

Microstructural study of enzymatically and non-enzymatically hydrolyzed potato powder

Ishtiaq Ahmad^{1,2}  | Zhouyi Xiong³ | Xiong Hanguo¹  | Fei Lyu² |
Rana Muhammad Aadil⁴  | Nauman Khalid⁵ | Noman Walayat² |
Muhammad Imran Taj¹ | Gaopeng Zhang² | Wei Tang² | Yan Li² | Minghui Li²

¹College of Food Science and Technology, Huazhong Agricultural University, Wuhan, PR China

²College of Food Science and Technology, Zhejiang University of Technology, Hangzhou, P.R. China

³Fisheries Research Institute, Wuhan Academy of Agricultural Sciences, Wuhan, PR China

⁴National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

⁵School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan

Correspondence

Xiong Hanguo, College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, PR China.
Email: xionghanguo@163.com

Abstract

In this study, potato powder (PP) was hydrolyzed with α -amylase and investigated the characteristics of PP and enzymatically hydrolyzed potato powder (EHPP). The PP was enzymatically hydrolyzed for 30 min at 50°C with different amounts of α -amylase (0 to 0.015 g/100 ml) for 30 min at 50°C. The swelling power, water solubility index, amylose contents, and dextrose equivalent values of EHPP were improved substantially in comparison to native PP. The α -amylase concentration of 0.015/100 ml with heat treatment boosted the scale of hydrolysis of PP. Zeta potential (ζ) and particle size distribution showed improved stability and dispersibility of nanocrystals after enzymatic hydrolysis. Further, X-ray diffraction and Fourier transform infrared analysis showed that the amorphous area of potato starch was effectively hydrolyzed by α -amylase. Transmission electron microscopy and scanning electron microscopy depicted enzymatic erosion accrued primarily at PP granules' surface. EHPP exhibited coarse surface and porous granules as compared to the native form of PP. The results suggest that enzymatic treatment using α -amylase could be feasible to proficiently prepare EHPP for product development. The results suggested that the EHPP hydrolyzed (0.015 g/100 ml α -amylase for 30 min at 50°C) has the potential for developing and stabilizing the new kind of food products with high values added.

Practical applications

It is recommended that enzymatically hydrolyzed potato powder (EHPP) with α -amylase provides a reference for food products with the addition of starch-based ingredients as well as added market value because of the potential functional properties of potato powder. Moreover, the novel EHPP not only improves the quality but also has numerous health benefits after combining it with other powder proteins. The results provide information about the use of EHPP for the development of potato-based products, such as ethnic foods and gluten-free products, with specific rheological and functional properties.

1 | INTRODUCTION

Potato (*Solanum tuberosum* L.) is a dicotyledonous plant and can grow on a range of soil types and conditions with strong growth ability, and this crop is termed an economic crop in several countries (Bovell-Benjamin, 2007; Rashid et al., 2021). It is the fourth largest cultivated crop after wheat, maize, and rice having 0.37 billion tonnes of production worldwide (Hu et al., 2020). Potato is rich in biomass-rich proteins that contain large amounts of vitamins B3, B6, and C, and it is a good source of minerals like calcium, iron, phosphorus, potassium, and copper (Andre et al., 2007; Yong et al., 2018). Lysine and tryptophan are available in handsome quantities that meet the human nutritional need. Recently, researchers able to produce antioxidant-rich potatoes with an appreciable quantity of phenolic compounds like anthocyanins and carotene can be spoiled quickly because it contains starch and other high nutritional value ingredients (Guo et al., 2019; Kim et al., 2019).

Potato powder (PP) is made from hot air-dried peeled potato slices subsequently ground and sieved while heat applied during the drying period impacts the quality of starch and hence affects the properties of PP (Cui et al., 2018). The protein content of PP is better than that of cassava and yam powder but equal to that of rice; however, its fiber content is higher than processed wheat powder, maize, and rice (Wang et al., 2020). Nutritionally PP is equivalent to potato tuber and supplies proteins, fiber, and carbohydrates and it is extensively used in bakery products, sauces, gravy, and snacks (Avula & Singh, 2009).

However, PP properties are dependent on several factors including, techniques of formation, heat intensity and duration, modifications, and occurrence of other constituents like protein and fiber (Van Hal, 2000). The powders have high sustainability and thus can withstand conditions such as high heating and cooling processes and their utilization in products entailing sterilization is possible. Potato powder, because of its high starch content, displays distinctive characteristics that identify in designing new product formulations (Pu et al., 2017). The data about the functional properties of PP is scarce and is different from that of starch and as well as other components like proteins, fats, and non-starch polysaccharides (Jangchud et al., 2003). The alterations or modifications in PP can also be made using chemicals and enzymes, which influence its dough rheology, gel-forming properties, paste viscosity, and swelling ability, while the enzyme-treated powder having high paste viscosities have shown to be good thickeners (Avula & Singh, 2009). The α -amylase enzymes are hydrolyzing enzymes that break glycosidic bonds of starch to form glucose, maltotriose, maltose, and dextrin (Mu et al., 2015). The α -amylase enzyme functions by catalyzing the breakdown of α -1,4-O-glycosidic bonds of polysaccharides while retaining the α -anomeric conformation in low molecular weight compound glucose, maltose (Aquino et al., 2003; Nguyen et al., 2002). The α -amylase enzymes fall in the category of glycosyl hydrolases and these are responsible for breaking glycosidic linkages between two carbohydrates or between carbohydrate and non-carbohydrate compounds (Polaina & MacCabe, 2007). The amylase enzymes have

wide usage across the industries like paper, food, textile, and detergent, which signifies their importance (Polaina & MacCabe, 2007; Soares & Távora, 1959).

A recent study provided information about the use of potato powder in the development of food products with desired quality characteristics (Hu et al., 2020). However, there is an available study on enzymatically hydrolyzed sweet potato flour with an α -amylase enzyme (Shariffa et al., 2009) but there is no study available on enzymatic hydrolysis of whole potato powder through α -amylase enzyme and makes it suitable for the development of food products.

The present work aimed to elucidate the effects of enzymatic hydrolysis with α -amylase enzyme on the structural, microstructural, and hydrolysis properties of PP by the analysis of Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy analysis, Particle size distribution measurement and Zeta potential (ZP) (ζ) measurement, amylose contents solubility, and swelling power and rheological properties. These results would provide useful information to stabilize and development of food products for desired properties.

