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ORIGINAL ARTICLE

The effects of alkaline extraction on the characteristics of lima bean (*Phaseolus lunatus*) starch

Os efeitos da extração alcalina nas características do amido de fava (Phaseolus lunatus)

Hapsari Titi Palupi[1,2], Teti Estiasih[3]*, Yunianta Subroto[3], Aji Sutrisno[4]

[1]Doctoral Program of Food Science, Faculty of Agricultural Technology, Universitas Brawijaya, Malang - Indonesia
[2]Universitas Yudharta, Faculty of Agriculture, Department of Food Science and Technology, Pasuruan - Indonesia
[3]Universitas Brawijaya, Faculty of Agricultural Technology, Department of Food Science and Biotechnology, Malang - Indonesia
[4]Universitas Brawijaya, Faculty of Agricultural Technology, Department of Food Science and Biotechnology, Malang - Indonesia

***Corresponding Author:** Teti Estiasih, Universitas Brawijaya, Faculty of Agricultural Technology, Department of Food Science and Biotechnology, Jalan Veteran, 65145, Malang - Indonesia, e-mail: teties@ub.ac.id; teties@yahoo.co.id

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Abstract

This study provides an overview of the use of lima bean (*Phaseolus lunatus* L.) flour as a raw material for starch extraction in consecutive steps for the preparation of lima bean protein concentrate. The starch from lima bean flour was extracted at different alkaline pH levels: 7, 8, 9, 10, and 11. The physicochemical, microstructural, crystallinity, pasting, and functional properties of the extracted starch were evaluated. The results showed that the purity of the starch increased with a higher extraction pH. However, a further increase in pH reduced the yield. The alkaline-extracted starch still contained other non-starch compounds. The extraction pH affected the amylose content, presumably due to the interaction of OH^- ions with the starch granular components, influencing the yield and amylose level. At lower pH levels, alkali promoted a more orderly granular structure, decreasing starch solubility and resulting in higher yield and amylose content. Meanwhile, at higher pH levels, alkali ions increased protein solubility and starch content. The pH had a slight influence on the starch granule morphology. Lima bean starch exhibited a crystallinity index of 29% to 34% depending on the alkaline extraction pH. All starches showed individual peaks at 2θ values of 15° and 23°, unresolved peaks at 2θ values of 17° and 18°, and a diffracted plane around 5.63°, indicating a C-type starch. After alkali treatment, the starch maintained its C-type structure, indicating no structural transformation in the orthorhombic and hexagonal structures. Increasing the extraction pH to 9 increased starch crystallinity, but a further increase in pH decreased it. Viscosity during pasting was affected by the intensive interaction of water with amylose in the amorphous regions. The extraction pH influenced peak, trough, setback, and final viscosity. The alkaline treatment might disrupt granular regions, altering the functional properties of the starch. Alkaline extraction of starch can be performed on lima bean flour.

Keywords: Alkali; Crystallinity; Pasting properties; Physicochemical properties; Starch extraction; Starch granule morphology.

Resumo

Este estudo fornece uma visão geral do uso da farinha de feijão-fava (*Phaseolus lunatus*) como matéria-prima para extração de amido em etapas consecutivas, para a preparação de concentrado proteico de feijão-fava. O amido da farinha de feijão-fava foi extraído em diferentes níveis de pH alcalino: 7, 8, 9, 10 e 11. As propriedades físico-químicas, microestruturais, a cristalinidade e de pasta, e as propriedades funcionais do amido extraído foram avaliadas. Os resultados mostraram que a pureza do amido aumentou com o maior pH de extração. No entanto, um novo aumento no pH reduziu o rendimento. O amido obtido por extração alcalina ainda continha outros compostos não amiláceos. O pH de extração afetou o teor de amilose, presumivelmente devido à interação dos íons OH- com os componentes granulares do amido, influenciando o rendimento e o nível de amilose. Em níveis de pH mais baixos, o álcali promoveu uma estrutura granular mais ordenada, diminuindo a solubilidade do amido e resultando em maior rendimento e teor de amilose. Enquanto isso, em níveis de pH mais altos, os íons alcalinos aumentaram a solubilidade da proteína e o teor de amido. O pH teve uma leve influência na morfologia dos grânulos de amido. O amido de feijão-lima apresentou um índice de cristalinidade de 29% a 34%, dependendo do pH de extração alcalina. Todos os amidos apresentaram picos individuais nos valores 2θ de 15° e 23°, picos não resolvidos nos valores 2θ de 17° e 18°, e um plano difratado em torno de 5,63°, indicando um amido tipo C. Após tratamento alcalino, o amido manteve sua estrutura tipo C, indicando ausência de transformação estrutural nas estruturas ortorrômbica e hexagonal. Aumentar o pH de extração para 9 aumentou a cristalinidade do amido, mas um aumento adicional no pH a diminuiu. A viscosidade durante a colagem foi afetada pela intensa interação da água com a amilose nas regiões amorfas. O pH de extração influenciou pico, vale, recuo e viscosidade final. O tratamento alcalino pode romper regiões granulares, alterando as propriedades funcionais do amido. A extração alcalina do amido pode ser realizada na farinha de feijão-fava.

Palavras-chave: Alcalino; Cristalinidade; Propriedades de pasta; Propriedades físico-químicas; Extração de amido; Morfologia dos grânulos de amido.

Highlights

- Lima bean starch was extracted at an alkaline and the starch characteristics were evaluated
- Alkaline extraction slightly affected starch characteristics and the starches are suitable for foods
- Alkaline starch extraction from lima bean is suitable to conduct as part of protein concentrate preparation and starch separation

1 Introduction

Lima bean (*Phaseolus lunatus* L.) is an edible legume that belongs to the *Fabaceae* family, spreads and adapts well to dry land, and is easily cultivated (Sandoval-Peraza et al., 2020; Diniyah et al., 2020). Although lima beans have been consumed for years, this bean is included as an underutilized and minor legume compared to important crops such as soybeans and mung beans which are more explored and well-studied. Because they are high in protein (14.24%-24.6%), lima beans are a good and inexpensive source of nutrition (Jayalaxmi et al., 2016; Ibeabuchi et al., 2019), providing essential amino acids like lysine and threonine (Chel-Guerrero et al., 2012), as well as high in complex carbohydrates (57.3%), mainly starch and fiber (Du et al., 2014). However, lima beans contain different antinutritional compounds such as trypsin inhibition, phytic acid, saponins, flatulence oligosaccharides, and cyanide-producing glucoside toxins (Mathew et al., 2017; Jayalaxmi et al., 2016).

