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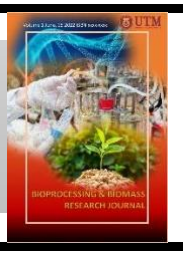

Research Article

Enzymatic Treatment of Enhanced Musa Peel Flour as Potential Low Digestible Starch

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ARTICLE INFO

Article History:
Received 22 February 2023
Received in revised form 13 June 2023
Accepted 14 June 2023
Available online 30 June 2023

Keywords:
Low digestible starch,
Resistant starch,
Enzymatic hydrolysis,
Banana peel flour

ABSTRACT

Musa peel flour has a great potential as a low digestible starch that is beneficial for diabetic patients. Low digestible starch prevents the rapid increase of blood-glucose level which is crucial to protect from internal organ from damage. The Enhanced Musa peel flour has been developed previously, however, the degrees of starch resistance is yet to be analyzed and the dark colored flour that corresponds to the phenolic compound oxidation in the cell wall has affected the sensory evaluation of consumers. In this work, the starch resistance and improved color of the Enhanced Musa peel flour were reported using xylanase enzymatic treatment. Enzymatic treatment was optimized by varying xylanase concentration and incubation duration. Next, starch digestibility analysis was conducted using amylase enzyme and color reduction was observed. The results show a significant increment of resistant starch content (14%) in the treated enhanced Musa peel flour. The reduction of 86.44% of rapidly digested starch content in the treated flour was also obtained. Color reduction was also observed although not significant. The cell wall disruption by degradation of hemicellulose component by xylanase was hypothesized to increase the resistant starch content in the enhanced Musa peel flour and remove the dark pigment.

INTRODUCTION

Banana is an edible fruit, produced by a large herbaceous flowering plant in the genus of *Musa* and they can be classified into four sections which are *Eumusa, Rhodochlamys, Australimusa and Callimusa*. This fruit is very popular with its texture, taste and can be obtained with low price in the city and rural area. In Malaysia, banana is one of the widely grown crops, covering a total land area of 8 084.50 hectares (annual statistic from Malaysian Ministry of Agriculture in 2019). The production increased from 260,911 tonnes in 2007 to 335,974 tonnes in 2012 (Razak et. al., 2021). The increased demand of the fruit indirectly increases the banana peel waste that becomes a threat to the environment. In the previous studies, banana peel waste has been utilized to produce bioethanol (Danmaliki et al., 2016), wine (Rodolphe, 2015) and the source of alkali in the production of soap (Udosen & Enang, 2000).

The production of banana peel flour is also one of the ways to utilize the waste and it is also a promising alternative for the wheat flour and has a great potential in the development of gluten-free products (Gutierrez, 2018). Besides, the flour with enhanced properties has excellent potential to substitute the wheat flour in the

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diet especially for the diabetic patients, as wheat flour has high glycemic index that leads to a drastic fluctuation in the blood glucose level after consumption. The drastic fluctuation of blood glucose is mainly caused by the food that contains high amount of rapidly digested starch (RDS). RDS was absorbed and digested rapidly in the small intestine by which the uncontrolled intake of this type of starch can give bad effects to the internal organ (Zhang & Hamaker, 2009). Consuming slowly digested starch (SDS) and resistant starch (RS) in daily diet can help in preventing these troublesome effects to occur. This is due to the low digestion properties of SDS and RS (Englyst et al., 1992, Zhang & Hamaker, 2009).

In our previous study, the enhanced Musa peel flour has been developed with the combination of mango and banana peel by a series of processes which include cooling, drying and grinding (Rosli, 2018). The flour is in the form of powder, odourless and dark in colour. The flour is potential as low digestible starch, however, the degree of starch resistance of the flour is yet to be analyzed. In addition, the colour of the flour is important to determine the quality of the flour and the sensory evaluation by the consumers. Therefore, in this work, enzymatic approach was utilized to enhance the starch resistance and to reduce the colour of the enhanced Musa peel flour. The potential of the flour as low digestible food source was determined via starch digestibility analysis.

MATERIALS AND METHOD

Materials

The enhanced Musa peel flour was a complimentary from Universiti Tun Hussein Onn, Malaysia. Xylanase, SIGMA(X2753-10G) and Diastase (α-amylase), SIGMA (0962-100G) were purchased from Sigma Aldrich, USA.