2 | MATERIAL AND METHODS

2.1 | Materials

PP (carbohydrates 81.7%, fat 1.0%, protein 8.6%, and sodium 35mg/100g) was obtained from the ZhangJiakou Yunbie Potato Development Co., (Hubei, China) and α -amylase (4000 U/g) was purchased from Beijing Culture Media Product Field (Beijing, China). All chemicals used in this study were of analytical grade and used without additional purification.

2.2 | Hydrolysis of potato powder, water-solubility index and swelling power, amylose content, dextrose equivalent (DE) value, and rheological analysis

The rapid hydrolysis of PP was done by treating PP with bacterial liquefying α -amylase at 50°C for 30 min following (Shariffa et al., 2009) with some modification. The potato powder was mixed with water (1:8), respectively, and adds α -amylase (0, 0.011, 0.012, 0.013, 0.014, and 0.015 g/100 ml) at 50°C with a magnetic stirrer water bath (DF-101S type, Wuhan Hengtaida Instrument Equipment Co., Ltd., Wuhan, P.R China) for 30 min and followed by heating for 10 min more on boiling water. The water solubility index (WSI) and swelling power (SP) of PP and enzymatically hydrolyzed potato powder (EHPP) samples were measured according to the method of Senanayake et al. (2013), with some modifications. Each sample of PP and EHPP was assessed for its amylose content with the method outlined by Shariffa et al. (2009), with some modifications. The reducing sugar maltose formed as a result of hydrolysis of PP and EHPP was estimated using the dinitrosalicylic (DNS) acid method

(Miller, 1959). Stable and dynamic rheological parameters of optimized EHPP and PP were assessed using the previously described methods (Ahmed et al., 2017). The optimized EHPP was also evaluated based on SEM analysis, TEM, XRD, FTIR, spectroscopy analysis, PSD measurement, and (ZP) (ζ) measurement along with PP.

2.3 | SEM analysis

PP and EHPP samples were freeze-dried for 48 h under 0.2 millibar pressure fixed in liquid nitrogen using a freeze dryer (Beta 2-8 LD Freeze Dryer, CHRIST Co., Ltd., Germany). These freeze-dried samples were placed on aluminum stubs and covered by a gold layer. Observations were carried out through a SEM with (X2000 10 μ m) magnification (Model: JSM-6390LV; NTC, Tokyo, Japan) and an accelerated voltage of 15 kV. The PP and EHPP pellets were fixed on aluminum specimen stubs with two-sided adhesive tape and sputtered with a 20–30 nm gold layer with a sputter coater (Polaron [Fisons] SC515, VG Microtech, Sussex, UK).

2.4 | TEM, XRD, and FTIR spectroscopy analysis

The suspension of PP and EHPP samples was prepared using distilled water and sonicated for 10 min. A drop diluted suspension droplet was placed on a carbon-coated copper grid and dry in at room temperature (25°C). Subsequently, the form of latex particles was observed using Hitachi (Japan) H-7650 TEM Microscope at 70 kV. Crystallinity patterns of PP and EHPP were performed using the XRD method as described earlier (Blazek & Gilbert, 2010), with a Shimadzu X-ray diffractometer in Tokyo Japan. The alterations in the secondary structure of protein and starch of PP were observed with an FTIR spectrometer (Nicolet 380, Thermo Scientific Inc. Waltman, MA, USA) at room temperature. PP and EHPP samples were freeze-dried at –80°C for 48 h and blended with potassium bromide (KBr) powder and pressed into pellets before measurements and scanning of all pellets were done 64 times ranging from 400 to 4000 cm^{-1} . The device was removed with nitrogen and spectra were taken over the 400–4000 cm^{-1} range. Omic version 8.3, professional software windows were used for the removal of background interference and finally, spectra obtained were analyzed using OMNIC software (Thermo Nicolet Madison, WI, and the USA) or Origin Pro 9 software (Microcal Northampton, MA, USA).

2.5 | PSD measurement

PSD measurement of starch granules was analyzed on a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The $d(4, 3)$ (volume-weighted mean diameter), and $d(0.5)$ (surface weighted mean diameter) were noted. For measurement (1 g) of the sample was added in 20 ml of distilled water proceeding with an ultrasonic bath for 10 min to homogenize properly. For analysis, a small amount

of sample (2 ml) was dispersed in a sampler at 25°C. Particle size was determined in triplicates to calculate the mean values.

2.6 | ZP (ζ) measurement

The ZP (ζ) of PP and EHPP particles in aqueous suspensions was estimated at 25°C using a Laser Doppler Electrophoresis (LDE) unit (Nano ZS90 Analyzer, Malvern UK). DTA1070- folded capillary cell was used for the measurement following the method reported (Teng et al., 2012). The PP and EHPP suspension were prepared by adding the specimen to deionized water to get the concentration of 0.01% (w/v). The ZP (ζ) for PP and EHPP was measured at neutral pH and in triplicates.

2.7 | Statistical analysis

All calculations were recorded in triplicates and the data was analyzed using ANOVA. The means significant difference was measured at $p \leq 0.05$ and the results were represented as means $\pm$ standard deviation using the LSD test.

3 | RESULTS AND DISCUSSION

The α -amylase significantly modified the PP solubility, SP, amylose contents, dextrose equivalence, and physical characteristics and made it suitable for product development.

3.1 | Water solubility index (WSI) and swelling power (SP)

The WSI and SP of PP and EHPP samples are shown in Table 1 and the WSI of EHPP is significantly higher ($p < 0.05$) than PP samples.

TABLE 1 Water solubility index, swelling power, and amylose contents of PP and EHPP

Samples α - amylase g/ml	Water solubility index g/g (%)	Swelling power g/g (%)	Amylose contents (%)
0	14.74 $\pm$ 0.06 ^g	11.77 $\pm$ 0.12 ^g	21.17 $\pm$ 0.15 ^e
0.01	16.61 $\pm$ 0.53 ^f	16.2 $\pm$ 0.72 ^f	21.50 $\pm$ 0.19 ^e
0.011	19.57 $\pm$ 0.50 ^e	20.27 $\pm$ 1.20 ^e	22.04 $\pm$ 0.16 ^d
0.012	27.07 $\pm$ 1.46 ^d	23.8 $\pm$ 0.26 ^d	22.60 $\pm$ 0.09 ^c
0.013	30.1 $\pm$ 0.62 ^c	25.8 $\pm$ 0.26 ^c	22.90 $\pm$ 0.09 ^c
0.014	37.2 $\pm$ 1.04 ^b	27.5 $\pm$ 0.50 ^b	23.47 $\pm$ 0.15 ^b
0.015	44.24 $\pm$ 1.41 ^a	30.00 $\pm$ 0.50 ^a	23.90 $\pm$ 0.09 ^b

Note: Mean values of solubility, swelling power, and amylose contents ($\pm$ standard deviation) within the same column sharing a common superscript (a, b, c, d, e, f) significantly different ($p < 0.05$) using LSD test.