Lima beans contain a significant amount of protein that could be processed into protein concentrate or isolate. Commercially, protein concentrates or isolates use legumes as protein sources, such as soy protein concentrate, or isolate that are extensively used in food industries (Shevkani et al., 2015; Fischer et al., 2020). One step in protein concentrate or isolate preparation from legumes is alkaline extraction (Boye et al., 2010; Jarpa-Parra et al., 2014; Du et al., 2018). During this extraction, starch from high starch-containing legumes is produced in a considerable amount. Hence, the characterization of starch from this alkali extraction is important as the basis for its utilization.

In lima beans, starch is a major carbohydrate component, thus comprising 37-44% (Du et al., 2014). Processing legumes into starch is a key strategy to increase the value-added utilization and reduce antinutritional compounds (Joshi et al., 2017) and toxins (Sandoval-Peraza et al., 2020). Compared to cereal starches, legume starch has a low glycemic index, which is one reason for carrying out the starch isolation process (Ma et al., 2017). Consequently, the food industry is becoming increasingly interested in using legume starches as alternative sources of conventional starch, such as corn and potato, in food formulations. Lima bean starch has great potential for industrial applications (Okekunle et al., 2020). Although some studies on lima bean starch have been conducted (Okekunle et al., 2020), it can be seen that information available to explain the chemical composition, morphological, pasting, and functional characteristics of lima bean starch from alkaline extraction were limited. Therefore, a comprehensive understanding of the physicochemical properties of lima bean starch is required to provide information about the potential for new product development. Moreover, this characterization serves as a basis for starch utilization, produced as the by-product during alkali extraction in the preparation of legume protein concentrates or isolates.

Alkali treatment affects the purity, structural, and functional characteristics of various starches, such as swelling capacity, retrogradation, and gelatinization. The extraction conditions should ideally cause little or no structural change to the extracted starch component (Jiang et al., 2014). Currently, the effect of extraction pH on the physicochemical changes of starches has received little attention. This extraction is part of the preparation of lima bean protein concentrates because an alkaline solution was used to dissolve the protein, which would be further processed into protein concentrate by precipitation. In contrast to soybean, residual starch in soy protein concentrate preparation was not of great interest due to its low concentration in the flour. This research aimed to evaluate the effect of different extraction pH on the physicochemical, pasting, functional, and morphological characteristics of lima bean starch.

2 Material and methods

2.1 Materials

Lima beans were obtained from local farmers in Malang, East Java, Indonesia. The lima bean seeds were cleaned of dirt and foreign matter and then stored at 20 °C in a plastic bag before further use. All of the reagents used in this research were of analytical grade (Merck Co.).

2.2 Lima bean flour preparation

The seeds were thoroughly washed under running water, and soaked for 8 h, then the kernels were removed and air-dried. The dehulled lima beans were dried in a hot oven at 50 °C for 8 h. Lima bean flour was prepared by milling the samples with a laboratory grinding machine, followed by then sieving through a 100 mesh sieve and packing the resulting flour into plastic bags.

2.3 Starch extraction

Starch was extracted using the wet method at an alkaline pH. Lima bean flour was dissolved in water (1:10 wt/vol), and the pH of the suspension was adjusted to 7, 8, 9, 10, and 11 using 1 N NaOH solution. The suspension was constantly mixed on a magnetic stirrer at 25 ± 2 °C for 1 h to solubilize the protein and separate the starch. Afterward, the suspension was allowed to stand for 2-3 h, and the supernatant was obtained by centrifugation twice at 3000 rpm for 15 min. The supernatant was separated, and the tailing starch was carefully collected from the bottom of the tube. The starch was then washed 2-3 times with distilled water, and the supernatant was decanted. Finally, the wet sediment starch was dried at 50 °C in an oven and sieved using a 100 mesh sieve. The diagram of the starch extraction process is shown in Figure 1.

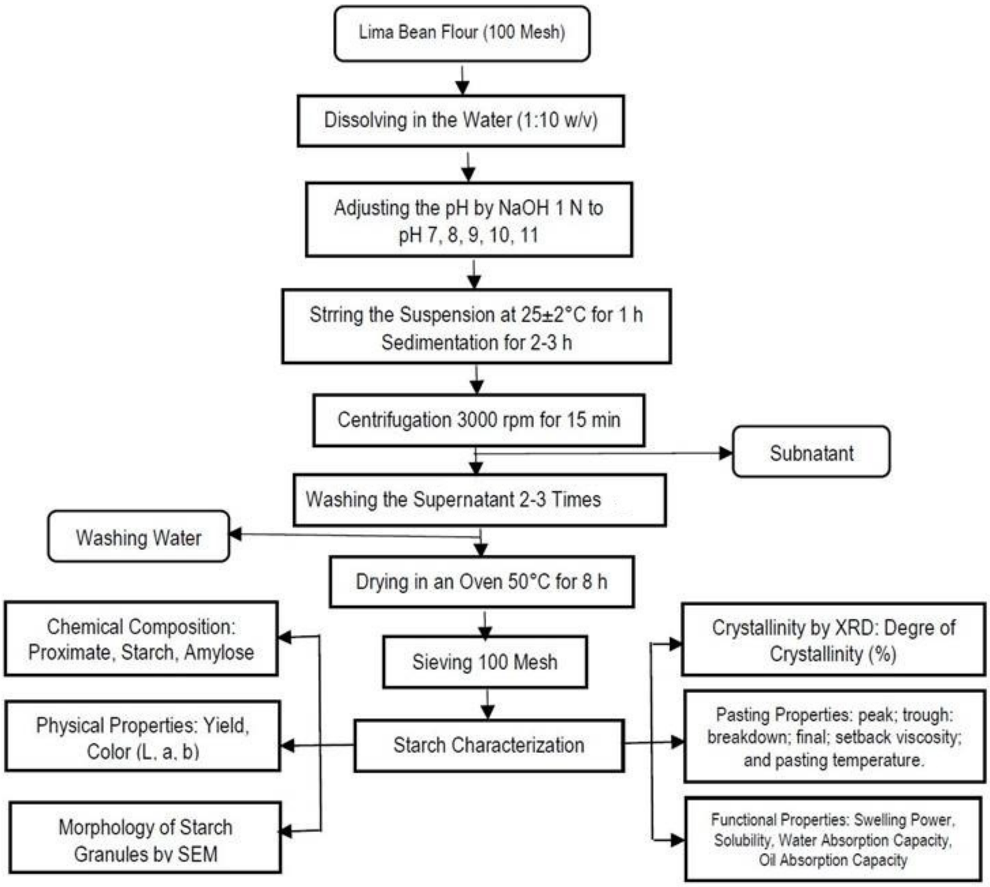

Figure 1. Alkaline extraction of starch from lima bean flour.

