

The Use of Transglutaminase Enzymes in Food: Is there Any Issue of Lawful and Unlawful in Islam?

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Abstract: In many years ago, consumers in Malaysia do not recognize the term of enzyme. However, the fact that today most consumers have known the term even though in general. Indirectly, it shows the spread out of the use of enzymes in food production in Malaysia. Enzyme is a kind of material which is consisting of protein and it is available in all living systems. Even living systems are in need of the enzyme function. It serves as a catalyst for reactions that occur in living systems. Catalyzing a reaction means the reaction can not occur or occurs at a slower rate in the absence of the enzyme. With the enzyme, the reaction can occur at a rate as required and finally produce a product that may be useful for the Muslim community. The finding of this study shows the importance of enzymes in the production of food products, medicine and so on. But however, the Muslim scholars decided that the enzyme must be derived from lawful sources if not any product containing it would be unlawful to be consumed. The importance of this study is very relevant to Muslim community because it will help to solve the problem of food products, medicine and the like in relating to legal decision.

Key words: Enzyme, transglutaminase, food processing, biotechnology, microorganism, genetic

INTRODUCTION

Enzyme: The term of enzyme becomes most popular word today. A decade ago, consumers especially in Malaysia have mostly never heard or known this term. But now when we ask them about it, mostly they will be able to explain even in a different rate of accuracy. This indirectly indicates the spread out of increasingly use of enzymes in the manufacturing industry in Malaysia. Enzymes are made up of protein compounds. Enzyme presents in all living systems. Even living systems are in need of the function of enzyme. This is because the function of enzyme is to carries out a duty as a catalyst against reactions that occur in living systems. A duty as a catalyst against a reaction means the reaction can not occur or it occurs at a slower rate in the absence of the enzyme. With the existing of enzyme, the reaction can occur at a normal rate as required.

The reaction can be stimulated not only by enzymes but also by chemicals. As an example of reaction which is catalyzed and stimulated by chemical is oxidation of the askorbic acid by copper. Enzymes are natural biological catalysts. Example of enzyme that catalyzes reactions in

the human body is glucose-6-phosphate dehydrogenase which catalyzes road base of phosphate pentosa. This enzyme catalyzes the exchange of reaction of glucose-6-phosphate to 6-phosfoglucono lacton. Individuals who are born without this enzyme are normally at risk for getting a hemolytic anemia that is the red blood cells rupture when taking medications or certain chemicals. Enzymes can be classified into six categories (Olsen, 2000) namely:

- Oksidoreduktase catalysing the oxidation reaction
- Transferase catalyzing the transfer of atoms from one molecule to another molecule
- Hidrolase catalyzing a chemical reaction with water. Among the most common reaction is a breakdown of large molecules into small such as hydrolysis of proteins
- Liase catalyzing reactions that produce double bonds
- Isomerase catalyzing the transfer of the group in the same molecule and producing a new structure for the molecule
- Ligase catalyzing the merger of molecules to form larger molecules

THE USE OF ENZYMES IN FOOD PROCESSING

Food industry in Malaysia has grown rapidly since the 1980's until now. The development of the food industry has been driven by Malaysia's economic development which has resulted in a higher purchasing power. The development of food industry is also contributed by the increase in demand for processed food either for the local market or international market. Among the factors that play a role in the increasing demand in local market is the increase of women involved in jobs. With increasing demand for processed food at the same time, there is a rising demand and expectation of consumers to quality food. Thus, the food industry has been driven to take steps to improve food quality. Among the measures adopted to improve the quality of food is the use of enzymes.

In fact, the use of enzymes in the processing or preparation of food in Malaysia has long been practiced by the community. However, this practice is quite unconsciously and is not associated with specific enzymes.

For example to soften the beef or chicken, customary practices in Malay society is that boiled and mixed with new leafages of papaya. Scientifically, shoots of papaya contains an enzyme called papain. Papain is a protease that reacts to breakdown proteins. The action of decomposition of proteins by papain is actually to cause a softening effect to beef or chicken (Nissen, 1993). At this point, there are various types of enzymes used in food processing (Table 1).

As mentioned, most of the reaction other than stimulated by the enzyme can also be stimulated by chemicals. Hence, a question arises of why to use the enzyme while in the aspect of cost, chemical substance is much less than the enzyme.

Using enzymes as bio-catalysts even at a higher cost has its own advantages. Firstly, the reaction which is stimulated by the enzyme is a very specific compared to chemical catalysis. Enzyme will only catalyzes specific reactions. Thus, the scope of changing which is more specific can be obtained and enabling the only desired changes have occurred. This can reduce the effects or side reactions on the other components in food systems.

