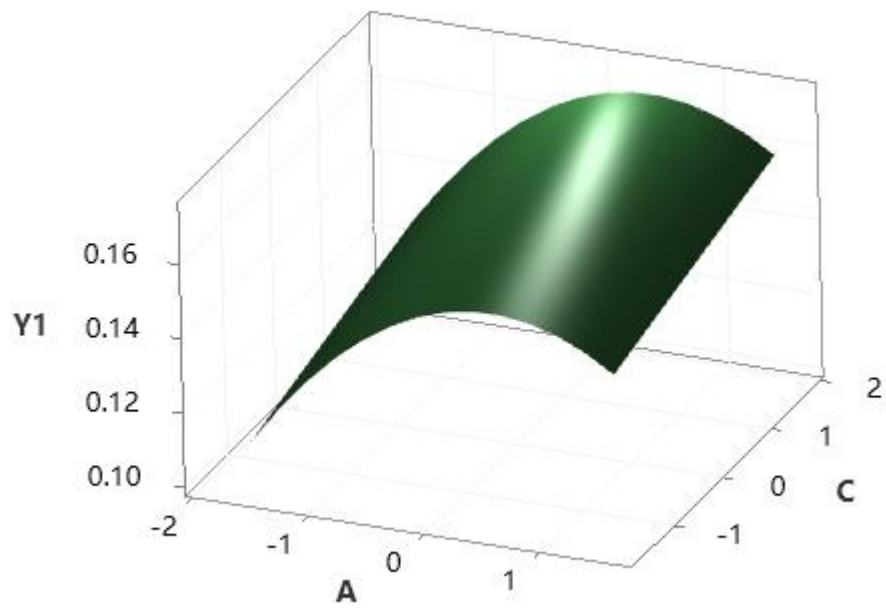
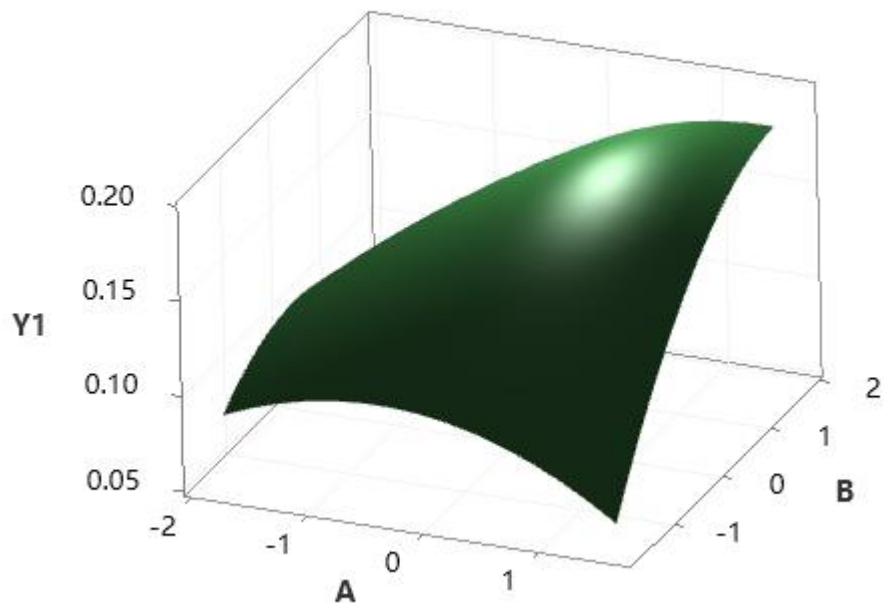
The R<sup>2</sup> of each model was 91.76%, 64.39%, and 89.99%, respectively. A larger R<sup>2</sup> indicates higher model accuracy.

The processing parameters and the results were presented in Table 8. Among the 20 treatments, soluble solids of oat milk vary from 10.95% to 18.91%, the highest solids yield was 59.23% while the lowest was 25.31%.