The starch yield was calculated using the method described by Palacios-Fonseca et al. (2013) as follows (Equation 1):

$$\text{Yield (\%)} = \frac{\text{weight of dry starch (g)}}{\text{weight of flour (g)}} x100 \quad (1)$$

2.4 Chemical composition analysis

Moisture content, crude protein, total fat, and ash were analyzed by the Association of Official Analytical Chemists (2005) standard methods. Amylose content was measured using the complex amylose-iodide methodology using an Ultraviolet/Visible (UV/Visible) spectrophotometer (Association of Official Analytical Chemists, 2011). The acid hydrolysis method (Association of Official Analytical Chemists, 2005) was used to determine the starch content.

2.5 Color measurement

The color was measured using a digital colorimeter (Chroma Meter CR-300 Konika Minolta Sensing, Japan). L (lightness), a (redness to greenness), and b (yellowness to blueness) were the parameters determined.

2.6 Starch granule morphology analysis

The morphology of lima bean starches was measured by a scanning electron microscope (SEM) (HITACHI TM 3000). The starch powder was sprinkled on a double-sided cellophane adhesive glued to aluminum stubs, magnification at 500×, with a max voltage of 15.0 kV.

2.7 Crystallinity analysis

The X-ray diffraction pattern of all samples was recorded using the X-ray Diffraction (XRD) instrument PANalytical, X'Pert3 Powder operating at 40 kV and a current of 40mA. The scanning region of the diffraction angle ranged from 3 to 35° (2θ), and the scanning speed was set at 2.0°/min. The degree of crystallinity of starch granules was calculated from the ratio of the area of the crystalline curve (upper diffraction peak area) to the total area (amorphous and crystalline) using Origin Pro 8.5 software package (Bharti et al., 2019; Wang et al., 2019).

2.8 Pasting properties analysis

The pasting properties of lima bean starches were analyzed using Rapid Visco Analyzer (RVA) equipment (TechMaster). The starch slurry with a concentration of 9% (wt/wt) was placed in an RVA instrument. The analysis was conducted with both heated and cooled phases at a constant rotation speed (160 rpm). During the heated phase, the starch suspension was heated from 50 to 95 ºC and held for 5 min at a heating rate of 6 °C/min. Subsequently, the starch paste was passed through to the cooling phase, where the temperature was reduced to 50 ºC at a constant rate, and then held for 3 min. The parameters observed included pasting temperature, peak viscosity, through viscosity, final viscosity, breakdown viscosity, and setback viscosity as determined from the viscogram.

2.9 Swelling power and solubility measurement

Swelling power and solubility were measured using the method described by Jiang et al. (2014) with minor modifications. Starch (1 g) was dispersed in 50 mL of distilled water in a pre-weighed centrifuge tube. The suspension was heated in a water bath at 90 °C for 30 min, then cooled to room temperature and centrifuged at 3000 rpm for 15 min. The supernatant was decanted into a pre-weight petri dish and dried at 110 °C. The wet starch sediment was weighed and recorded. Solubility was calculated as the percentage by weight of the dried supernatant to the weight of dry starch. The swelling power was determined as the ratio of wet sediment weight to the dried starch weight, deducting the amount of soluble starch (Equations 2 and 3).

$$\text{Solubility (\%)} = \frac{\text{Weight of dried supernatant}}{\text{Weight of starch}} x100 \quad (2)$$

$$\text{Swelling power (g/g)} = \text{Weight of wet sediment x } \frac{100}{\text{Weight of starch (100-\% solublity)}} \quad (3)$$

2.10 Water and oil absorption capacity

The water and oil absorption capacities were determined with a method by Okekunle et al. (2020) with a slight modification. One gram of starch and 10 mL of distilled water or/oil were mixed in a pre-weighed centrifuge for 1 min and allowed to stand at room temperature for 30 min. Subsequently, they were centrifuged at 3000 rpm for 30 min, and the supernatant was decanted. The mass of water or oil absorbed is expressed as the absorption capacity of water and oil.

2.11 Statistical analysis

The results obtained in the study were expressed as the mean value of 3 replications with standard deviation. Data obtained were analyzed by single-factor analysis of variance (ANOVA) using MINITAB 17 software. Analysis of variance was performed to calculate significant differences, and comparisons of means were made using Tukey's test at the 5% significance

3 Results and discussion

3.1 Physicochemical characteristics

The protein content of the lima flour was 19.22%, indicating that this legume contains a considerable amount of protein and potential to be a good source of nutrition. The yield of lima bean starch was from 29.02 to 36.12% (Table 1). These results are in the range of legume starch yields (16.40 to 47.10%) reported by Wani et al. (2016). Increasing the pH of extraction to 10 resulted in a significantly higher ($p < 0.05$) yield compared to pH 7, but the yield was not significantly different from pH 11. The highest yield at an alkaline pH of 8 is possibly related to the increasing molecular interaction inside starch granules that is facilitated by OH^-. Qin et al. (2019) reported that a 2% NaOH treatment enhanced the order of starch molecules rearrangement and formed the crystal structure of E_h-type. The higher alkaline pH tended to decrease extracted starch yield which was contributed by increasing protein solubility and lowered the residual protein in the starch (Table 1). In alkaline conditions, proteins are more soluble because alkali ions break down the matrix containing proteins and make them more soluble. Proteins carry a higher charge at a pH far from their isoelectric point (pI), thus their solubility in aqueous media increases (Deleu et al., 2019).