Table 1: Some examples of enzymes used in food processing (Riaz and Chaudry, 2004)

Sources	Sort	Usage
Bacterium (Bacillus)	Protease	Soften the meat
Fungus (Aspergillus)	Protease	Production of cheese
Yeast (Kluyveromyces)	Rennin	Production of cheese
Plant (Papaya)	Protease	Soften the meat
Animals (Ruminants)	Rennin	Production of cheese

Using a chemical catalyst will produce a less specific reaction. Thus, catalysis by using enzymes is better selected so long as it is economic. Other than that using enzyme as a catalyst is also more friendly to environment. The reaction by using chemical catalysts often produce toxic byproducts and also not friendly to environment. Using enzymes in the processing reaction is one approach to green technology which should be encouraged to food industries. It is also giving another advantage where it does not involve the addition of chemicals into the food system as it does when using chemical catalysts.

WHAT IS TRANSGLUTAMINASE?

Transglutaminase is an enzyme that is used to form cross-links or bonds in the protein food for the increase in texture. Transglutaminase is an enzyme called protein-glutamine glut amyl transferase with code of EC2.3.2.1.3. This enzyme catalyzes transfer reaction of acyl group in which the δ -carboksemida on glutaminil residues which bound in the peptides is the acyl contributor (Renzetti *et al.*, 2008). Transglutaminase has been used to catalyze a formation of cross binding for several types of protein. For example, the formation of cross binding between the protein of wei, soy, gluten, miosin and aktomiosin. Modifications through the formation of cross bonds between proteins by transglutaminase can produce a product with better texture features which protect the amino acid that is lysine, encapsulation of fat and fat-soluble materials, form a heat susceptible film, increase flexibility and holding water (Matheis and Whitaker, 1987; Kitabake and Doi, 1993; Motoki and Seguro, 1998). Transglutaminase has been used for the formation of cross bonds between protein isolate which is mixed into bread dough which using rice flour (Renzetti *et al.*, 2008; Marco and Rosell, 2008).

Apart from the formation of cross bonds, transglutaminase can also modify the protein in the food system through the merger of nitrogeneous groups (amines) or remove the nitrogeneous (Motoki and Seguro, 1998). Transglutaminase can be found in most animal tissues and is involved in several biological phenomena such as blood clotting, wound healing, quarantining epidermis and hardening of the outer layer of red blood cells.

THE USE OF TRANSGLUTAMINASE IN THE FOOD SYSTEM

In line with the functions of transglutaminase to form cross-bonds, it can be used in food processing to improve

or achieve particular physical characteristics. Studies have shown the potential use of transglutaminase in the food system. Ikura *et al.* (1992) reported to have been success in modifying the feature of casein milk that is a kind of protein in milk and globular protein in soybeans using transglutaminase. Studies on wei protein and aktomiosin from cattle, pigs, poultry or fish also showed the ability of transglutaminase to form cross-bonds. Protein in the emulsion oil-in-water has also been acted on by transglutaminase to form a gel. This shows that transglutaminase is able to form cross-bonds in proteins from different sources (Yokoyama *et al.*, 2004). Based on this reactions, transglutaminase has proven to be a potential enzyme to be used as a modification of products that contain protein.

In fact, the use of transglutaminase in the food system has traditionally been practiced by Japanese society in making suwari that is fish meat which is mixed with salt. In the manufacturing process of suwari, the transglutaminase used is the intrinsic transglutaminase which is found in the fish. In this suwari production, minced fish meat which has been mixed with salt being mingling or associating after letting for some time as a result of the formation of cross bonds from the reaction of transglutaminase (Motoki and Seguro, 1998).

Transglutaminase can also be used in meat or beef products in which transglutaminase may cause binding of meat pieces to form a solid product. Binding ability of meat pieces for a new product is very suitable for use in the production of reconstituted product from meat such as burgers, sausages, chicken nuggets and so on. The formation of cross bonds allows products reconstructed from mince meats are able to heat resistant and also pressure during the preparation and cooking. Although, chemicals such as sodium chloride and phosphate can be used to produce this reconstituted material, the use of transglutaminase is seen as a new approach that can produce food products that less salt and healthier (Yokoyama *et al.*, 2004).

Apart from products of meats or beefs, transglutaminase can also be used in the processing of fish products. As for meat products, transglutaminase can be used in the production of fish flesh which is reconstituted. Examples of the reconstituted products of fish flesh are as surimi, fish balls and fish cakes. Imitation foods can also be produced by using transglutaminase. For example, transglutaminase has been used in producing of imitation shark fin (Tani *et al.*, 1990). The use of transglutaminase is able to improve texture characteristics in the aspect of shape and elasticity (Zhu *et al.*, 1995). Transglutaminase can also be used in some dairy products. Casein, a milk protein will form a

heat-resistant gel when mixed with transglutaminase but it will not be it without transglutaminase (Yokoyama *et al.*, 2004). Yogurt that is very popular food at this time can be improved its quality by the use of transglutaminase.

