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Sugar Conversion of Immature Green Rice Syrup under Solid State Fermentation

Chainarong Chuayjum[1*], Wiriya Onsaard[1] and
Chuenjit Prakitchaiwattana[2]

[1] *Department of Agro-Industry, Faculty of Agricultrure, Ubon Ratchathani University Warinchamrap, Ubon Ratchathani, 34190, Thailand*

[2] *Department of Food Technology, Faculty of Science, Chulalongkorn University Pathumwan, Bangkok, 10330, Thailand*

[*] *Corresponding Author: Chainarong.ch.57@ubu.ac.th*

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Abstract-Immature green rice contains high content of γ–oryzanol as well as γ -aminobutiric acid (GABA) and found that several bioactive active compounds such as phenolic compounds, chlorophyll, ß-carotene, tocopherol as well antioxidant activities were reported. Thus, this research is aimed to add more value of immature green rice as a substrate for Isomaltooligosaccharides (IMOs). The scope is studied a sugar conversion from immature green rice during liquefaction using solid state fermentation (SSF) of *Aspergillus oryzae* TISTR 3102. The process included, gelatinization of immature green rice starter inoculation as solid state with *A. oryzae* and under 30 °C for 8 days. The highest syrup volume was obtained after 7 days of fermentation ($p \leq 0.05$) with increasing of total soluble solids (°Brix) ($p > 0.05$). Moreover, reducing sugar found increased and the highest value was observed at the 4th day of fermentation ($p \leq 0.05$) and tended to constant through fermentation period. The highest total solid content (%) was found at the 6th day of fermentation ($p \leq 0.05$) and tended to decreasing. pH value was decreased from 5.27±0.01 to 4.73±0.01 after 7 days of fermentation ($p \leq 0.05$). Ultra-High-Performance liquid Chromatography (UHPLC) was conducted for sugar and isomaltose analysis and the highest amounts of glucose, maltose and isomaltose were observed in 3 and 8 days of fermentation. As monosaccharide was produced by SFF of *A. oryzae,* it is agreed with increasing of syrup volume, total soluble solid and reducing sugar content and dextrose equivalent.

Keywords: Dough rice stage, solid state fermentation, monosaccharide, isomaltooligosaccharide

1. Introduction

In northeastern part of Thailand, it have been found that immature green rice is a rice product producing from an immature green paddy or dough stage rice using a conventional method similar to parboiling process. This rice product is normally produced and consumed among Asian countries in particular of Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam and Cambodia. However, different countries produce different rice varieties with different process methods, thus providing different characteristics of the final product. Basically, the immature green paddy is harvested at the 19-21 days after flower blooming as it is called "dough stage". This rice stage was reported to have high sugar content than a maturity stage (Singh & Juliano, 1977) giving a special characteristic of rice product. Unique and good attributes of immature rice product are green color, gummy-like texture, earthy and green odor with a little bit sweet taste. Moreover, (Ekasit and Jiraporn, 2013) have reported that immature green rice product or young flatten rice using a traditional method contains high value of γ–oryzanol (56-113 mg/100g) as well as γ-aminobutiric acid (GABA) was observed as high as 4-8 mg/100 g for this processed rice at dough stage. (Phomkong *et al*., 2014) have found that several bioactive active compounds such as phenolic compounds, chlorophyll, ß-carotene, tocopherol as well antioxidant activities have been observed in this rice product. It could be suggested that young flatten rice has been produced by conventional method partially of parboiling process, thus high nutritional compound could be moved from embryo and brown layer to endosperm and thus high nutritional rice product is obtained. Moreover, it has been reported that rice has been estimated to have a higher protein digestibility and biological value compared to other cereals (i.e., wheat, corn, barley, millet and sorghum (Eggum, 1979, Young & Pellett, 1994), Amagliani *et al*., 2016). Thus, this research is aimed to add more value of immature green rice as a substrate for Isomaltooligosaccharides (IMOs) which the amount of imported IMO could be decreased.

