# Enzymatic production of maltodextrins derived from sago flour using heat-stable alpha-amylase and pullulanase

**A Laga, A Syarifuddin and A Dirpan**

Department of Agricultural Technology, Hasanuddin University, Jalan Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

E-mail: *amranlaga@yahoo.co.id*

**Abstract**. Maltodextrins are produced by starch modification in a partial hydrolysis thus altered physical sago properties. Sago as one of starch resources has characteristic with high amylopectin that influences high viscosity during cooking. Partial hydrolysis or liquefaction will influences starch hydrolysis and the size of maltodextrin produced. The aim of this study was to analyze the degree of sago starch hydrolysis during the enzymatic process using single α-amylase and combination with pullulanase The starting solids content was 20% (w/v), with adjusted pH to 6.5, and calcium ($Ca_2$+ ions) addition as high as 50 ppm. The majority of starches used in the study contain 0.2 % (w/v), to combination of 0.2 % (w/w) and 0, 3 gram per kg of sago. The sago suspension temperatures were started from 105 °C lowered to 60 °C for 30 minutes, respectively. Optimum liquefied starch yields, which accounted for virtually all of the starch present, were obtained at temperatures of 80°C and above, for 120 minutes, with each sampling every 20 minutes. Observed parameters were levels of reducing sugars, degree of hydrolysis, and refined sago starch. The result showed that there was a significant increase in reducing sugars, degree of hydrolysis during 120 minutes until liquefaction process for both enzymatic treatments. The amount of reducing sugars was 95.76 g/L at 120 min for the single α-amylase and 98.84 g/L combination with pullulanase. The degree of hydrolysis was 37.93 % at 120 minutes for the single α-amylase and 37.32 % combination with pullulanase, whereas 0.035 % and 0.038 % for refined sago starch value respectively.

## 1. Introduction

Sago (*Metroxylon sagu*) is an Indonesian indigenous plant species which can produce sago starch. In part of Indonesia, sago has been consumed as a traditional staple food. For example, Kapurung in South Sulawesi and Papeda in Papua. Because of its cheap, food manufacturer has encouraged to create sago starch-based food industry such as maltodextrin. In food, maltodextrin can be used as an ingredients because it offers many advantages such as consistency, viscosity, mild texture and stability. In addition, as it was added into food the soluble solids increased allowing better inhibit crystallization and control the freezing point [1]. Maltodextrin are enzymatic and/or acid hydrolysis products of starch, consisting of a-(1,4) linked D-glucose oligomers and/or polymers, which are normally defined as having a dextrose equivalent (DE) value < 20 [2]. A number of maltodextrin have been produced from different starches. In the manufacture of maltodextrins, the long chain of starch molecules was hydrolyzed by thermal, acid and enzymes treatments. Various components have been used to account for the efficiency of starch hydrolysis to produce sugars and maltoolligosaccharides which are α-amylase, glucoamylase, and pullulanase enzymes action [3].

Due to enzymatic modifications have improved the functional properties of starch, most of the maltodextrin production enzymatically was carried out by liquefaction of starch using α-amylase obtained from bacteria. With enzymatic method, sago starch can be modified by α-amylase which first attach to the granule surface and peel away section of granule [4] and then pullulanase, an important debranching enzyme, has been widely utilised to hydrolyse the α-1,6 glucosidic linkages in starch, amylopectin, pullulan, and related oligosaccharides, which enables a complete and efficient conversion of the branched polysaccharides into small fermentable sugars during saccharification process [5]. The size of maltodextrin molecule can be adjusted by adjusting the degree of starch hydrolysis to the duration of the required enzymatic reaction. Commercial maltodextrin generally ranges from DE 3 – 25 in which the DE value of the maltodextrin is dependent on its use [6].

In the present work, sago starch was hydrolyzed by commercial α-amylase and pullulanase. The aim of this work was to investigate the effect of α-amylase and α-amylase combined with pullulanase on the degree of hydrolysis of sago starch during liquefaction reaction.

## 2. Material and Method

### *2.1. Materials*

Sago starch was supplied from local market, α-amylase and pullulanase (Novo Nordisk A/S) in the form of liquid concentrate were used as hydrolysis enzyme.

