JTE HCMUTE
ISSN: 2615-9740
JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

Effect of Hydrolyzed Lima Bean Protein on the Quality of Low - Fat Ice Cream Products

Huu Khang Nguyen, Hoan Pham-Thi*
Ho Chi Minh City University of Technology and Education, Vietnam
*Corresponding author. *Email:* hoanpt@hcmute.edu.vn

ARTICLE INFO

Received:	16/10/2024
Revised:	06/11/2024
Accepted:	08/11/2024
Published:	28/05/2025

KEYWORDS

Ice cream;
Protein hydrolysis;
Protein;
Phaseolus lunatus L.;
Alcalase.

ABSTRACT

In this study, the possibility of replacing skim milk powder with hydrolyzed lima bean (*Phaseolus lunatus* L.) protein by alcalase (APPC) in low-fat ice cream formulations was evaluated at replacement levels of 0, 6, 12, 18, 24 and 30%. Increasing APPC substitution resulted in higher protein content (3.96–4.81%) and reduced carbohydrate (25.21–22.94%) and fat (2.20–1.72%) levels. APPC addition positively affected the physical properties by enhancing overrun and melting resistance while decreasing hardness. Sensory evaluations showed that substitutions between 6% and 18% did not significantly affect the ice cream's sensory attributes, whereas higher substitutions (24–30%) introduced mild bitterness and reduced sensory quality. Moreover, substituting skimmed milk powder with APPC improved ice cream stability by decreasing bubble size and promoting even air distribution. The 18% APPC substitution provided the best balance of nutritional and sensory properties, indicating that APPC is suitable for large-scale low-fat ice cream production and can contribute to product diversification in the market.

Doi: https://doi.org/10.54644/jte.2025.1694

1. Introduction

Ice cream is a widely consumed frozen dairy product favored by all age groups, especially during hot weather. However, its high sugar and fat content can contribute to health issues like diabetes and obesity [1]. This has led to increasing consumer demand for low-fat ice cream alternatives to reduce caloric intake and associated health risks [2], [3]. In response, researchers have focused on developing fat substitutes using lipid-based, protein-based, and carbohydrate-based alternatives to lower fat content by replacing traditional ingredients with lower-calorie components [4], [5].

Legumes are a promising plant-based protein source, rich in essential amino acids and offering health benefits such as reduced cholesterol and lower risks of certain cancers and heart diseases [6]. Their cost-effectiveness and functional properties make them attractive for use in food products, including ice cream. Hydrolyzed legume proteins using Alcalase®, enhance their functional properties, such as solubility and emulsification, while producing bioactive peptides with additional health benefits [7].

Among legumes, lima bean (*Phaseolus lunatus* L.) is rich in easily digestible protein but is underutilized in food products [8]. This study explores the use of alcalase-hydrolyzed lima bean protein (APPC) as a partial replacement for skimmed milk powder in low-fat vanilla ice cream. The effects of APPC on the ice cream's nutritional, physical, and sensory properties were evaluated to create a healthier product without compromising quality.

2. Materials and Methods

2.1. Materials

The lima bean protein concentrate was extracted from lima beans following the method of Pham et al. with some modifications [9]. The obtained protein concentrate powder (PPC) contained 65.4% protein, 0.8% fat, 7.1% ash, and 5.41% moisture.

Alcalase 2.5 LPF is a hydrolytic enzyme from Novozymes® (Denmark). Enzyme activity reached 2.5 AU/g, brown color with a density of 1.19 g/ml.

Skim milk powder from NZMP (New Zealand) contained 32.7% protein, 1.0% fat, 7.5% ash, and 3.9% moisture. Whipping cream (35% of fat) from Anchor Dairy (New Zealand), sugar from Bienhoa Pure (Vietnam), vanilla flavour (Rayner's, UK), emulsifier and stabilizer (STAB 2000 Louis FRANCOIS, France).

2.2. Hydrolyzed lima bean protein preparation

Hydrolyzed lima bean protein was obtained following the method of G. Zhao et al. [10] and Betabcur-Ancona et al. [11] with some modifications. First, lima bean protein concentrate powder (PPC) was dissolved in distilled water at a ratio of 2:100 (w:w). The suspension was gently stirred for 1 hour and adjusted to pH 8.0 using 1N NaOH. Alcalase (0.3 AU/g protein) was then added to initiate hydrolysis. The process was carried out at 50℃, with continuous shaking at 120 rpm for 45 minutes. Upon completion of hydrolysis, the alcalase was inactivated by heating the solution to 85℃ for 15 minutes. The hydrolysate was condensed using condensation system and then dried at 50℃ for about 5 hours until the moisture content of the protein powder reached below 8.6%. Finally, the hydrolyzed lima bean protein was ground and passed through a 200-mesh sieve, packaged in sealed containers, and stored in a dry environment. The obtained hydrolyzed protein powder (APPC) contained 59.2% protein, 0.8% fat, and 8.4% ash. The degree of hydrolysis was determined to be 36.85%.

2.3. Ice cream preparation

In this study, a portion of skim milk powder was replaced with hydrolyzed lima bean protein at substitution levels of 0, 6, 12, 18, 24, and 30% (w/w). The ice cream ingredients were accurately weighed and mixed according to the formulations in Table 1. The prepared mixture was pasteurized at 90°C for 15 minutes and then mechanically homogenized using an IKA T25 digital Ultraturrax® homogenizer (IKA, Germany) at 10,000 rpm for 5 minutes. The ice cream mix was aged at 5 ± 1°C for 24 hours. Subsequently, it was pre-frozen in a batch ice cream machine (Unold 48895, Germany) with continuous stirring and a machine temperature of -23 ± 3°C for approximately 45 ± 5 minutes, until the core temperature of the product reached -5 ± 1°C. Finally, the ice cream product was packaged in plastic containers and stored at -18 ± 2°C [12].