IMOs, are non-digestible prebiotic oligosaccharide which pass through the gastrointestinal tract without digestion by digestive enzymes, have been found to increase bifidobacterial number in faces and the ratio in fecal microflora and balancing the human gut (Kaneko *et al*., 1994). IMOs production includes 3 steps i.e. liquefaction, saccharification and transglucosidation. Most of liquefaction and saccharification process were aimed to obtain high amount of maltose syrup by enzymatic hydrolysis (Lee *et al*., 2008, Rudeekulthamrong *et al*., 2013, Sorndech *et al*., 2017). Most of liquefaction and saccharification process starting with starch slurry as a substrate which takes a period of time for preparing since the raw materials are in solid form (Wang *et al*., 2007). Thus, an alternative method was proposed by using a substrate as a solid form i.e. rice grain (Sawangwan and Saman, 2016 and Kim *et al*., 2017), rice crumbs (Pan and Lee, 2004). Moreover, it was found that solid state fermentation for IMOs production from rice has been successful as the glutinous rice syrup exhibited high prebiotic properties by using solid-state fermentation of *Aspergillus oryzae* (Sawangwan & Saman, 2016). It is well recognized that *Aspergillus oryzae* is an endo-enzyme producing α-amylase target to hydrolyze α-1,4 glycosidic bond

of branched/linear starch during liquefaction step. Thereafter, β-amylase plays a role to obtain maltose syrup in saccharification stage followed by IMO synthesis is ocurred during transglucosidation step by α-transgucosidase. Thus, a low degree of starch liquefaction (DE ~ 6-8) is preferred for high maltose content during saccharification process (Niu *et al*., 2017). Moreover, *Aspergillus oryzae* preparing as a solid state for fermentation is more practical in industrial application and it is recognized as safe and has been approved by WHO to apply for food industry. Therefore, the aim of the research is focusing of sugar conversion from immature green rice during liquefaction using solid state of *Aspergillus oryzae* to obtain low dextrose equivalent (DE) during starch liquefaction stage.

2. Materials and Methods

2.1 Material

Aspergillus oryzae TISTR 3102 were kindly provided from Thailand Institute of Scientific and Technological Research. Glutinous rice (RD6) includes dough stage

(October, 2019) and maturation stage (November, 2019) was obtained from Pho Sai district, Ubon Ratchathani province, Thailand. All other chemicals used were analytical grade and the sugar standards were HPLC grade.

2.2 Substrate preparation

Immature green rice was washed 3 times, and soaked in 2 % sodium chloride (NaCl) solution for 2 hours, then steamed for 20 minutes and dried until 12 % moisture content of paddy was obtained before milling. . The rice grain was then furthur soaked in distilled water overnight in the raio of water:rice gain at 1:2 and steamed for 40 minutes until cooked by using rice cooker.

2.3 Preparation of Fungal Spore Inoculum

Spore suspensions of A. oryzae TISTR 3102 were prepared from a fully sporulated (7 days old) PDA slant culture using 10 ml of 0.85% NaCl solution (Saman *et al*., 2012) This spore suspension was used as the master suspension. Spore concentration in the inoculum was estimated by a haemacytometer (Wanichsan *et al*., 2015).

2.4 Preparartion of Solid State of Fungal Spore

Glutinous rice (50 g) was placed into an erlenmeyer flasks (koji) containing 10% (v/v) distilled water. The contents of the flasks were mixed thoroughly and autoclaved at 121°C for 20 minutes. Then spore suspension of *Aspergillus oryzae* TISTR 3102 (10^6 spores/ml) was inoculated in autoclaved rice and the mixture was incubated at 30°C for 3 days.

2.5 Fermentation Process

A rice sample was soaked in distilled water overnight in the raio of 1:2 (rice grain: water) and steamed for 40 minute by using rice cooker. Then the substrate was mix with koji as prepared in 2.4 before incubated at 30°C for 8 days using incubator. The syrup was collected everyday for 8 days duringincubation for further analysis.

2.6 Analytical Medthods

2.6.1 Spore concentration

The spore concentration was estimated using a haemacytometer according to the method reported by Wanichsan *et al*., (2015).

2.6.2 Syrup volume

Volume of syrup was measure by filtered through filter cloth before measuring using a 200 milliliter measuring cylinder.