Yogurt is a milk gel resulting from the increase of the content of lactic acid during fermentation by using lactic acid bacteria. But the milk gel or yogurt is easy to become unstable then to release of liquid and produces two separate layers consisting of liquid and gel milk. This change occurs when there are temperature changes or impacts on the yogurt system. Addition of transglutaminase to the yogurt will produce a more stable milk gel formation as a result of cross ties. A more stable gel will keep the structure that holds water and prevents the secretion of fluids (Yokoyama *et al.*, 2004).

The use of transglutaminase in noodles and pasta products have been reported. Adding the transglutaminase in processing has resulted in noodles with a better and more resistant to the effects of cooking, even though the noodles are made from a lower grade of flour. The use of transglutaminase in making of bread has been conducted. Cross bonding between the flour protein in bread has enabled to produce a greater volume of bread (Sakamoto *et al.*, 1992). Kato *et al.* (1991) has been using transglutaminase during tofu processing. The resulting tofu was reported as stable and could be retorted involving heating at very high temperatures.

Takagaki *et al.* (1991) also reported as to the use of transglutaminase as preservative. They have coated vegetables and fruits with transglutaminase and protein. Results showed that fresh vegetables and fruits are able to be sustained.

TRANGLUTAMINASE RESOURCES

Transglutaminase can be obtained from most of the living system. This is not surprising in view of transglutaminase plays an important role in living systems such as blood clotting and wound healing. Thus, the transglutaminase may be produced by extraction from various sources. However whether a source is suitable for extracting, it depends on *inter alia*, the available quantity of transglutaminase. Among the studies that were conducted to extract transglutaminase are shown in Table 2. In general, there are three approaches to produce transglutaminase.

The first approach is through the extraction and purification of transglutaminase directly from tissue or body fluids of animals such as cows, pigs and fish. In Europe, transglutaminase has commercially been extracted from the blood of cattle and pigs which is processed at the meat processing factory (Yokoyama *et al.*, 2004).

Table 2: The studies of extraction of transglutaminase that have been performed

Sources	Species name	Researchers
Fish	Tilapia fish (<i>Oreochromis niloticus</i>)	Worratao and Yongsawatdigul (2005)
Fish	Pollock fish (<i>Theragra chalcogramma</i>)	Seki <i>et al.</i> (1990)
Fish	Red sea bream (<i>Pagrus major</i>)	Yasueda <i>et al.</i> (1995)
Bacteria	Streptovorticillium S-8112	Ando <i>et al.</i> (1989)
Bacteria	<i>S. griseocarneum</i>	Moteki <i>et al.</i> (1989)
	<i>S. cinnamomeum</i>	
	<i>S. mobaraense</i>	
Animals	Mencit	Brookhart <i>et al.</i> (1983)
Plants	<i>Helianthus tuberosus</i>	Falcone <i>et al.</i> (1993)

Other than blood, transglutaminase is also commercially extracted from mouse liver (guinea pig) (Zhu *et al.*, 1995). Extraction process to produce the transglutaminase is a complicated process. There are also reports on the production of transglutaminase from tilapia (*Oreochromis niloticus*) (Worratao and Yongsawatdigul, 2005) as shown in Table 2. But these studies are still in the laboratory.

The second approach is to extract the transglutaminase from natural microorganism which containing transglutaminase. To use this approach, the first step is to conduct reviews to determine the potential microorganism which contains high levels of transglutaminase. For example, Ando *et al.* (1989) have carried out the reviews on the 5000 microorganisms from the land taken from various places before found Streptovorticillium S-8112.

After identifying a specific microorganism, the next step is to carry out the breeding of such a microorganism on a large scale. The action of breeding may use of fermentation technology that already developed. This approach is a direct approach that does not involve a very sophisticated technology and it is already available in laboratory. At the same time, the risk of failure is not too high. However, the filtering process to select the appropriate microorganism in producing the transglutaminase will take a quite longer and a significant cost. In addition, the microorganism should not in kind of pathogens. Microorganism pathogens are microorganisms that can cause harm to consumers. Examples of pathogenic microorganisms are *Escherichia coli*, *Staphylococcus aureus*, *Clostridium botulinum* and many more. Not all microorganisms will cause harm to consumers. There are several microorganisms to be good and useful to the human beings like *Rhizopus oligosporus* which is used in tempeh manufacturing and lactic acid bacteria which is used to process the yogurt. Thus, any non-pathogenic microorganism which can produce the transglutaminase may be considered for large scale fermentation for the commercial production.