The optimization of xylanase concentration for the colour reduction of enhanced Musa peel flour.

For optimization of concentration of enzyme, the sample was prepared by mixing 1 g of flour with 0.05 M of sodium citrate buffer. The concentration of enzyme was varied from 0 U to 1200 U for each sample. Each of the mixture was incubated at 55 °C and pH of 6.0 with 60 rpm shaking for 1 hour. Then, the enzymes were inactivated by 10-minute incubation of enzyme-treated flour sample in a boiling water bath. The optimum concentration of enzyme was obtained based on the highest removal of the dark pigment on the flour which was observed by using colorimetric method.

The optimization of incubation period for the colour reduction of enhanced Musa peel flour

To find the optimum time required for the maximum xylanase activity, the optimum concentration of enzyme was added into 1 g of flour with 0.05 M sodium citrate buffer. Each of the mixture was incubated at 55°C and pH of 6.0 with 60 rpm shaking. The duration of incubation was varied from 1 hour to 4 hours. The optimum duration of the enzymatic bleaching process was based on the highest removal of the dark pigments.

Colorimetric method

The analysis of colour reduction of the flour sample was done by Chroma meter Minolta CR300 (Konica Minolta colorimeter, sensing, Japan) and the results were expressed in accordance with the CIELAB system. The meter was calibrated with white background (L* = 63.15, a* = 2.21and b* 2.44). The flour sample was placed into the glass petri dish, which is suitable for powder and liquid. The measurements were determined in triplicates and mean and standard deviation was determined. The colour attributes were determined by colour coordinates of L* (L* = 0 [black] and L* = 100 [white]), a* (−a* = greenness and +a* = redness), and b* (−b* = blueness and +b* = yellowness (Szabó et. al., 2016).

Analysis of the starch fraction by using Guraya method.

The analysis of the low digestible starch was a mimic of the process of starch digestion in human stomach. The optimized treated flour sample (15 mL) was placed in the beaker. Diastase α-amylase (129 U) in a phosphate buffer (0.1 M, 5 mL pH 6) containing sodium chloride (100 mL of 0.9%) was added into the beaker. The digestion of the starch was done at 25 ℃ which is the optimum temperature of the Diastase α-amylase. The starch of the flour was digested with sampling every hour until no further digestion noticed. The 3,5-Dinitrosalicylic acid (DNS) assay was used to measure and calculate the maltose released. This analysis was also performed by using untreated flour to compare starch digestibility before and after the enzymatic treatment process

The starch content was calculated by using formula of Guraya method as follows:

%RDS = (D-E)/F x 100
D = mg maltose produced on digestion at 1 hour
E = mg maltose at 0 hour of digestion
F = total starch (mg)

%SDS = (G-H)/F x 100
G = maximum mg maltose produced until no further digestion
H = mg maltose at 1 hour digestion
F = total starch (mg)

RS = (F-G)/F x 100
F= total starch (mg)
G = maximum mg maltose produced until no further digestion.

RESULTS AND DISCUSSION

In this work, two different parameters (incubation time and concentration of enzyme) were studied for the xylanase treatment for color reduction and starch digestibility of the enhanced Musa peel flour. Only one parameter was varied at one time to see its effect alone without considering other parameters. After the optimization of these parameters, the starch fraction of xylanase-treated flour and untreated flour was analyzed to study the effect of the enzyme activity on the starch content.

The effect enzyme concentration and incubation period on the colour reduction of the flour

The effect of xylanase on the colour reduction of the flour was initially done by varying the concentration of enzyme from 0 U to 1200 U. At this time, the temperature, pH and incubation time were fixed at 55 ℃, pH 6.0 and 1 hour. **Figure 1** shows that colour reduction increased as the concentration of xylanase increased. The colour unit indicates the lightness of the colour for the enhanced Musa peel flour samples. The range value of colour unit from 50 to