According to Jiang et al. (2014) and Wang & Copeland (2012), OH^- in an alkali solution diffused into the amorphous regions of the granules, breaking intermolecular bonds and leaching the amylose. The amorphous region of the starch granule consists of amylose chains and amylopectin branches. Meanwhile, the crystalline area is formed by amylopectin double helices (Dome et al., 2020). At a very high pH of 11, the alkaline ions diffused into the amorphous regions of the granules and then leached starch out from the granules into the solution, thus decreasing the yield. Ionization of the starch chain seemed to occur at a very high pH extraction, which increased starch solubility. Alkali treatment decreases the relative crystallinity, associated with the disruption of double-helix structures and the disorder of starch granule regions (Chávez-Esquivel et al., 2022).

Table 1. Yield, chemical composition, and color of lima bean starches extracted in alkaline pH.

Parameter	Flour	Starch Extracted at				
		pH 7	pH 8	pH 9	pH 10	pH 11
Yield (%)	-	29.02 ± 0.43^b	36.12 ± 0.05^a	34.89 ± 1.03^a	34.11 ± 2.20^a	28.94 ± 0.27^b
Moisture (%) (wb)	7.51 ± 0.61	11.69 ± 0.73^{ab}	11.16 ± 0.10^b	11.49 ± 0.41^{ab}	11.39 ± 0.24^{ab}	12.71 ± 0.44^a
Ash (%) (db)	3.67 ± 0.22	0.43 ± 0.08^a	0.24 ± 0.10^{ab}	0.19 ± 0.12^{ab}	0.18 ± 0.05^{ab}	0.15 ± 0.03^b
Protein (%) (db)	19.22 ± 2.09	2.37 ± 0.45^a	1.95 ± 0.30^b	1.49 ± 0.18^c	1.28 ± 0.33^c	1.23 ± 0.42^c
Lipid (%) (db)	1.33 ± 0.11	0.10 ± 0.02^a	0.08 ± 0.01^a	0.08 ± 0.01^a	0.11 ± 0.07^a	0.10 ± 0.02^a
Starch (%) (db)	50.10 ± 1.70	86.42 ± 2.18^b	87.44 ±1.09^b	89.20 ± 0.44^{ab}	89.43 ± 0.16^{ab}	91.79 ± 1.45^a
Amylose (%) (db)	20.06 ± 1.40	44.03 ± 0.93^b	48.13 ± 1.13^{ab}	50.65 ± 1.48^a	48.48 ± 1.18^{ab}	48.23 ± 1.21^{ab}
Color						
L*	83.47 ± 0.11	89.33 ± 0.12^c	91.07 ± 0.09^b	91.43 ± 0.05^a	91.63 ± 0.05^a	91.36 ± 0.08^a
a*	-0.40 ± 0.11	-0.88 ± 0.05^c	-1.63 ± 0.05^{ab}	-1.75 ± 0.05^a	-1.55 ± 0.05^b	-1.53 ± 0.05^b
b*	14.40 ± 0.20	9.83 ± 0.11^a	8.63 ± 0.15^b	8.42 ± 0.02^{bc}	8.53 ± 0.05^{bc}	8.23 ± 0.23^c

The results were expressed as mean ± standard deviation. Means in a row with different superscript letters are significantly different ($p < 0.05$). db = dry basis; wb = wet basis.

The starch content of alkaline-treated lima bean starch increased significantly ($p < 0.05$) by increasing pH, which contributed to the lowering of non-starch components such as protein, fat, and ash (Table 1). The protein content of lima bean starches was significantly reduced ($p < 0.05$) when the pH was increased. A comparable finding was reported by Jiang et al. (2014) that the protein content of quinoa starch decreased by increasing alkali concentration during the steeping step. Palacios-Fonseca et al. (2013) found that soaking in alkali softened the protein-starch matrix, leading to a reduction in protein content. The alkali pH adjustment (pH 8.0 to 12.0) was designed to solubilize proteins from flour and separate insoluble components (primarily carbohydrates and other substances) from the solubilized proteins. Alkaline condition improved protein solubility in the extraction solution (Momen et al., 2021). The highest moisture content was found in the starch extracted at pH 11, which differed significantly from other pH values ($p < 0.05$). At high pH levels, some OH^- groups tended to ionize (Israkarn et al., 2014), making it easier for water to bind, thus rendering it more challenging to remove during drying. The increasing pH in starch extraction significantly reduced the ash content of the lima bean starches, possibly due to the higher solubility of the mineral at alkaline pH.

Lima bean starch has a starch content ranging from 86.42 to 91.79%, indicating the presence of other non-starch components in the alkaline extracted starch (Tabel 1). This content was lower than cowpea and mungbean starch (93.0 to 98.2%) (Kim et al., 2018) and falls within the range reported by Marquezi et al. (2016) for *Phaseolus vulgaris* L. (83.60 to 91.40%). The starch content increased significantly ($p < 0.05$) by increasing pH, with the lowest content observed at an extraction pH of 7. As a result, the purity level of starch is based on a lower level of residual protein (Jan et al., 2017). The amylose content of lima bean starch ranged from 44.03 to 50.65%, higher than cowpea starch (Ratnaningsih et al., 2020) and Bambara groundnut starch (Oyeyinka & Oyeyinka, 2018). The amylose content of legume starches has been reported to range between 17.0 and 51.69% (Wani et al., 2016). The amylose content of lima bean starch significantly increased ($p < 0.05$) from extraction pH 7 to 9, while further increasing the pH resulted in a decrease in amylose content. At very high pH levels, the amorphous regions containing amylose chains were disrupted. The alkaline ions were distributed into amylose-rich amorphous regions of the granules breaking down intermolecular bonds, swelling the granules, and eluting amylose out of the granules into the solution (Rafiq et al., 2016; Jan et al., 2017).

The L, a, and b values of lima bean starch color were significantly influenced by pH (Table 1). Higher starch purity resulted in increased lightness (L), and decreased redness (+a) and yellowness (+b). This finding was also observed by Kim et al. (2018) that starch lightness was affected by its purity. An increase in L value was observed for lima bean starches extracted at pH levels from 7 to 9, indicating that higher pH levels led to increased starch purity and decreased protein and ash content.

3.2 Starch granule morphology

Legume starch granules were mainly oval, with some being spherical and elliptical, and they had a smooth surface without pinholes and cracks (Ma et al., 2017). Figure 2 shows that the starch granules of lima beans were generally round or oval with a smooth surface, which aligns with the findings reported by Okekunle et al. (2020). The sizes of the lima bean starch granules ranged from 9.09 to 40.54 µm in width and from 22.97 to 48.52 µm in length. These values are larger than those found for mungbean starch (17.0 to 17.6 µm) but comparable to those found in cowpea starch (20.9 to 48.6 µm) (Kim et al., 2018). Starch granules extracted at pH 7 appeared intact and had some residual protein (Figure 2A). Increasing the pH to 9 revealed that lima bean starch granules remained intact and oval with a smooth, thus indicating no damage.