Table 1. *Formulation of ice cream production with substituted hydrolyzed lima bean protein*

Ingredient	Samples					
	DC	**APPC6**	**APPC12**	**APPC18**	**APPC24**	**APPC30**
Skim milk powder, g	11	10.34	9.68	9.02	8.36	7.70
Hydrolyzed lima bean protein (APPC), g	0	0.66	1.32	1.98	2.64	3.30
Protein substitution ratio, %	0	6	12	18	24	30
Whipping cream, g	4	4	4	4	4	4
Stabilizers and emulsifiers*, g	0.5	0.5	0.5	0.5	0.5	0.5
Sugar, g	18	18	18	18	18	18
Vanilla flavour, g	0.2	0.2	0.2	0.2	0.2	0.2
Water, g	66.3	66.3	66.3	66.3	66.3	66.3
Total, g	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

**STAB 2000 Louis FRANCOIS (France), includes Locust Bean Gum, Sodium Alginate, Carrageenans, Glycerol Monostearate.*

2.4. Analytical methods

Overrun (OR, %) of ice cream samples was determined by comparing the mass of the ice cream mixture after aging and the finished ice cream product at the same volume (30 mL) using Equation (1) [13]. The overrun was monitored at 1, 30, and 45 days of storage.

$$OR\ (\%) = \frac{(M_0 - M_f)}{M_f} \times 100 \qquad (1)$$

where M_0 (g) is the mass of the ice cream mixture after aging, and M_f (g) is the mass of the finished ice cream product at 1, 30, and 45 days of storage.

Hardness of ice cream was measured according to the method described by Kaleda et al. [6], with some modifications. Ice cream samples (2 × 2 × 1 cm) were removed from the freezer (-18 ± 2°C) immediately before testing. The hardness was measured using a CT3 Texture Analyzer (Brookfield, USA) equipped with a cylindrical probe TA-MTP 3R. The test parameters included a penetration depth of 0.5 mm, a trigger force of 100 mN, and a test speed of 2 $mm \cdot s^{-1}$.

Melting rate was evaluated at temperature of 25 ± 2 °C, using ice cream samples of fixed shape and mass (M_0, 30 ± 5 g), placed on a wire mesh with 1 × 1 cm openings. The following parameters were recorded: time to first drip, time to complete melting, and the mass of melted ice cream every 5 minutes (M_n). The melting rate is calculated using Equation (2) [14]:

$$MR(\%) = \frac{M_n}{M_0} \times 100 \qquad (2)$$

Rheological properties of ice cream samples were determined through two main characteristics: steady shear rheological properties and dynamic rheological behavior, following the method of Aloğlu et al. [14] with some modifications. The ice cream mixture after aging for 24 hours was subjected to rheological tests with a RheoStress600 rheometer (HAAKE, Germany) equipped with a C35/4° TiL probe (diameter 35mm, angle 4°).

For the steady shear rheological properties, tests were performed at shear rate range from 1 to 100 s^{-1} at 5°C. The flow behaviour of the ice cream samples was described using the Herschel–Bulkley model:

$$\tau = k \times (\dot{\gamma})^n \qquad (3)$$

Where k: consistency index, $\dot{\gamma}$: shear rate, s^{-1}; n: the flow index; τ: shear stress, Pa

For dynamic rheological behavior, frequency sweep tests were conducted at a constant stress of 0.1 Pa over a frequency range from 0.1 to 20 Hz at 5 °C. The elastic of storage modulus (G') and the viscous or loss modulus (G'') were recorded.

The microstructure of ice cream was analyzed using scanning electron microscopy (SEM) to compare the microstructural differences among the ice cream products. Ice cream samples (1 × 1 cm) were frozen at –45 °C for 24 hours and then freeze-dried using a Scanvac freeze dryer (Denmark) at 30 ± 2 °C under a pressure of 10–20 Pa for 36 hours. SEM images were captured using a Hitachi S-4800 FESEM (Japan) at an accelerating voltage of 10 kV.

Microbiological analysis. The ice cream samples were tested for microbial criteria according to the quality requirements specified in FAO/WHO and TCVN 7402:2004, which include total aerobic mesophilic bacteria, Coliforms, and *Salmonella* spp [15], [16].

Sensory evaluation. A descriptive sensory evaluation was conducted by 11 panellists (9 females and 2 males), who underwent training over three sessions of four hours each. The evaluation criteria included aroma, colour, texture, and taste of the ice cream samples, assessed using a standardized scoring scale ranging from 0 to 5 points. On this scale, scores from 5 to 3 represented the extent to which the product meets technical specifications, while scores from 2 to 0 indicated that the product didn't meet the sensory properties. The scale for evaluating the quality of the ice cream samples was developed based on TCVN 10565-2:2015 (ISO 22935-2:2009) and TCVN 7402:2004 [17], [16].

Chemical analyses including protein, carbohydrate, fat, moisture, and ash were determined according to ISO 8968-1:2014, ISO 7328:2008, ISO 3728:2004, and AOAC 923.03, respectively. The total carbohydrate content in the ice cream was calculated using Equation (4) [18]:

$$\%\text{Total carbohydrate} = 100\% - (\%\text{protein} + \%\text{fat} + \%\text{moisture} + \%\text{ash}) \quad (4)$$

Total energy (TE, kcal/100 g) of the analyzed ice cream samples was determined using Equation (5) [19]:

$$\text{TE (kcal/100 g)} = (9 \times \text{m}_{\text{fat}}) + (4 \times \text{m}_{\text{protein}}) + (4 \times \text{m}_{\text{carbohydrate}}) \quad (5)$$

where m_{fat}, $m_{protein}$, $m_{carbohyrate}$ are the masses (g) of fat, protein, and carbohydrate in 100 g of ice cream sample; and 9, 4, and 4 are the energy conversion factors for fat, protein, and carbohydrate, respectively.

