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Optimization of process variables for the preparation of oat milk using the Box–Behnken response surface model and studying the effect of enzyme hydrolysis on structural and thermal properties of oat starch

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ABSTRACT

Lactose intolerance and the growing number of vegan and plant-based lifestyles are the main causes of the growing popularity of dairy substitutes, including sesame, soy, almond, coconut, cashew, oat, and peanut milk. Oat milk, a popular alternative, is created by blending oats with water. Many individuals highly favor this plant-based beverage due to its milk-like taste and its provision of essential nutrients. An oat milk substitute has been developed through the enzymatic hydrolysis process utilizing alpha-amylase. The Box–Behnken design of response surface methodology (RSM) was used to investigate the combined impact of the enzyme concentration (2–10% v/w), oat slurry concentration (10–15% w/w), and liquefaction time (15–45 mins). The optimization of process parameters was conducted by considering many criteria including yield %, viscosity, total soluble solids, total solids, zeta-potential, and particle size. The optimized oat milk is also compared to traditionally prepared raw oat milk to analyze the structural and thermal alterations in starch following enzymatic treatment. An investigation was conducted to examine the disparities in the subjects' structural characteristics, physicochemical attributes, and thermal properties. The XRD, DSC, and FTIR analyses revealed that the oat milk exhibited significantly higher relative crystallinity, and a more organized and stable double-helix structure, compared to the untreated raw oat milk.

1. INTRODUCTION

In the last decade, there has been a strong emphasis on developing new healthy food products, focusing on functional and specialty drinks. Green consumerism or vegan diet, which views plant-based products as more sustainable and low-impact on the environment, is on the rise as a result of growing environmental consciousness. The process of urbanization has expedited these requirements, resulting in an increasing inclination toward drinks that specifically address individual lifestyle preferences and medical conditions such as lactose intolerance. One such important functional necessity to address issues with lactose intolerance, animal milk allergy, milk protein allergy, calorie conscience, and the prevalence of hypercholesterolemia is milk substitutes [1]. Milk is an essential dietary element that is necessary for people of all age groups, ranging from newborns to the elderly. Despite their self-sufficiency, individuals are progressively opting for vegan milk or non-dairy substitutes, which have seen a surge in popularity in recent years. Following the advent of lifestyle choices such as lacto-vegetarianism, vegetarianism, ovo-vegetarianism, and veganism, plant-based milk substitutes are increasingly required for vegan food products such as yogurt, curd, cheese, ghee, kefir, probiotic drinks, butter, and frozen dessert. People who are suffering from lactose intolerance or allergies to cow's milk also need these replacements. The plant-based beverage industry is seeing growth as a result of these modifications and advancements. In 2019, the plant-based milk segment grew significantly in the global retail market, making up 40% of all milk products [2]. Due to the nutritional advantages of soy milk, it has become more popular as a cow's milk substitute. Nevertheless, current studies have prioritized the use of oilseeds, nuts, and cereals for novel culinary applications due to their advantageous functional characteristics. Various milk substitutes are available on the market such as rice, sesame, peanut, coconut, oat, almond, hazelnut, hemp, tiger nut, quinoa, and lupin milk [3].

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The flavor and nutritional advantages of cereal-based oat (*Avena sativa*) milk make it one of the most popular and widely used plant-based milk substitutes. The environment is a significant contributing factor to the acceptance of oat milk. Compared to milk, oat milk has a lesser climatic impact since it emits between 16 and 41% fewer direct greenhouse gases [4]. In terms of fostering carbon peak and carbon neutrality, which ultimately aid in maintaining a carbon footprint, oat milk performs better than animal-based milk, according to the research [5]. Commercial cereal-based milk can be divided into two classes based on the product and processing features: those that mimic milk (like oat milk) and those that look and feel more like milk (like corn milk) but still have the original cereal color and texture [6]. The market for oat milk has seen a 71% gain in sales volume between 2017 and 2019, with a valuation of $17 billion in 2018 and projected to : reach $18.9 billion by 2028 [7,8]. Oat milk alternative, which is also known as oat beverage, is a water extract of oats rather than a milk derivative. It is a well-liked option for a plant-based beverage that not only offers vital nutrients but also encourages a healthy lifestyle because of its creamy, milk-like flavor [9]. To make oat milk, the conventional method involves blending oats with water and filtering through a cloth. Oats contain between 50 and 60% starch. This starch gelatinizes at a temperature between 44.7 and 73.7°C [10] causing an issue when oat milk is heated to a high temperature because the liquid milk becomes gel. The application of oat milk to various food product preparations is restricted due to the gelatinization of oat starch. There are two main approaches to starch gelatinization: acid hydrolysis and enzyme hydrolysis. An often-used procedure in the starch industry is acid hydrolysis, which yields thin boiling starches suitable for use in printing, textiles, food as well as many other industries [11]. The standard process for producing acid-thinned starch is to apply mineral acid to a concentrated slurry of starch (36–40% solids) at a temperature in the range of 40–60°C, which is a temperature lower than the starch's gelatinization temperature for a certain amount of time [12]. After achieving the necessary degree of conversion or rheological properties (viscosity), the starch is recovered after the neutralization of acid. Hydrolysis is influenced by temperature, length of reaction, and acid content. The present understanding of how acid hydrolysis affects the physicochemical characteristics and structures of different types of starch [13]. It is possible to alter the starch structure chemically, physically, or enzymatically. In general, the modified starches exhibit improved stability, paste clarity, better freeze–thaw stability, and resistance to retrogradation [14].

The enzymatic technique involves applying a liquefying enzyme, ideally during the gelatinization process, as it has been discovered that this causes the starch to become completely amorphous for amylases to be able to digest it [15]. It has been shown that the enzymatic liquefaction procedure increases the production of tuberous roots, i.e., jicama (*Pachyrhizus erosus*) and the saccharification of maize starch [16,17]. Oat starch's enzymatic liquefaction reduces viscosity and enhances the yield of oat milk while streamlining the filtration process [15,18]. Viscosity and solubility are two of the physicochemical properties of oat dextrin (OD), which is the hydrolysis product of oat starch with a dextrose equivalent (DE) value of less than 20. These products are largely used in the food industry, especially in dairy products, as a fat substitute [19,20]. The developed beverage's sensory and rheological attributes play a major role in determining customer approval. Food's sensory characteristics are influenced by its rheological properties. Rheological data is used to calculate fluid flow in several processes, including extraction, pump sizing, filtration, purification, and extrusion. To analyze the flow conditions in food operations, including evaporation, dehydration or drying, pasteurization, and aseptic processing, it is also essential [21,22]. The particular oat grain variety used and the processing parameters affect the final composition of oat milk. These factors affect the nutritional value of the oat milk in addition to its sensory attributes, yield, and rheological properties. The heat-induced gelatinization of the starch in the milk influences the rheological properties, sensory attributes, and yield of oat milk [23-27]. Presently, there is a deficiency in the available literature regarding comprehensive investigations that demonstrate the influence of the composition, formulation, and physical attributes of the oats used as a raw material in the preparation of oat milk on the quality attributes of the end product. Several investigations were conducted, taking into account limited quality and quantity attributes. The optimization of process parameters is more reliable when it is planned by considering several responses. This research focuses on optimizing the process parameters by taking into account many factors such as yield percentage, viscosity, total soluble solids (TSS), total solids, zeta-potential, and particle size. The aim of this study was to examine the impact of oat slurry concentration, alpha-amylase concentration, and reaction time on oat starch to get a thorough understanding of the effects of raw material properties. The optimized oat milk is also compared with traditionally prepared raw oat milk to understand the structural and thermal changes in starch after enzymatic treatment.