The WSI of EHPP increased with increased the α -amylase concentration and the treatment containing 0 g/100ml of α -amylase has 14.74% while the samples containing 0.015 g/ml have 44.24%. EHPP samples showed higher values of WSI as compared to PP. The results of WSI were consistent with the previous study of Takata et al. (1996), in which high-branch polymers are highly water-soluble due to their molecular mass and low amylose content, while a higher number of short-chain and degree of branching were attained. The increase in WSI is due to branch points of amylose that restrain the recrystallization of starch (Jensen et al., 2013). In addition, the increase of new branch points in amylopectin inhibited the formation of highly ordered structures and inhibits the realignment of the glucan chain (Guo, 2018).

The SP of EHPP was observed to be higher than PP (Table 1). These variations are likely due to the different experimental conditions and different growing conditions of potatoes. The high SP of EHPP is most likely due to the existence of charge phosphate monoester groups in granules of PP (Hemar et al., 2007). The previous study has explained that starch is influenced by several elements including granule integrity, amylose constitution, proteins and peptides, the chemical arrangement of amylopectin and the interactions between starch molecules (Srichuwong & Jane, 2007; Vamadevan & Bertoft, 2015). In this study, we have noticed that short α -chains and longer α -chains of amylopectin were positively correlated for SP. Short α -chains contribute to the development of building blocks while longer α -chains are meant to connect the smaller building blocks (Zhu et al., 2011). This proposes that shorter inter-block chain length promotes disordered arrangement of the double helices in granules (Vamadevan et al., 2013). The resulting disordered arrangement leads to enhanced hydration, swelling, and solubility of PP during hydrolysis.

3.2 | Amylose contents

The results of amylose contents were given in Table 1. The amylose contents of EHPP were significantly higher than PP. Amylose contents (21.50% to 23.90%) were found in EHPP treatment (0.01 to 0.015 g/ml of α -amylase) while the lowest amylose contents were found in control PP (without α -amylase enzymatic hydrolysis). This may be attributed to the core amylose levels and the consequent breaking of α -1,-4 linkages during hydrolysis. In all EHPP samples, we noticed a higher SP value that is less degraded and can retain the integrity of granules. The amylose of EHPP treatments was higher than the control PP value, which is quite relevant to the previous study of Wang et al. (2020).

3.3 | DE value

As shown in Figure 1, DE values were increased after increasing the concentration of α -amylase for hydrolysis. It was further noticed that subjecting PP to mild heat before enzyme hydrolysis improved

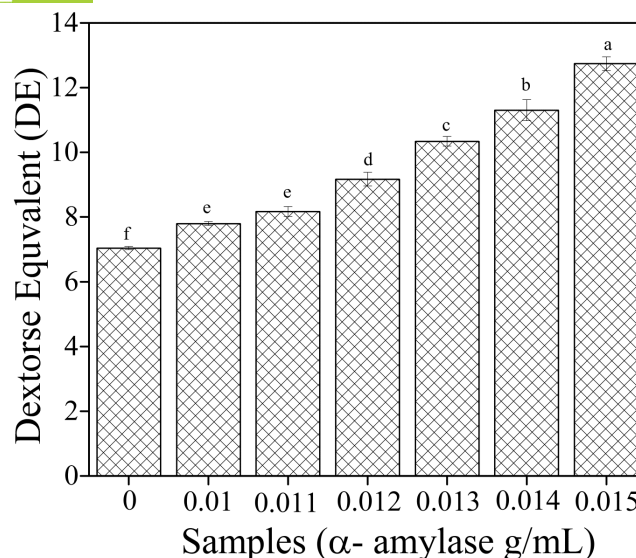


FIGURE 1 Mean values of dextrose equivalent (DE) ($\pm$ standard deviation) within the same column sharing a common superscript (a, b, c, d, e, f) significantly different ($p < 0.05$) using the LSD test.

the hydrolysis substantially. Keeping PP at 50°C for 30 min resulted in a partially irreversible swelling of an amorphous section of the granules, which helps the enzyme to access promptly and act on PP granules. It is also suggested that swelling of granules may enlarge the pinholes and internal spaces in PP granules thus permitting the enzyme to infiltrate swiftly and effortlessly into the granules. Juszczak et al. (2003), also suggested the pores on the surface of PP granules could serve as hubs of enzyme action. Another strong possibility for increased enzyme activity on EHPP granules might be due to the heat treatment of PP that impacts the weaker areas of granules, thus allowing more enzymatic action.

3.4 | SEM of optimized EHPP and PP

The enzymatic hydrolysis and its effects on PP and EHPP samples were observed under SEM (Figure 2a). The susceptibility of PP and EHPP mainly depends on the severity and way the granules are eroded and corroded (Das & Kayastha, 2019). The results of SEM showed that the enzyme primarily invaded the granular starch surface and eroded it. Then, the surface of EHPP granules was rougher and eroded (Figure 2a) as compared to PP granules (Figure 2a), which were less eroded. The heat treatment of PP caused a disturbance in hydrogen bonding among the polymer chains resulting in weaker granules subsequently contributing to easy penetration of enzyme and swift degradation of starch (Soottitawat et al., 2007). This caused the surface of PP to be rougher and corroded in contrast to EHPP. A large number of small spores were seen on EHPP granules (Figure 2a) in contrast to control PP granules (Figure 2a) when observed under magnification (X2000 10 μ m), we can state that α -amylase triggered the PP swelling and consequently the pinholes on the surface enlarged. This facilitated enhanced enzyme entry

FIGURE 2 (a) Scanning electron microscopy (SEM) of potato powder and optimized enzymatically hydrolyzed potato powder (EHPP) with magnification (X2000 10 μm) and (b) transmission electron microscopy (TEM) of potato powder and optimized enzymatically hydrolyzed potato powder with 1 μm magnification.