2.6.3 pH, Total Soluble Solid (TSS) and Total Solid (TS)

The pH value of syrup was determined using a pH meter (METTLER TOLEDO AG, Schwerzenbach, Switzerland). Total Soluble Solids (TSS) was determined using a hand refractometer (ATAGO, Model G-50 Brix 0-50, Japan). Total solid (TS) values were measured using (AOAC, 2000) method and calculated as follows:

$$(\%)TS = \frac{\text{weight of sample after drying}}{\text{weight of sample before drying}} \times 100 \quad (1)$$

2.6.4 Reducing sugar content

Reducing sugar content were performed according to dinitrosalicylic colorimetric method (DNS method) (Miller,1959)

2.6.5 Sugar content

Both qualitative and quantitative of sugar collected from syrup were analyzed using Ultra-High-Performance liquid Chromatography, UHPLC (DIONEX, Ultimate 3000, Sunnyvale, CA, USA) with Refractive Index Detector (Refracto Max 521). YMC-Pack Polyamine II column (4.6×250 mm) (YMC japan) was used. A mixture of solvent (Ethanol: Ethyl acetate: Acetronitrile: DI water) was used as mobile phase at constant flow rate of 1.0 ml/minute with controlling temperature at 30°C. All standard compounds (glucose, maltose, fructose) were HPLC grade (DR ehrenstorfer) and isomaltose (Megazyme) Dextrose equivalent (DE value) were calculated as described by Klanarong and Kuakoon (2007)

$$\text{DE value} = \frac{\text{reducing sugar}}{\text{total solid}} \times 100 \quad (2)$$

2.7 Data Analysis

Analytical measurements and all data were presented by mean values with standard deviation. Each parameters were analysed by analysis of variance (ANOVA) by using completely randomized design (CRD) as expermental design. Duncan's new multiple range test was used as determining of differences between the treatment at the 95% confidentail ($p \leq 0.05$).

3. Results and Discussion

Solid state of *A. oryzae* preparing as a koji was obtained with a number of spore forming ($2x10^5$ spores/ml) after 3 days incubation. The koji was mixed with cooked green rice before fermentation under 30 ^oC incubation for 8 days. Syrup volume was rapidly increased to 172 ml since day 4th ($P \leq 0.05$) and tended to constant after 5 days of fermentation ($p > 0.05$), and a slightly decreasing was observed at day 8th of incubation with non-significantly difference with day 7th (Figure 1) These results agreed with the changing of total soluble solid contents but the amount of total soluble solid content was increased since day 3rd of fermentation and tended to constant until

8 days of fermentation. The changing of syrup volume and total soluble solid could be described that the fungal penetrates across the rice kernel before hydrolysis takes place by fungal amylolytic enzymes such as α-amylase (Sivaramakrishnan *et al*., 2007) and glucoamylase (Zambare, 2010). This α-amylase catalyzethe hydrolysis of internal α-1,4-glycosidic linkages at the inner part of the amylose or amylopectin in starch as a random manner (Nielsen, 2000) Moreover, glucoamylase hydrolyzes α-1,4 and α-1,6 glycosidic bonds from the non-reducing ends of starch, resulting in the production of glucose or disaccharide such as maltose and isomaltose (Uhling, 1998). Therefore, an increasing of syrup volume was obtained. But after the 3rd day of fermentation, the amount of substrate might be decreased, thus the changing of syrup volume and total soluble content were constant. However, the syrup volume and total soluble solid content were found coincident with reducing sugar content as shown in(Figure 1) by using DNS method. It was found that the amount of reducing sugar rapidly increased ($p \leq 0.05$) and the highest amount was observed at 3 days of fermentation due to hydrolysis by fungal amylolytic enzymes. In addition, preparing of immature green rice by starch gelatinization resulting of enzyme hydrolyzed substrate easily breaks the hydrogen bonds within molecules inside of starch in the amorphous part, causing of unfolding of the amylose and amylopectin polymer chains and water binding is increased, causing starch granule to swell. Thereafter, the leaching amylose/ amylopectin from amorphous region after gelatinization were degraded by amylolytic enzymes producing from fungi to release oligosaccharides by hydrolysis (Nielsen, 2000). Moreover, as a long period of fermentation process, inducing more digestion activities of α-amylase and glucoamylase enzyme as a result of increasing of the total sugart and reducing sugar (Kong *et al*., 2018). However, reducing sugar content tended to constant after day the 5th of fermentation ($p>0.05$). It could be due to a decreasing of fungal synthesis as there might be a decreasing of substrate due to a long period of fermentation process (Yanfang *et al*., 2008).