2. MATERIALS AND METHODS

Rolled oats were purchased from True Elements, India. Uniform-sized, 100% whole grain rolled oats are also known as old-fashioned oats. Oats are processed by steaming the whole grains until they become soft and malleable, and then pressing them until they become flat. Food-grade fungal alpha-amylase enzyme was purchased from Bioven Ingredients, India, with 170,000 U/mL activity in liquid form. All the chemicals and reagents were purchased from Sigma-Aldrich, India.

2.1 Experimental Design and Statistical Analysis

Response surface methodology, or RSM, is a set of statistical and mathematical techniques for figuring out which component combinations will produce the desired responses and how important each influencing factor is to the others, even when there are intricate interconnections present. Three overlapping 22 factorial designs with points are located on the surface of a sphere encircling the center of the Box–Behnken design [28]. The center point and the center of the cube's corners that the sphere surrounds make up the spherical Box–Behnken rotating surface model. To find the optimal system response, a model that fits the experimental data is developed. Due to the linear or quadratic influence of important variables, the design causes the graphs to rebuild [29].

The Box–Behnken model was applied to investigate the influence of enzyme concentration *X1* (2–10% v/w), oat slurry concentration *X2* (10–15% w/w), and the time for liquefaction *X3* (15–45 min) on yield *Y1* (% w/w), viscosity *Y2* (m.Pa.s), TSS *Y3* (°Brix**.**), total solids *Y4* (% w/w), zeta-potential *Y5* (mV), and particle size *Y6* (nm). The experiment was designed using the E Design Expert software, 23.1 version (Stat-Ease Corporation, USA). To estimate the pure error, the software generated a total of 17 runs with three replicates at the central point. In order to enhance precision, every trial was conducted thrice. The determination of coefficient R2 is used to check the goodness of the model. In addition, R2 can be used to assess the model's suitability. The higher R2 values provided a superior model.

2.2 Preparation of Oat Milk

Rolled oats were roasted at 180°C for 10 min and ground into flour in a laboratory food processor (Bosch, India). The process parameters were optimized using the Box–Behnken design. Water was added to oat flour in various ratios (10–15% w/w) and the mixture was heated at 85°C to form a slurry. The enzyme was diluted 1:1000 in distilled water before being added to the slurry at different concentrations (2–10% v/w). A total of 0.2% calcium chloride per weight of oat flour was used to activate the amylase as a catalyst, and pH was maintained at 6.2. The slurry was allowed to liquefy for 15–45 min. After treatment, the enzyme was deactivated by boiling it for 10 min at 100°C. The cooled oat slurry was filtered through a muslin cloth to collect the oat milk. All the samples were tested for total yield, TSS, viscosity, and stability. Optimized oat milk was compared with untreated oat milk to determine the starch profile.

A new batch of raw oat milk was made without the use of enzymatic treatment. A mixture was created by combining roasted rolled oats with water at an optimized slurry concentration of 13.17%. The slurry was thoroughly blended and then strained using a muslin cloth. The unprocessed oat milk was compared to the optimized oat milk produced using enzymes for further examination.

2.3 Yield

Yield % of the oat milk was calculated after the enzymatic treatment and filtration of the slurry. The yield was calculated in w/w % of the oat slurry.

2.4 Viscosity

The viscosity studies were performed by using the Anton Paar rheometer (Model-MCR 92, Anton Paar GmbH, Graz, Austria). All samples were tested at 25°C. Before being placed in the rheometer, the samples were well mixed using a magnetic stirrer to achieve a uniform consistency. A 60-mm space was established between the parallel plates.

2.5 TSS and Total Solids

The samples' total solids were measured using a hand refractometer (Erma), and the results were given in °Brix. The total solids of the oat milk were estimated by dehydrating the samples at 103°C to 105°C [30].

2.6 Particle Size and Zeta-Potential

Lite sizer DLS (Anton Paar) was employed to determine the oat milk sample's average particle size at 25°C. Zeta sizer Nano SZ (Malvern, Worcestershire, United Kingdom) was used to measure the samples' zeta potentials at 25°C. All the oat milk samples were diluted in Milli Q water and homogenized before the analysis.

2.7 Optimization of the Process Parameters for Oat Milk Production

The standardization or optimization of the process parameters was achieved by considering the desirability of the oat milk. The oat milk prepared by an optimized process was compared to raw oat milk that was made without any enzymatic treatment. The comparison of these two milks may provide insights into the structural alterations that occur in starch as a result of enzymatic treatment.

2.8 X-ray Diffractometer

At a wavelength of 0.154 nm, the X-ray diffractometer (PANalytical, the Netherlands) was used to record the starch patterns of raw and optimized oat milk samples. The following formula was used to determine the relative crystallinity (RC) of starch:

$$RC\ (\%) = Area\ under\ peaks/Total\ area \times 100 \quad (1)$$

2.9 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectrophotometer (SHIMADZU, IRTRACER 100, Japan) was used to acquire the infrared spectra of samples of raw oat milk and optimized oat milk, having a 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.10 Thermal Properties

Thermal analyses of both raw and optimized oat milk were conducted using a differential scanning calorimeter (DSC) (NETZSCH, Germany). The onset temperature (To), peak temperature (TP), conclusion temperature (TC), and gelatinization enthalpy (ΔH) of starches were studied. The optimized oat milk was compared to raw oat milk that was made without any enzymatic treatment. The comparison of these two milks may provide insights into the structural alterations that occur in starch as a result of enzymatic treatment.

3. RESULTS AND DISCUSSION

3.1 Box–Behnken Design

The physical and rheological properties of oat milk are shown in Table 1 for all 17 runs. Statistical analysis was performed on all the responses using the Design Expert, Version 13.0.5 software (Stat-Ease Corporation, USA). The data obtained from the tests was analyzed to optimize the method. An ANOVA (analysis of variance) was conducted to assess the impacts and regression coefficients of individual quadratic, linear, cubic, and reduced cubic interaction factors. P-values were used to statistically assess each term's significance up to a 5% significance level. The model terms are considered significant when P-values are less than 0.05. The model terms are not significant if the value is greater than 0.1. The coefficient of determination (R^2) was used to determine the quality of fit of the model. The reduced cubic model was chosen after analyzing the data and prioritizing the model that maximizes the adjusted R^2 and the predicted R^2. Adequate precision quantifies the ratio of the signal strength to the noise ratio. A ratio over 4 is preferable. All the responses had a ratio that suggested a signal that was more than 4, which is considered sufficient.