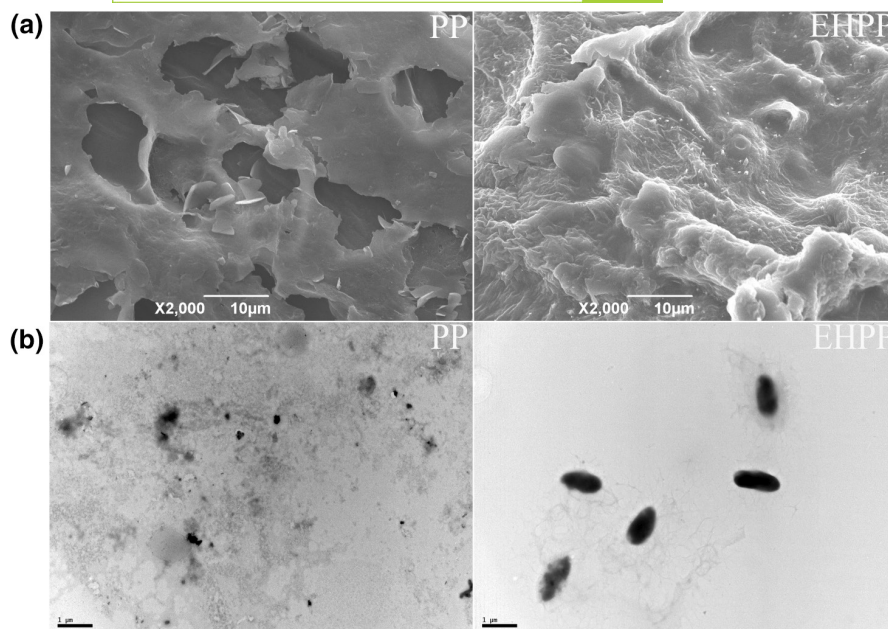
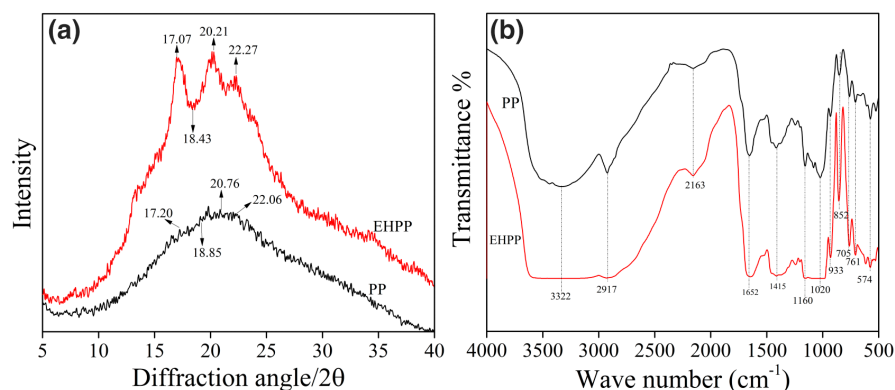


FIGURE 3 (a) X-ray diffraction pattern of potato powder (PP) and optimized enzymatically hydrolyzed potato powder (EHPP) and (b) FTIR spectrum of potato powder (PP) and optimized enzymatically hydrolyzed potato powder (EHPP).



into the granules and resulted in the pore and channel formation (Kurakake et al., 1996). It was also noticed that EHPP has more amylase-digested areas than control PP. When outside the granule, α -amylase performs surface changes and corrodes the external surface of the granule but when it enters inside the granule it starts corroding internally through the tiny poles from where the enzyme entered (Aggarwal & Dollimore, 1998). It was noticed that granules of both PP and EHPP were rough and eroded (Figure 2a) and especially in control PP, large pores were prevalent on its surface.

3.5 | TEM of optimized EHPP and control PP

Transmission analysis is a very useful and commonly used technique for studying the form of colloidal systems. TEM micrograph of the latex particles from this research has shown in Figure 2b. The latex system presented small and polydispersed particles as illustrated by the well-defined TEM images. The characteristics of spherical polymer colloids in all micrographs of the latex system. Moreover, the particle was aggregated without α -amylase hydrolyzed indicated as represented in Figure 2b. In contrast, latex particle was evenly

distributed for EHPP indicating a polydispersed system depending upon the hydrolysis. This might be attributed that hydrolyzed α -amylase readily absorbed on the surface of the dispersed particles consequently, then the aggregation of latex particles was prevented (Rajisha et al., 2014). These dents and pores facilitate the penetration of α -amylase into the interior of the PP particles, thereby reducing the preparation duration of EHPP (Figure 2b) and presenting the micro-morphological and phasic images of EHPP with round edges. It was assumed that EHPP was composed of platelet-like nanoparticles. In addition, grape-like aggregates of EHPP were also found in Figure 2b with a particle size of 1 μm . Observation by TEM of PP and EHPP originated from the hydrolysis after (Figure 2b), respectively. The particles were linked in larger aggregates composed of smaller granules, which conformed to the results of the SEM image (Figure 2a).

3.6 | X-ray diffraction

XRD configurations of PP and EHPP were presented in Figure 3a. The XRD can be employed to discriminate between PP and EHPP. It can

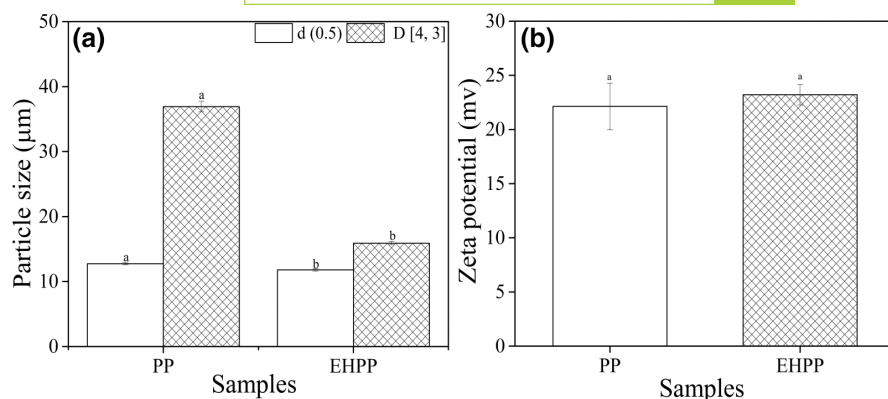


FIGURE 4 (a) Particle size of potato powder (PP) and optimized enzymatically hydrolyzed potato powder (EHPP) and (b) zeta potential (ζ) of potato powder (PP) and optimized enzymatically hydrolyzed potato powder (EHPP). (the values of zeta potential [ζ] from 5 to 30 = -5 to -30).