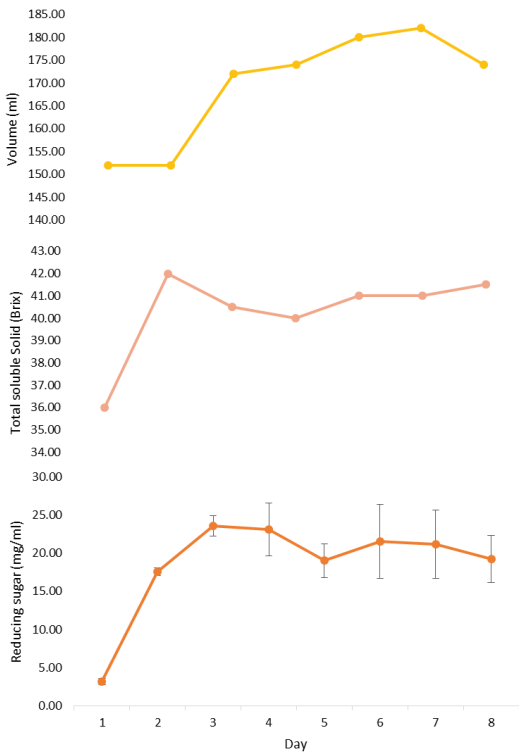

Figure 1. Changing of chemical properties of immature green rice syrup during solid-state fermentation at 30°C for 8 days.

Moreover, the highest value of total solids content (TS) (Figure 2) were found on the 6th days of fermentation and slightly decreased after day 8th of fermentation ($p \leq 0.05$). Generally, total solids include total soluble solids and insoluble solid i.e carbohydrate, protein, lipid etc. Moreover, enzyme containing in fungals (such as protease) as well as amylolytic enzyme play

a role for hydrolysis (Saman *et al.,* 2012), thus total solid content can be increased after 3 days of fermentation. However, a decreasing of total solid was observed after 5 days of fermentation. It could be suggested that a substrate for fungal synthesis is decreased.

The changing of total solid content was agreed with the changing of pH value. It was observed that pH value of dough rice syrup were decreased from 5.27±0.01 to 4.73±0.01 through a 7-day period of solid stage fermentation (P≤0.05). This could be due to fungal metabolism (Yanfang *et al*., 2008). Moreover, during fermentation using solid-state of *A. oryzae* produces the other compounds such as organic acid (Konlam *et al*., 2014) resulting decreasing of pH value.

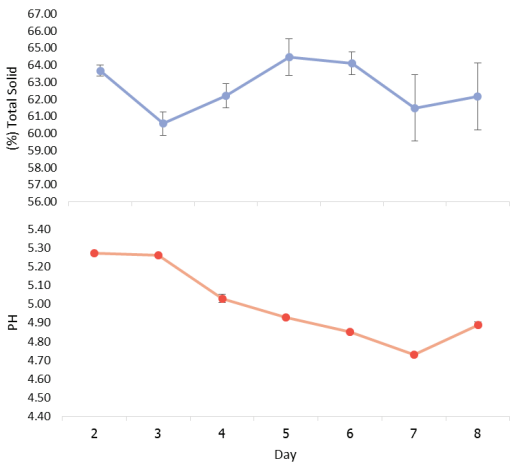

Figure 2. Changing of reducing sugar and pH of immature green rice syrup during fermentation at 30°C for 8 days

UHPLC analysis

The immature green rice syrups were daily withdrawn and analyzed for both quantitative and qualitative analysis of sugar by UHPLC techniques. A chromatogram of sugar analysis of immature rice flour fermentation (Figure 3a) exhibited monosaccharide compounds as peak of fructose, glucose and maltose were found at retention time of 8, 11 and 13 minutes, respectively. This result agreed with (Hu *et al*., 2017) which soluble sugar profile of rice were found such as glucose, fructose, sucrose, raffinose and maltose. However, glucose and maltose in immature rice syrup was observed after 4 day of fermentaion by SSF of *A. oryzae* TISTR 3102 (Figure 3b), but a peak of fructose was no longer appear. It is suggested that during fermentation process, there is metabolism of fungals by using fructose as a substrate for synthesis. These results agreed with total solid and pH value through a fermentation process. Moreover, UHPLC chromatogram exhibited isomaltose peak at retention time between 15-16 minutes.