pH value was determined using a pH meter (Hanna HI98100, Italy). ***Titratable acidity (TA, %)*** was measured according to TCVN 6509:2013 (ISO 11869:2012) by titrating 10 g (m_0) of the sample with 0.1 N NaOH using 0.1% phenolphthalein as an indicator. The titratable acidity was calculated using Equation (6):

$$\text{TA (\%)} = \frac{V_{NaOH} \times 0{,}9}{m_0} \quad (6)$$

Statistical analysis. Each experiment was performed in triplicate. Data are presented as mean ± standard deviation. Statistical analyses were conducted using one-way analysis of variance (ANOVA) to assess differences among samples ($p < 0.05$), followed by Tukey's test using SPSS 27 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Chemical composition

As shown in Table 2, substituting skimmed milk powder with hydrolyzed lima bean protein in the ice cream formulations resulted in statistically significant differences in chemical compositions, including protein, carbohydrate, and dry matter content among the ice cream samples ($p < 0.05$). The protein content showed an increasing trend as the APPC substitution ratio increased from 0% to 30%. This increase in protein content can be attributed to the higher protein content in the APPC preparation (59.2%) compared to that in skimmed milk powder (32.7%). However, the carbohydrate content decreased (from 25.21% to 22.94%) as the protein substitution ratio increased. The lipid content also decreased (from 2.20% to 1.72%) when replacing skimmed milk powder with APPC; the control sample (DC) had the highest lipid content, and the APPC substituted samples did not show significant differences ($p < 0.05$). The ash content remained unchanged with the inclusion of APPC in the formulations ($p > 0.05$). The dry matter content showed a decreasing trend from 32.25% to 30.37%, which corresponded to an increasing APPC substitution ratio of 0% to 30%. This reduction is due to the lower moisture content in skimmed milk powder (3.9%) compared to that in the APPC preparation (8.6%). Changes in the chemical composition of the ice cream samples led to variations in the energy values provided by the products. Analysis results indicated that the energy value decreased from 136.52 kcal/100 g to 126.53 kcal/100 g as the APPC substitution ratio increased from 0% to 30%. Based on Goff's classification, all samples qualify as low-fat ice cream [20].

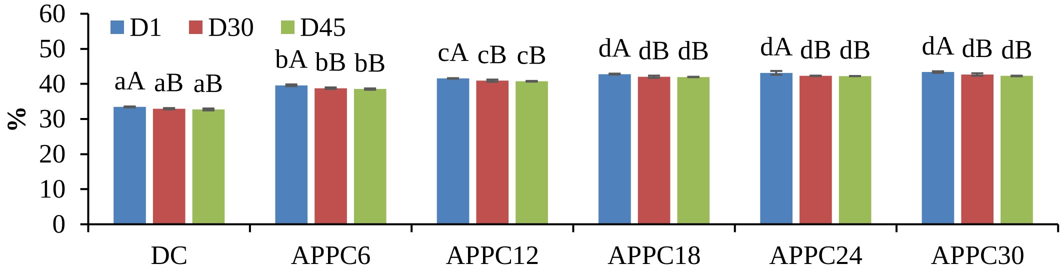
Table 2. *Chemical composition and energy value of ice cream samples*

Samples	Protein (%)	Carbohydrate (%)	Lipid (%)	Ash (%)	Dry matter (%)	Total energy (kCal/100gr)
DC	3.96 ± 0.07^{f}	25.21 ± 0.20^{a}	2.20 ± 0.03^{a}	0.87 ± 0.02^{a}	32.25 ± 0.28^{a}	136.52 ± 1.20^{a}
APPC6	4.30 ± 0.03^{e}	24.82 ± 0.01^{b}	1.74 ± 0.01^{b}	0.88 ± 0.02^{a}	31.74 ± 0.03^{b}	132.22 ± 0.21^{b}
APPC12	4.38 ± 0.00^{d}	24.42 ± 0.05^{c}	1.74 ± 0.04^{b}	0.88 ± 0.04^{a}	31.42 ± 0.01^{c}	130.90 ± 0.29^{c}
APPC18	4.62 ± 0.03^{c}	23.85 ± 0.06^{d}	1.74 ± 0.03^{b}	0.89 ± 0.03^{a}	31.10 ± 0.01^{d}	129.54 ± 0.15^{d}
APPC24	4.71 ± 0.03^{b}	23.40 ± 0.03^{e}	1.73 ± 0.02^{b}	0.90 ± 0.02^{a}	30.74 ± 0.02^{e}	128.02 ± 0.18^{d}
APPC30	4.81 ± 0.03^{a}	22.94 ± 0.02^{f}	1.72 ± 0.03^{b}	0.90 ± 0.02^{a}	30.37 ± 0.02^{f}	126.53 ± 0.20^{e}

In the same column, values with different letters (a, b, c, …) are statistically different (p<0.05)

3.2. Overrun

Air is an essential component in ice cream, significantly influencing its physical properties such as hardness, melting rate and storage stability. The amount of air incorporated into ice cream is quantified by overrun value [20]. In this study, the overrun of ice cream samples at different storage times exhibited an increasing trend with higher substitution ratios of skimmed milk powder by APPC (0–30%). Specifically, the overrun values ranged from 33.45% to 43.38% on day 1 (D1), from 32.94% to 42.69% on day 30 (D30), and from 32.73% to 42.27% on day 45 (D45) (Figure 1). The substitution of skimmed milk powder with APPC increased the viscosity of the ice cream mixture after temperature reduction (see more in Section 3.6). This increase in viscosity led to a higher incorporation of air, resulting in greater overrun values in the ice cream samples [21], [22]. Additionally, according to Sivasankari et al. (2019), the enhanced overrun could be attributed to the improved foaming capacity of the protein, which facilitates the formation of a larger number of air bubbles [23]. However, when the APPC substitution ratio increased from 18% to 30%, no significant differences in overrun were observed among the ice cream samples ($p > 0.05$). This might be due to the protein content reaching a threshold level, creating a more viscous gel network that impeded air incorporation during the freezing process [21]. The overrun values of ice cream samples after 30 and 45 days of storage showed a decreasing trend compared to day 1 ($p < 0.05$, Figure 3). However, no significant differences were observed between the samples on day 30 and day 45 ($p > 0.05$).



