CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

APPLICATION OF STARCH PROCESSING ENZYMES IN FOOD TECHNOLOGY-A REVIEW

Saeideh Esmaeili[1], Zohreh Noorolahi[2*]

[1]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, ShahidBeheshti University of Medical Sciences, Tehran, Iran.
[2]Department of Food Science and Technology, Faculty of Agriculture, TarbiatModares University, Tehran, Iran.
**z.noorolahi@modares.ac.ir*

Article history:
Received:
18 April 2017
Accepted:
8 September 2017

Keywords:
Starch;
Processing Enzymes;
Amylases (endo, exo);
De-branched enzymes;
Transferses.

ABSTRACT

Starch is an important storage compound in plants that has many benefits for human beings. It has complex structure and made of two polymers: amylose and amylopectin; so the combination of enzymes is needed for its decomposi.tion Many of these enzymes exist in natural resources.

In industry, starch is used in producing different compounds. Some of them are made by chemical methods but others are produced by just enzymes. Starch processing enzymes are categorized into two groups based on their operation. The first group are hydrolases that hydrolyze glycoside bonds by water as endo and exo. The second group are glocanohydrolases which break one glycoside α-1.4 bond and creates a new glycoside α-1.4 or α-1.6 linkages. The aim of this article is a general review of these enzymes operation and application in food industry.

1. Introduction

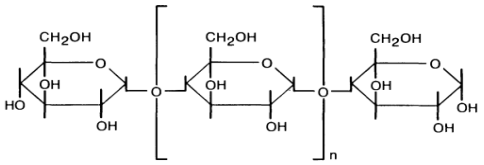
The starch is an important nutrition carbohydrate for human being which is stored in leaves, glands, seeds and roots of plants. This compound is the second plentiful heterogeneous polysaccharide after cellulose that produced by the plants (Yamada et al., 2009, Zeeman et al., 2010, Roy Goswamiet al., 2015). The most known sources of starch are cereals, potato, and tapioca which are used for starch extraction moreover direct consumption (Whitehurst and van Oort, 2010). Corn, wheat and potato are mostly used for producing industrial starch in Europe and America, whereas in Asia, casava, rice, sweat potato, yam and arrow root are used too. The annual production of starch is around 48 $\times 10^6$ ton in the world which nearly 70% of this is used in food industry (Daiuto et al., 2005).

Starch is stored in granolas. The shape and size of the granolas are varying in different sources. There are several methods to extract starch. Each method has different efficiency of extraction and the obtained starch has different practical properties too. During extraction, every damages to the crystalline phase of starch and depolymerizing of it is undesirable (Lee et al., 2007). The most common method of starch extraction is grinding the soaked raw substances for breaking cells' wall, separating substances by different sieves and finally separating starch from slurry by decanters or centrifuges (Daiutoet al., 2005; Zhang et al., 2009). In another method, fractionation is used for separating starch from protein. In this method, grinding is done for dry substances and then air is used for separating starch. This method is efficient and easy and there is no need to moving plenty amount of starch solution (Lee et al., 2007) but the produced starch shows weaker practical properties comparing with the starch from moist method

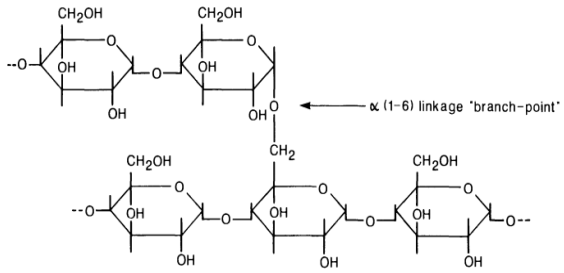
(Tian et al., 1999). In some methods, alkali is used for solubilizationof protein and extraction of starch (Cardosoa et al., 2007). The produced starch from this method has got high purity (Puchongkavarinet al., 2005) but comparing with the one produced from moist method, it shows lower pasting temperature and higher pasting viscosity (Dokicet al., 2010; Lai et al., 2004; Han and Hamaker, 2002).

2. Types of starch processing enzymes

The natural starch is made of about 20% amylose and 80% amylopectin. Amylose is linear polymer of glucose with α-1.4 bounds and its molecular weight is around 10^6 Dalton whereas amylopectin has 5% α-1.6 links in addition to mentioned bonds and its weigh is about 10^8 Dalton. In this way amylopectin has a lot of small branches but amylose is composed of fewer long branches (Srichuwong et al., 2005; Yoo and Jan., 2002). The structure of starch components is shown in Figure 1. The construct, molecular weight and proportion of amylose to amylopectin is different in extracted starch from different plant sources. This leads to diverse characteristics invarious types of starch (Syaharizaet al., 2010; Srichuwong et al., 2005). The waxy starches consist of plenty amount of amylopectin but the hylon consists of more than 50% of amylose (van der Maarel and Leemhuis. 2013). At the moment, near 30% of worldwide enzyme production has allocated to starch processing enzymes.In Table 1 most commercial applications of starch processing enzymes in food industry has been .shown



Amylose: linear polymer of glucose with α -1,4 links



Amylopectin; glucose polymer with α -1,4 links and branched with α -1,6 links

Figure 1. Chemical structure of amylose and amylopectin (Tucker and Woods, 1995)

Table 1. The most common commercial application of starch processing enzymes in food industry

Application	Enzyme/Enzymes
Starch liquefaction	α-amylase
Starch saccharification	α-amylase, glucoamylas,pullulanase,isoamylase, maltogenic amylase
Baking industry	α-amylase,

Anti-staling	α-amylase, β-amylase, pullulanase, debranched enzymes, branching enzymes, maltogenic amylase, glucoamylase, cyclodextrin glucanotransferases
Cycloamylose production	α-glucanotransferases

As starch polymer has complex constructer, combination of enzymes is needed for decomposing it to smaller parts or transferring oligoglycosidic bonds or creating new bonds (Bertoldo and Antranikian, 2002). The important enzymes which are usually used for biotechnology field and also starch process are called amylase. In one category, starch enzymes are classified in to two groups: a) hydrolases which hydrolyze glycosidic bonds by water in two ways endo and exo; b) glocanotransferases which break one α-1.4 bound and create new α-1.4 or α-1.6 bound.