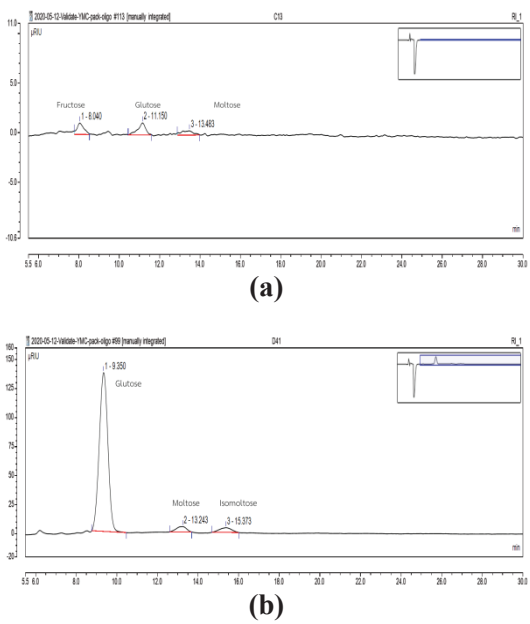

Figure 3. HPLC chromatogram of immature green rice (a) (peak 1: fructose, 2: glucose and 3: maltose), and glutinous rice syrup from SSF by *A. oryzae* TISTR 3102 at 30°C 4 day (b) (peak 1: glucose, 2: maltose, 3: isomaltose)

The peak area of immature green rice syrup was analyzed by comparing with sugar and isomaltose standard curve (R^2=0.99). As shown in (Table 1), it was found that the highest glucose content was obtained in the 3rd day of fermentation period which agreed with reducing sugar content measured by DNS method (Figure 1) and tended to constant throughout fermentation period (P>0.05). On the other hand, maltose tended to increase with fermentation period was increased, which the maximum content was obtained on the 8th day of fermentation (P≤0.05). However, the increasing of isomaltose content was found through a period fermentation process. It could be due to an activity of α-glucosidase produced from SSF of *A. oryzae* by catalyses liberation of glucose from non-reducing ends of oligosaccharides and polysaccharides. This enzyme is able to transfer sugar or groups of sugar residues from one compound to another with the formation of a similar or a distinct type of linkage. Thus, an α-(1,4) link in a chain might be broken and the separated end could be joined to the same or different chain via either of α-(1,4) or α-(1,6) link resulting to produce molecules of maltose, isomaltose, or long chain of oligosaccharides. (Saman, 2012).

Resulting of an increasing of reducing sugar content, thus high dextrose equivalent (DE-value) was obtained up to 55.13 It could be suggested that high glucose syrup was obtained under solid-state of *A. oryzae* fermentation, using immature green rice as a substrate. In addition, there is a production of oligosaccharide such as isomaltose during fermentation by SSF of *A. oryzae* which the maximum content was observed on the 8th day of fermentation (P≤0.05).

However, as a result of high DE-value was obtained in this study, it is suggested that this fermentation process is not suitable for IMOs synthesis. Generally, a requirement for IMOs production to obtain high maltose syrup after saccharification process is required DE value between 6 to 8 (Niu *et al*., 2017). Therefore, high glucose syrup was found under solid-state of *A. oryzae* fermentation, using immature green rice as a substrate under 30°C, is not suitable for IMOs production. Therefore, the production process may need to be modified by changing of fermentation conditions to be a suitable environment for beta-amylase activity such as temperature and pH or can be using both crude enzyme or SSF of *A. niger*. Since, β-amylase enzyme catalyzes the hydrolysis of the α-1,4 glycosidic bond at specific manner, cleaving off two glucose units (maltose) at a time (Beck & Ziegler, 1989).