The 3D surface plots of the response were presented in Figure 8 and 9. From those Figures, it can be seen that as the volume of enzyme added increases, the yield increases as well. However, when the amount of enzyme added continues to increase, the yield actually decreases. This may be because under a certain substrate concentration, when the enzyme amount reaches a certain level, as the enzyme amount increases, the per unit enzyme act with substrate decreases, result in the degree of starch hydrolysis decreases. Moreover, the amount of enzyme added also affects production costs. The research of (Subramani et al., 2022) showed that when the enzyme concentration increased from 4% to 5%, the extraction rate also decreased.

Table 8-Matrix of Central Composite Rotatable Design

Trial number	Volume of enzyme(ul)	Slurry concentration	Incubation temperature (°C)	Total Solids	Extracted Yield	Solids Yield
1	23	13%	65	14.96%	376	43.26%
2	7	13%	65	12.03%	302	27.94%
3	15	13%	65	16.17%	407	50.62%
4	15	13%	65	16.29%	410	51.37%
5	15	13%	77	17.50%	440	59.23%
6	10	16%	74	12.73%	320	25.46%
7	15	7.9%	65	10.95%	418	57.53%
8	15	13%	65	16.49%	405	51.37%
9	15	18.1%	65	16.61%	275	25.31%
10	10	16%	56	13.49%	339	28.58%
11	15	13%	65	16.53%	405	51.50%
12	10	10%	74	12.16%	406	49.37%
13	15	13%	53	14.58%	366	41.04%
14	20	10%	56	12.03%	415	49.93%
15	20	16%	56	17.76%	310	34.41%
16	20	16%	74	18.91%	290	34.27%
17	20	10%	74	12.54%	395	49.52%
18	15	13%	65	16.29%	410	51.38%
19	10	10%	56	11.52%	415	47.80%
20	15	13%	65	16.66%	406	52.03%



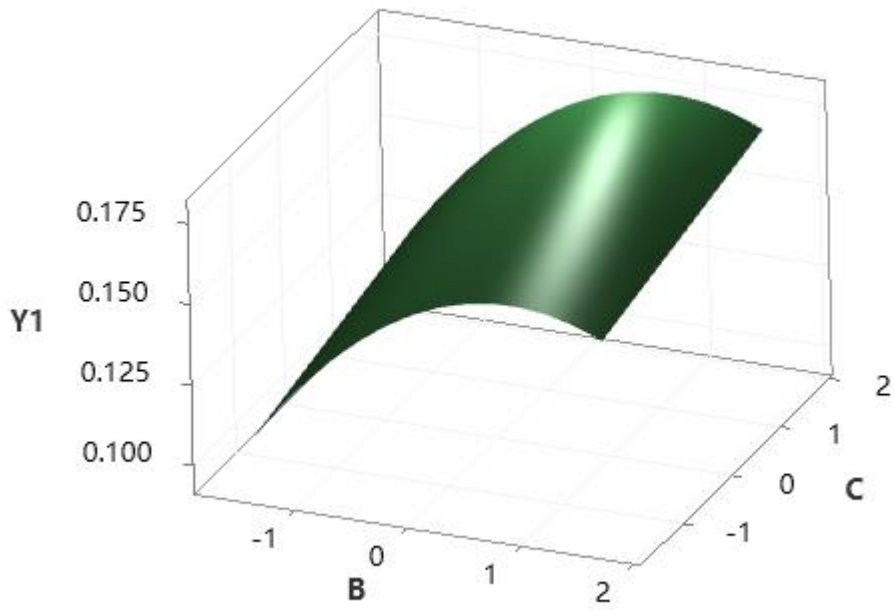
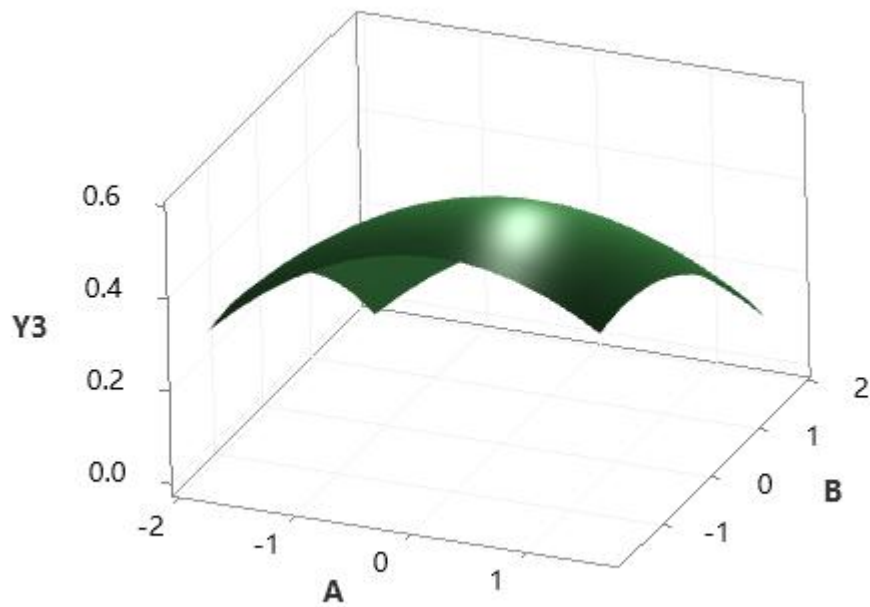