Generally, the effective enzymes on starch can be categorized into four groups: endo-amylases, exo-amylases, de-branched enzymes and transferses (Hii et al., 2012). The best-known enzymes affecting on starch belong to glycoside hydrolases families 13, 15 and 28 (Whitehurst and van Oort, 2010). Specificationsof starch processing enzymes have been shownin Table 2. Table 3alsoincludes enzymessubstrates and products derived from the effects of these.

Table 2. Characteristics of starch processing enzymes

Activity	Enzyme name	EC	Enzyme Group
Hydrolysis of α-1,4 linkages	α-amylase	3.2.1.1	Endoamylases
Hydrolysis of α-1,4 and α-1,6 linkages	Glucoamylase	3.2.1.3	Exoamylases
Hydrolysis of α-1,4 linkages	α-Glucosidase	3.2.1.20	
Hydrolysis of α-1,4 linkages	β-amylase	3.2.1.2	
Hydrolysis of α-1,6 linkages	Amylo-1-6-glucosidase	3.2.1.33	De-branched enzymes
Hydrolysis of α-1,6 linkages	Pullulanase type I	3.2.1.41	
Hydrolysis of α-1,4 and α-1,6 linkages	Pullulanase type II	3.2.1.41	
Hydrolysis of α-1,4 linkages	Pullulan hydrolase types I (neopullulanase)	3.2.1.135	
Hydrolysis of α-1,4 linkages	Pullulan hydrolase types II (isopullulanase)	3.2.1.57	
Hydrolysis of α-1,6 linkages	Isoamylase	3.2.1.68	
Hydrolysis of α-1,4 linkages and formation of new α -1,4 linkages	Cyclodextrin glucanotransferases	2.4.1.19	α-glucanotransferases
Hydrolysis of α-1,4 linkages and formation of new α -1,4 linkages	4-α-glucanotransferase	2.4.1.25	
Hydrolysis of α-1,4 linkages and formation of new α -1,6	Branching enzyme (Q-enzyme)	2.4.1.18	

linkages			

Table 3. Substrates and products obtained from acting ofstarch processing enzymes

Product/products	Mainsubstrate	Enzyme name
Linear and branched oligosaccharides, α-limit dextrins	Starch	α-amylase
High glucose syrup	Starch	Glucoamylase
1- Glucose, isomaltose, isomaltortiose 2-Isomalto-oligosaccharides (IMO)	1-Starch 2-Oligosaccharidescontainingmaltose 3-Branched Oligosaccharides likely panose and isopanose	α-Glucosidase
β-limit dextrins	Starch	β-amylase
	Side chain containing of one glucose (product of transglycosylase enzyme)	amylo-1-6-glucosidase
Maltotriose	Pullulan, Amylopectin	Pullulanase type I
Maltose, maltotriose	Pullulan, Amylopectin	Pullulanase type II
Maltose, maltotriose	Amylopectin, glycogen	Isoamylase
α، β و ϒ-cyclodextrins, gluco-oligosaccharides with cycle α-1,4 bonds (scchardinger sugars)	Compounds containing of someconsecutive α-1,4 glycosidic bond	Cyclodextrin glucanotransferases
Glycogen, amylopectin	Compounds containing of someconsecutive α-1,4 glycosidic bond	4-α-glucanotransferase
Amylopectins and glycogen with more side chains	Starch, glycogen	Branching enzyme (Q-enzyme)

2.1. Endo-amylases

These enzymes can break α-1.4 glucosidic bonds that exist in inner part of amylase or amylopectin chain. Among these enzymes, there is α-amylase (EC1.1.2.3) which breaks mentioned bonds randomly but when reaches to α-1.6 linkages, these operation is stopped. In this way, they produce linear and branched oligosaccharides and α-limit dextrins (Kammoun et al., 2008; Van der Maarel et al., 2002). These enzymes are industrially obtained from bacterial and fungal sources. The bacterial types of α-amylases are really resistant to high temperature, react in normal pH and need to calcium ion for their activity. While the fungal α-amylases are nearly sensitive to temperature. These enzymes are maltogenic and their main product are maltose and other oligomers; so they are not used for liquefaction but are used as substitute of β-amylase for producing high maltose syrups (Souza and Magalhaes, 2010; Tucker and Woods, 1995).

2.2.Exo-amylases

These enzymes which consist of glucoamylase (EC 3.2.1.3) and α glucosidase (EC 3.2.1.2) break α-1.4 and α-1.6 bonds between glycosides from non-reducing end of amylase and amylopectin and therefore, they produce glucose. β-amylases (EC 3.2.1.2), which also belong to this group, break α-1.6 bonds exclusively from the end of non-reducing and therefore produce maltose and β-limit dextrins (Haki and Rakshit, 2003; Bertoldo and Antranikian, 2002).

2.2.1. β-amylases

These enzymes are exo-acting. They cannot separate α-1.6 branches like α-amylases, and can't turn the obstacles; so they produce high molecular weight dextrin that is well-known as β-limit (Li and Yu, 2011). These enzymes accompany with de-branched

enzymes are used for producing syrups in industry. The maltose syrups are hygroscopic and have more constant color than glucose syrups. Moreover, they are used in confectionary industry and producing frozen desserts because of less crystallization and adhesion (Tucker and Woods, 1995).

2.2.2. Glucoamylase

These enzymes which are known as amylo-glucosidase or sugar-making amylase separate α-1.4 bonds and in lower extent α-1.6 bonds from non-reducing end, similar pullulanase type II. (Whitehurst and van Oort, 2010). The high activity of this enzymewould produce high glucose syrups. This activity is done by using a lot of enzymes but this amount of enzyme may cause undesirable side reactions such as return reaction and producing isomaltose and maltose and therefore may decrease final output. These enzymes are acid like and sensitive to temperature. In industry, they are used for hydrolysis dextrins to glucose syrup in saccharification (Hii et al., 2012; Tucker and Woods, 1995).