Table 1. Sugar content and dextrose equivalent using UHPLC analysis frome solid-state by *A. oryzae* for immature green rice under 30°C for 8 days of fermentation.

Day	Fructose (mg/ml)	Glucose (mg/ml)	Maltose (mg/ml)	Isomaltose (mg/ml)	Dextrose equivalent (DE)
0	0.89 ±0.14	2.81 ±0.0.33^{f}	2.01 ±0.50^{d}	N.D.	0.63 ±0.10^{d}
2	N.D.	325.58 ±2.93^{d}	6.02 ±0.31^{c}	27.79 ±4.64^{c}	47.04 ±0.54^{c}
3	N.D.	353.02 ±5.63^{a}	12.64 ±1.54^{b}	32.87 ±1.04^{abc}	55.13 ±2.29^{a}
4	N.D.	340.01 ±3.68^{bc}	13.91 ±1.75^{b}	35.18 ±1.93^{abc}	51.61 ±2.01^{ba}
5	N.D.	336.83 ±5.84^{c}	14.14 ±1.06^{b}	29.35 ±3.47^{c}	48.46 ±0.42^{bc}
6	N.D.	345.00 ±0.91^{b}	13.84 ±0.62^{b}	30.05 ±3.79^{bc}	48.30 ±1.55^{bc}
7	N.D.	290.37 ±1.11^{e}	13.46 ±0.62^{b}	37.01 ±1.10^{ab}	45.30 ±2.16^{c}
8	N.D.	343.40 ±1.52^{bc}	16.61 ±1.00^{a}	39.37 ±1.02^{a}	53.45 ±3.09^{a}

Note: - The data shows the average and standard deviation.
a-f Mean values in the same column with different letters expressed significant differences ($p \leq 0.05$) all among samples.
N.D. means not detectable.

4. Conclusion

The production of green rice syrup by solid-state of *A. oryzae* TISTR 3102 at 30 ^{o}C for 8 days fermentation was observed. It was found that glucose and maltose were increased since 3 days of fermentation period analysed by Ultra-High-Performance liquid Chromatography (UHPLC). These results correspond with increasing of dextrose equivalent, syrup volume, total soluble solid and reducing sugar content. The amylolytic enzymes plays a role for starch hydrolysis under solid-state fermenation of *A. oryzae* under a suitable condition of fungal metabolism by using immature green rice as a substrate resulting of decreasing of total solid and pH value. However, isomaltose was also obtained under solid-state fermenation of *A. oryzae*, but a low value was obtained which is not suitable for further IMOs synthesis.

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6. References

Amagliani, L., O'Regan, J., Kelly, A.L. & O'Mahony, J.A. (2016). Chemistry, structure, functionality and applications of rice starch. *Journal of Cereal Science, 70*, 291-300.

AOAC. (2005). *Official methods of Analysis of AOAC international*. AOAC International.

Beck, E. & Ziegler, P. (1989). Biosynthesis and degradation of starch in higher plants.Annu. Rev. *Plant Physiol. 40*, 95-117.

Eggum, B.O. (1979). The nutritional value of rice in comparison with other cereals. *Proceedings of Workshop on Chemical Aspects of Rice Grain Quality* (pp. 91-111).

Ekasit, O. & Jiraporn, B. (2013). Some physical characteristics and bioactive compounds of young flattern rice (Khao-Mao). *Int. Food Res. J., 20*(3), 1627-1632.

Hu, X., Fang, C., Lu, L., Hu, Z., Shao, Y., & Zhu, Z. (2017). Determination of soluble sugar profile in rice. *Journal of Chromatography B*, *1058*, 19-23.

Kaneko, T., Kohmoto, T., Kikuchi, H., Shiota, M., Iino, H. & Mitsuoka, T. (1994). Effects of Isomaltooligosaccharides with different degrees of polymerization on human fecal bifidobacteria. *Bioscience, Biotechnology, and Biochemistry*, *58*(12), 2288-2290.

Kim, H.H., Lee, W.P., Oh, C.H. & Yoon, S.S. (2017). Production of a fermented organic rice syrup with higher isomalto-oligosaccharide using Lactobacillus plantarum. *Food Science and Biotechnology*, *26*(5), 1343-1347.