also perceive the alterations in the crystallinity in the granules of EHPP, which are caused by the α -amylase enzyme. We observed distinctive α -type arrangements in granules of PP and EHPP with clear peaks at 2θ about 17.07, 18.43, 20.21, and 22.27 in EHPP and 17.20, 18.85, 20.76, and 22.06 for PP. Similar α -type configurations were also noticed by Shariffa et al. (2009), on tapioca starch that was subjected to α -amylase and amyloglucosidase enzymes at 37°C. In another study, sweet potato starches were observed for XRD and noticed configuration on granules with three primary peaks at $2\theta = 15.31$, 17.11, and 23.51 (Moorthy, 2002). Although starches from different sources give comparable XRD patterns EHPP showed more crystalline features. The peaks obtained with EHPP are sharper and well-shaped in contrast to PP. This implied that α -amylase has extensively performed hydrolysis of amorphous regions of EHPP as compared to control PP and sharper XRD peaks designate that these regions are extensively interrupted by α -amylase (Mu et al., 2015). Our results are also supported by the findings of Mu et al. (2015), who reported more enzyme activity on amorphous domains of potato starches.

3.7 | FTIR spectroscopy

FTIR spectra of the samples were carried out and the results are shown in Figure 3b. In the PP spectrum fingerprint zone, four typical peaks that appeared between 930 and 1159 cm^{-1} are credited to C–O bond stretching (Jiang et al., 2015). The peaks were observed at 946, 1020, 1068, and 1132 cm^{-1} , while 950 and 1132 cm^{-1} observed in EHPP are linked to organized PP starch and primarily attributed to anhydroglucose ring C–O stretch of C–O–H in starch. Interestingly, a peak at 1020 cm^{-1} was recorded in control PP but this peak vanished in EHPP at 1058 cm^{-1} , which is due to the vibrations in the skeleton of α -(1 $\rightarrow$ 4) glycosidic linkage (C–O–C), and it is also sensitive to water and responsible for hydrophilicity of starch (Fang et al., 2002). Another sharp peak was recorded at 1652 cm^{-1} which is described to be vibrations caused by the distortion of the hydroxyl group in water and corroborates the occurrence of tightly bound water in PP and EHPP. At 3100 to 3600 cm^{-1} an extensive band emerged, which is attributed to the intricate vibrational expansion of free, inter and intra-molecular attached hydroxyl group that makes the overall

assembly of the PP starch. In both PP and EHPP, a discrete band was noticed at 2910–2917 cm^{-1} which is due to the typical $-\text{CH}_2$ expansion vibrations linked to the ringed methane hydrogen bonds. The sharper peak found in PP at 3322 cm^{-1} and this peak has been disappeared at EHPP at 3336 cm^{-1} . The band observed between 856 and 867 cm^{-1} is representative of organized morphology of starch and band that responded to amorphous starch was found at 1020 cm^{-1} (Mu et al., 2015). It was further noticed that there was a change in intensity of the bands after the hydrolysis was performed and the peaks were less sharp. The outcomes of the FTIR analysis suggested that other than the amorphous area, it is likely that a fraction of amylopectin in the crystalline region was also disintegrated. The results of FTIR are a little dissimilar from that of XRD analysis which indicates hydrolysis of the amorphous region only. Similar observations were recorded at the amorphous and crystalline regions during hydrolysis (Das & Kayastha, 2019).

3.8 | PSD of EHPP and PP

PSD analysis of PP and EHPP was performed and median particle dimensions are presented in Figure 4a. The purpose of analyzing the median size of particle helped to divide the population into even halves and give a picture of the average size of the particles. When PP is pre-treated for enzyme hydrolyzing, the smallest particles are obtained. Due to steric hindrance disorders in the crystal region, it is difficult to change the composition of the chair from the chair to the D-glucose during the hydrolysis process of the glucosidic bonds, resulting in a lower hydrolysis rate in the crystal region with enzyme hydrolysis (Li et al., 2007). The pretreatment of PP with α -amylase resulted in the least sized particles. We also noticed that α -amylase alters the PSD of the PP and is also influenced by the conditions of hydrolysis. In this study, we further noticed that pretreatment of PP reduced the time needed to acquire the smallest size of particles but also subsequently produced smaller granules. In pretreatment, the particle size was 11.80 μm , whereas the median size of particle in PP was 15.90 μm . This difference in size distribution may be attributed to the granule hardness that was changed after enzymatic hydrolysis (Hu et al., 2020).

3.9 | ZP (ζ) of EHPP and PP

The ZP (ζ) of PP and EHPP were presented in Figure 4b. It was observed that for both PP and EHPP, the zeta potential (ζ) value was negative, which is possibly due to the negative charges at the surface of the granules. ZP (ζ) also holds the feature of explaining the force of attraction and/or repulsion between the granules to explain the stability of the colloidal suspension. Our results displayed more dispositions toward negative ZP (ζ) after carrying out enzymatic hydrolysis, which might be due to the loss of a proton from carboxylate and sulfonic groups. Likewise, an ample quantity of phosphate esters was also found in aggregates of amylopectin in crystalline starch and hence in EHPP. As a result of hydrolysis of these phosphate esters in an alkaline environment with α -enzyme, ZP (ζ) of PP reduced from 21.20 mv (-21.20 , mV) to 23.20 (-23.20 mV). As the values of ZP (ζ) of EHPP were less (more negative) than that of PP, this inferred that nanoparticles of EHPP granules could be very well suspended during hydrolysis in deionized water. Also, we found a higher absolute ZP (ζ) value of PP before hydrolysis and lower after hydrolysis, probably due to

alterations on the surface of nanocrystals and ionization equilibrium (Hao et al., 2018).