3.1.1 Yield

One crucial factor in large-scale production is the oat milk yield. Since a higher yield is preferred for industrial production, the modeling constraint was set to the maximum yield. The minimum and maximum yields of oat milk were 68.38 ± 0.23 and 85.72 ± 0.32%, respectively [Table 1]. The determination coefficient R2 value is 0.9923 [Table 2]. For yield, *X1, X2, X3, X1X2, X2X3, X3², X1²X3, X1X2²* terms are significant model terms [Table 2]. Increasing the concentration of oats to get a higher yield is obvious and evident in this context. The slurry concentration (X1) has significant adverse effects on the yield due to its reduction in the available surface area for enzyme–starch molecule interactions. The oat milk output is strongly influenced by the concentration (*X2*) and time (*X3*) of the enzyme. The correlation between the slurry (*X1*) and enzyme concentration (*X2*) strongly

Table 1: The data represents the responses to the optimization process of oat milk production using the Box–Behnken model.

Run	Factor 1 A: Oat Slurry Concentration (*X1*)	Factor 2 B: Enzyme Concentration (*X2*)	Factor 3 C: Time (*X3*)	Response 1 Yield (*Y1*)	Response 2 Viscosity (*Y2*)	Response 3 Total Soluble Solids (*Y3*)	Response 4 Total Solids (*Y4*)	Response 5 Stability (Zeta-potential) (*Y5*)	Response 6 Particle Size (*Y6*)
	(%) w/w	(%) v/w	minutes	% (w/w)	m.Pa.s	°Brix.	% (w/w)	mV	Nm
1	15	2	30	69.58 ± 0.2	37.7 ± 0.21	10.8 ± 0.01	12 ± 0.22	−6.5 ± 0.01	188.82 ± 0.9
2	15	10	30	68.38 ± 0.23	33.5 ± 0.2	11.2 ± 0.02	12.6 ± 0.23	−12.33 ± 0.01	220.1 ± 1.2
3	10	6	15	83.43 ± 0.3	13.53 ± 0.1	7.3 ± 0.01	10 ± 0.27	−11 ± 0.04	187.22 ± 1.9
4	12.5	6	30	75.25 ± 0.36	23.7 ± 0.15	9.8 ± 0.021	10.4 ± 0.46	−14.13 ± 0.08	195.7 ± 0.88
5	12.5	2	15	72.92 ± 0.20	66.24 ± 0.24	9.7 ± 0.019	19.38 ± 0.31	−14.15 ± 0.01	183.03 ± 1.6
6	12.5	10	15	73.23 ± 0.34	22.21 ± 0.32	9.8 ± 0.02	11.2 ± 0.21	−10.3 ± 0.32	181.07 ± 2.0
7	12.5	2	45	75.1 ± 0.25	37.18 ± 0.52	9.9 ± 0.01	11.2 ± 0.53	−11 ± 0.09	250.4 ± 1.3
8	15	6	45	71.36 ± 0.21	69.1 ± 0.68	11.3 ± 0.03	7 ± 0.51	−14.65 ± 0.04	183.23 ± 1.0
9	10	2	30	79.33 ± 0.12	23.55 ± 0.19	7.3 ± 0.02	9.6 ± 0.34	−9.23 ± 0.36	250 ± 2.2
10	12.5	6	30	74.02 ± 0.35	25.52 ± 0.49	8.9 ± 0.01	11.2 ± 0.27	−14 ± 0.04	190.3 ± 2.1
11	12.5	6	30	72.99 ± 0.34	21.23 ± 0.41	9.3 ± 0.02	10.31 ± 0.35	−13.2 ± 0.21	185.02 ± 0.9
12	12.5	10	45	85 ± 0.41	18.7 ± 0.85	9 ± 0.01	13.5 ± 0.26	−15.6 ± 0.8	360.5 ± 2.3
13	10	6	45	78.3 ± 0.37	44.9 ± 0.28	7.8 ± 0.021	9.8 ± 0.19	−12.02 ± 0.021	175.54 ± 0.6
14	10	10	30	85.72 ± 0.32	38.26 ± 0.10	6.1 ± 0.02	6.4 ± 0.34	−12.23 ± 0.021	170.89 ± 0.62
15	12.5	6	30	73.25 ± 0.36	25.68 ± 0.38	9.3 ± 0.03	9.8 ± 0.23	−15.53 ± 0.022	189.63 ± 72
16	12.5	6	30	74.23 ± 0.28	30.12 ± 0.34	9.5 ± 0.03	9.8 ± 0.21	−14 ± 0.012	192.34 ± 31
17	15	6	15	74.02 ± 0.19	58.74 ± 0.8	11.5 ± 0.02	12.8 ± 0.1	−14.92 ± 0.31	190.52 ± 0.77

inhibits the yield. The correlation between *X2* and *X3*, as well as *X1* and *X3*, has a beneficial impact on the yield. However, only the interaction between *X2* and *X3* is statistically significant.

The yield is influenced by the concentration of the enzymes and the oat slurry when they are combined [Figures 1A and 1B]. It is observed that increasing the duration of the reaction and the amount of enzyme present results in greater production. The surge in dextrins following starch liquefaction is the main factor behind the enhanced yield seen upon increasing the enzyme concentration. These dextrin molecules may be filtered easily and are less heavy than starch molecules. Extended reaction periods lead to an increased yield of oat milk by generating a greater quantity of dextrin molecules [31,32]. Deswal et al. found that the extraction yield of oat milk generated by starch liquefaction with α-amylase treatments varied between 53.92% and 78.87% [32]. According to Salama et al., the various organic matter treatments resulted in yields ranging from 87.6% to 91.25% [33]. Also, the oat milk production obtained from various treatments, such as individual and combination acid, alkaline, α-amylase, and sprouting treatments, varied between 82.73% and 91.70% [34]. The results of this study demonstrate a substantial increase in yield after enzymatic treatment, thereby supporting prior research findings. While milk yield is an essential characteristic, it alone is not an adequate criterion for determining the quality of milk. The confirmation of a quality product cannot be just determined by the greatest yield. Therefore, it is crucial to examine additional physical factors to get the desired product.