Values with different letters (a, b, c, …) indicate differences between samples (p < 0.05)

Values with different capital letters (A, B, ...) indicate differences at storage days (p < 0.05).

Figure 1. *Overrun of ice cream samples during storage*

3.3. pH and titratable acidity

The analysis of titratable acidity and pH of the ice cream samples, as presented in Table 3, revealed that substituting skimmed milk powder with hydrolyzed protein tended to slightly increase the acidity of the samples. Specifically, the titratable acidity increased from 0.25% to 0.29%, while the pH exhibited a gradual decrease from 6.37 to 6.25. This decline in pH may be attributed to the presence of free amino acids in the hydrolyzed lima protein [24].

JTE HCMUTE
ISSN: 2615-9740
JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

Table 3. *Titratable acidity, pH and hardness according to storage days (D1, D30) of ice cream samples*

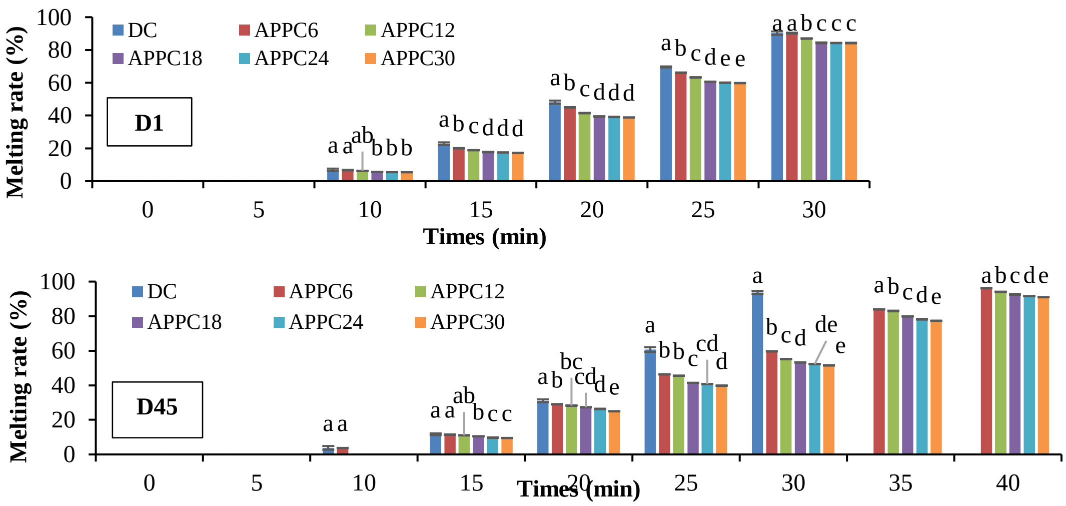
Samples	TA (%)	pH	Hardness (mN)	
			D1	D30
DC	0.25 ± 0.00^{a}	6.37 ± 0.01^{a}	8514.2 ± 594.78^{aA}	8604.2 ± 464.59^{aA}
APPC6	0.26 ± 0.01^{a}	6.36 ± 0.01^{a}	4252.2 ± 681.71^{bA}	5021.0 ± 584.07^{bA}
APPC12	0.27 ± 0.01^{ab}	6.32 ± 0.01^{b}	1925.2 ± 250.44^{cA}	2283.0 ± 405.60c^{A}
APPC18	0.28 ± 0.01^{ab}	6.29 ± 0.01^{c}	1876.0 ± 224.18^{cA}	2207.4 ± 413.68^{cA}
APPC24	0.29 ± 0.01^{b}	6.26 ± 0.01^{d}	1911.0 ± 269.49^{cA}	2154.6 ± 532.66^{cA}
APPC30	0.29 ± 0.01^{b}	6.25 ± 0.01^{d}	1908.4 ± 343.55^{cA}	2175.2 ± 346.60^{cA}

In the same column, values with different letters (a, b, c, ...) indicate differences between samples ($p < 0.05$)
In the same row, values with different capital letters (A, B, ...) indicate differences at storage days ($p < 0.05$).

3.4. Hardness

Replacing skimmed milk powder with APPC significantly affected the hardness of ice cream (Table 3). The DC sample exhibited the highest hardness values (8514 mN) and as the APPC content increased (0–12%), the hardness of the ice cream tended to decrease from 4252 to 1908 mN. However, no significant differences were observed in hardness among the APPC12, APPC18, APPC24, and APPC30 samples ($p > 0.05$). According to Sofjan & Hartel, there is an inverse relationship between overrun and hardness in ice cream, which is consistent with the results reported in Section 3.2 [20]. After 30 days of storage, an insignificant increase in hardness was observed for all samples ($p > 0.05$), indicating that extended freezing contributed to a slightly firmer ice cream structure.

3.5. Melting rate



Values with different letters (a, b, c, ...) indicate differences between samples ($p < 0.05$)

Figure 2. *Melting rate of ice cream samples on day 1 (D1) and day 45(D45) of storage period*

Ice cream is a complex frozen food comprising multiple phases, including ice crystals, air cells, fat globules, and partially coalesced fat clusters suspended in an unfrozen serum phase containing sugars, proteins, and stabilizers. This intricate microstructure significantly affects the melting rate of ice cream [20]. Based on Figure 2, across all storage days, the control sample (DC) exhibited the fastest melting rate, while the APPC30 sample melted the slowest. Previous studies have indicated that the viscosity of the ice cream mix, overrun, and ice crystal size influence the melting rate [13], [22].