100 shows that the sample has light colour which can be seen as white, contrary to the values range from 0 to 50 indicating dark colour sample and it was observed as black by using naked eyes. The treatment with xylanase resulted in increased of color unit for each sample, which indicates the removal of the dark pigment. Optimum concentration of xylanase obtained for the colour reduction of enhanced Musa peel flour is 800 U. Increased of enzyme concentration allowed the cell wall disruption at higher degree. Sridevi et. al. (2017) in their study also reported the same result where the increased in concentration of enzyme has been proven to increase the effectiveness of lignin removal of wood pulp for paper bio-bleaching. However, at concentration of 1000 U and 1200 U, the colour reduction obtained is almost the same. This is because the substrates are saturated with the enzyme. At this condition, the increasing of the xylanase may not result any higher degree of colour reduction as the concentration of substrate, xylan may become the limiting factor. Xylanase cannot bind to substrate as all the substrate has formed enzyme-substrate complex. Therefore, based on the result obtained for this investigation, the enzyme concentration of 800 U has been chosen for the next experiment.

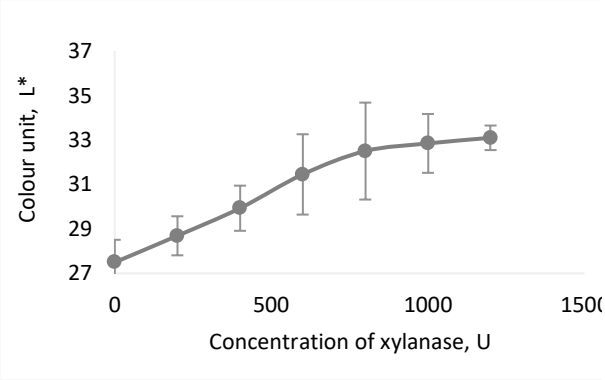

Figure 1 The optimization of concentration of xylanase for colour reduction of enhanced Musa peel flour. The colour unit indicated the lightness of the colour for the enhanced Musa peel flour samples

The second parameter studied was the incubation period of the enhanced Musa peel flour with the xylanase in which the flour samples were incubated for 4 hours. The colour reduction of the flour samples was measured for every 1 hour. Other parameters used were fixed (800 U of xylanase, pH 6.0, 55 °C). Based on **Figure 2**, as the incubation period increased, the color reduction of the flour also increased. The study by Sridevi et al (2017) also achieved the same result where the xylanase activity to bleach the wood pulp also increased as the day of incubation increased. Nonetheless, after 2 hours, the colour reduction of the enhanced Musa peel flour decreased. This is because during the incubation period, the xylanase structure may be degraded or denatured due to long exposure to the heat. The same result was also achieved by the previous studies where at high temperature, the hydrolysis rate of protein extract by enzyme, alcalase decreased (Salwanee et. al., 2013). The oxidation of the phenolic compound also increased during this incubation period. So, by observing these two experiments, it can be concluded that the colour reduction of enhanced Musa peel flour was the highest using 800 U of xylanase with incubation period of 2 hours.

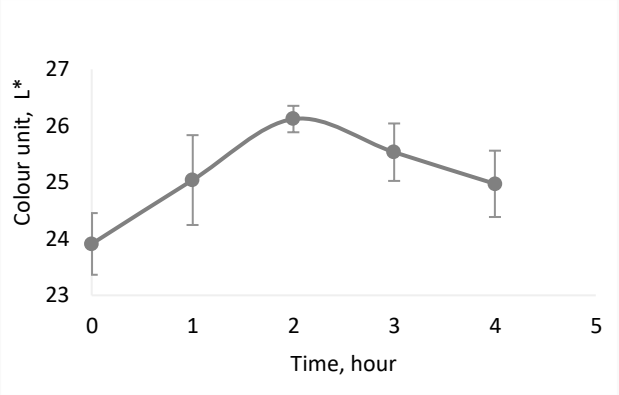

Figure 2 The optimization of incubation time for colour reduction of enhanced Musa peel flour. The colour unit indicated the lightness of the colour for the enhanced Musa peel flour samples

It was hypothesized that the oxidation of the phenolic compounds will produce the dark pigment that was attached on the cell wall. The cell wall consists of hemicelluloses, celluloses and lignin (Kabenge et. al., 2018). Previous study has reported that Musa peels have hemicellulose that consists of xylan structure (Zhang et. al., 2004). Hydrolysis of xylan by using xylanase in this study disrupted the structure of the cell wall and resulted in the removal of the dark pigment. The key reaction of xylanase is the release of its monomer, xylose from linear polysaccharide of xylan by cleaving their β-1,4 linkages (Moreira & Ferreira Filho, 2016).