3.1.2 Viscosity

The viscosity of the oat milk ranged from 13.53 ± 0.01 to 69.1 ± 0.68 m.Pa.s [Table 1], which is due to the gelatinization of its starch content. The amount of dietary fiber in oat milk is normally rather low, with around 0.8 g of dietary fiber per 100 mL in commercial goods. This is because β-glucan, which is responsible for increasing the thickness of the product, causes a significant portion of the dietary fiber to either break down or be removed during filtration [31]. The high viscosity of oat milk poses a significant challenge for many product development endeavors, making it both undesirable and problematic. In the context of the viscosity of oat milk, *X1, X2, X3, X1X2, X1X3, X2X3, X1², X3², X1²X2, X1²X3, X1X2²* are significant model terms. The concentration of oat slurry (*X1*), concentration of enzyme (*X2*), and time (*X3*) were significant process parameters to regulate the viscosity (*Y2*). The viscosity was significantly controlled by the oat slurry concentration, whereas the enzyme concentration and time had a significant negative effect on viscosity [Table 2].

The correlation between the concentration of slurry and enzyme has a substantial impact on reducing the viscosity of oat milk, resulting in a negative effect. The relationship between the concentration of the enzyme (*X2*) and the duration of time (*X3*) has a considerable positive influence on the viscosity (*Y2*), as seen in Figures 1C and 1D and Figure 2C. The interaction first decreases viscosity to a certain level, but as time and concentration increase, it subsequently increases. The quadratic relationship between *X1* and *X3* has a positive and statistically significant impact on viscosity.

Various products, including oat milk, exhibited an increase in yield acceleration and a decrease in viscosity. This effect mostly arises from the enzymatic breakdown of the starch present in the oat flour [35,36]. The viscosity measurements of many samples of oat milk exhibited substantial variability. The viscosity of commercial oat milk was determined to be 6.8 mPa·s when subjected to a shear rate of 10 1/s [37]. The consistency index of oat milk preparation following starch liquefaction varied from 10.22 to 1.03 Pa.s [32]. In another investigation, the viscosity data showed values within the range of

Table 2: ANOVA results for yield, viscosity, total soluble solids, total solids, zeta-potential, and particle size for each response variable in the optimization process.

Variation Source	Yield *Y1* (% w/w)		Viscosity *Y2* (m.Pa.s)		Total Soluble Solids *Y3* (°Brix.)		Total Solids *Y4* (% w/w)		Zeta-potential *Y5* (mV)		Particle Size *Y6* (nm)	
	p-value (prob>F)	Sum of Squares	*p-value* (prob>F)	Sum of Squares	*p-value* (prob>F)	Sum of Squares	*p-value* (prob>F)	Sum of Squares	*p-value* (prob>F)	Sum of Squares	*p-value* (prob>F)	Sum of Squares
Model	0.0012	408.78	0.0019	4320.36	0.0028	36.06	0.0021	127.35	0.0169	92.85	<0.0001	33874.64
X1	0.0008	66.83	0.0004	1204.44	0.0003	14.82	1.0000	1.421E−14	0.0178	10.73	0.2328	30.20
X2	0.0046	26.06	0.0007	976.88	0.2904	0.1600	0.0069	8.64	0.6798	0.1406	0.0002	2923.56
X3	0.0014	48.65	0.0075	265.20	0.4130	0.0900	0.0069	8.64	0.2718	1.16	<0.0001	15227.56
X1X2	0.0131	14.40	0.0441	89.40	0.0716	0.6400	0.0297	3.61	0.1690	2.00	0.0001	3046.49
X1X3	0.2386	1.53	0.0322	110.36	0.3469	0.1225	0.0082	7.84	0.4874	0.4160	0.6046	4.82
X2X3	0.0058	22.99	0.0173	163.20	0.2028	0.2500	0.0008	27.46	0.0075	17.85	0.0001	3139.36
$X1^2$	0.0810	4.29	0.0043	359.78	0.1185	0.4244	0.0019	17.45	0.0107	14.52	0.0004	1925.37
$X2^2$	0.1416	2.66	0.4784	6.49	0.2957	0.1560	0.0025	14.95	0.0055	21.17	<0.0001	6156.56
$X3^2$	0.0139	13.94	0.0016	613.79	0.0541	0.7876	0.0043	11.24	0.1129	2.92	0.0014	936.47
$X1^2X2$	0.1176	3.15	0.0014	666.49	1.0000	0.0000	0.1137	1.34	0.0277	8.16	0.0001	3040.83
$X1^2X3$	0.0010	59.08	0.0013	690.06	0.3878	0.1013	0.9447	0.0018	0.5891	0.2450	<0.0001	8829.21
$X1X2^2$	0.0131	14.42	0.0029	450.30	0.3878	0.1013	0.0061	9.24	0.0184	10.53	0.1066	65.90
R^2	0.9923		0.9903		0.9882		0.9897		0.9702		0.9982	
Adjusted R^2	0.9691		0.9610		0.9526		0.9590		0.8809		0.9928	
Adeq precision	22.2221		19.4866		18.7903		25.8379		12.3285		55.4308	

6.0–19.1 mPa·s [38]. The enzymatic treatment decreases the viscosity of oat milk, which is desirable for further processing and product development since a lower viscosity is preferred. Oat milk with a high viscosity provides a creamy mouthfeel without the need for thickeners or additives. Nevertheless, the oat milk has a significantly increased thickness, making it challenging to pour. This presents a significant problem in industrial environments, especially during the milk pumping procedure for further processing. Therefore, the constraints were established to limit the viscosity of oat milk.

3.1.3 Total Soluble Solids

TSS are a measure of the concentration of dissolved substances in a sample. The TSS in the experiment ranged from 6.1 ± 0.02 to 11.56.1 ± 0.05 °Brix. The only significant term for TSS is the slurry concentration of oats [Table 2], which positively influences the TSS. The interaction between oat slurry (*X1*) and enzyme concentration also positively controls the TSS but does not have a significant effect [Figure 2B].

The TSS of milk, as well as plant-based milk, is essential to the production of evaporated goods such as milk powder and condensed milk. Several production parameters and the raw material's soluble solid content are directly correlated. In particular, as the production yield rises, the evaporation time reduces, and the manufacturing costs fall with an increase in the soluble solid concentration [39]. The presence of soluble solid material in *dulce de leche* (evaporated milk) helps in managing the production process, identifying the manufacturing completion point, ensuring consistent product quality, and prolonging its shelf life [40]. The mean TSS of raw milk and high-pressure pasteurized milk was 10.02 ± 0.2 °Brix [41]. The TSS in the development of a new plant-based milk employing various combinations of coconut and chickpea changed from 1.77 to 4.04 °Brix. The TSS of the product decreased in proportion to the increase in the quantity of coconut milk used in the formulation [42]. The TSS range was observed in the range 4–5 °Brix and 10 °Brix for broken rice [43] and soy–cow milk [44], respectively. There is due to scarcity of research on the TSS of oat milk. Upon evaluating the TSS composition of several types of milk, including both dairy and plant-based alternatives, it can be inferred that oat milk is within the acceptable TSS limit. This is necessary to maintain the TSS content in different dairy products, including frozen desserts. Increased TSS levels will facilitate the production of oat milk-based flavored beverages and frozen dessert alternatives to evaporated milk.