Figure 8-Effect of Enzyme Volume(A), Slurry concentration(B) and Incubation Temperature on Soluble Solids(Y1)



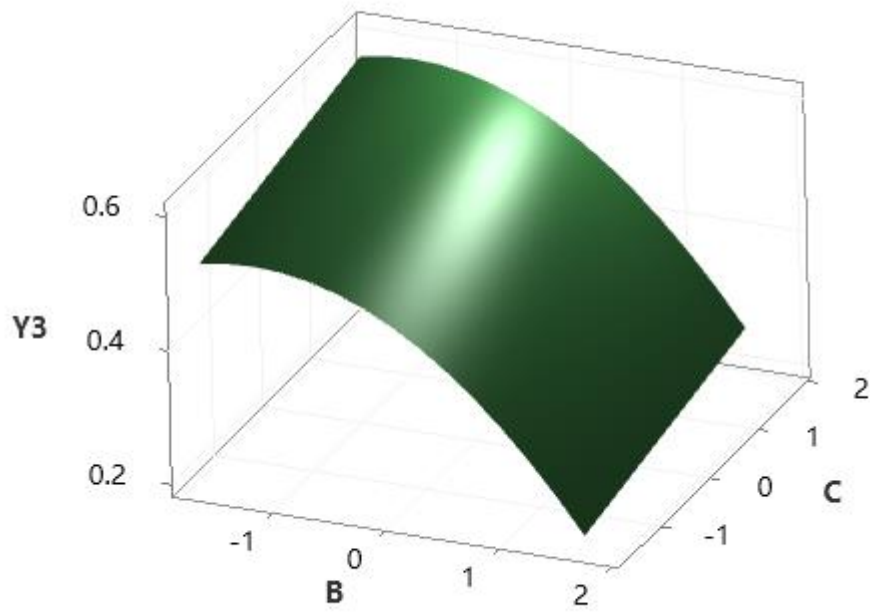
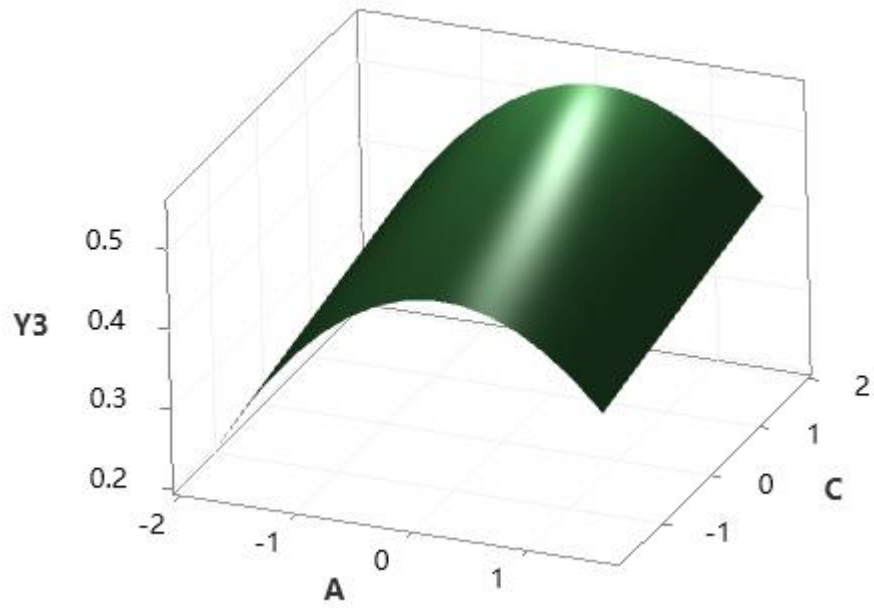