The effort of fermentation of microorganism in order to produce transglutaminase is basically similar to the fermentation of the other microorganisms. The sources of carbon which can be used are glucose, sucrose, starch,

glycerin and dextrin. While the source of nitrogen, it could be organic or inorganic. Example of nitrogen sources are NH_4NO_3 , NaNO_3 , soybeans, rice, maize, flour and so on. In addition should also be supplied with mineral salts such as phosphate, magnesium, potassium, iron, copper and vitamins. Temperature for growing that can be used is between 25-35°C for 2-4 days. Transglutaminase will be secreted by the microorganism and it should be refined. Purification using solvents such as ethanol, acetone, alcoholic isopropil and other solvents. The resulting enzyme should be stabilized by using stabilizers such as protein, fats and surfactants (Sakamoto *et al.*, 1992). The third approach is the more recent approach that is by using genetic transformation. The process begins by determining the microorganism that can produce a satisfactory rate of transglutaminase. This step is followed by cloning gene from the microorganism to another microorganism that can secrete enzymes at a higher rate or the non-pathogenic. Other than the microorganism, genetic resources that may express transglutaminase enzymes can also be used as shown in Table 2. Several studies have succeeded in producing transglutaminase by using cloning approach.

Washizu *et al.* (1994) have cloned a gene that produces transglutaminase from Streptovorticillium and carried out the transformation into *Streptomyces lividans*. Takehana *et al.* (1994) has been transferring genes for the production of transglutaminase from Streptovorticillium into *Escherichia coli*. The cloning approach also enables the production of transglutaminase from pathogenic microorganism genetic resources that have been transformed into a non-pathogenic microorganism. However, the approach based on genetic manipulation and cloning resources may be difficult to be accepted by consumers in view of the resistance by the publics to accept Genetically Modified Organisms (GMOs) as food or ingredient of food.

ISSUE OF HALAL IN USING OF TRANSGLUTAMINASE IN FOOD

The use of transglutaminase in food processing has successfully proven to enhance the important features of a product. The increase of these important features are

then able to produce higher quality products. Thus, it is not surprising if the use of transglutaminase would be wider in product processing. At this point, it is difficult to determine the extent to which degree the use of transglutaminase in the processing of food products in the Malaysian market.

In general, the halal issue concerning the use of transglutaminase in food processing can be divided into two. Firstly, in terms of resources of extracted transglutaminase. For the transglutaminase which is extracted from animal tissue, the next issue that arises is whether this animal comes from lawful group of animals or not. As aforementioned, some transglutaminases are extracted from swine tissues and they are certainly not halal for consumption by Muslims.

But however if the animals are from halal group for consumption by Muslims, the relevant question in this case is whether the animal has been slaughtered according to Islamic law. If the animal was slaughtered as required by the Islamic rule of law so, there is no further question as to the issue of halal for transglutaminase. Another scenario is a transglutaminase which was produced from components that are not halal for consuming even though they are from halal animals. As discussed before, transglutaminase may also be produced from cow's blood. Although, cattles are halal animals if they have been slaughtered according to Islamic law but blood is a forbidden component for consumption. Even the blood is considered as unclean and impure by the Islamic law. Thus, transglutaminase which is extracted from the blood in which transglutaminase is one of the components in the blood also should be non-halal for Muslims. Thus, to produce a lawful transglutaminase by direct extraction from animal tissues, it is essential to make sure that the animals per se and their components are halal to be consumed.

The second issue of halal is in the case of the transglutaminase derived from the genetic manipulation and cloning. Several aspects should be observed to enable the production of transglutaminase being halal or lawful to be eaten. Firstly, needs to ensure the resources of genetic which would like to be cloned or transformed into a host microorganism. If the genetic resources originate from lawful or legitimate sources such as halal animals, plants, fish, etc., then there is no doubt of halal at this stage in respect of transglutaminase, it is surely halal to be consumed. But if the genetic resources derived from animals that are non-halal to be consumed then the transglutaminase should be avoided to be consumed by Muslims. It is status of halal is very doubtful and can be arguable. After successfully cloned, microorganism which has been modified will be bred and reproduced via the process of fermentation on a large

scale for the production of transglutaminase. This fermentation process causes the growth of the microorganism in the growth medium. This growth medium should also be sure that it is halal. The use of non-halal medium would make the resulting transglutaminase is not halal. This happened in Indonesia in 2001 in which a manufacturer of Monosodium Glutamate (MSG) has sparked anger and enragement of consumers when they used a growth medium that has been produced by using enzymes from pigs (Roderick, 2001). Lastly, all materials used as solvents, preservatives and the like as briefly discussed above must be lawful according to Islamic law. The usage of non-halal ingredients in turn will result in transglutaminase would be non-halal.

CONCLUSION

Transglutaminase is a potential enzyme for improving the quality of protein products such as fish balls, fish cakes, yogurt and so on. Although, the actual quantity of this enzyme in products in Malaysia is unknown, the possibility of using it by food manufacturers can not be denied. It is important therefore for food manufacturers to ensure that the transglutaminase used by them is halal according to Islamic law so that the food products would be halal for Muslims.

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