2.2.3. α-Glucosidase

α-glucosidases are also exo-acting enzymes that separate amylose, amylopectin and oligosaccharides with maltose from non-reducing end and produce glucose (Latorre-Garcia *et al*., 2005; Tatsumi and Katano, 2005). When the maltose concentration is high enough, transglycosylation reaction is done by this enzyme and therefore maltose changes to isomaltose (two glucose with 1, 6 linkage). In the next stage isomaltose will be glycosylated and will produce isomaltotriose. Branched oligosaccharides like panose and isopanose can also be glycosylated by this enzyme. These compounds are generally well- known as isomalto-oligosaccharides (IMO) which are produced as prebiotic fibers in China and Japan (Whitehurst and van Oort, 2010).

2.3. De-branched enzymes

These enzymes catalyze hydrolysis of α-1.6 bonds and tend to separate amylopectin branches.This tendency is the main difference between de-branched enzymes and other hydrolysis enzymes because the main place of other hydrolysis enzymes is α-1.4 bonds (Hii*et al*., 2012). These enzymes are categorized into two main groups: direct de-branched and indirect de-branched enzymes (Fogarty and Kelly, 1990).

2.3.1. Indirect de-branched enzymes

Amylo-1,6-glucosidase (EC 3.2.1.33) is an indirect de-branched enzyme. In the beginning , the substrate of this enzyme has to be reformed by another enzyme because this enzyme can hydrolyze just the side chain which consists of one glucose molecule. For this reason, at first oligosaccharide was taken with transglucosylase enzyme (4-α-glucanotransferase, EC 2.4.1.25) and substrate was ready for acting of this enzyme (Nakamura, 1996). This enzyme does not widelyused in industry (Hii et al., 2012).

2.3.2. Direct de-branched enzymes

These enzymes directly hydrolyze α-1.6 bonds in unreformed substrates. Among these enzymes are pullulanases and isoamylases.

2.3.2.1. Pullulanases

Other names ofpullulanases EC 3.2.1.41 are α-dextrine 6-glucanohydrolase, amylopectine 6-glucanohydrolase, pullulan 6-glucanohydrolase and limit dextrinase. These enzymes are extracted from microbial sources and arehydrolyzed α-1.6 bonds in pullulan (the repeated units contain of three glucose residues with α-1.4 bonds which are linked together by one α-1.6 bond) (Zareian et al., 2010; Kuroiwa*et al*., 2005; Roy and Gupta, 2004). The final product by these enzymes is maltotriose (Kunamneni and Singh, 2006). Pullulanases type I hydrolyze α-1.6 bonds and produce branched dextrins. These enzymes are like isoamylases. Pullulanases type II hydrolyze both α-1.4 and α-1.6 bonds andoften

generate maltose and maltotriose. Recent enzymes are similar toglucoamylase and are commercially used in saccharification (Whitehurst and van Oort, 2010).

,So far five groups of pullulan hydrolyzing enzymes are reported that are categorized based on characteristics of the substrate and reaction of products:

Pullulanases type I: they hydrolyze α-1.6 bonds in pullulan and branched polysaccharides. Pullulanases type II (amylopullulanase): they hydrolyze α-1.4 or α-1.6 linkages and are used vastly in starch processing industry. Both enzymes don't affect on cyclodextrins (Roy et al., 2003; Ben Messaoudet al., 2002; Duffner et al., 2000). Neopullulanases (pullulan hydrolase type I) and isopullulanases (pullulan hydrolase type II) can break α-1.4 bonds and affect on cyclodextrins but not starch. The enzymes which analyze cyclodextrins faster than starch are called cyclodextrinase (Kaharet al., 2013). Since these enzymes are able to recognize the structural differences between α-1.4 bonds and α-1.6 bonds exactly, they are used vastly for analyzing polysaccharide and oligosaccharide structures (Roy et al., 2003). Pullulan hydrolase type III which were known by Niehause et al, unlike other pullulan hydrolyzing enzymes, can hydrolyze α-1.4 and α-1.6 bonds in pullulan that produce a mixture of maltotriose, panose, maltose, and glucose (Niehause et al., 1999). These enzymes can also analyze starch, amylose and amylopectin and produce maltose and maltotriose (Hii et al., 2012).

2.3.2.2. Isoamylase

Isoamylases (EC 3.2.1.68) can hydrolyze α-1.6 bonds. These enzymes are the only known ones which can debranche glycogen completely. They are made of two subunits with 45000 molecule weight (Zobel, 1992).

2.3.2.3. Comparing pullulanase and isoamylase

The main difference between these enzymes is in their substrates so that pullulanase hydrolyzes α-1.6 bonds in pullulan and amylopectin whereas isoamylase hydrolyzes these linkages in amylopectin and glycogen (Mikami et al., 2006). In other words, pullulanase needs two chains for actions containing at least two glucose residues with α-1.4 bonds which are joined with α-1.6 bonds whereas isoamylase prefers high molecule weight substrates. Another difference is their source so that the de-branched enzymes in yeasts and molds are mostly isoamylase but bacterial de-branched are mostly pullulanases (Hii et al., 2012; Van der Maarel et al., 2002).

Application of only α-amylase or β-amylase during starch saccharification processes produces plenty of α-limit and β-limit dextrins respectively and reduces the final viscosity of glucose. For this reason, the use of de-branched enzymes increases efficiency of glucose production. The hydrolysis of amylopectin and limit dexterins like produced dexterins by *Klebsiella planticola* and *Bacillus acidopullyticus* can be done by using bacterial pullulanase, but using fungal isoamylase is limited because of the lower temperature stability and lower pH adaptation with β-amylase.

Another problem for using isoamylase in saccharification is its inability in hydrolysis of side chains consisted of 2 and 3 glucose unit in α-limit and β-limit dextrins. Therefore the simultaneous action of β-amylase and isoamylase cannot change amylopectin to maltose. On the other hand, β-amylase is considered as isoamylse inhibitor. The existence of maltotrios and maltotetraose also inhibits isoamylse action competitively (Hii et al., 2012).