JTE HCMUTE
ISSN: 2615-9740
JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

Additionally, the higher viscosity of the ice cream mixes improved melt resistance, leading to a slower melting rate [20]. After the first day of storage, the melt resistance increased (Figure 2) and remained stable throughout the storage period. This phenomenon might be explained by the increased viscosity of the samples during low-temperature storage and the formation of new ice crystals due to temperature fluctuations [12].

According to Table 4, the initial dripping time and complete melting time of the ice cream samples indicate that the DC sample had the quickest onset of dripping and fastest complete melting. The samples supplemented with APPC demonstrated slower melting times, which tended to increase with higher APPC substitution ratios. On the first day, there were no significant differences in initial dripping time and complete melting time among the APPC12, APPC18, APPC24, and APPC30 samples ($p > 0.05$). However, by the 30th and 45th days of storage, the differences between the samples became more pronounced. This might be attributed to the influence of overrun, the distribution of ice crystals, and the increased viscosity of the ice cream during storage. In the study by Chen et al., the hydrolysis of soy protein was found to create appropriately sized fat clusters, reducing the melting rate of ice cream [25]. Another reason why ice cream with higher overrun melts more slowly is due to differences in heat transfer rates; the larger amount of air acts as an effective insulator, slowing down the heat transfer process [20], [25].

Table 4. *First dripping time and complete melting time of ice cream samples during storage*

Sample	First dripping time (min)			Complete melting time (min)		
	D1	D30	D45	D1	D30	D45
DC	7.29 ± 0.41^{aA}	7.70 ± 0.19^{aA}	7.84 ± 0.22^{aA}	31.22 ± 0.16^{aA}	31.26 ± 0.10^{aA}	31.28 ± 0.18^{aA}
APPC6	7.94 ± 0.10^{bA}	9.11 ± 0.18^{bB}	9.50 ± 0.09^{bC}	32.08 ± 0.22^{bA}	40.62 ± 0.08^{bB}	40.81 ± 0.10^{bB}
APPC12	8.57 ± 0.13^{cA}	10.27 ± 0.21^{cB}	10.68 ± 0.06^{cC}	32.69 ± 0.18^{cA}	41.72 ± 0.11^{cB}	41.84 ± 0.15^{cB}
APPC18	8.66 ± 0.04^{cA}	11.55 ± 0.19^{dB}	11.90 ± 0.11^{dC}	32.74 ± 0.11^{cA}	42.77 ± 0.06^{dB}	43.11 ± 0.15^{dC}
APPC24	8.86 ± 0.14^{cA}	12.17 ± 0.10^{eB}	12.51 ± 0.18^{eB}	32.87 ± 0.08^{cA}	43.24 ± 0.08^{eB}	43.52 ± 0.14^{eC}
APPC30	9.02 ± 0.06^{cA}	13.28 ± 0.07^{fB}	13.52 ± 0.14^{fC}	33.01 ± 0.17^{cA}	43.69 ± 0.06^{fB}	43.88 ± 0.07^{eB}

In the same column, values with different letters (a, b, c, …) indicate differences between samples ($p < 0.05$)
In the same row, values with different capital letters (A, B, ...) indicate differences at storage days ($p < 0.05$).

3.6. Rheological properties

The consistency index (k) and the flow behavior index (n) exhibited opposite trends with increasing levels of skimmed milk powder replacement by hydrolyzed protein. An increase in the k value was observed as the concentration of hydrolyzed protein in the formulation increased, whereas the n value decreased with higher concentrations (Table 5). Specifically, when the substitution ratio of skimmed milk powder with hydrolyzed protein increased from 0% to 30%, the viscosity index of the ice cream mix increased from 2.2130 to 8.9643, indicating an increase in viscosity. The rheological properties of dispersion systems may be related to the water absorption capacity of their constituent proteins; proteins with better water-holding capacity confer higher viscosity [21]. In all samples, the flow behavior index ranging from 0.3667 to 0.4880 ($0 < n < 1$), indicating that all ice cream samples demonstrated pseudoplastic (shear-thinning) properties [26]. Furthermore, increasing the APPC content in the ice cream formulation led to a gradual decrease in the n value, resulting in a greater deviation from Newtonian flow (as n deviates further from 1.0). Therefore, samples with higher APPC content exhibited greater pseudoplasticity.

Table 5. *Herschel - Bulkley regression equations of ice cream samples*

Sample	Equation	*k*	*n*	Sample	Equation	*k*	*n*
DC	$\tau = 2.2130\dot{\gamma}^{0.4880}$	2.2130	0.4880	APPC18	$\tau = 5.9890\dot{\gamma}^{0.4353}$	5.9890	0.4353
APPC6	$\tau = 3.6384\dot{\gamma}^{0.4267}$	3.6384	0.4267	APPC24	$\tau = 7.0852\dot{\gamma}^{0.4125}$	7.0852	0.4125
APPC12	$\tau = 4.8668\dot{\gamma}^{0.4297}$	4.8668	0.4297	APPC30	$\tau = 8.9643\dot{\gamma}^{0.3667}$	8.9643	0.3667