### *2.2. Experimental procedure*

First of all, a suspension containing sago starch (20% w/v) was adjusted to pH 6.5 (Dakton pH 510 Series). $CaCl^{-2}$ 50 ppm was added into the suspension and the reaction was started by adding α-amylase 0.01%. The suspension was heated at 105 °C for 7 minutes. After gelatinization reached, the reaction temperature was adjusted at 60 °C for 30 min and then added pullulanase 0.3 g/kg of substrate and α-amylase of 0.1% w/w. Then, the process of liquefaction was started by increasing the reaction temperature at 80 °C. The liquefaction reaction was carried out in a stirred fermentor for 120 minutes with specified sampling time were 20 minutes.

### *2.3. Chemical analysis*

Parameters observed in this work were reducing sugars (DNS method), degree of hydrolysis [7], remaining iodine content, viscosity modified from [8].

### *2.4. Data analysis*

An experimental design with type of enzyme: α-amylase and combining α-amylase with pullulanase and time of liquefaction varying from 0 to 120 with specified sampling time 20 minutes were used. Data analyses were performed using SPSS 16. One way ANOVA was used to determine significant differences among parameters. Three replications were used. For all data analyses, the effects were considered significant when $p<0.05$.

## 3. Results and discussion

### *3.1. Reducing sugar*

The reducing sugar is a sugar having the ability to reduce an electron receiving compound because it has an aldehyde and ketone free group. Except sucrose and starch, all monosaccharides (glucose, fructose, galactose) and disaccharides (lactose, maltose) were reducing sugar. In this study, the reducing sugar obtained by combine α-amylase and pullulanase was higher (64.49 g / L - 103.61 g / L) than reducing sugar obtained by α-amylase alone (58.74 g / L - 101.51 g / L). ANOVA showed that time liquefaction was significant effect on reducing sugar ($p<0.05$) or in the other words the reducing sugar increase with increasing time of liquefaction (Fig.1). It was caused by the length of contact time between the enzyme and the substrate allowing hydrolysis process in the α-1.4 glycosidic bond in

short chain formation is increasing. Jane and Chen [8] explains that reaction time of starch hydrolysis enzymatically will affect the duration of enzyme and substrate contact. This phenomenon is then further elaborated by Jane *et al.* [9]. The authors reported that the reaction time increases, the more hydrolyzed starch leading to increase enzyme activity to break the α-1.4 glycosidic bonds into simple oligosaccharide chains. The more glycosidic bonds, the amount reducing sugar is increasing. However, type of enzyme and interaction between type of enzymes and time liquefaction did not show significant.