Although slight colour reduction were observed, this optimization resulted in insignificant data pattern even though very high concentration of xylanase was used and incubation period was prolonged. This happened because the oxidation process increased from time to time due to the high content of phenolic compound in the enhanced Musa peel flour. Based on the research by Ramli et. al. (2010), banana peel has the highest phenolic compound compared to banana pulp. Total phenolic compound in banana peel ranges widely from 75.01 to 685.87 mg GAE/100 g of dry matter. The polyphenol enzyme catalyzes the transfer of electrons from phenolic compound in the presence of oxygen and this will result in the formation of quinone. Another interaction between quinone and the protein in the enhanced Musa peel flour leads to the formation of dark-colored compound. The reduction of color by reducing the xylan using xylanase may not be enough. For further study, it is recommended to extract the phenolic compound first before treating the flour sample with xylanase.

Digestibility test of enhanced Musa peel flour by in vitro enzymatic starch digestion

The digestibility test of the enhanced Musa peel flour has been done to determine the starch fraction of the flour sample. By performing this test, the starch fraction of treated and untreated enhanced Musa peel flour samples was determined and compared. This experiment was set up mimicking the human digestive system by using α-amylase (Diastase) as the digesting enzyme. Phosphate buffer and 0.9% of NaCl were used along with the α-amylase in this experiment. Phosphate buffer was used to control the pH of the solution to be as same as our body (pH 6.0). 0.9% of NaCl was functioned to maintain the right balance of the fluid in the solution. Starch structure consists of amylose and

amylopectin. Amylose consists of α-1,4 glycosidic linkages while amylopectin consists of α-1,4 and of α-1,6 glycosidic linkages. α-amylase was able to cleave the glycosidic linkage of both amylose and amylopectin to release its monomers such as glucose, maltose, maltotriose and dextrin.

Starch fraction of the food consists of three different type of starch which are resistant starch (RS), rapidly digested starch (RDS) and slowly digested starch (SDS). RS is the starch that cannot be digested by the enzyme in the small intestine, but it will produce short chain fatty acid (SCFA) via microbial fermentation that beneficial for the health of the colon (Lockyer et. al., 2017). RDS can be digested and absorbed rapidly in the small intestine resulted in the large fluctuation of glucose in the blood (Zhang & Hamaker, 2009). SDS is the intermediate of RDS and RS and this type of starch will be digested slowly but complete in our body. So, the release of glucose with a low initial glycemia can be sustained (Englyst et. al., 1992, Zhang & Hamaker, 2009).

Obviously, the digestion properties of these three types of starch are different and this is closely related to the ratio of amylose and amylopectin. Usually, RS has high amount of amylose since amylose is a linear structure, so it consists of limited surface area for the α-amylase digestion. This limitation contributed to the low digestion properties (Jeevetha, 2014). Based on previous research by Ao et. al. (2007), the enrichment of α-1,6 glycosidic linkage and the short amylopectin branch structure will produce a high content of SDS starch. Not only that, the structure of RDS consists of high amount of amylopectin compared to amylose. Amylopectin is a highly branched structure so it can provide large surface area for the digestion of the α-amylase and resulted in the rapid digestion of the starch in the human digestive system (Byrnes et. al., 1995).

Table 1 shows the comparison between starch fraction of the enhanced Musa peel flour before the treatment of xylanase and after xylanase treatment with the data of starch fraction of green banana peel, oat starch and rice starch from previous research. It was observed that the RS content of the enhanced Musa peel flour before the treatment of xylanase and green banana fruit peel is higher than SDS and RDS. This is because the green banana peel flour has a high level of dietary fibre and resistant starch. Mango peel flour also has a high level of dietary fibre which is at the range of 40.6% to 72.5%. The addition of mango peel flour to the banana peel flour will increase the dietary fibre in the enhanced Musa peel flour. Resistant starch is classified as dietary fibre where it has resistant to enzyme digestion properties (Lockyer et. al., 2017).