The biggest advantage of using pullulanase instead of isoamylase in saccharification is that adding stages of isoamylse is very critical. For example adding this enzyme to the hydrolysis system before amyloglucosidase can cause depolymerisation of amylopectin and therefore acceleration of the retrogradation (Guzman-Maldonado and Paredes-Lopez, 1995).

2.3.3. Application of de-branched enzymes

During saccharification process, α-1.6 bonds in starch act as an inhibitor for action of starch hydrolyzing enzymes and for this reason, hydrolysis continues with the usage of de-branched enzymes and efficiency of glucose production increases.

Application of glucoamylase alone can produce plenty of isomaltose. Since this compound is resistant to hydrolysi,s the efficiency of final production decreases (Crabb and Shetty, 1999; Uhlig, 1998). Using glucoamylase and pullulanase simultaneously can prevent by-product isomaltose. In this situation, pullulanase hydrolyses branch points specifically and then glucoamylase hydrolyses α-1.4 bonds in line chain (Hii et al., 2012). With utilization of de-branched enzymes together with glucoamylase, the speed of saccharification increases, the amount of needed glucoamylase decreases and less activity of glucoamulase is needed. In this way, the cost of pullulanase usage will be compensated by saving in vaporization cost and glucoamylase cost. Application of these two enzymes in fructose syrup process decreases the isomerization expenses too because of increasing capacity of factory as the result of decreasing saccharification time (Poliakoff and and Licence, 2007).

Nowadays de-branched enzymes are used in saccharification of starch, production of high maltose corn syrup, production of detergents and with CGTAs in production of cyclodextrins (Hii et al., 2012).

2-4. α-glucanotransferases

Application of a glucanotrnsferases or AGTAs (EC 2.4.2.XX) is made prevalent in two recent decades. These enzymes do not hydrolyze starch as amylases but at first break some parts of amylose and amylopectin molecules and then makes new bonds again (Van der Maarel and Leemhuis, 2013).

Substrates of α glucanotrnsferases are the compounds consist of some consecutiveα-1.4 glycosidic linkages like amylose, amylopectin, maltodextrins and glycogen. α-glucanotransferases belong to glycoside hydrolases (GH) family. Members of this family break glycosidic bonds and move them to acceptor (usually water). These enzymes can also link separate parts to other sugar and make new glycosidic bonds (so-called disproportionation reaction) (Oh et al., 2008). α-glucanotransferase used in industry belong to GH 13,57,77 families. All these enzymes are as α-retaining; it means that anomeric configuration of new made glycoside bonds is similar to broken bonds in substrate (Vocadlo and Davies, 2008). Reaction of these enzymes at first is started by breaking one α-1.4 glycosidic linkage in substrate and an enzyme-glycosyl intermediate is produced. In the next stage and after existing the fragmented sugar part from acceptor, non-reducing end from another glucan can enter acceptor site and the new bond is made. On the base that whether non-reducing end attack from 4-hydroxyle or 6-hydroxyle, the new bonds are as α-1.4- and α-1.6 respectively (Stam*et al*., 2006; Zona et al., 2004).

2.4.1. The types of α-glucanotransferases

Tree types of α-glucanotransferases have been known so far: I. Cyclodextrin α-glucanotransferase (CGTAs; EC 2.4.1.19), II. 4-α-glucanotransferase (4αGT; EC 2.4.1.25) which is sometimes shown as amylo-maltase, disproportionating or D-enzyme too, III. Branching enzyme (BE; EC 2.4.1.18) which is known as Q-enzyme (Van der Maarel and Leemhuis, 2013; Leemhuis et al., 2010; Kaper et al., 2004). In Table 4 there is industrial application of these enzymes briefly.

2.4.1.1. Cyclodextrin glucanotransferases (CGTAs)

For this group of enzymes the dominant reaction is intra-molecular transglycosylation which causes cycle product with 6, 7, 0r 8

glucose residues with α-1.4 bonds that are called α-, β- and γ-cyclodextrins (Vollu et al., 2008; Qi and Zimmermann, 2005). When the amount of glucan acceptor containing one free 4-hydroxile group ishigh , CGTAs can shift the glucan intermediate to another glucan chain and do inter molecular transglycosylation. In this way, a circular product is created (Whitehurst and van Oort, 2013; Van der Maarel and Leemhuis, 2013).

2.4.1.1.1. Application of cyclodextrin glucanotransferases (CGTAs)

These enzymes exist in bacteria and archaeas which areactive in high temperature. They are considered asthe first industrial AGTAs (Biwer*et al*., 2002; Leemhuis *et al*., 2010). The main usage of these enzymes is production of industrial cyclodextrins. α-, β-, and γ-cyclodextrins are considered asgluco oligosaccharides with cyclic α-1.4 bonds which are known as sacchardinger. These compounds have hydrophobic inner part and hydrophilic outer part because the sequence of their glucose residues. Therefore, they can complex with hydrophobic compounds and change their physical and chemical properties. These compounds are used as antiseptic factor (Whitehurst and van Oort, 2010). In food industry, α-, β-, and γ-cyclodextrins are also used vastly for taking cholesterol, stabilizing aromatic and sensitive compounds, solubilizing hydrophobic compounds in water, and eliminating undesirable taste and odor (Astray et al., 2009; Szente and Szejtli, 2004; Reineccius et al., 2003).

Wacker Chemie Company has offered α-cyclodextrins as indigestible diet fiber. This product is obtained from the effect of cyclodextrin glucanotransferases enzyme on the liquefied starch.Mentioned enzyme is extracted from *K.oxytoca*. This product has been produced with KAWAMAX W6 trade name and is used as a diet fiber in carbonated and noncarbonated soft drinks, dairy products, and bakery products (Whitehurst and van Oort, 2010).

CGTAs are also used for preventing of bread staling. Since the structure of this enzyme is highly similar to the structure of anti-stalingenzyme, Novomyl, the structural change in later enzyme changes it to a CGTAs. There are some reports which show that the mutant CGATs types improve the quality of bakery production too (Shim et al., 2007; Kelly et al., 2009).