Figure 9-Effect of Enzyme Volume(A), Slurry concentration(B) and Incubation Temperature(C) on Solids Yield(Y3)

The desirability value of the experiment generated by response optimization was Total solids = 16.5%, Extracted Yield = 400g and Solids Yield = 56.9%, it would be observed at 16ul of volume of  $\alpha$ -amylase, 76°C of incubation temperature and 8% of slurry concentration.

### 4.3 Experimental validation of optimal conditions

Three replicated experimental results of oat milk produced under the optimal condition was shown in Table 9, the result was well fitted with the response prediction of the model (95% PI:0.14808, 0.18250; 334.00, 466.62; 0.4841,0.6536).

Table 9-Validation results

	Total Solids	Extracted Yield	Solids Yield
1	17.89%	404g	59.57%
2	17.86%	418g	61.53%
3	17.83%	412g	60.57%

The optimized process flowchart for production of oat milk was shown as follow.

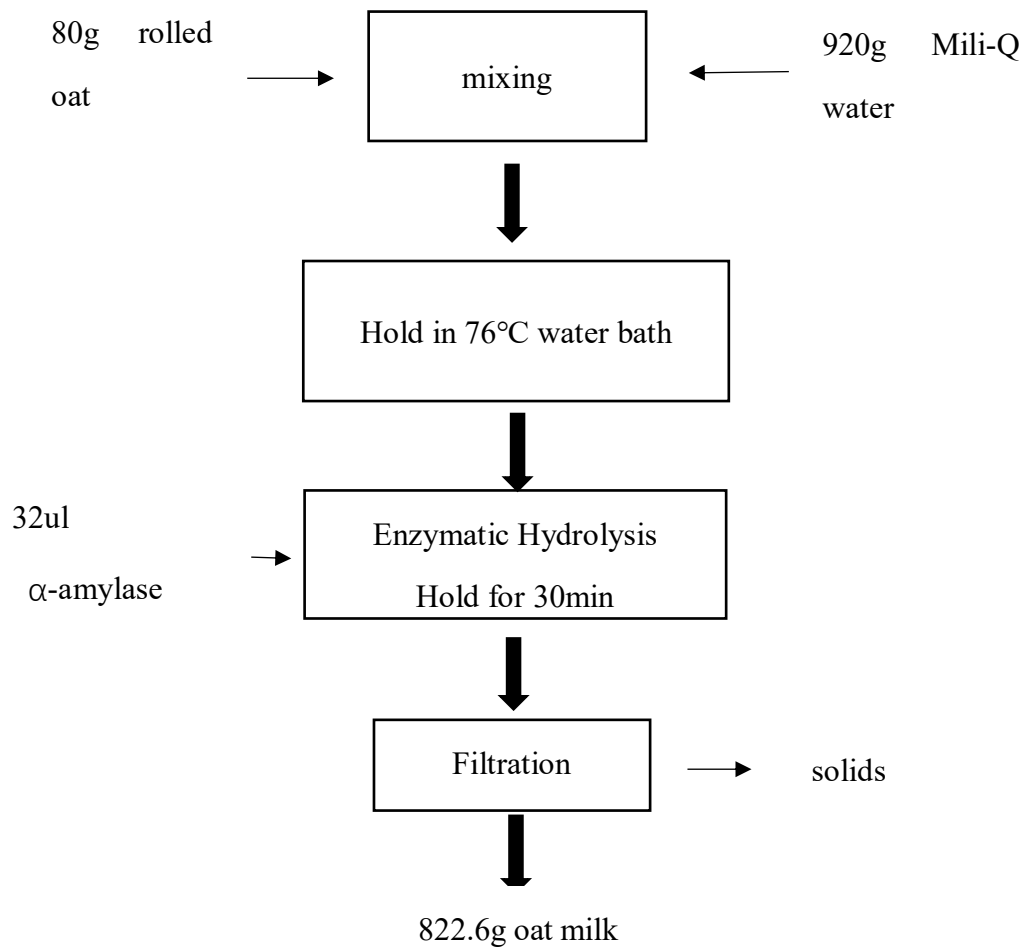


Figure 10-Optimal production