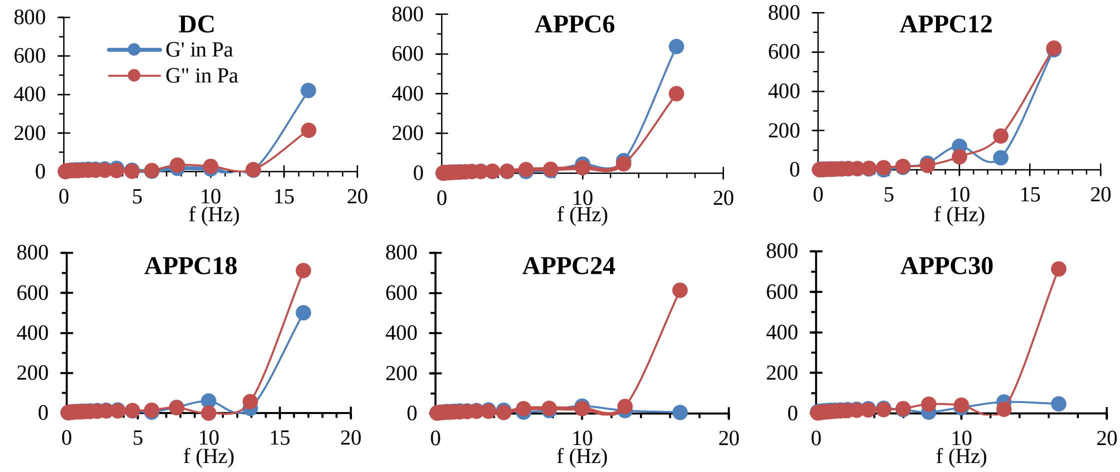


Figure 3. *Change of G' and G'' values of ice cream samples with frequency sweep*

The analysis presented in Figure 3 illustrates the dependence of the storage modulus (G') and the loss modulus (G") by frequency sweep, which was a typical indication of viscoelastic properties [27]. In the low-frequency range, no significant differences were observed in the G' and G" values among the samples. However, as the frequency increased, the G' and G" values of the ice cream samples exhibited different trends, and differences between the samples became apparent at frequencies between 12.92 and 16.68 Hz. Within this frequency range, the DC and APPC6 samples demonstrated dominant elastic behavior over viscous behavior (G' > G"), whereas the APPC12, APPC18, APPC24, and APPC30 samples exhibited predominant viscous behavior (G' < G"). This phenomenon can be attributed to the fact that samples with higher fat content exhibit greater elastic characteristics, while the addition of protein increased the viscosity of the mixture [27]. The DC sample had the highest fat content, with no significant differences in this parameter compared to the other ice cream samples, and the protein content tended to increase as the APPC substitution ratio for skimmed milk powder increased. Therefore, the DC sample exhibited more pronounced elastic properties compared to the other samples; however, there was not much difference when compared to the APPC6 sample. As the APPC substitution ratio increased (12–30%), the viscosity of the ice cream samples increased. The results of this analysis indicated that when the fat content in the mixture decreased and the protein content increased, viscosity became the predominant characteristic of ice cream [27].

3.7. Microstructure

Scanning electron microscopy images (Figure 4) revealed distinct structural differences corresponding to varying levels of hydrolyzed lima bean protein substitution. The control, APPC6, and APPC12 samples displayed large air pockets, whereas APPC18, APPC24, and APPC30 exhibited smaller and more numerous air pockets, with APPC18 showing the most significant reduction in bubble size and increased density. These structural variations were attributed to three primary mechanisms: Ostwald ripening, coalescence, and drainage which interacted and influenced each other to varying extents [27]. Increased APPC substitution reduced surface tension and elevated viscosity, which inhibited Ostwald ripening by maintaining smaller bubble sizes and limiting gas diffusion between bubbles [22]. Additionally, the higher fat content in the DC sample promoted coalescence, leading to larger air pockets, while the increased surface-active agents and viscosity in APPC-substituted samples reduced drainage, enhancing bubble uniformity [28]. Although APPC24 and APPC30 also benefited from higher viscosity, they exhibited some larger bubbles due to slowed bubble formation processes [29]. Overall, APPC substitution improved ice cream stability by reducing bubble size and ensuring even air distribution.

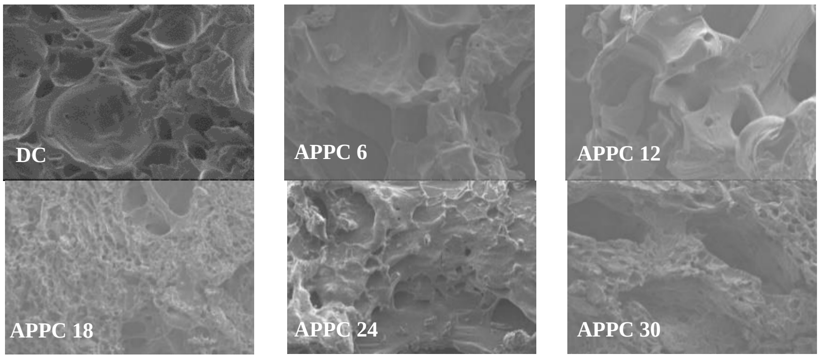


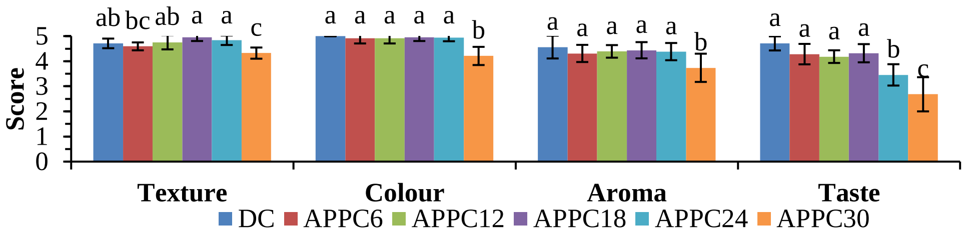
Figure 4. *Microstructural images of ice cream samples*

3.8. Microbiological analysis

The microbiological analysis of the ice cream samples supplemented with hydrolyzed lima bean protein indicated full compliance with FAO/WHO and TCVN 7402:2004 standards. Specifically, the total aerobic mesophilic bacteria count was well within the permissible limit, with a result of 1.8 x 10^4 cfu/g compared to the standard of ≤ 5 x 10^4 cfu/g. Additionally, coliforms were not detected, adhering to the limit of ≤ 10^2 cfu/g, and *Salmonella spp*. was absent in 25g samples, meeting the strict requirement of zero presence [15], [16]. These results confirmed that ice cream products are microbiologically safe for consumer consumption, ensuring compliance with food safety regulations.