3.10 | Rheological analysis of EHPP and PP

The rheological analysis of PP and EHPP was performed and the results were presented in Figure 5. We have noticed a considerable decline in the apparent viscosity of PP and EHPP with enhancing shear rate. The PP demonstrated greater apparent viscosity in comparison to EHPP. The decline in apparent viscosity of EHPP is likely to cause a reduction in the entanglements among amylose chains as the complexly branched amylopectin is unable to form efficient entanglements (Ai & Jane, 2015; Surendra Babu et al., 2015). Moreover, having more branch density the interchain linkage is also reduced by alterations in the enzyme that consequently reduce the matter of junction zones in the gel network causing more shear-thinning ability (Li et al., 2016). It was observed that EHPP revealed almost coinciding curves (Figure 5a). Similarly, in the case of apparent viscosity, PP possessed considerably higher shear stress than EHPP which proposes that PP and EHPP

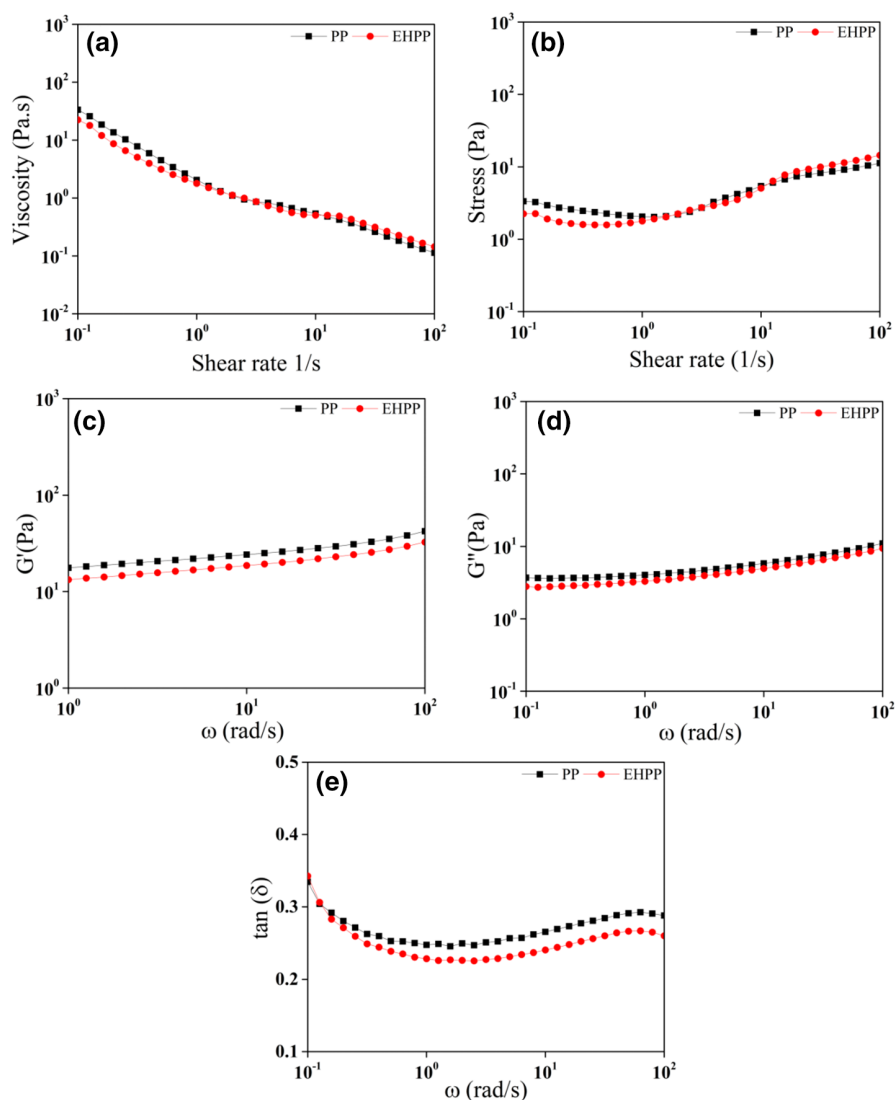


FIGURE 5 Rheological properties of potato powder (PP) and optimized enzymatically hydrolyzed potato powder (EHPP). Viscosity versus shear rate (a) stress versus shear rate (b) (G') storage modulus versus (ω) angular frequency (c) loss modulus versus (G'') (ω) angular frequency (d) $\tan \delta$ (G''/G') versus (ω) angular frequency (e)

suspensions are non-Newtonian fluids and can be regarded as pseudo-plastic fluids. Figure 5c–e highlight the difference of G' , G'' , and $\tan \delta$ as a purpose of angular frequency (ω) of PP and EHPP. It was noticed that for PP, the escalation of G' and G'' is linear and equal because of the growing connections between amylose and amylopectin generating double-helix clusters. G' and G'' rely on the frequency and no crossover point among the two moduli was observed during the frequency spans implying a conventional three-dimensional weak gel (Ngwuluka et al., 2013). In the case of EHPP, we noticed a straight viscoelastic section in which G' was almost invariable (minor deformation), and a non-linear section where G'' decreased (huge deformations) with intensifying angular frequency (ω). The pastes of PP and EHPP showed solid-like ($G' > G''$) features in the viscoelastic region (Figure 5c,d). On the other hand, G' was observed to be greater than G'' beyond the flow point and EHPP displayed more liquid-like features. The values of G'' and G' of PP pastes were greater than that of EHPP. The values of G' and G'' reduced after treatment with an enzyme which implies the associated factors like firmness, hardness, and viscoelasticity of the samples. The primary purpose of the analysis of $\tan \delta$ (G''/G') is to check rheological behavior because of interacting ingredients in the system. As a rule, the value of $\tan \delta < 1$ denotes the material to be solid-like and elastic while $\tan \delta > 1$ indicates materials to be viscous and liquid-like. In this study, the values of $\tan \delta$ of PP and EHPP are less than 1 signifying the behavior to be elastic (Figure 5e). We also noticed that $\tan \delta$ values of PP and EHPP were low at low frequencies but as the angular frequency was raised the value of $\tan \delta$ also increased. As mentioned earlier, with the increasing value of $\tan \delta$ the materials behave more like liquids and the three-dimensional network of gels becomes weaker (Hsu et al., 2000). The values of $\tan \delta$ of PP were greater than the values of EHPP and hence it implies that EHPP is relatively more elastic than control PP.

4 | CONCLUSION

This study primarily aimed at preparing an effective enzyme hydrolyzed PP, using an α -amylase enzyme that can be utilized in food products. The improvement in PP starch by α -amylase enhances the α -1-4 glycosidic bonds but on the other hand reduces chain length in PP thus consequently giving lower relative crystallinity, viscosity, storage modulus, loss modulus, and higher solubility and amylose content. Only in comparison with EHPP the ratio of $1047 \text{ cm}^{-1}/1022 \text{ cm}^{-1}$ and relative crystallinity gradually increase with enzymatic treatment. A reasonable justification in this regard is that number building blocks (increased branching density) and enhanced regular short chains in amylopectin are formed during and after the enzymatic hydrolysis that may end up in giving symmetric short-chain branches with equal chain length facilitating the formation of the ordered structure of PP starch. The outcomes of the study revealed that pores and indentations on the granule surface can facilitate the entry of α -amylase resulting in prompt hydrolysis in less time. It was corroborated that the noticeable improvement in crystallinity was ascribed to the hydrolysis of the amorphous region first. The

results demonstrated improved EHPP stability and dispersion in water obtained subject to enzymatic pretreatment, which was approved by ZP (ζ) measurement and dispersion experiment. In conclusion, the results suggest that enzymatic treatment using α -amylase could be feasible to proficiently prepare EHPP for product development. In this study, we did not focus on the mechanism and effect of branched-chain structures of amylopectin on the physicochemical properties and molecular structure of PP, which is under consideration for future studies since the impact is yet not clear.