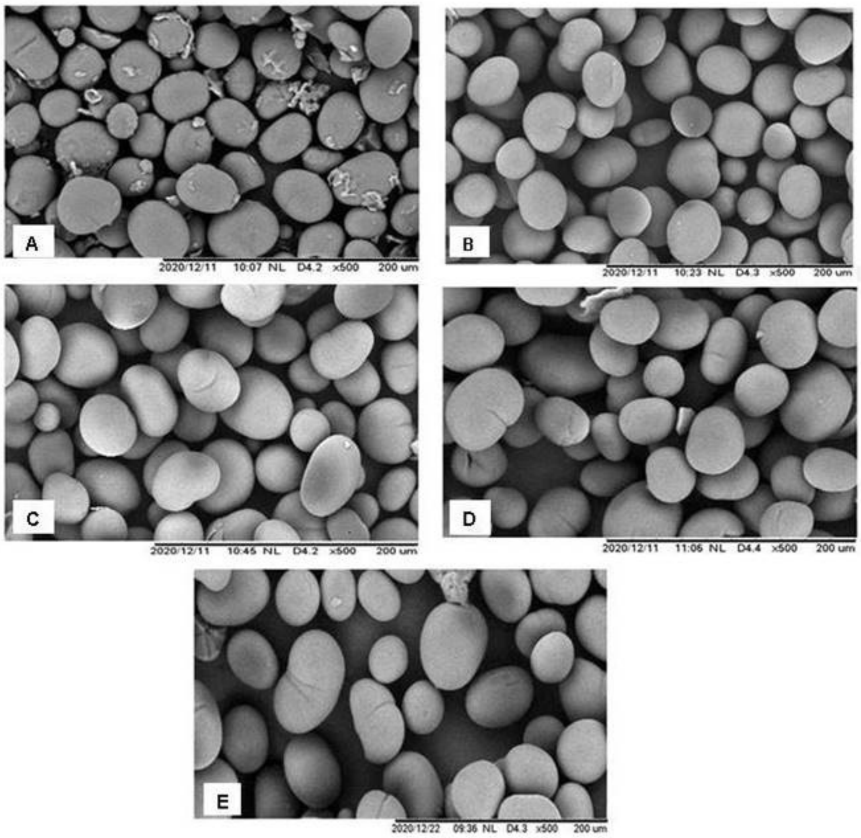

Figure 2. Scanning electron micrograph of lima bean starch granules at magnification of 500x. (A) pH 7; (B) pH 8; (C) pH 9; (D) pH 10; (E) pH 11.

Higher extraction pH levels (10 and 11) resulted in some damage to the starch granule surface, with some granules exhibiting grooves of varying depths. This observation is consistent with the findings of Bharti et al. (2019) and Nadiha et al. (2010), who reported that alkaline-treated starches displayed grooves on their surfaces. At extraction pH levels of 10 and 11, some corrosive damages were observed (Figures 2D-2E), but the overall shape of the starch granules remained intact. The surface damage or depressions on the starch granules were attributed to the leaching of amylose, which increased at higher extraction pH levels. According to Sun et al. (2022), strong alkalis, such as NaOH penetrate the starch granules and react with lipids bound to amylose, leading to chemical instability in the amylose-lipid complex.

Furthermore, the leaching of amylose chains contributed to changes or losses in the starch granule shape. Amylose is primarily located in the amorphous regions of legume starch granules, with some amylose molecules scattered among the amylopectin groups. The leaching of amylose from within the granules may cause internal structure changes and surface depression after alkali treatment. Similar findings were reported by Cai et al. (2014) and Nadiha et al. (2010) when alkali concentration and steeping were increased.

3.3 Starch crystallinity

Starches exhibit semi-crystalline structures consisting of amorphous and crystalline regions that could be observed by XRD. The study by Li & Gong (2021) showed that the thickness of amorphous lamellae is determined by the amounts of amylose and amylopectin and their chain lengths. The crystalline phases in starch correspond to the occurrence of the orthorhombic crystalline structure for A-type starches and the hexagonal structures for B-type starches (Rodriguez-Garcia et al., 2021). Crystalline parts are indicated by sharp peaks in the XRD patterns, meanwhile, the dispersive curves correspond to amorphous regions (Chen et al., 2022). Starch granules contain orthorhombic and hexagonal crystalline structures that can be identified in the X-ray diffraction patterns. This pattern is also influenced by crystal sizes and lattice disorder distribution (Singh et al., 1995). The diffraction peaks (2θ) at 15.018, 17.1435, 17.959, and 23.008° (Figure 3) correspond to the orthorhombic and hexagonal crystalline structures, identifying this starch as C-type (Dome et al., 2020). The XRD patterns of lima bean starch at different pH extractions are shown in Figure 3 (2θ (Degrees) vs Intensity (Arbitr. Unts)). All starches showed individual peaks at 2θ values of 15° and 23°; along with unresolved peaks at 17°(17.1435 corresponds to the (103) direction of the orthorhombic crystalline structure; unresolved 18° (17.959 corresponds to the (121) direction of the orthorhombic crystalline structure), and unresolved 5.63° (corresponding to the (100) 5.5056 for the hexagonal structure).

All treatments of extracted lima bean starch had a specific X-ray diffraction pattern, with main or strong peaks at 2θ at 15.1029°-15.1530° and 23.1076°-23.2079° and double peaks at 17.1470°-17.2085° and 18.0107°-18.0942°. Lima bean starch also exhibits a very weak peak at 5.2098°-5.4004°, reflecting orthorhombic and hexagonal diffraction patterns. The extraction pH treatment did not affect the crystalline structure. The pH treatment did not produce significant changes in hexagonal and orthorhombic nanocrystals. This diffraction pattern is similar to that of mung bean and cowpea starches (Kim et al., 2018). The extraction pH treatment did not affect the XRD pattern but did affect the peak intensity and crystallinity for the five treatments, and it could be noted that similar observations were reported by Bharti et al. (2019). The lima bean starch is classified as a C-type starch, a common characteristic among legume starches (Kim et al., 2018).