3.9. Sensory evaluation

The results of the descriptive sensory evaluation indicated that substituting skimmed milk powder with APPC at levels ranging from 0% to 30% affected the sensory properties of the ice cream products (Figure 5). Regarding texture, all ice cream samples exhibited smoothness and fluffyness without ice crystals. Notably, samples with APPC substitution ratios of 0% to 24% demonstrated better texture and received higher scores compared to the APPC30 sample. In terms of colour, all samples displayed the characteristic hue of the ingredients (milky white to ivory). The substitution levels of APPC between 6% and 24% did not significantly impact on the product's colour (4.95 to 5.00 points). However, increasing the APPC substitution to 30% resulted in a darker and less uniform colour, leading to a lower score for the APPC30 sample (4.21 points). This observation aligned with the colourimetric measurements presented above. Aroma scores were similar for 0–24% APPC samples ($p > 0.05$), noted for strong vanilla and milk scents with minimal bean odour. The 30% sample scored lower (3.74 points) due to a pronounced bean aroma and reduced vanilla/milk notes. Taste scores were comparable for 0–18% APPC (4.32 to 4.71 points; $p > 0.05$), but significantly declined at 24–30% (2.68 to 3.45 points) due to bitterness from amino acids in the hydrolyzed protein. Overall, APPC substitution up to 18% did not significantly affect sensory properties.



Values with different letters (a, b, c, ...) indicate differences between samples ($p < 0.05$)

Figure 5. *Descriptive scofres of ice cream samples*

4. Conclusions

This study demonstrated that replacing skimmed milk powder with hydrolyzed lima bean protein by alcalase in low-fat ice cream formulations significantly affected product properties. Increasing APPC substitution levels (0–30%) resulted in higher protein content and lower carbohydrates, fat, total dry matter, and energy values. Rheological analysis showed enhanced viscosity and pseudoplastic behavior with higher APPC concentrations. Physically, APPC incorporation improved overrun and melt resistance, reduced hardness, and led to smaller, more uniformly distributed air bubbles, due to the functional properties of the hydrolyzed protein. Sensory evaluations indicated that up to 18% APPC substitution maintained acceptable taste without bitterness, while higher levels adversely affected sensory quality. These findings suggested that APPC is a viable ingredient for producing nutritious, low-fat vanilla ice cream with improved technological properties, suitable for industrial scale manufacturing.

Acknowledgments

The authors sincerely thank Ho Chi Minh City University of Technology and Education for providing the facilities needed to complete this study. We sincerely thank the following students for their contributions in conducting the experiments and for their honesty in obtaining these valuable results: Pham Thi My Duyen (Student ID 20116278), Tran Thi Thu Nga (Student ID 20116111).

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] N. F. Nik Hassan, N. Ahmad, A. R. Abu Kassim, and Y. Hamzah, “The Customers’ Consumption Trends on Healthy Ice Cream,” *Jurnal Intelek*, vol. 16, no. 2, pp. 89–98, Jul. 2021, doi: 10.24191/ji.v16i2.419.

[2] F. Javidi, S. M. A. Razavi, F. Behrouzian, and A. Alghooneh, “The influence of basil seed gum, guar gum and their blend on the rheological, physical and sensory properties of low-fat ice cream,” *Food Hydrocoll*, vol. 52, pp. 625–633, Jan. 2016.

[3] M. Bastien, P. Poirier, I. Lemieux, and J.-P. Després, “Overview of Epidemiology and Contribution of Obesity to Cardiovascular Disease,” *Prog Cardiovasc Dis*, vol. 56, no. 4, pp. 369–381, Jan. 2014, doi: 10.1016/j.pcad.2013.10.016.

[4] R. Liu, L. Wang, Y. Liu, T. Wu, and M. Zhang, “Fabricating soy protein hydrolysate/xanthan gum as fat replacer in ice cream by combined enzymatic and heat-shearing treatment,” *Food Hydrocoll*, vol. 81, pp. 39–47, Aug. 2018.

[5] A. Tiwari, H. K. Sharma, N. Kumar, and M. Kaur, “The effect of inulin as a fat replacer on the quality of low-fat ice cream,” *Int J Dairy Technol*, vol. 68, no. 3, pp. 374–380, Aug. 2015, doi: 10.1111/1471-0307.12176.

[6] M. J., Messina and C. L. Loprinzi, “American Institute for Cancer Research 11th Annual Research Conference on Diet,” *Nutr Cancer*, 2001.

[7] M. D. Magaña, M. Segura-Campos, G. Dávila-Ortiz, D. Betancur-Ancona, and L. Chel-Guerrero, “ACE-I inhibitory properties of hydrolysates from germinated and ungerminated Phaseolus lunatus proteins,” *Food Science and Technology (Campinas)*, vol. 35, no. 1, pp. 167–174, Mar. 2015, doi: 10.1590/1678-457X.6551.

[8] J. A. Adebo, “A Review on the Potential Food Application of Lima Beans (Phaseolus lunatus L.), an Underutilized Crop,” *Applied Sciences*, vol. 13, no. 3, p. 1996, Feb. 2023, doi: 10.3390/app13031996.

[9] H. Pham, V. T. Nguyen, and K. S. Trinh, “Physico-Chemical and Functional Properties of Protein Concentrate from Lima Beans (Phaseolus lunatus),” *Journal of Technical Education Science*, no. 70B, pp. 48–56, Jun. 2022, doi: 10.54644/jte.70B.2022.1180.

[10] G. Zhao, Y. Liu, M. Zhao, J. Ren, and B. Yang, “Enzymatic hydrolysis and their effects on conformational and functional properties of peanut protein isolate,” *Food Chem*, vol. 127, no. 4, pp. 1438–1443, Aug. 2011.