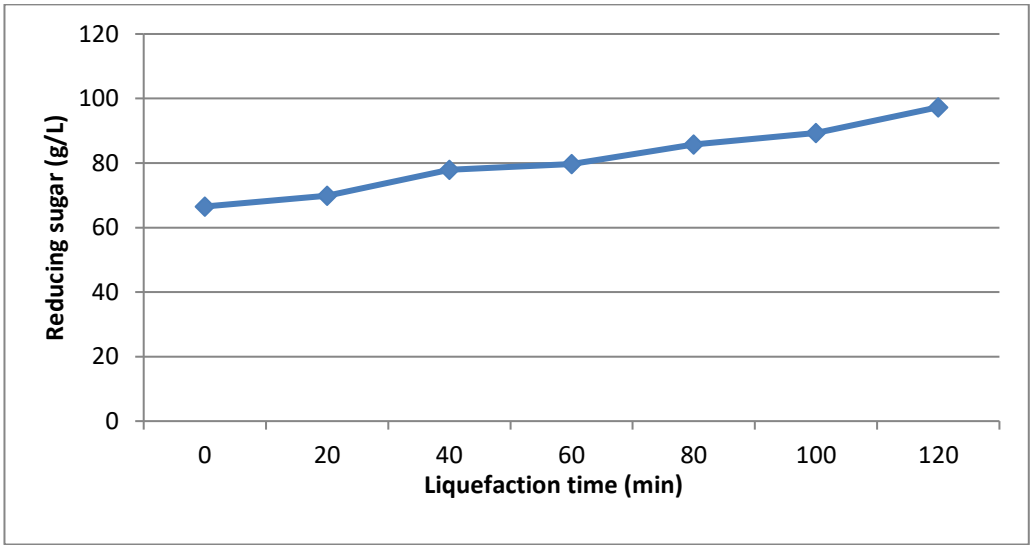


**Figure 1**. Relation between liquefaction time (min) with reducing sugar (g/L)

The use of single α-amylase enzyme and α-amylase and pullulanase combined were not significantly on reducing sugar. It was due to time for debranching process was only for 30 minutes, so that the cutting branching process α-1,6 glycosidic was ineffective resulting in the formation of reducing sugar not significant. Leong *et al.* [10] reported that the best debranching on sago substrate was 8 hours. This phenomenon also explained that the time to cut the α-1,6 glycosidic bond required 30 time more longer than to bypass α-1,4 glycosidic.

*3.2. Degree of Hydrolysis (DH) Relative Humidity*

Another property measured was degree of hydrolysis. The product of hydrolyzed starch is generally characterized by the degree of hydrolysis which is expressed by the DH value (hydrolysis degree), the percentage of pure dextrose in the total of solid of the hydrated substrate. In this study, the DH of α-amylase was 27.26% - 39.10%. and the DH of combined α-amylase and pullulanase was 27.99% - 38.92%.

ANOVA showed that time liquefaction was significant effect on the DH ($p<0.05$) or in the other words the degree of hydrolysis (DH) increased with increasing time of liquefaction (figure 2). According to Nakamura [11] the longer the hydrolysis time, the more hydrolyzed molecules leading to increase reducing sugar and DH. The same results was also reported by [12]. The authors reported that the longer hydrolysis time the more the hydrolyzed component.

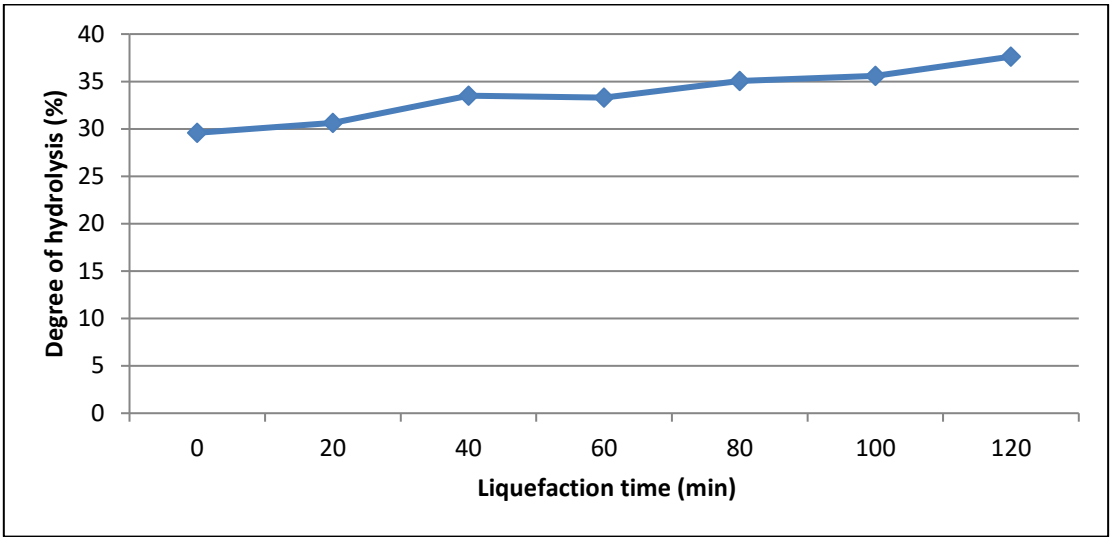


**Figure 2**. Relationship between liquefaction time (min) with degree of hydrolysis (%)

The use of single α-amylase enzyme and combined α-amylase and pullulanase did not significant on the DH. α-amylase activity cuts the α-1.4 glycosidic bond in a straight chain while the pullulanase cuts the α-1.6 glycosidic bond with each product in the form of a straight chain or oligosaccharide. This phenomenon shows that the cutting activity of α-1.6 glycosidic did not significant in the DH. It is caused by the condition or timing of the cutting of the unfavorable branching chain. According to Griffin and Brooks, pullulanase will work well under suitable conditions such as pH 5.2 [13].