## 4.4 Physical and Chemical Properties of Oat Milk

### 4.4.1 Rheological properties of oat milk

The viscosity of oat milk varies with shear rate at different temperatures as shown in the Figure 11. It can be seen from the graph that oat milk is a non-Newtonian fluid and the viscosity of oat milk is low when the temperature is high.

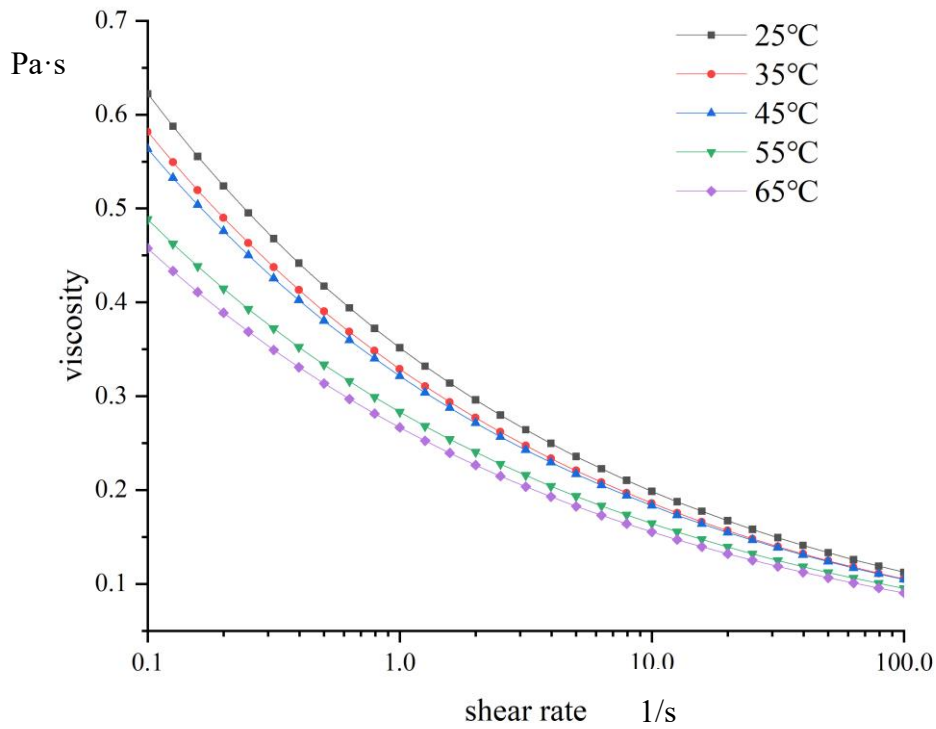


Figure 11-Rheological curve of oat milk

The oat milk is shear-thinning fluid. At higher temperatures, the intermolecular forces of the liquid decrease, therefore the friction of substances in milk decreases during flow, resulting in a decrease in oat milk viscosity (Deswal et al., 2014a). The power law equation was used to fit the rheological properties of oat milk, and the relevant parameters of each equation were shown in Table 9. The power law equation is  $\tau = K\gamma^n$  ( $\tau$  is the shear stress,  $\gamma$  is shear rate,  $K$  is consistency index and  $n$  are the flow behavior index). The  $n$  (flow behavior index) of the fitted equation at all temperatures is less than one. This indicates oat milk in the experiment is pseudoplastic fluid. Deswal et al. (2014b) found that the rheological behavior of oat milk was not linear and oat milk obtained exhibited a pseudo-plastic behavior.