Interestingly, the digestion properties of the enhanced Musa peel flour after the treatment of xylanase shows that the resistant starch obtained is higher than before the treatment of xylanase. There is 14% increment of resistant starch content while the rapidly digested starch content declined at 86.44%. Li et. al. (2018) also reported the same result where the the RS content is the highest followed by SDS and RDS. But, the studies by Kaur et al (2018) shows that the RS content in the oat and wheat starch are the lowest. The SDS and RDS contents in these starches are almost the same. This is because the structure of banana peels and the starch granules are different. Banana peel consists of lignocellulosic compound and has a high content of dietary fiber while the starch granules of wheat and rice consist of a high content of B-type starch (Kaue et. al., 2018). B-type starch commonly displayed a small spherical shape (Ao et al, 2007). Due to its small size, it can be digest rapidly by the digestive enzyme.

Table 1 The digestion properties of enhanced Musa peel flour before and after xylanase treatment with other studies

Sample	RDS (%)	SDS (%)	RS (%)	Ref.
Enhanced musa peel flour before xylanase treatment	7.30 ±1.43	8.43 ±1.55	84.28 ± 2.89	This study
Enhanced musa peel flour after xylanase treatment	0.99 ±0.82	3.28 ±0.80	95.73 ±1.15	This study
Green banana fruit peel	1.4 ±0.3	3.4 ±0.2	95.2 ±0.3	Li et al, 2018
Oat starch	52.7 ±0.85	40.0 ±0.61	7.1 ±0.55	Kaur et al., 2018
Rice starch	54.4 ±0.67	39.5 ±0.84	6.1 ±0.27	Kaur et al., 2018

RDS: rapidly digested starch SDS: slowly digested starch RS: resistant starch

The increment of resistant starch content and the decline of rapidly digested starch in xylanase-treated enhanced Musa peel flour could be due to the xylan-degrading activity of enzyme. **Figure 3** illustrates the structure of the cell wall and the location of the starch granules which are inside the cell wall. The disruption of the cell wall by xylanase releases the resistant starch granules (Horner et al., 2007). On the other hand, the increase in amount of free resistant starch (RS) molecule indirectly reduces the percentage of the rapidly digested starch (RDS) and slowly digested starch (SDS)

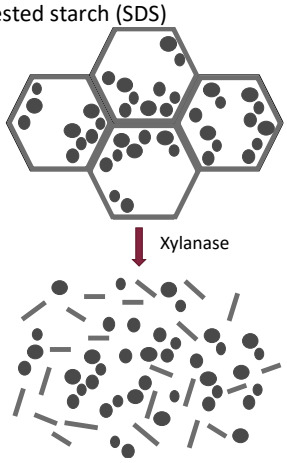

Figure 3 The xylanase treatment that disrupts the cell wall and releases the resistant starch granules.

In this work, the free resistant starch granule cannot be further modified by using enzymatic approach such as debranching enzymes since this type of starch can resist the activity of the enzyme on them. Usually, the starch modification will be performed by using rapidly digested starch molecules such as normal maize starch and waxy rice starch as substrate. These types of substrate react rapidly with the enzymes and obvious result can be observed after the alteration of starch structure was done.

CONCLUSION

The enhanced Musa peel flour has great potential as low digestible starch. The enzymatic treatment of enhanced Musa peel flour by using xylanase resulted a further increment of resistant starch content (14%) and the decline of rapidly digested starch (86.44%) compared to before xylanase treatment. The increment of resistant starch content in the xylanase-treated enhanced Musa peel flour could be due to the xylan-degrading activity of enzyme that allow the release of resistant starch granules from the cell wall. The colour of the enhanced Musa peel flour, although was not significant, reduce after the xylanase treatment. The enhanced Musa peel flour produced in this study is suitable for use as an alternative to the wheat flour especially for diabetic patient.

Acknowledgement

The authors acknowledge the Ministry of Higher Education Malaysia and Universiti Teknologi Malaysia for giving full of support in this research activity. The study was supported by Fundamental Research Grant Scheme (FRGS) (Grant No. FRGS/1/2020/TK0/UTM/02/6).

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