3.1.4 Total Solids

The quantity of total solids is important since it has a direct effect on the prepared product's final quality [45]. In this case, *X2, X3, X1X2, X1X3, X2X3, X1², X2², X3², X1X2²* are significant model terms. The enzyme concentration (*X2*) and time (*X3*) significantly regulate the total solids in the oat milk [Figure 2A]. The interaction between *X1, X2* and *X2, X3* positively regulates the total solids in the oat milk. The interaction between *X1* and *X3* negatively regulates the total solids in the oat milk. The total solids in oat milk ranged from 6.4 ± 0.34 to 19.38 ± 0.31%w/w. Minimum reaction time and minimum enzyme concentration resulted in maximum total solids. More reaction time and enzyme concentration are responsible for starch hydrolysis,

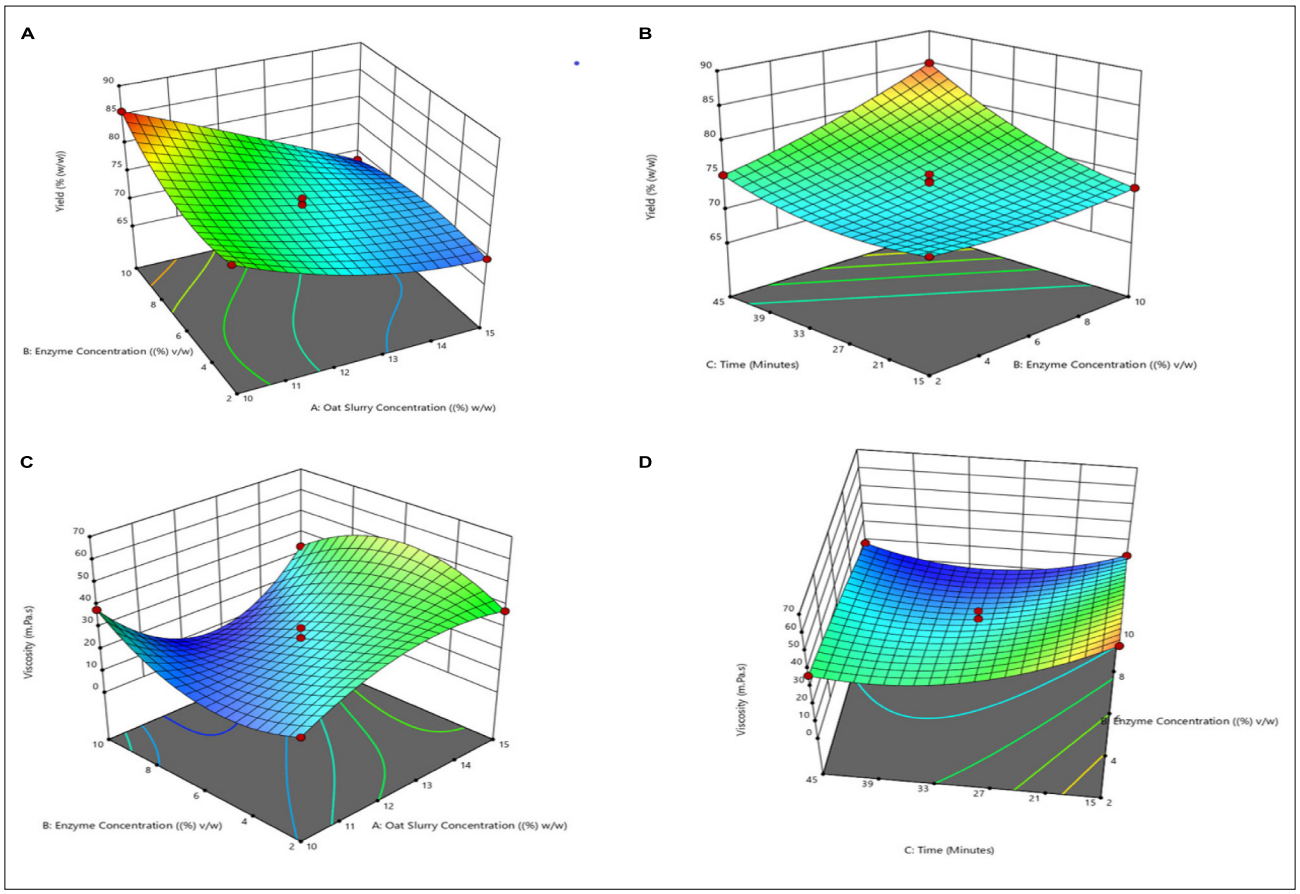

Figure 1: (A) Effect of enzyme concentration and slurry concentration on yield %; **(B)** effect of enzyme concentration and time on yield %; **(C)** effect of enzyme concentration and slurry concentration on viscosity; and **(D)** effect of enzyme concentration and time on viscosity.

which ultimately reduces the total solids by converting larger starch molecules into dextrins.

The total solid content in plant-based milk derived from almond milk and soy milk ranged from 8.11% to 27.96% [46]. The total solids in the oat milk were 25.01 ± 0.15. The study also found that increasing slurry concentration in oat milk increases solids, leading to challenges in manufacturing and increased raw material costs. Lower slurry concentrations can achieve enzyme saturation levels, while higher concentrations make filtering more challenging. Optimizing independent variables is crucial for achieving desired solid levels [32].

3.1.5 Stability (Zeta-Potential or ζ Potential)

The electrical potential difference between the stationary layer of the dispersion and the mobile dispersion medium is known as the ζ-potential. The medium's adhesion to the dispersed particle indicates the physical stability of the result [47]. A ζ-potential more than 60 mV, whether positive or negative, indicates excellent stability of the solution or emulsion. A value less than 20 mV indicates moderate stability, whereas a value less than 5 mV leads to aggregation and coagulation [48]. In this case, *X1, X2X3, X1², X2², X1²X2, X1X2²* are significant model terms. The stability in terms of zeta-potential of the oat milk is negatively influenced by the interaction between enzyme concentration (*X2*) and time (*X3*). The slurry concentration is also responsible for the instability of the oat milk. The stability of oat milk ranged from −6.5 ± 0.01 to −15.53 ± 0.022 mV, which gradually improved after increasing the enzyme concentration and time.

The coconut drink had a ζ-potential of −16 mV [49], whereas the almond and hazelnut beverages displayed ζ-potentials of −18 mV and −22 mV, respectively [50]. With the use of α-amylase (0.3 mL/100 g grains), glycosylase (0.5 mL/100 g grains), and β-glucanase (0.05 mL/100 g grains), the ζ-potential of oat milk was determined in the study to be −31.93 ± 0.74 mV. A minute of 5000 rpm oat milk dispersion was followed by two cycles of 45 MPa high-pressure homogenization [51]. The stability of this plant-based milk is comparable to other plant-based milk. However, by using various kinds and combinations of enzymes, the zeta-potential of oat milk may be enhanced. The stability of oat milk was significantly increased by chemical processes such as enzymatic treatment and mechanical processing techniques including homogenization.