The apparent viscosity plays an important role in the sensory attributes and quality of the food from consumer perspective (Szczesniak, 1979). Normally milk exhibits non-Newtonian behavior as well at low shear rates and temperature below 40°C (Heartwin

A. Pushpadass, 2019). The study of oat milk rheology would help the production process control and product handling.

Table 10-Parameters of oat milk at different temperature estimated by Power law Model

Temperature °C	K	n	R <sup>2</sup>
25	0.3475	0.4539	0.9815
35	0.3289	0.4521	0.9921
45	0.3214	0.4213	0.9765
55	0.2832	0.3847	0.9839
65	0.2666	0.3756	0.9861

#### 4.4.2 Physical Properties and Chemical Composition of Oat Milk

The protein content of the sample is 1.021% (SD = 0.001), which is lower than the protein content in bovine milk (Plamada et al., 2023). The average particle size was also analyzed. The specific Surface Area of the sample was 3.17m<sup>2</sup>/g with the Surface Weighted Mean (D[3,2]) = 1.892µm and the Weighted Mean (D[4,3]) = 7.309µm. The volume distribution of the sample was 2.791µm.

The large particle size of the sample indicates the instability profile of oat milk. Generally, smaller particle sizes would observed in stable plant-based milk (McClements et al., 2019).

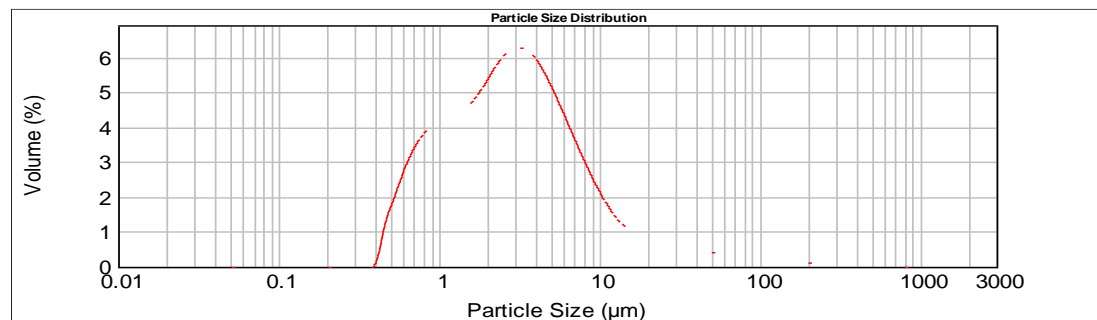


Figure 12-Particle Size distribution

## 4.5 Stability of Oat Milk

Both sets of samples showed phase separation on the second day of storage, and the speed of phase separation was slower for sample stored at 4°C than for the sample stored at 25°C. However, the phase separation of both sets of samples did not increase after being stored for more than 20 days. After 30 days of storage, there can be seen phase separation with complete sedimentation in the sample (Figure 16). The main components of oat kernels are starch and protein, and the influence of starch was negatively correlated with the protein content. The high density of other components in oat milk can lead to phase separation as well (Patra et al., 2021).