AUTHOR CONTRIBUTIONS

Ishtiaq Ahmad: Conceptualization; Writing—original draft; Methodology. **Zhouyi Xiong:** Writing—review & editing. **Xiong Hanguo:** Methodology; Supervision; Writing—review & editing. **Fei Lyu:** Writing—review & editing. **Rana Muhammad Aadil:** Writing—review & editing. **Nauman Khalid:** Writing—review & editing. **Noman Walayat:** Writing—review & editing. **Muhammad Imran Taj:** Writing—review & editing. **Gaopeng Zhang:** Writing—review & editing. **Wei Tang:** Writing—review & editing. **Yan Li:** Writing—review & editing. **Minghui Li:** Writing—review & editing.

ACKNOWLEDGMENTS

This work was financially supported by the Nature Science Foundation of Hubei Province (2018CFB269).

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Ishtiaq Ahmad  <https://orcid.org/0000-0003-0553-4725>

Xiong Hanguo  <https://orcid.org/0000-0002-8677-7261>

Rana Muhammad Aadil  <https://orcid.org/0000-0002-0185-0096>

REFERENCES

- Aggarwal, P., & Dollimore, D. (1998). A thermal analysis investigation of partially hydrolyzed starch. *Thermochimica Acta*, 319, 17–25.
- Ahmed, J., Mulla, M. Z., & Arfat, Y. A. (2017). Particle size, rheological and structural properties of whole wheat flour doughs as treated by high pressure. *International Journal of Food Properties*, 20, 1829–1842.
- Ai, Y., & Jane, J. (2015). Gelatinization and rheological properties of starch. *Starch - Stärke*, 67, 213–224.
- Andre, C. M., Oufir, M., Guignard, C., Hoffmann, L., Hausman, J.-F., Evers, D., & Larondelle, Y. (2007). Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β -carotene, α -tocopherol, chlorogenic acid, and petanin. *Journal of Agricultural and Food Chemistry*, 55, 10839–10849.
- Aquino, A., Jorge, J., Terenzi, H., & Polizeli, M. (2003). Studies on thermostable α -amylase from thermophilic fungus *Scytalidium thermophilum*. *Applied Microbiology and Biotechnology*, 61, 323–328.