[11] D. B. Ancona, R. M. Rosado, A. Corona-Cruz, A. Castellanos-Ruelas, M. E. Jaramillo-Flores, and L. Chel-Guerrero, “Functional properties of hydrolysates from Phaseolus lunatus seeds,” *Int J Food Sci Technol*, vol. 44, no.1, pp.128–137, Jan. 2009.

[12] M. B. Guler-Akin, F. Avkan, and M. S. Akin, “A novel functional reduced fat ice cream produced with pea protein isolate instead of milk powder,” *J Food Process Preserv*, vol. 45, no. 11, Nov. 2021, doi: 10.1111/jfpp.15901.

[13] M. R. Muse and R. W. Hartel, “Ice Cream Structural Elements that Affect Melting Rate and Hardness,” *J Dairy Sci*, vol. 87, no. 1, pp. 1–10, Jan. 2004, doi: 10.3168/jds.S0022-0302(04)73135-5.

[14] H. Ş. Aloğlu, Y. Özcan, S. Karasu, B. Çetin, and O. Sağdiç, “Influence of transglutaminase treatment on the physicochemical, rheological, and melting properties of ice cream prepared from goat milk,” *Mljekarstvo*, vol. 68, no. 2, pp. 126–138, 2018.

[15] FAO/WHO, “Report of 2nd Joint FAO/WHO Expert Consultation,” 1977.

[16] TCVN 7402:2004 Edible ices cream - Technical requirements. VietNam: The Commission for the Standards, Metrology and Quality of Viet Nam, 2004.

[17] TCVN 10565-2:2015 (ISO 22935-2:2009) Milk and milk products - Sensory analysis - Part 2: Recommended methods for sensory evaluation. VietNam: The Commission for the Standards, Metrology and Quality of Viet Nam, 2015.

[18] FAO/WHO, “Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation,” FAO Food Nutr Pap, vol. 66, pp. 1–140, 1998, Accessed: Oct. 01, 2024. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/9743703/

[19] W. S. Arbuckle, “Energy Value and Nutrients of Ice Cream,” in *Ice Cream*, Boston, MA: Springer US, 1986, pp. 9–18.

[20] R. P. Sofjan and R. W. Hartel, “Effects of overrun on structural and physical characteristics of ice cream,” *Int Dairy J*, vol. 14, no. 3, pp. 255–262, Mar. 2004, doi: 10.1016/j.idairyj.2003.08.005.

[21] G. das G. Pereira, J. V. de Resende, L. R. de Abreu, T. M. de Oliveira Giarola, and I. T. Perrone, "Influence of the partial substitution of skim milk powder for soy extract on ice cream structure and quality," *European Food Research and Technology*, vol. 232, no. 6, pp. 1093–1102, Jun. 2011, doi: 10.1007/s00217-011-1483-z.

[22] Y. Chang and R. W. Hartel, "Stability of air cells in ice cream during hardening and storage," *J Food Eng*, vol. 55, no. 1, pp. 59–70, Nov. 2002, doi: 10.1016/S0260-8774(01)00242-4.

[23] R. Sivasankari, G. Hemalatha, S. Amutha, M. Murugan, C. Vanniarajan, and T. Umamaheswari, "Physical and Sensory Properties of Ice Cream as Influenced by Pulse Protein Concentrates," *Eur J Nutr Food Saf*, pp. 322–328, Jul. 2019.

[24] S. M. Rutherfurd, "Methodology for Determining Degree of Hydrolysis of Proteins in Hydrolysates: A Review," *J AOAC Int*, vol. 93, no. 5, pp. 1515–1522, Sep. 2010, doi: 10.1093/jaoac/93.5.1515.

[25] W. Chen *et al.*, "Effects of soy proteins and hydrolysates on fat globule coalescence and meltdown properties of ice cream," *Food Hydrocoll*, vol. 94, pp. 279–286, Sep. 2019, doi: 10.1016/j.foodhyd.2019.02.045.

[26] B. C. Petit, "Food texture and viscosity—concept and measurement," *LWT - Food Science and Technology*, vol. 36, no. 2, p. 281, Mar. 2003, doi: 10.1016/S0023-6438(02)00182-2.

[27] S. Adapa, H. Dingeldein, K. A. Schmidt, and T. J. Herald, "Rheological Properties of Ice Cream Mixes and Frozen Ice Creams Containing Fat and Fat Replacers," *J Dairy Sci*, vol. 83, no. 10, pp. 2224–2229, Oct. 2000.

[28] P. Walstra, "Principles of Foam Formation and Stability," 1989, pp. 1–15, doi: 10.1007/978-1-4471-3807-5_1.

[29] C. W. D. Engelsen, J. C. Isarin, H. Gooijer, M. M. C. G. Warmoeskerken, and J. G. Wassink, "Bubble size distribution of foam," *AUTEX Research Journal*, vol. 2, no. 1, 2022.

Pham Thi Hoan received the BE degree in food technology from Tver State Technical University, Russia in 2010 and the Ph. D degree in Technology and commodity science of food products, functional and specialized products and public catering from Moscow State University of Food Production, Russia in 2014. From 6/2015 till now, she has been being a lecturer in Ho Chi Minh city University of Technology and Education, Ho Chi Minh city, Vietnam. Her research interest includes food processing; acquisition and application of active substances in food processing; application of enzymes in food processing.

Email: hoanpt@hcmute.edu.vn. Tel.: (081) 97 220 93 96. ORCID: https://orcid.org/0000-0002-8525-6317

Nguyen Huu Khang graduated with a Bachelor of Engineering degree in food technology from Ho Chi Minh City University of Technology and Education in 2020. He's currently studying for a master's degree in food technology at the same university.

Email: 2231208@student.hcmute.edu.vn. ORCID: https://orcid.org/0009-0001-4508-6261