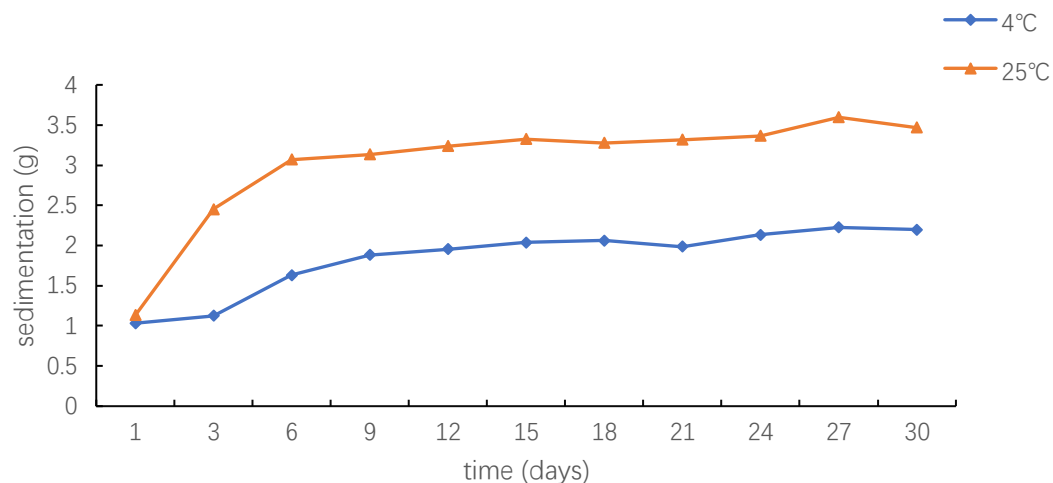


Figure 13-Curve of Sedimentation amount over time at different temperature

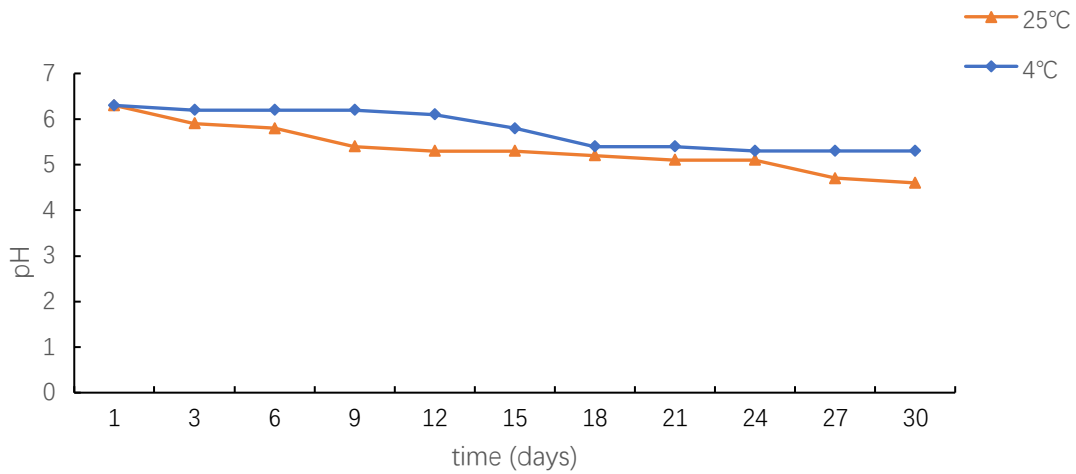


Figure 14-Change of pH over time at different temperatures

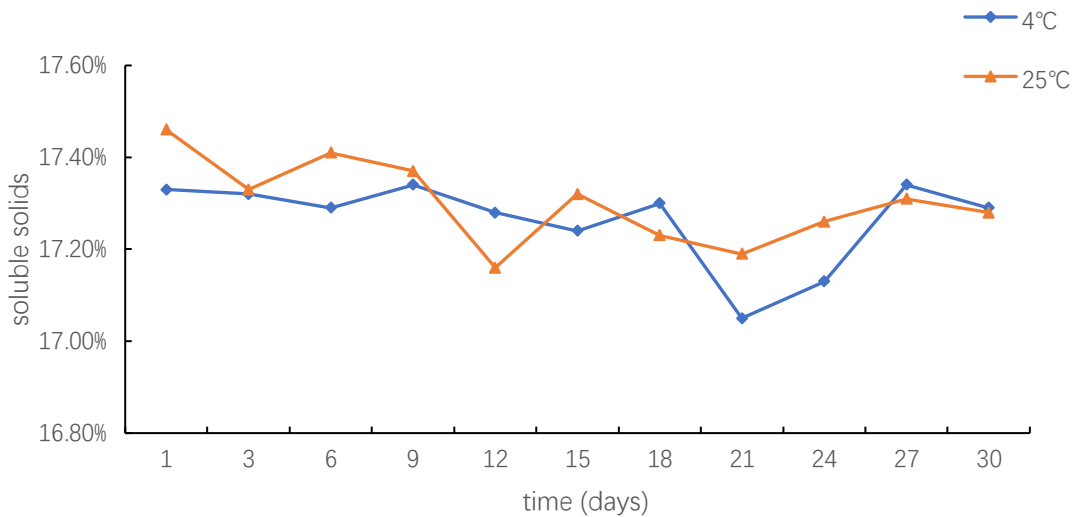


Figure 15-Curve of soluble solids amount over time at different temperatures

Stability of the oat milk was analyzed as well, from Figures 13, 14, and 15, it can be seen that the sedimentation amount of oat milk increases with the prolongation of storage time. After a rapid increase in the first 6 days, the increase rate slows down and the sedimentation increase is more obvious when stored at ambient temperature. The pH of the sample decreases when the storage time increases, possibly due to the rapid deterioration of oat milk at higher temperatures, resulting in a decrease in pH value of the sample (Yao et al., 2022). However, the soluble solids content in oat milk does not

change significantly with storage time increase, ranging from 17% to 17.5%.

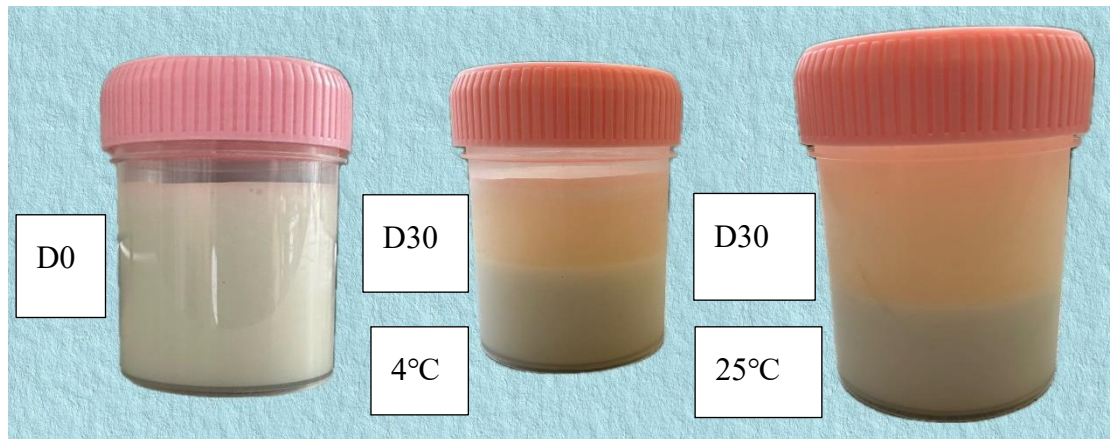


Figure 16-Pictures of oat milk at day 0 and day 30 stored at 4 and 25 °C

## 5 Conclusions

The production conditions of oat milk were successfully optimized by Response surface methodology. The yield of oat milk is maximum when the enzyme concentration is 16ul/500ml oat slurry, the incubation temperature is 76 °C, and the slurry concentration is 8%. Oat milk is a pseudoplastic fluid, and its viscosity decreases with increasing the temperature. Furthermore, oat milk is an unstable liquid that phase separation would be easily occurred.

Compared to the production of milk, the cost of producing oat milk is much higher because it requires enzymatic hydrolysis. Therefore, increasing the processing yield and efficiency of oat milk can reduce the costs and make it more competitive in the market.

## 6 Recommendations

- The optimal process developed can be scaled to produce larger amounts of oat milk
- The stability of the oat milk needs to be improved which can be achieved by adding stabilizers.
- The sensory properties of the milk need to be assessed.

## 7 Reference

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