- Avula, R., & Singh, R. (2009). Functional properties of potato flour and its role in product development—A review. *Food*, 3, 105–112.
- Blazek, J., & Gilbert, E. P. (2010). Effect of enzymatic hydrolysis on native starch granule structure. *Biomacromolecules*, 11, 3275–3289.
- Bovell-Benjamin, A. C. (2007). Advances in food and nutrition research. In *Sweet potato: A review of its past, present, and future role in human nutrition* (pp. 1–59). Academic Press.
- Cui, L., Tian, Y., Tian, S., Wang, Y., & Gao, F. (2018). Preparation of potato whole flour and its effects on quality of flour products: A review. *Grain & Oil Science and Technology*, 1, 145–150.
- Das, R., & Kayastha, A. M. (2019). Enzymatic hydrolysis of native granular starches by a new β -amylase from peanut (*Arachis hypogaea*). *Food Chemistry*, 276, 583–590.
- Fang, J. M., Fowler, P. A., Tomkinson, J., & Hill, C. A. S. (2002). The preparation and characterisation of a series of chemically modified potato starches. *Carbohydrate Polymers*, 47, 245–252.
- Guo, K., Liu, T., Xu, A., Zhang, L., Bian, X., & Wei, C. (2019). Structural and functional properties of starches from root tubers of white, yellow, and purple sweet potatoes. *Food Hydrocolloids*, 89, 829–836.
- Guo, L. (2018). Sweet potato starch modified by branching enzyme, β -amylase and transglucosidase. *Food Hydrocolloids*, 83, 182–189.
- Hao, Y., Chen, Y., Li, Q., & Gao, Q. (2018). Preparation of starch nanocrystals through enzymatic pretreatment from waxy potato starch. *Carbohydrate Polymers*, 184, 171–177.
- Hemar, Y., Hardacre, A., Hedderley, D. I., Clark, S., Illingworth, D., Harper, J. W., & Boland, M. (2007). Relationship between the pasting behaviour and the phosphorus content of different potato starches. *Starch - Stärke*, 59, 149–155.
- Hsu, S., Lu, S., & Huang, C. (2000). Viscoelastic changes of rice starch suspensions during gelatinization. *Journal of Food Science*, 65, 215–220.
- Hu, C., Xiong, Z., Xiong, H., Chen, L., Zhang, M., Wang, P., Nawaz, A., & Walayat, N. (2020). Effects of granule size on physicochemical and digestive properties of potato powder. *Journal of the Science of Food and Agriculture*, 100, 4005–4011.
- Jangchud, K., Phimolsiripol, Y., & Haruthaithanasan, V. (2003). Physicochemical properties of sweet potato flour and starch as affected by blanching and processing. *Starch - Stärke*, 55, 258–264.
- Jensen, S. L., Zhu, F., Vamadevan, V., Bertoft, E., Seetharaman, K., Bandholm, O., & Blennow, A. (2013). Structural and physical properties of granule stabilized starch obtained by branching enzyme treatment. *Carbohydrate Polymers*, 98, 1490–1496.
- Jiang, T., Cai, M., Huang, M., He, H., Lu, J., Zhou, X., & Zhang, Y. (2015). Characterization of a thermostable raw-starch hydrolyzing α -amylase from deep-sea thermophile *Geobacillus* sp. *Protein Expression and Purification*, 114, 15–22.
- Juszczak, L., Fortuna, T., & Krok, F. (2003). Non-contact atomic force microscopy of starch granules surface. Part I. potato and tapioca starches. *Starch - Stärke*, 55, 1–7.
- Kim, J., Soh, S. Y., Bae, H., & Nam, S.-Y. (2019). Antioxidant and phenolic contents in potatoes (*Solanum tuberosum* L.) and micropropagated potatoes. *Applied Biological Chemistry*, 62, 17.
- Kurakake, M., Tachibana, Y., Masaki, K., & Komaki, T. (1996). Adsorption of α -amylase on heat-moisture treated starch. *Journal of Cereal Science*, 23, 163–168.
- Li, W., Corke, H., & Beta, T. (2007). Kinetics of hydrolysis and changes in amylose content during preparation of microcrystalline starch from high-amylose maize starches. *Carbohydrate Polymers*, 69, 398–405.
- Li, W., Li, C., Gu, Z., Qiu, Y., Cheng, L., Hong, Y., & Li, Z. (2016). Relationship between structure and retrogradation properties of corn starch treated with 1,4- α -glucan branching enzyme. *Food Hydrocolloids*, 52, 868–875.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31, 426–428.
- Moorthy, S. N. (2002). Physicochemical and functional properties of tropical tuber starches: A review. *Starch - Stärke*, 54, 559–592.
- Mu, T. H., Zhang, M., Raad, L., Sun, H. N., & Wang, C. (2015). Effect of α -amylase degradation on physicochemical properties of pre-high hydrostatic pressure-treated potato starch. *PLoS One*, 10, 1–15.
- Nguyen, Q. D., Rezessy-Szabó, J. M., Claeysens, M., Stals, I., & Hoschke, Á. (2002). Purification and characterisation of amylolytic enzymes from thermophilic fungus *Thermomyces lanuginosus* strain ATCC 34626. *Enzyme and Microbial Technology*, 31, 345–352.
- Ngwuluka, N. C., Choonara, Y. E., Kumar, P., Modi, G., Du Toit, L. C., & Pillay, V. (2013). A hybrid methacrylate-sodium carboxymethylcellulose interpolyelectrolyte complex: Rheometry and in silico disposition for controlled drug release. *Materials*, 6, 4284–4308.
- Polaina, J., & MacCabe, A. P. (2007). *Industrial enzymes: Structure, function and applications*. Springer.
- Pu, H., Wei, J., Wang, L., Huang, J., Chen, X., Luo, C., Liu, S., & Zhang, H. (2017). Effects of potato/wheat flours ratio on mixing properties of dough and quality of noodles. *Journal of Cereal Science*, 76, 236–242.
- Rajisha, K. R., Maria, H. J., Pothan, L. A., Ahmad, Z., & Thomas, S. (2014). Preparation and characterization of potato starch nanocrystal reinforced natural rubber nanocomposites. *International Journal of Biological Macromolecules*, 67, 147–153.
- Rashid, M. H., Khan, M. R., Roobab, U., Rajoka, M. S. R., Inam-ur-Raheem, M., Anwar, R., Ahmed, W., Jahan, M., Ijaz, M. R. A., Asghar, M. M., Shabbir, M. A., & Aadil, R. M. (2021). Enhancing the shelf stability of fresh-cut potatoes via chemical and nonthermal treatments. *Journal of Food Processing and Preservation*, 45(6), e15657.
- Senanayake, S., Gunaratne, A., Ranaweera, K., & Bamunuarachchi, A. (2013). Effect of heat moisture treatment conditions on swelling power and water soluble index of different cultivars of sweet potato (*Ipomea batatas* [L.] Lam) starch. *ISRN Agronomy*, 2013, 1–4.
- Shariffa, Y. N., Karim, A. A., Fazilah, A., & Zaidul, I. S. M. (2009). Enzymatic hydrolysis of granular native and mildly heat-treated tapioca and sweet potato starches at sub-gelatinization temperature. *Food Hydrocolloids*, 23, 434–440.
- Soares, I., & Távorá, Z. (1959). Enzymes in the food industry. *Nature*, 184, 1366–1367.
- Soottitantawat, A., Yujiro, I., Hidefumi, Y., Takeshi, F., Myllärinen, P., Forssell, P., & Poutanen, K. (2007). Visual observation of hydrolyzed potato starch granules by α -amylase with confocal laser scanning microscopy. *Starch - Stärke*, 59, 543–548.
- Srichuwong, S., & Jane, J.-L. (2007). Physicochemical properties of starch affected by molecular composition and structure: A review. *Food Science and Biotechnology*, 16, 663–674.
- Surendra Babu, A., Parimalavalli, R., & Rudra, S. G. (2015). Effect of citric acid concentration and hydrolysis time on physicochemical properties of sweet potato starches. *International Journal of Biological Macromolecules*, 80, 557–565.
- Takata, H., Takaha, T., Okada, S., Hizukuri, S., Takagi, M., & Imanaka, T. (1996). Structure of the cyclic glucan produced from amylopectin by *Bacillus stearothermophilus* branching enzyme. *Carbohydrate Research*, 295, 91–101.
- Teng, Z., Luo, Y., & Wang, Q. (2012). Nanoparticles synthesized from soy protein: Preparation, characterization, and application for nutraceutical encapsulation. *Journal of Agricultural and Food Chemistry*, 60, 2712–2720.
- Vamadevan, V., & Bertoft, E. (2015). Structure-function relationships of starch components. *Starch - Stärke*, 67, 55–68.
- Vamadevan, V., Bertoft, E., & Seetharaman, K. (2013). On the importance of organization of glucan chains on thermal properties of starch. *Carbohydrate Polymers*, 92, 1653–1659.
- Van Hal, M. (2000). Quality of sweet potato flour during processing and storage. *Food Reviews International*, 16, 1–37.

- Wang, H., Yang, Q., Gao, L., Gong, X., Qu, Y., & Feng, B. (2020). Functional and physicochemical properties of flours and starches from different tuber crops. *International Journal of Biological Macromolecules*, 148, 324–332.
- Yong, H., Wang, X., Sun, J., Fang, Y., Liu, J., & Jin, C. (2018). Comparison of the structural characterization and physicochemical properties of starches from seven purple sweet potato varieties cultivated in China. *International Journal of Biological Macromolecules*, 120, 1632–1638.
- Zhu, F., Yang, X., Cai, Y.-Z., Bertoft, E., & Corke, H. (2011). Physicochemical properties of sweetpotato starch. *Starch - Stärke*, 63, 249–259.

How to cite this article: Ahmad, I., Xiong, Z., Hanguo, X., Lyu, F., Aadil, R. M., Khalid, N., Walayat, N., Taj, M. I., Zhang, G., Tang, W., Li, Y., & Li, M. (2022). Microstructural study of enzymatically and non-enzymatically hydrolyzed potato powder. *Journal of Food Processing and Preservation*, 00, e16998. <https://doi.org/10.1111/jfpp.16998>