Konlam, P., Bovonsombut, S., Bovonsombut, S. & Juwattanasamran, R. (2017). Role of Aspergillus oryzae M-01 in the production of amylase and protease enzymes on soybean Koji prepared for brine fermentation of Tamari soy sauce. In 55. *Kasetsart University Annual Conference, Bangkok (Thailand)*.

Lee, Min Sung, Seung Kee Cho, Hyun-Ju Eom, So-Young Kim, Tae-Jip Kim & Nam Soo Han. (2008). Optimized substrate concentrations for production of long-cahin isomlatooligosaccharides using Dextransucrase of *Lueconostoc mesenteroides* B-512F. *Journal of Microbiology and Biotechnology, 18*(6), 1141-1145.

Lieberman, M. & Marks, A.D. (2009). *Marks' basic medical biochemistry: a clinical approach*. Lippincott Williams & Wilkins.

Miller, G. (1959). Use of dinitrisalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry 31*, 426-429.

Nielsen JE. (2000). Borchert TV. Protein engineering of bacterial α-amylases. *Biochim Biophys Acta, 1543*, 253-74.

Niu, D., Qiao, J., Li, P., Tian, K., Liu, X., Singh, S. & Lu, F. (2017). Highly efficient enzymatic preparation of isomalto-oligosaccharides from starch using an enzyme cocktail. *Electronic Journal of Biotechnology, 26*, 46-51.

Pan, Y.C. & Lee, W.C. (2005). 'Production of high-purity isomalto-oligosaccharides syrup by the enzymatic conversion of transglucosidase and fermentation of yeast cells. *Biotechnology and Bioengineering, 89*, 797-804.

Saman, P., Chaiongkarn, A., Moonmangmee, S. & Artjariyasripong, S. (2012). Prebiotic isomalto-oligosaccharide production from economic crops of Thailand. *Asia-Pacific Journal of Science and Technology, 17*(5), 794-799.

Sawangwan, T. & Saman, P. (2016). Shelf life enhancement isomalto-oligosaccharide from glutinous rice syrup with prebiotic properties. *International Journal of Probiotics & Prebiotics, 11*(2).

Singh, R. & Juliano, B.O. (1977). Free sugars in relation to starch accumulation in developing rice grain. *Plant physiology, 59*(3), 417-421.

Sivaramakrishnan, S., Gangadharan, D., Nampoothiri, K.M., Soccol, C.R., & Pandey, A. (2007). *Alpha amylase production by Aspergillus oryzae employing solid-state fermentation.*

Sriroth, K. & Piyachomkwan, K. (2005). *Technology of Starch*. Starch, Kasetsart University.

Uhling, H. (1998). *Industrial Enzymes and Their Applications. Translated and updated by Elfried M. Linsmaier-Bednar* (Doctoral dissertation, Ph.D.).

Wang, P., Singh, V., Xue, H., Johnston, D.B., Rausch, K.D. & Tumbleson, M.E. (2007). Comparison of raw starch hydrolyzing enzyme with conventional liguefaction and saccharification enzymes in dry-grind corn processing. *Cereal Chemistry, 84*(1), 10-14.

Young, V.R. & Pellett, P.L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *The American journal of clinical nutrition, 59*(5), 1203S-1212S.

Wanichsan, D., Ninchawee, N., Rattanakom, T. & Kongjuk, P. (2015). An algorithm for hemocytometer cell counting based on image processing technique and DBSCAN. *Inf. Technol. J, 11*(2), 56-61.

Wiriya Phomkong, Ekasit Onsaard, Apinya Ekpong, Jittra Singthong, Ratchadaporn Oonsiwilai, Jirawan ard & Lamul Wiset. (2014). *Final report of "Process development of young flatten rice and its application as functional products*. Final report, Department of Agro-Industry, Ubon ratchathani University.

Yanfang, Z., lijuan, W. & Wenyi, T. (2009). Biochemical changes in low-salt fermentation of solidstate soy sauce. *African Journal of Biotechnology, 8*, 7028-34.

Zambare, V. (2010). Solid state fermentation of Aspergillus oryzae for glucoamylase production on agro residues. *International Journal of life sciences, 4*, 16-25.