The kind of plant used to prepare vegan milk is crucial, as is the choice of processing using machinery. The stability of milk from plants and animals that had been exposed to commercial ultrahigh temperature (UHT) was compared, and the results showed that oat milk was less stable than soy and bovine milk but more stable than rice milk. The manufacturing method may employ high-intensity ultrasound irradiation (UHPH), ultrahigh-pressure homogenization, and ultrasonic homogenization to overcome the stability issue [52,53]. The research conducted by [54] found that the ultrasound treatment increased the ζ-potential electronegativity of pure coconut milk from −0.446 mV to −9.396 mV due to cavitation caused by ultrasonic bubbles. This led to the dissociation of water molecules and the production of hydrogen, hydroxyl radicals, and free radicals. The electronegativity of the

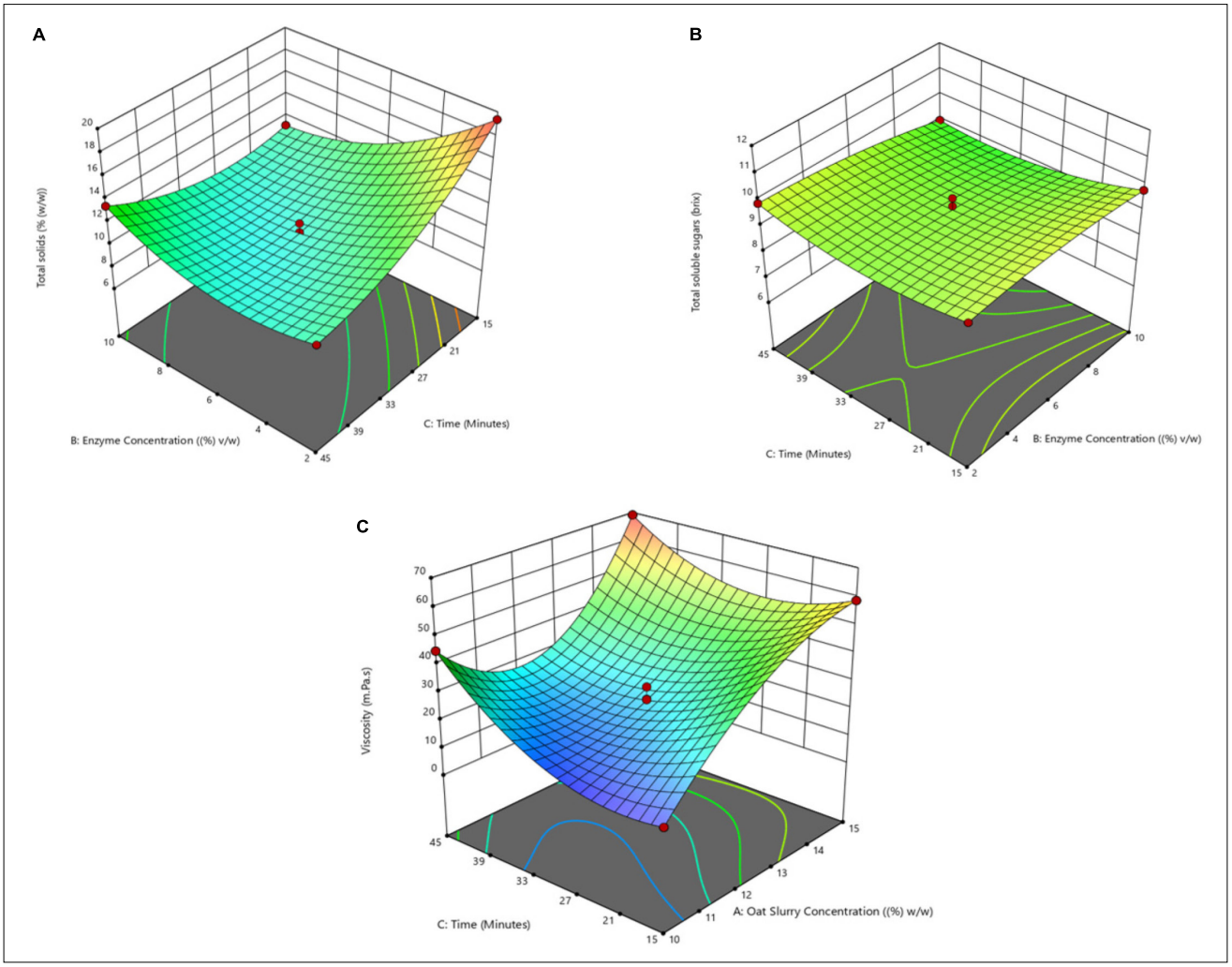

Figure 2: (A) Effect of amylase enzyme concentration and time on total solids; **(B)** effect of amylase enzyme concentration and time on total soluble solids; and **(C)** effect of oat slurry concentration and time on viscosity.

composite system of maize kernel and coconut milk was measured to be −34.573 mV, while the system with high-amylose maize starch and coconut milk had an electronegativity of −26.286 mV. These values showed significant variation, resulting in enhanced system stability. The possible reason for the alteration in stability might be the transformation of the starch granule's surface proteins from being hydrophilic to hydrophobic during heat treatment [55]. In the future, methods such as ultrasonic homogenization, ultrahigh-pressure homogenization, and high-intensity ultrasound irradiation could be combined with the usage of α-amylase, glycosylase, and β-glucanase to produce more stable oat milk. The nutritional profile of the oat milk can be preserved by using nonthermal processing techniques.

3.1.6 Particle Size

Previously, a distribution with two distinct modes has been documented in plant-based beverages. The system may become more sedimented as a result of the larger particles. Typically, it is preferable to have a smaller particle size and dispersion to achieve stable plant-based drinks. The separation of oat milk based on density is impacted not only by particle size, but also by factors such as density, viscosity, and gravity in the continuous phase [52,56,57] The sedimentation process may be elucidated by using Stokes' law, which states that unsound samples will exhibit a larger particle size, resulting in a higher sedimentation rate due to gravity compared to sonicated samples with a smaller particle size during storage [58]. Even a slight reduction in sedimentation is a significant accomplishment for a representative sample of plant-based milk on the market.

In this case, *X2, X3, X1X2, X2X3, X1², X2², X3², X1²X2, X1²X3* are significant model terms [Table 2]. Notably, 170.89 ± 0.62 nm and 360.5 ± 2.3 nm are the smallest and largest particle sizes observed in the oat milk. The interaction between enzyme concentration (*X2*) and time (*X3*) helped to reduce the particle size in the formulation. An irregular pattern was observed in the interaction between oat slurry concentration (*X1*) and the enzyme concentration (*X2*). To reduce sedimentation and improve stability, the smallest particle size was set in desirability for optimization.