From a crystallographic perspective, each diffraction is independent, some time they are very close. C-type crystalline structure does not exist. Orthorhombic crystalline structure is found in A-type starch, hexagonal structure in B-type starch, and C-type starches have both crystalline structures, but this is not a new crystalline phase. The C-type starch was intermediate between the A and B-types (Ratnayake et al., 2002). Rodriguez-Garcia et al. (2021) reported the A-type orthorhombic and B-type hexagonal crystalline structures. The XRD pattern of C-type crystalline structures might contain various superpositions of diffraction peaks, which is affected by the ratio of the content of these polymorphs (Dome et al., 2020). The only difference was in the diffraction intensity at the dominant peak. The intensity of any X-ray pattern depends on intrinsic and extrinsic properties, such as the amount of sample. It seemed that the amylose content did not affect the crystallinity degree.

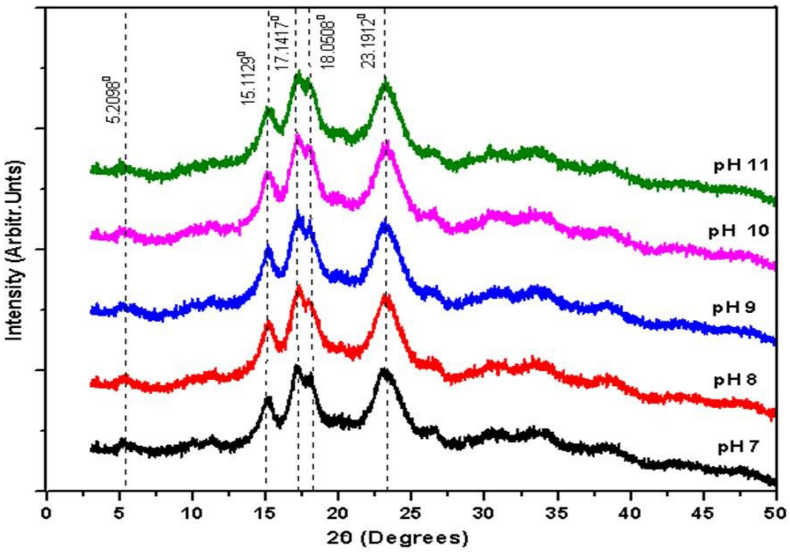

Figure 3. XRD pattern of lima bean starch extracted at alkaline pH.

The diffraction patterns of all lima bean starches from different extraction pH levels remained unchanged and exhibited C-type starch. The only difference was in the diffraction intensity of the dominant peak. Extraction at pH 7 resulted in a lower diffraction intensity at the dominant peak, which enhanced at extraction pH 8 and 9 but decreased slightly at extraction pH 10 and 11. A similar finding was reported by Wang & Copeland (2012) that pea (*Pisum sativum* L.) starch, after alkali treatment, retained its C-type starch characteristics, indicating that there are no significant structural transformations in the orthorhombic and hexagonal structures. The presence of gradual changes in the diffraction pattern can be attributed to fluctuations in the amylose content over a small range change in crystallinity accompanied by changes in intensity. Starches have super-molecular structures, consisting of amylose with a left-handed α-helical structure, and the building nanocrystals dimension. The nanocrystals have relatively uniform chain lengths (DP 16.5–25.2) (Zhang et al., 2021), therefore the changes in crystallinity are relatively small after alkaline pH extraction.

The crystallinity of starch as affected by extraction pH treatment was from 29.32 to 34.46% (Table 2). Similar values were reported by Fu et al. (2020) for *Vicia sativa* L. starches (22 to 34%). Extraction pH significantly affected the crystallinity degree of lima bean starches. The increase of extraction pH from 7 to 9 significantly ($p < 0.05$) increased the crystallinity degree, and further increase of extraction pH decreased the crystallinity degree. It might be affected by the small changes in moisture content, thus causing the crystalline lamellae to break down and decrease the crystallinity. According to Yang et al. (2021), changes in crystal peaks indicated the destruction of hydrogen bonds between starch chains. Possibly, an increase in the extraction pH to 10 and 11 caused the ionization of the hydroxyl group into OH^-, thus disrupting intermolecular chain interaction in the crystalline region. Meanwhile, the surface of the starch granules could be eroded by the alkali solution and form a cavity (Jiang et al., 2014), thus causing the crystalline lamellae to break down and decrease the crystallinity. Starch granules exhibit a structure "onion-like" governed by semicrystalline growth rings as an alternating stack of crystalline and amorphous lamellae (Zhong et al., 2020a). The highly ordered organization of starch granules is mainly contributed by amylopectin, whereas the defects of lamellae structure are in conjunction with amylose (Bertoft, 2017). A study by Zhong et al. (2020a) demonstrated that the main contributors to crystalline lamella are short amylopectin chains (DP 6–18), and longer chains (DP 19–30) showed a positive correlation with decreased crystallinity. It seemed that the alkaline OH^- increases the interaction of starch with water, slightly affecting the XRD peak intensity. Increasing extraction pH from 7 to 9 enhances the degree of starch crystallinity that might be related to the increasing starch interaction with water. However, higher extraction pH at 10 and 11 decreases crystallinity due to the ionization of the starch chain by OH^-, thus breaking down the starch molecule interchain interaction. However, when the moisture content effect is removed, the starch crystallinity remains unchanged. No changes were observed in the structural properties because pH did not affect the starch nanocrystals (orthorhombic and hexagonal).

Table 2. Degree of crystallinity, pasting properties, and functional properties of lima bean starches.