2.4.1.2. Amylo-maltases or 4αGT

This enzyme in contrast to CGTAs does intermolecular transglycosylation. In this way, they *break* α-1.4 bonds and create new bonds. These new bonds are in the form of α-1.4 too. If the concentration of glucan acceptor containing a free 4-hydroxile group is little, these enzymes do intermolecular transglycosylation and create cyclic molecules containing 15 glucose residues with -1,4α bonds which are known as Large-ring cyclodextrins LR-CD (Srisimarat et al., 2011; Kaperet al., 2004; Terada et al., 1999).

2.4.1.2.1. Application of Amylo-maltase or 4αGT

In nature, these enzymes participate in glycogen metabolism in bacteria and also foundation of amylopectin in plants (Kaper et al., 2004). But in industry, the amylo-maltases of thermal resistant bacteria are used to transfer a part of amylose to non-reducing end of amylopectin. The obtained substance is composed of the amylopectins with long chains which can produce a white opaque gel with ability of thermal returning. Therefore, this substance is a suitable vegetal gel and can be used instead of gelatin which is an animal compound (Euverink and Binnema, 1998). The starch which is used in this purpose has to contain amylose (Hansen et al., 2009; Hansen et al., 2008). In this process, α-1.4 linkage between two glucose is broken and a new α-1.4 linkage is formed (Oh et al., 2008). The new substance is similar to primary substance in reducing power, percebranches, and average of molecular weight (van der Maarel et al., 2005).

Etenia is a trade name for a thermal reversible gel which is produced by AVEBE, a Dutch company. This substance is obtained by the effect of extracted amylo-maltase enzyme from *Thermus thermophiles* bacteria on potato starch. It is free of amylose and unlike starch, produces thermal reversible gel starch because it has amylopectin with side chains containing more than 35 glucose. The only difference between this gel and starch is its opal. This compound is used for making creamy texture in low fat products. Altinget *al.* applied this enzyme for increasing ceramin in yogurt. The mentioned enzyme is produced from B.amyloquefaciens bacteria by DSM Company (Altinget al., 2009; Munet al., 2009). In contrast to most enzymes which are used for starch processing, this enzyme doesn't secreted out of cells so the downstream process of its extraction is difficult (Whitehurst and van Oort, 2010). All modified starch with this enzyme contain amylopectins with long chains (Hansen et al., 2008).

When the high amount of amylose with high molecule weight is incubated with high amount of amylo-maltase, some compounds called cycloamyloses are produced. Cycloamyloses are large cycle compounds containing at least 16 glucose residues. Because of the large size of these compounds, they make two or more unparalleled spiral. In this way a hydrophobic channel is made. This channel is used for conservation of different compounds like drugs and also protection of proteins from deforming (Tomonoet al., 2002; Machida et al., 2000).

The starch that is resistant to digestion have been produced by using of amylo-maltase accompany with a de-branched enzyme like pullulanas. These products are called Promitor RS60 and RS75 and are linearmalto-oligosaccharids with different polymerization degrees which are made in crystal form. Pancreatic and saliva α-amylases has no effect on these crystals so usage of these compounds comparing with normal starch increases blood insulin and glycemic index less (Van der Maarel and Leemhuis, 2013).

2.4.1.3. Q- Enzymes (branched enzymes)

This group of enzymes is completely different as they break α-1.4 bonds and link produced α-glucan to 6-hydroxile groupin a linear glucan chain which has α-1.4 bonds itself. Therefore, one branch is made (van der Maarel and Leemhuis, 2013; Whitehurst and van Oort, 2010). These enzymes are in GH 13 and GH 57 families. GH 13 family affects on amylopectin and amylose whereas the members of GH 57 family affect on just amylose (Palomo*et al*., 2009; Palomo*et al*., 2011).

2.4.1.3.1. Application of Q- Enzymes (Branched enzymes)

Innature these enzymes are involved in constructingof side branches in amylopectin and glycogen (Zeeman et al., 2010; Murakami et al., 2006). Glycogen branched enzymes differs from starch branched ones in number of synthesized α-1.6bonds so that all types of starch branched enzymes make about 3.5 to 5% of α-1.6 bonds whereas the construction of these bonds in glycogen is more than 10 % (Whitehurst and van Oort, 2010).

The ability of these enzymes in breaking α-1.4 bonds and creating α-1.6 instead is lead to products which lack long α-1.4 chains. Therefore, they produce dextrins contain short side chains and as a result do not retrograde and have low viscosity (Spendler and Jorgensen, 1997). Novozym company has extracted a glycogen branched enzyme from *Rhodothermus obamensi s*thermophile bacteria that produces branched dextrin. Since this enzyme is stable up to 80°C, it seemed to be suitable for starch process (Shinohara et al., 2001). So far no branched dextrin has been produced as anti-stalling factor because of non-commercial production of enzymes (Van der Maarel and Leemhuis, 2013). The constant viscosity derivatives of starch are used for paper and texture industry (Bisgaard-Frantzen et al., 1999). Kim et al. in 2008 extracted a

glycogen branched enzyme from *Streptococcus mutans* that delayed retrogradation by changing the starch to more branched structure.

Q- Enzymes arealso used for producing branched dextrins which are digested slowly. The higherthe amount of branch in starch, the harder pancreatic amylose can degrade starch, therefore the level of blood glucose remains low (Whitehurst and van Oort, 2010). *Deinococcus radiodorans* is able to produce branched enzyme which changes amylopectin to a branched maltodextrin. This enzyme accompany with pancreatic amylase releases glucose slowly in vitro condition (Palomo et al., 2009). Roquete company has also produced a starch with low digestibility by a branched enzyme accompany with β-amylase (EC 3.2.1.2) (Dermaux et al., 2007). Using maltogenic amylase instead of β-amylose showed similar effect (Le et al., 2009).