The mean particle size of the oat milk obtained from different cultivars was in the range of 0.48 ± 0.009 µm to 0.54 ± 0.02 µm achieved using homogenization. The average size of the particles was smaller than that of UHT milk, which was measured at 2.18 ± 0.02 µm. At 147.41 ± 4.001 µm, the commercially available oat milk had the largest particle size [51]. When compared to untreated milk, peanut milk treated with ultrasound at various power levels (200 W, 300 W, and 400 W)

considerably decreased in particle size. The untreated and sonicated samples did not significantly differ in particle size, according to the study. At 300 W and 400 W, there was a significant size reduction, yielding values of 0.21 ± 0.02 µm and 0.02 µm, respectively [59]. The particle size of the oat milk in this study was attained without any processing, such as homogenization. The reduction of starch in the form of dextrins was mostly facilitated by the enzymatic treatment, which contributed to the particle size of the oat milk. The stability will be improved by reducing the particle size after using other processing techniques such as UHT or homogenization.

3.1.7 Process Parameter Optimization

By projecting contour plots of response data, it was possible to determine the ideal process parameters and pinpoint locations where actual operating conditions were almost perfect. The desirability function was employed to amalgamate multiple responses into a singular outcome, determined by assigning a value ranging from 0 (representing unfavorable product characteristics) to 1 (representing all product attributes falling within the desired range) [60]. This approach is appealing due to its simplicity and innate comprehension. The inputs include mean response estimates, goal value, as well as lower and upper bounds of acceptability. The individual desirability is determined by computing the geometric mean. The desirability function technique utilizes mathematical modifications to transform a problem with several responses into a problem with a single, ideal answer.

The optimization was carried out with a technique known as desirability, which involves considering numerous responses. This optimization approach integrates preferences and priorities for each variable, establishing a mechanism to define the correlation between expected answers on a dependent variable and the attractiveness of such responses. The results demonstrate that oat milk production was not dependent on a single independent factor. Instead, the optimization of the process parameters of oat milk production was influenced by the interplay of many variables. The constraint selection in the Box–Behnken model facilitates the selection of an optimized formulation that yields the desired outcomes. The major aim of this study is to optimize the manufacturing process of oat milk while minimizing its viscosity. Maximum stability of oat milk is required, namely in terms of zeta-potential. The procedure was ultimately optimized by taking into account these limiting circumstances.

The optimization led to the following projected values for the responses: 13.17% (w/w) oat slurry concentration, 9.9% (w/w) enzyme concentration, and a time of 45.0 min. In total, 0.92 was the calculated desirability for the formulation of oat milk. The suitability of the model equations in estimating the optimal response values was assessed by using a 13.17% (w/w) concentration of oat slurry, a 9.9% (w/w) concentration of enzymes, and a duration of 45.0 min. The RSM optimization technique using Box–Behnken design found that this combination of factors is optimal. It was then empirically validated and utilized to forecast the values of the responses using the model equation. The findings demonstrated that the observed values corresponded with the anticipated value [Table 3].

Table 3: Predicted value and actual value of the responses after optimization of process parameters for oat milk.

Responses	Predicted Value	Experimental Value
Yield (%)	82.41	81.32 ± 0.23
Viscosity (m.Pa.s)	19.99	20.12 ± 0.18
Total soluble solids (°Brix.)	9.634	9.26 ± 0.09
Total solids (%)	13.86	14.02 ± 0.52
Zeta-potential (mV)	15.51	16.01 ± 0.96
Particle size (nm)	356.202	396.23 ± 9.66

3.1.8 X-ray Diffraction

The irreversible physical changes that happen to starch when it is heated in water are referred to as "gelatinization". These alterations include granular expansion, melting of crystallites, disruption and loss of molecular order, and solubilization of starch. Cereals and tuber starch have different X-ray diffraction patterns. The two X-ray diffraction pattern types are associated with the two polymorph forms, the A-type in grain starches. The partially crystalline and amorphous parts of the granules are composed of amylose and amylopectin. The short chains of amylopectin arranged in crystallites are thought to be the cause of the native granule's crystallinity [14].

Cereal starches have a type of crystalline structure, which is characterized by a more closely packed, stable monoclinic structure. Peaks at 20° are typical V-type pattern peaks that show the creation of an amylose–lipid complex and the transition of enzyme-modified starches from an A-type to an A + V-type crystallinity pattern. Typically, an amylose–lipid combination strengthens starches' defenses against attacks by digestive enzymes [61]. The study's findings, as depicted in Figure 3, are comparable. The X-ray pattern of untreated raw oat starches displayed an A-type pattern, characterized by significant reflection peaks at 20°, and 33° of 2θ angles, as well as a peak of lesser strength. The starches from oat milk that had been enzyme-modified formed a peak at 20° and a significantly sharper peak at 33° [Figure 3]. Peaks at 20° are representative of the V-type pattern and show the creation of the amylose–lipid complex. They also show the shift in the crystallinity pattern of enzyme-modified starches from A type to A + V type. The RC increased after enzymatic treatment from 40.56% to 47.65%. Through enzymatic hydrolysis, the oat milk that had been enzyme-treated generated a significant amount of amylose and subsequently created a huge number of new double-helix structures. Additionally, the polymer's structure was tighter, and there were more crystals and more crystallinity overall [62]. The improved RC suggests that the enzymatic hydrolysis process may encourage amylose to reorganize in both non-crystalline and crystalline regions, resulting in a more stable structure of oat starch, which is crystalline in nature.

3.1.9 FTIR

Resistance starch's short-range order and molecular structure may be investigated using FTIR spectroscopy, which can identify the primary functional group features through stretching, bending, and deformation [62]. FTIR analysis of the raw and enzymatically treated oat milk revealed structural differences. Raw oats and enzyme-modified oat milk both showed absorption peaks in their FTIR spectra at 3300 cm^{-1}. These are identified as stretching–absorption bands of hydrophilic hydroxyl groups (–OH). An intense peak at 2929 cm^{-1} is associated with the $–CH_2$ functional group's stretching vibrations.

RC (%) = relative crystallinity.