Parameter	Starch Extracted at				
	pH 7	pH 8	pH 9	pH 10	pH 11
Degree of crystallinity (%)	29.32 ± 0.62^{b}	33.51 ± 1.24^{a}	34.46 ± 0.06^{a}	31.67 ± 1.26^{b}	30.48 ± 1.05^{b}
PV (cP)	3173.00 ± 115^{c}	3415.50 ± 152^{bc}	3905.50 ± 63^{a}	3846.00 ± 119^{ab}	3725.50 ± 36^{ab}
TV (cP)	2919.50 ± 64^{b}	3019.00 ± 63^{ab}	3473.00 ± 88^{a}	3403.00 ± 114^{ab}	3207.50 ± 11^{ab}
VBD (cP)	254.00 ± 179^{a}	396.50 ± 73^{a}	431.50 ± 25^{a}	443.00 ± 30^{a}	518.00 ± 25^{a}
FV (cP)	5714.00 ± 42^{c}	6160.50 ± 133^{a}	5968.50 ± 60^{b}	6136.50 ± 50^{a}	6037.00 ± 52^{a}
SBV (cP)	2795.00 ± 21^{ab}	3014.00 ± 96^{a}	2495.50 ± 28^{b}	2732.00 ± 65^{b}	2829.50 ± 41^{ab}
PT (°C)	84.00 ± 0.00^{a}	83.25 ± 0.07^{a}	82.80 ± 0.67^{a}	82.75 ± 0.57^{a}	83.20 ± 0.00^{a}
Functional properties:					
Swelling power (g/g)	11.80 ± 0.10^{c}	13.20 ± 0.51^{b}	17.41 ± 0.35^{a}	17.25 ± 0.25^{a}	16.83 ± 0.05^{a}
Solubility (%)	12.01 ± 0.42^{c}	12.60 ± 0.10^{bc}	14.26 ± 0.20^{a}	14.07 ± 0.20^{a}	13.55 ± 0.43^{ab}
WAC (g/g)	0.87 ± 0.01^{a}	0.86 ± 0.01^{ab}	0.85 ± 0.06^{bc}	0.85 ± 0.01^{bc}	0.84 ± 0.01^{c}
OAC (g/g)	0.85 ± 0.01^{a}	0.83 ± 0.01^{b}	0.83 ± 0.01^{b}	0.83 ± 0.01^{b}	0.81 ± 0.01^{c}

Results expressed as mean ± standard deviation. Means in a row with different superscripts are significantly different ($p < 0.05$). PV: peak viscosity; TV: trough viscosity; VBD: breakdown viscosity (BD = PV - TV); FV: final viscosity; SB: setback viscosity (SB = FV - TV); PT: pasting temperature. (centipoise = cP); WAC: water absorption capacity; OAC: oil absorption capacity.

3.4 Pasting properties

During heating, when starch is present in the presence of water, hexagonal and orthorhombic nanocrystals might not be completely soluble, which allows the formation of lamellae. Lamellae are solvated planes derived from the original crystal structure, which is the most responsible for the interpretation of starch physicochemical properties including pasting profile. Starch could be defined as a submicron or micro-particle containing amylose, amylopectin, free and linked water, minerals, hexagonal and orthorhombic nanocrystals, proteins, and lipids. Each of these compounds plays a central role in the changes in the pasting profile (Esquivel-Fajardo et al., 2022). Table 2 and Figure 4 describe the starch pasting properties of lima beans obtained at different extraction pH levels. Extraction pH resulted in a significantly different ($p < 0.05$) peak, trough, setback, and final viscosity.

Peak viscosity indicates the end of the stable hydrogel formation whereas the temperature at peak viscosity is the temperature at which the gel finalizes the formation, and at this point no starch granules are integer. Peak viscosity also indicates the capacity of the starch granules to absorb water and is marked by a halt in swelling. At this point, an equilibrium occurs between the swollen granules and the leachate polymers (Rincón-Londono et al., 2016). The peak viscosity of alkaline-treated lima bean starch was 3173-3905 cP. The starch extracted at pH 7 exhibited the lowest peak viscosity. Increasing extraction pH to 9 significantly increased peak viscosity. The lower peak viscosity of starch extracted at pH 7 can be attributed to the presence of protein at the surface of the starch granule (Figure 2) and ash content (Table 1), limiting the starch granules from reaching their maximum swelling. According to Nadiha et al. (2010), removing lipids and protein from the surface of starch granules may cause swelling during pasting, thus increasing viscosity. Peak viscosity is also affected by amylose content, which is influenced by the high rigidity and integrity of amylose in the granular structure (Rafiq et al., 2016). Hence, at pH 10 and 11, the peak viscosity slightly decreased. Excessive alkali treatment at pH 10 and 11 compromised the amorphous structure, which reduced granular structure integrity and limited swelling capacity, thus decreasing peak viscosity (Wang & Copeland, 2012; Bharti et al., 2019).

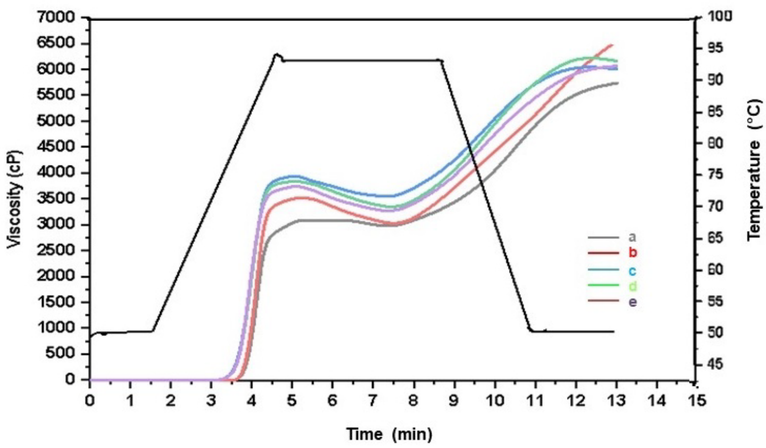

Figure 4. Pasting characteristics of lima bean starch from different extraction pH. (a) pH 7; (b) pH 8; (c) pH 9; (d) pH 10; (e) pH 11.

Trough viscosity was obtained after starch granule rupture, representing the lowest viscosity obtained after retaining at the highest temperature (Karakelle et al., 2020). The trough viscosity ranged from 2919 to 3473 cP, with the highest value observed at pH 9, thus corresponding to the highest amylose content, demonstrating a positive correlation between both amylose content and trough viscosity. Presumably, the alkaline treatment modified the amylose structure by ionization of the OH groups, disrupting the interaction of amylose chains due to negatively charged chains. Consequently, amylose retained viscosity by retaining water through interactions with the negatively charged chains.