Other products yielded by branched enzymes are cluster cyclodextrins (CCD). These compounds are used in sport drinks. This substance increases the time of being empty in stomach and can affect positively on body perseverance. Ezaki Glico Company extracted a branched enzyme from *Aquifexaeolicus* thermophile bacteria and applied it for converting corn starch to a cluster cyclodextrins. At present, this enzyme is produced by Nagase Company (Whitehurst and van Oort, 2010).

3. Conclusions

In this research the starch processing enzymes that are used for modification and conversion of starch and production of various compounds were studied. Some of these enzymes as alpha amylase and glocoamylase are used in large scale but the others are applied in smaller scale. Producing ofsweeteners is the most common application of these enzymes. Some other produced compounds by these enzymes have functional properties and their usage will have high potential influence on users' health. Despite performance ofvide researches and production ofvarious compounds, all starch processing enzymes have not been known thoroughly. Therefore many studying in the field of recognition and application of these enzymes for new products is being continued so that production of new refined and practical starch will be seen in future.

4. References

Alting, A.C., van de Velde, F., Kanning, M.W., Burgering, M., Mulleners, L., Sein, A. (2009). Improved creaminess of low-fat yoghurt: The impact of amylomaltase-treated starch domains. *Food Hydrocolloids*, 23, 980–987.

Astray, G., Gonzalez-Barreiro, C., Mejuto, J.C., Rial-Otero, R., Simal-Gandara, J. (2009). A review on the use of cyclodextrins in foods. *Food Hydrocolloids*, 23, 1631–1640.

Ben Messaoud, E., Ben Ammar, Y., Mellouli, L., Bejar, S. (2002). Thermostable pullulanase type I from new isolated *Bacillus Thermoleovorans* US105: cloning, sequencing and expression of the gene in *E. coli*. *Enzyme and Microbial Technology*, 31, 827–832.

Bertoldo, C., Antranikian, G. (2002). Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. *Current Opinion in Chemical Biology*, 6, 151–160.

Bisgaard-Frantzen, H., Svendsen, A., Norman, B., Pedersen, S., Kjaerulff, S., Outtrup, H., Borchert, T.V. (1999). Development of Industrially Important α-Amylases. *Journal of Applied Glycoscience*, 46, 199-206.

Biwer, A., Antranikian, G., Heinzle, E. (2002). Enzymatic production of cyclodextrins. *Applied Microbiology and Biotechnology*, 59, 609–617.

Cardosoa, M.B., Putaux, J.L., Samios, D., Silveira, N.P. (2007). Influence of alkali concentration on the deproteinization and/or gelatinization of rice starch. *Carbohydrate Polymers*, 70, 160–165.

Crabb, W. D., Shetty, J.K. (1999). Commodity scale production of sugars from starches. *Current Opinion in Microbiology*, 2, 252-256.

Daiuto, E., Ceredaa, M., Sarmento, S., Vilpoux, O. (2005). Effects of Extraction Methods on Yam (*Dioscoreaalata*) Starch Characteristics. *Starch*, 57, 153-160.

Dermaux, L., Peptitjean, C., Wills, D. (2007). Soluble, highly branched glucose polymers for enteral and parenteral nutrition and for peritoneal dialysis. World Intellectual Property Office (WIPO) patent, WO/2007/099212**.**

Dokic, L., Dapcevic, T., Krstonosic, V., Dokic, P., HadnaCev, M. (2010). Rheological characterization of corn starch isolated by alkali method. *Food Hydrocolloids*, 24, 172–177.

Duffner, F., Bertoldo, C., Andersen, J. T., Wagner, K., Antranikian, G. (2000). A new thermoactivepullulanase from *Desulfurococcus mucosus*: cloning, sequencing, purification, and characterization of the recombinant enzyme after expression in *Bacillus subtilis*. *Journal of Bacteriology*, 182, 6331–6338.

Euverink, G.J.W., Binnema, D.J. (1998). Use of modified starch as an agent for forming a thermoreversible gel. U.S. Patent Application

Fogarty, W.M., Kelly, C.T. (1990). Recent advances in microbial amylases. Microbial Enzymes and Biotechnology, pp. 71–132, Elsevier Applied Science Publishers, London, UK, 1990.

Guzman-Maldonado, H., Paredes-Lopez, O. (1995). Amylolytic enzymes and products derived from starch: a review. *Critical Reviews in Food Science and Nutrition*, 35(5), 373–403.

Han, X.Z., Hamaker, B.R. (2002). Partial leaching of granules associated proteins from rice starch during alkaline extraction and subsequent gelatinization. Starch-Starke, 54, 454–460.

Hansen, M.R., Blennow, A., Pedersen, S., Nørgaard, L., Engelsen, S.B. (2008). Gel texture and chain structure of amylomaltase-modified starches compared to gelatin. *Food Hydrocolloids*, 22, 1551–1566.

Hansen, M.R., Blennow, A., Pedersen, S., Engelsen, S.B. (2009). Enzyme modification of starch with amylomaltase result in increasing gel melting point. *Carbohydrate Polymers*, 78, 72-79.

Haki, G.D., Rakshit, S.K. (2003). Developments in industrially important thermostable enzymes: a review. *Bioresource Technology*, 89, 17–34.

Hii, S.L., Tan, J.S., Ling, T.C., Ariff, A.B. (2012). Pullulanase: Role in Starch Hydrolysis and Potential Industrial Applications. *Enzyme Research*, 2012, 921362.

Kahar, U.M., Chan, K. G., Salleh, M.M., Hii,S.M., Goh, K.M. (2013). A high molecular-mass anoxybacillus sp. SK3-4 Amylopullulanase: characterization and its relationship in carbohydrate utilization. *International Journal of Molecular Sciences*, 14, 11302-11318.

Kammoun, R., Naili, B., Bejar, S. (2008). of a statistical design to the optimization of parameters and culture medium for alphaamylase production by *Aspergillus oryzae* CBS 819.72 grown on gruel (wheat grinding by-product). *Bioresourc Technology*, 99, 5602-5609.

Kaper, T., van der Maarel, M. J. E. C., Euverink, G. J., Dijkhuizen, L. (2004). Exploring and exploiting starch-modifying amylomaltases from thermophiles. *Biochemical Society Transactions*, 32, 279–282.