The functional groups COO are linked to the absorption peak at 1600 cm^{-1} [Figure 3]. When the oat starch was treated with enzymes, the absorption peaks at 2929 and 1600 cm^{-1} changed to lower wave numbers, indicating that the strength of the hydrogen bonding between the starch molecules had decreased. Moreover, the crystalline portions

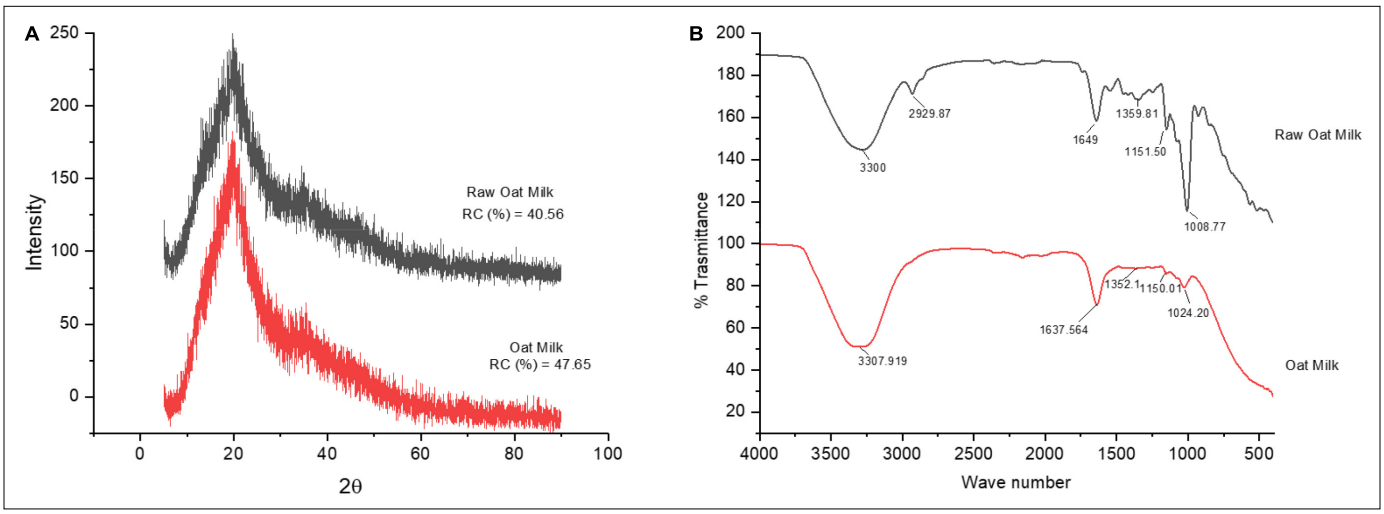

Figure 3: (A) XRD pattern of representative raw oat milk and enzymatically treated oat milk; **(B)** FTIR spectra of representative raw oat milk and enzymatically treated oat milk.

Table 4: DSC data table—Thermal properties of oat samples.

Samples	T_o (°C)	T_p (°C)	T_c (°C)	T_c–T_o (°C)	ΔH (J/g)
Raw oat milk	76.0 ± 0.02	84.5 ± 0.03	127.2 ± 0.03	51.2 ± 0.02	19.84 ± 0.23
Oat milk	35.3 ± 0.03	78.5 ± 0.04	131.5 ± 0.02	96.2 ± 0.03	258.9 ± 0.19

of the starch are seen in bands at 1008.77 cm^{-1}, and an amorphous zone is indicated by the absorption peak at 1024.20 cm^{-1} [63]. The alteration in the peak at 1022 cm^{-1}, which results in the amorphous region's deterioration prioritizing or degrading more quickly, is due to the loss of the original crystal arrangement mode during sample preparation by enzymatic treatment.

3.1.10 Thermal Properties

The crystalline portions within the starch granule melt, causing the X-ray diffraction pattern and the starch granules' birefringence seen under a polarizing microscope to vanish. A simple method for assessing the degree of starch gelatinization is DSC. The primary cause of the gelatinization enthalpy is the starch granules' loss of molecular order [64]. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH) of the enzymatically treated and raw oat milk are shown in the DSC data [Table 4]. The primary cause of the gelatinization enthalpy is the starch granules' loss of molecular order. In comparison to the oat milk that had been enzyme-treated (258.9 J/g), the raw oat milk starch had a reduced gelatinization enthalpy (19.84 J/g). The stability and uniformity of the microcrystals in starch are represented by T_c–T_o. A wider range of temperatures for gelatinization implies a higher concentration of microcrystals within the crystalline portion of starch [65]. In this study, T_c–T_o has increased, which supports the previous studies of Xia et al. [62] that suggested that the modest amount of free fatty acids produced during the hydrolysis phase delayed the subsequent gelatinization and granule swelling. Monoglycerides were found to prevent starch from gelatinizing in previous investigations [14]. The melting of the double-helix structure is reflected in the gelatinization enthalpy or ΔH. The enthalpy value of oat milk that had been enzyme-treated rose as a result of an increase in the number of double-helix structures and an increase in the energy required to melt them. Greater thermal stability, tighter packing, and more double helices were all suggested by higher enthalpy values. This outcome was further supported by the high crystallinity of oat milk that had been enzyme-treated.

Shah et al. enzymatically hydrolyzed oat starch twice, yielding a modified starch with a low ΔH value (11.06 J/g). The continuous enzymatic digestion by the two enzymes may have led to an increase in the fraction of short amylopectin chains and a decrease in their length, reducing the number of double helixes and lowering the energy required to melt the crystal structure [66].

4. CONCLUSION

The current investigation involved the production of oat milk substitutes through enzymatic processing using alpha-amylase. The process parameters that were optimized utilizing the Box–Behnken model of RSM included a 13.17% (w/w) concentration of oat slurry, a 9.9% (w/w) concentration of enzymes, and a period of 45.0 min. The process was optimized by taking into account many factors such as yield percentage, viscosity, TSS, total solids, zeta-potential, and particle size. The optimized oat milk, which was treated with enzymes, was compared to the raw oat milk prepared using traditional methods.

An analysis was conducted to examine the physicochemical attributes and structural aspects of the subject. Out of all the options, oat milk that underwent enzymatic treatment had the greatest RC and the best organized and enduring double-helix structure.

Simultaneously, the thermal stability of oat milk treated with enzymes was also greatly enhanced. The enhanced RC indicates that the enzymatic hydrolysis process promotes the reorganization of amylose in both crystalline and non-crystalline areas, leading to a more stable crystalline structure of oat starch. The gelatinization enthalpy, or ΔH, indicates the dissolution of the double-helix structure. After being treated with enzymes, the enthalpy of oat milk rose because more double-helix structures were formed and more energy was required to melt them. Greater thermal stability, tighter packing, and a higher quantity of double helices were all indicated by higher enthalpy values. The notable crystallinity in the oat milk treated with enzymes, which was directly linked to the structural characteristics of resistant starch,

further supported this conclusion. The developed alternative to oat milk can be used to make baked goods, frozen desserts, and beverages.

5. ABBREVIATIONS

ANOVA: Analysis of variance. DE: Dextrose equivalent. DSC: Differential scanning calorimetry. FTIR: Fourier transform infrared spectroscopy. RC: Relative crystallinity. RSM: Response surface methodology. TSS: Total soluble solids. UHPH: High-intensity ultrasound irradiation. UHT: Ultrahigh Temperature. XRD: X-ray diffraction.

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7. AUTHORS' CONTRIBUTIONS

The authors have been involved in the planning, implementation, data collecting, and interpretation of the study, as well as in the writing and editing of the article to uphold its intellectual integrity. They have consented to submit the manuscript to the journal and have assumed accountability for all facets.

8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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