The final viscosity of lima bean starches was 5744-6180 cP. The lowest final viscosity was found in starch extracted at pH 7. Starch extracted at higher pH levels also exhibited significantly higher final viscosity ($p < 0.05$). Amylose played a significant role in the final viscosity, which was higher for starch extracted under alkaline conditions. Wang et al. (2014) reported that an increase in final viscosity could be caused by amylose molecule aggregation. The setback viscosity was an indicator of starch retrogradation or syneresis upon cooling (Wang et al., 2014). The setback viscosity was the difference in the pasting curve between the final and trough viscosity (hot viscosity) (Nadiha et al., 2010). The setback viscosity of lima bean starch was 2494 to 2895 cP, which was higher than *Vicia sativa* L. (1314 to 2097cP) (Fu et al., 2020) and oxidized lima bean starch (844 to 2050 cP) (Okekunle et al., 2020). The lowest setback viscosity was found in starch extracted at pH 9, with no significant difference ($p < 0.05$) from that extracted at pH 10. A decrease in setback viscosity reflected a reduced retrogradation rate. The presence of OH^- ions prevented starch molecules from realigning after cooling, resulting in lower setback viscosity. A reduced setback viscosity was also found in alkaline-treated corn starch (Nadiha et al., 2010). Increasing pH to 11 also increased setback viscosity. Ionization of the hydroxyl group at high alkaline pH enhanced the ability of starch molecules to bind water and retain water on their structure; the final viscosity was also high. This binding and retention implied high setback viscosity, although viscosity also increased due to starch's ability to retain viscosity during stirring at the highest constant temperature.

Breakdown viscosity showed no significant difference, indicating no difference in the shear resistance of swollen starch granules among all lima beans treated. In this study, lima bean starches had lower breakdown viscosity (254 to 518 cP) than kidney beans and green gram starch reported by Andrabi et al. (2016). Previous findings by Bharti et al. (2019) and Okekunle et al. (2020) showed that the lower values of breakdown viscosity indicate restricted starch granule swelling and to withstand greater stress distribution and heating.

Pasting temperatures of lima bean starch were unaffected by different extraction pH levels. The pasting temperature ranges from 82.75 to 84.00 °C, depending on the extraction pH. Compared to the previous reports, pasting temperatures in this study were within the range for native lima bean starch (Okekunle et al., 2020) and similar to that observed for *Lablab purpureus* L. starch (Prazeres et al., 2021). The higher viscosity temperature indicates its great resistance to swelling (Prazeres et al., 2021). Therefore, in this study, amylose content exhibited a positive correlation with the viscosity parameters of pasting properties. Modification of alkali treatment by ionization of starch chain molecules resulted in different phenomena of pasting.

3.5 Functional properties

The swelling power and the solubility of lima bean starch extracted at various alkaline pH levels are shown in Table 2. The swelling power of alkali-extracted lima bean starches ranged from 11.80 to 17.41 g/g, which was higher than oxidized lima bean starches (11.37 to 15.37 g/g) (Okekunle et al., 2020) and pea starches (10.1 to 11.5) (Wang & Copeland, 2012). The lowest swelling power was observed in starch extracted at pH 7, and increasing the extraction pH also increased the swelling power significantly ($p < 0.05$), but a slight decrease was found in starch extracted at pH 11. Hence, alkali extraction might disrupt amorphous regions within the granule. Heat treatment of starch granules in excessive water weakened the crystalline structure and increased the hydrogen bonds formed between amylose and water, thus increasing the swelling power (Kaur et al., 2011). This finding was in accordance with Nadiha et al. (2010) who reported that the increase in swelling power was believed to be due to the interruption of the amorphous region in the granule, thus allowing the granule to expand more freely.

The solubility of lima bean starch ranged from 12.01 to 14.26%. This solubility was in agreement with the previous report of native lima bean starch (Okekunle et al., 2020), but lower than alkali-modified horse chestnut starches (Rafiq et al., 2016). The lowest solubility was found in the starch extracted at pH 7. Increasing pH to 9 and 10 increased solubility significantly ($p < 0.05$). Alkali treatment disrupted intermolecular hydrogen bonds between starch chains (Kaur et al., 2011; Jiang et al., 2014), thus increasing the solubility. The granule structure's amylose content and the organization influenced swelling power and solubility (Singh et al., 2005; Bharti et al., 2019). In this study, amylose content exhibited a positive correlation with solubility. Starches with higher amylose content had lower water solubility due to the amylose inhibition effect on swelling (Zhong et al., 2020b).

Water and oil absorption capacities of alkaline extracted lima bean starches are shown in Table 2. The water absorption capacity (WAC) varied from 0.84 to 0.87 g/g, which was comparable to kidney bean starches (Andrabi et al., 2016). Increasing the extraction pH significantly reduced WAC ($p < 0.05$). The WAC analysis was conducted without heating. The crystalline nature of starch granules affected the starch's ability to absorb water. The decreased WAC value with increasing pH extraction is likely due to the granulator's associative forces following alkali treatment. Our research findings were comparable to those described on mango kernel starches (Bharti et al., 2019). The presence of OH groups in hydrogen formation and covalent bonds between starch elements may decrease the capacity to bind water. The oil absorption capacity (OAC) of alkaline-extracted lima bean starches ranged from 0.81 to 0.85 g/g. Oil binding capacity decreased significantly ($p < 0.05$) when extraction pH was increased. According to Bharti et al. (2019), the decrease in oil binding capacity can be induced by disruption of the amorphous region, which decreases the amount of oil binding sites. Increasing extraction pH significantly decreased OAC ($p < 0.05$). A similar phenomenon to that observed in WAC was also found in OAC.

4 Conclusion

The present research has provided an overview of the impact of alkali extraction pH on various properties of lima bean starch. It has been found that extraction pH significantly affects the physicochemical, morphological, pasting, and functional characteristics of lima bean starch, while the crystallinity remains unchanged. Increasing the extraction pH resulted in a higher level of purity. Lima bean starch exhibited a higher amylose content ranging from 44.03% to 50.65%. However, a slight decrease in amylose content was observed at higher extraction pH levels. The amylose content influenced the morphological, crystallinity, pasting, and functional properties of starch. Morphological characteristics of alkaline-treated starches showed deformation with corrosive damage to the granular surface at higher extraction pH levels. Extraction pH caused no changes in the XRD pattern but resulted in a slight change in intensity and crystallinity. The pasting properties of lima bean starches varied significantly with different extraction pH levels. Generally, starch showed a high pasting temperature and lower values of breakdown viscosity. High swelling power, WHC, OAC, and pasting properties are all excellent, providing an opportunity to utilize the potential of starches in unexplored industrial applications.

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