Kelly, R.M., Dijkhuizen, L., Leemhuis, H. (2009). The evolution of cyclodextrin glucanotransferase product specificity. *Applied Microbiology and Biotechnology*, 84, 119–133.

Kim, E.J., Ryu, S.I., Bae, H.A., Huong, N.T., Lee, S.B. (2008). Biochemical

characterization of a glycogen branching enzyme from *Streptococcus mutans*: enzymatic modification of starch. *Food Chemistry*, 110, 979-984.

Kunamneni, A., Singh, S. (2006). Improved high thermal stability of pullulanase from a newly isolated thermophilic *Bacillus* sp. AN-7. *Enzyme and Microbial Technology*, 39, 1399–1404.

Kuroiwa, T., Shoda, H., Ichikawa, S., Sato, S., Mukataka, S. (2005). Immobilization and stabilization of pullulanase from *Klebsiella pneumonia* by a multipoint attachment method using activated agar gel supports. *Process Biochemistry*, 40, 2637–2642.

Lai, L.N., Karim, A.A., Norziah, M.H., Seow, C.C. (2004). Effects of Na_2CO_3 and NaOH on pasting properties of selected native cereal starches. *Food Chemistry and Toxicology*, 69, 249-256.

Latorre-Garcia, L., Adam, A.C., Manzanares, P., Polaina, J. (2005). Improving the amylolytic activity of *Saccharomyces cerevisiae* glucoamylase by the addition of a starch binding domain. *Journal of Biotechnology*, 118, 167–176.

Le, Q.T., Lee, C.K., Kim, Y.W., Lee, S.J., Zhang, R., Withers, S.G., Kim, Y.R., Auh, J.H., Park, K.H. (2009). Amylolytically-resistant tapioca starch modified by combined treatment of branching enzyme and maltogenic amylase. *Carbohydrate Polymers*, 75, 9–14.

Lee, H.C., Htoon, A.K., Paterson, J.L., (2007). Alkaline extraction of starch from Australian lentil cultivars Matilda and Digger optimized for starch yield and starch and protein quality. *Food Chemistry*, 102, 551-559.

Leemhuis, H., Kelly, R.M., Dijkhuizen, L. (2010). Engineering of cyclodextrin glucanotransferases and the impact for biotechnological applications. *Applied Microbiology and Biotechnology*, 85, 823–835.

Li, X and Yu. H. Y. (2011). Extracellular production of beta-amylase by a halophilic isolate, *Halobacillus* sp. LY9. *Journal of Industrial Microbiology and Biotechnology*, 38, 1837–1843.

Machida, S., Ogawa, S., Xiaohua, S., Takaha, T., Fujii, K., Hayashi, K. (2000). Cycloamylose as an efficient artificial chaperone for protein refolding. FEBS Letters, 486, 131–135.

Mikami, B., Iwamoto, H., Malle, D., Yoon, H. J., Demirkan-Sarikaya, E., Mezaki, Y., Katsuya, Y. (2006). Crystal structure of pullulanase evidence for parallel binding of oligosaccharides in the active Site. *Journal of Molecular Biology*, 359, 690–707.

Mun, S., Kim, Y.L., Kang, C.G., Park, K.H., Shim, J.Y., Kim, Y.R. (2009). Development of reduced-fat mayonnaise using 4GTase-modified rice starch and xanthan gum. *International Journal of Biological Macromolecules*, 44, 400–407.

Murakami, T., Kanai, T., Takata, H., Kuriki, T., Imanaka, T. (2006). A novel branching enzyme of the GH-57 family in the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. *Journal of Bacteriology*, 188, 5915–5924.

Nakamura, Y. (1996). Some properties of starch debranching enzymes and their possible role in amylopectin biosynthesis. *Plant Science*, 121, 1–18.

Niehaus, F., Bertoldo, C., Kahler, M., Antranikian, G. (1999). Extremophiles as a source of novel enzymes for industrial application. *Applied Microbiology and Biotechnology*, 51, 711–729.

Oh, E. J., Choi, S.J., Lee, S.J., Kim, C.H., Moon, T.W. (2008). Modification of granular corn starch with 4-α-glucanotransferase from *Thermotogamaritima*: effects on structural and physical properties. *Journal of Food Science*, 73, 158-166.

Palomo, M., Kralj, S., van der Maarel, M.J.E.C., Dijkhuizen, L. (2009). The unique branching patterns of Deinococcus glycogen branching enzymes are determined by their N-terminal domains.

Applied and Environment Microbiology, 75, 1355–1362.

Palomo, M., Pijning, T., Booiman, T., Dobruchowska, J.M., van der Vlist, J., Kralj, S., Planas, A., Loos, K., Kamerling, J.P., Dijkstra, B.W., van der Maarel, M.J.E.C., Dijkhuizen, L., Hans Leemhuis. (2011). *Thermus thermophilus* glycoside hydrolase family 57 branching enzyme: Crystal structure, mechanism of action, and products formed. *Journal of Biological Chemistry*, 286, 3520-3530.

Poliakoff, M., Licence, P. (2007). Sustainable technology: green Chemistry. Nature, 450, 810–812.

Puchongkavarin, H., Varavinit, S., Bergthaller, W. (2005). Comparative study of pilot scale rice starch production by an alkaline and an enzymatic process. *Starch*,57, 134-144.

Qi, Q., Zimmerman, W. (2005). Cyclodextrin glucanotransferase: from gene to applications. *Application of Microbiology and Biotechnology*, 66, 475-485.

Reineccius, T.A., Reineccius, G.A., Peppard, T.L. 2003). Flavor release from cyclodextrin complexes: comparison of alpha, beta, and gamma types. *Food Chemistry and Toxicology*, 68, 1234-1239.

Roy, A., Messaoud, E. B., Bejar, S. (2003). Isolation and purification of an acidic pullulanase type II from newly isolated *Bacillus* sp.US149. *Enzyme and Microbial Technology*, 33, 720–724.