*3.3. Residual starch content*

Residual starch represents un-hydrolyzed starch content into a simple form of starch. The residual starch test is to find out the content of enzymatic digestibility of raw starch granules due to the differences in amylose content and branch-chain length of the amylopectin. The Residual starch content of α-amylase enzymatic usage was 0,021% - 0,042%. Whereas the combination of α-amylase and pullulanase 0,029% - 0,044%. The starch content in of α-amylase enzymatic is lower than α-amylase and pullulanase combined (figure 3).

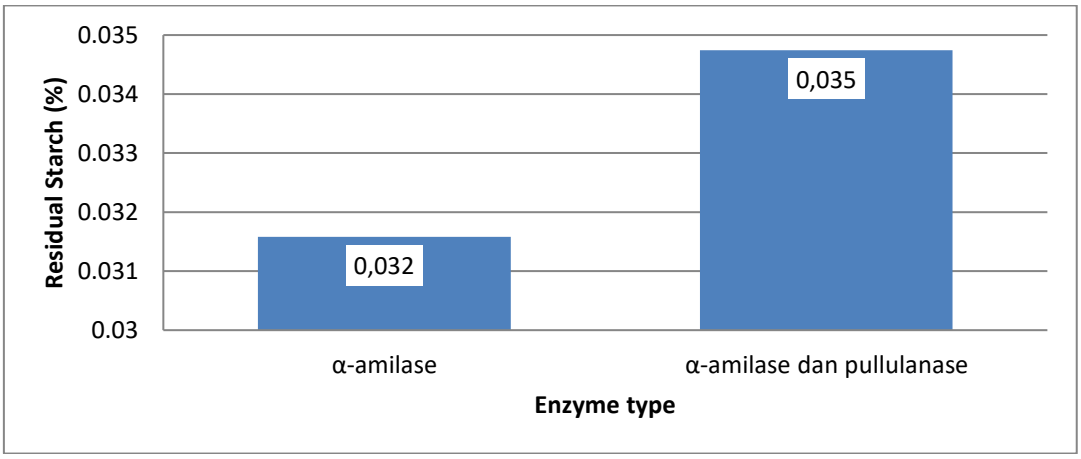


**Figure 3.** Correlation of different enzymatic usage towards the Residual starch (%) of Maltodextrins

The analysis of variance (ANOVA) for the Residual starch (%) of Maltodextrin showed that susceptibility to the enzyme production and liquefaction, or both interactions were not significant to the amount produced.

Susceptibility of the residual starch during enzyme hydrolysis for α-amylase and pullulanase combined showed the least amount of enzyme. The residue value was stable during increased of liquefaction time. The residual starch contributes the highest amount of enzyme units during liquefication 120 minutes was for 0,037%. The combination of different enzymatic usage towards the residual starch (%) showed that the basic gelatinization step at 105 ° C for 7 min was not sufficient to convert crystalline sago starches formed. Therefore there are many of crystalline starches, but it's fine granular structure.

Starch crystallinity affects thermal properties of the starch granules due to the dense packing of cellulose chains due to its resistance to enzymatic hydrolysis. Breaking down the intermolecular bonds of starch crystallinity can be done under high temperature and pressure. The starch crystallinity of the starch granule is difficult to breakdown by the production of starch hydrolyzing enzymes, hence slow breakdown during hydrolysis. The stronger the starch crystallinity, the higher temperature and energy total needed [10].

### *3.4. Viscocity*

The data for the production of viscosity test was presented quantitatively. The viscosity can be measured for a product thickness. Viscosity from maltodextrins are usually supplied with the dextrose equivalent value (DE) as the only information from the source of the starch or hydrolysis of starch.

Viscosity value obtained from α-amylase enzyme utilization was around 46 – 80 cP, for the lowest of 100 to 120 min reaction. While the viscosity number from a combination of α-amylase and pullulanase enzyme was between 56 – 74 cP, with the same time duration as a single enzyme. Figure 4 showed the relationship between liquefaction reaction (min) and viscosity (cP) of maltodextrin viscosity.