Roy, I., Gupta, M. N. (2004). Hydrolysis of starch by a mixture of glucoamylase and pullulanase entrapped individually in calcium alginate beads. *Enzyme and Microbial Technology*, 34, 26–32.

Roy, G.S., Dumont, M. J., Raghavan, V. (2015). Starch to value added biochemicals. Starch-Stärke, 67, 1-2, 14-29.

Shim, J.H., Seo, N.S., Roh, S.A., Kim, J.W., Cha, H., Park, K.H. (2007). Improved bread-baking process using *Saccharomyces cerevisiae* displayed with engineered cyclodextrin glucanotransferase. *Journal of Agricultural and Food Chemistry*, 55, 4735–4740.

Shinohara, M.L., Ihara, M., Abo, M., Hashida, M., Takagi, S., Beck, T. C. (2001). A novel thermostable branching enzyme from an extremely thermophilic bacterial species, *Rhodothermuso bamensis*. *Applied Microbiology and Biotechnology*, 57, 653–659.

Souza, P.M., Magalhaes, P.O. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology*, 41, 850-861.

Spendler, T., Jorgensen, O. (1997). Use of a branching enzyme in baking. WO97/41736.

Srichuwong, S., Sunarti, T.C., Mishima, T., Isono, N., Hisamatsu, M. (2005). Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydrate Polymers*, 60, 529–538.

Srisimarat, W., Powviriyakul, A., Kaulpiboon, J., Krusong, K., Zimmermann, W., Pongsawasdi, P. (2011). A novel amylomaltase from *Corynebacterium glutamicum*and analysis of the large-ring cyclodextrin products. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 70, 369–375.

Stam, M.R., Danchin, E.G.J., Rancurel, C., Coutinho, P. M., Henrissat, B. (2006). Dividing the large glycoside hydrolase family 13 into subfamilies: Towards improved functional annotations of α-amylase-related proteins. Protein Engineering, Design and Selection: PEDS. 19, 12, 555–562.

Syahariza, Z.A., Li, E., Hasjim, J. (2010). Extraction and dissolution of starch from rice and sorghum grains for accurate structural analysis. *Carbohydrate Polymers*, 82, 14-20.

Szente, L., Szejtli, J. (2004). Cyclodextrins as food ingredients. *Journal of Food Science and Technology*, 15, 137–142.

Tatsumi, H., Katano, H. (2005). Kinetics of the surface hydrolysis of raw starch by glucoamylase. *Journal of Agricultural and Food Chemistry*, 53, 8123-8127.

Terada, Y., Fujii, K., Takaha, T., Okada, S. (1999). *Thermusaquaticus* ATCC 33923 amylomaltase gene cloning and expression and enzyme characterization: Production of cycloamylose. *Applied and Environment Microbiology*, 65, 910–915.

Tian, S., Kyle, W. S. A., Small, D. M. (1999). Pilot scale isolation of proteins from field peas (*Pisumsativum* L.) for use as food ingredients. *International Journal of Food Science and Technology*, 34(1), 33–39.

Tomono, K., Mugishima, A., Suzuki, T., Goto, H., Ueda, H., Nagai, T. (2002). Interaction between cycloamylose and various drugs. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 44, 267–270.

Tucker, G. A., Woods, L.F.J. Enzymes in Food Processing. 2nd edition. UK. Springer Science. 1995. P 328.

Uhlig, H. Industrial Enzymes and Their Applications, WileyInterscience, New York, NY, USA, 1998.

van der Maarel, M. J. E. C., Van der Veen, B., Uitdehaag, J. C. M., Leemhuis, H., Dijkhuizen, L. (2002). Properties and applications of starch-converting enzymes of the α-amylase family. *Journal of Biotechnology*, 94, 137–155.

van der Maarel, M. J. E. C., Capron, I., Euverink, G. J. W., Bos, H. Th., Kaper, T., Binnema, D. J. (2005). A novel thermoreversible gelling product made by enzymatic modification of starch. *Starch*, 57, 465-472.

van der Maarel, M. J. E. C., Leemhuis, H. (2013). Starch modification with microbial alpha-glucanotransferase enzymes. *Carbohydrate Polymers*, 93, 116-121.

Vocadlo, D. J., Davies, G. J. (2008). Mechanistic insights into glycosidase chemistry. *Current Opinion in Chemical Biology*, 12, 539–555.

Vollu, R. E., da Mota, F. F., Gomes, E. A., Seldin, L. (2008). Cyclodextrin production and genetic characterization of cyclodextrin glucanotranferase of *Paenibacillusgraminis.Biotechnology* Letters, 30, 929–935.

Yamada, R., Bito, Y., Adachi, T., Tanaka, T., Ogino, C., Fukudab, H., Kondo, T. (2009). Efficient production of ethanol from raw starch by a mated diploid *Saccharomyces cerevisiae* with integrated α-amylase and glucoamylase genes. *Enzyme and Microbial Technology*, 44, 344-349.

Yoo, S.H., Jane, J. (2002). Structural and physical characteristics of waxy and other wheat starches. *Carbohydrate Polymers*, 49, 297-305.

Zareian, S., Khajeh, K., Ranjbar, B., Dabirmanesh, B., Ghollasi, M., Mollania, N. (2010). Purification and characterization of a novel amylopullulanase that converts pullulan to glucose, maltose and maltotriose and starch to glucose and maltose. *Enzyme and Microbial Technology*, 46, 57–63.

Zeeman, S. C., Kossmann, J., Smith, A. M. (2010). Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, 61, 209-234.

Zobel, H. F. (1992). Starch: Sources, Production and Properties.*Starch Hydrolysis Products*, 23-44.

Zona, R., Chang-Pi-Hin, F., O'Donohue, M. J., Janecek, S. (2004). Bioinformatics of the glycoside hydrolase family 57 and identification of catalytic residues in amylopullulanase from *Thermococcus hydrothermalis*. *European Journal of Biochemistry*, 271, 2863–2872.

Whitehurst, R.J., van Oort, M. Enzymes in Food Technology. Second edition. USA. Wiley-Blackweel. 2010. P, 388.