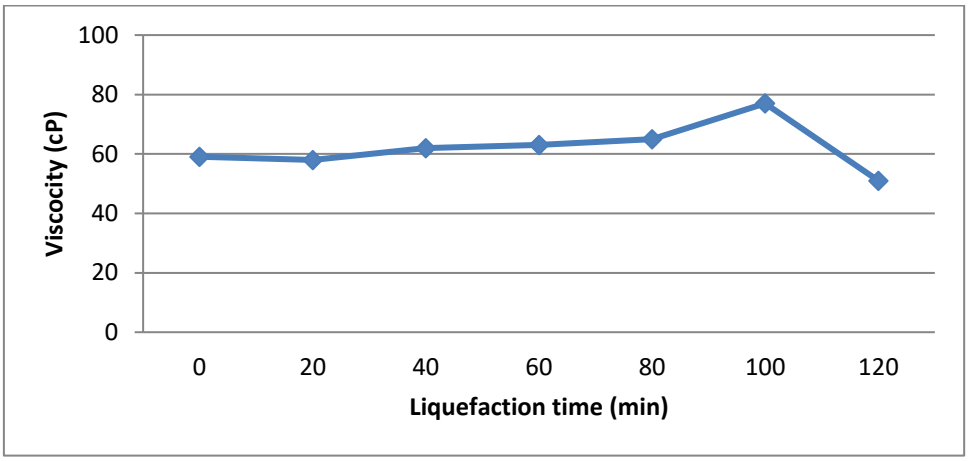


**Figure 4**. The relationship between liquefaction reaction (min) and viscosity (cP) of maltodextrin viscosity.

The viscosity value increased during the reaction time of 100 minutes due to the starch fraction, i.e. amylopectin and a portion of the amylose extracted out of the granules and suspended in water thereby increasing the viscosity. The fraction of amylopectin is faster out compared with amylose because it has higher molecular weight. Suspected amylopectin fractions have crystalline regions with tight structures, raising viscosity because of their ability to bind strongly with water through intermolecular hydrogen bonds.

The presence of water as a solvent and sufficient heat energy causes the starch granules to swell to the point where the starch granules can absorb water. Furthermore, the starch granules ruptured causing the amylose and amylopectin extracted out of the granules. It is clear that compounds that have high molecular weight would come out faster than compounds that have low molecular weight. Amylopectin has a higher molecular weight than amylose so that amylopectin will break faster than

amylose. Nakamura [11] described the increase in the viscosity of the paste that increases the viscosity of the paste due to the development of starch granules that form molecular bonds through hydrogen bonds so that the starch soles form of an opaque and firm gel. The formation of the opaque gel is caused by the intermolecular hydrogen bonds that make up the aggregate in the water-insoluble crystalline region.

Furthermore, the viscosity decreases in the duration of the 120-minute reaction are due to the amylose fraction remaining in the outgoing starch granules and suspended in maltodextrin. The more amylose that is suspended will decrease the viscosity. Decreased viscosity also occurs quickly due to the hydrolysis of the starch fraction by the enzyme. The amylose fraction and some suspended amylopectin are hydrolyzed by the enzyme into fractions with lower molecular weights such as short-chain dextrins. The formation of the dextrin unit causes the DH value to rise, and the viscosity decreases at a reaction time of 120 min.

The effect of the amylose fraction on viscosity decrease can be influenced by the higher proportion of amylose and cause lower product density. Nakamura [11] further explained that the viscosity of the starch solution rapidly decreases in the event of hydrolysis by α-amylase (liquefaction of starch). The decrease in viscosity is a change in physical properties of natural starch when experiencing enzymatic hydrolysis. The enzyme will bypass the amylose and amylopectin fractions from the starch granules to the simpler sugars thus decreasing the viscosity of the starch suspension.

## 4. Conclusion

Hydrolysis degree evaluation was not significant to make a difference between maltodextrin obtained from the use α-amylase and combination α-amylase with pullulanase on sago starch. The highest yield of maltodextrin determined from degree of hydrolysis reveals that the yield depends on the viscocity at 120 min of liquefaction time.

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