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Review

Halal status of enzymes used in food industry

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ABSTRACT

Background: Enzymes are extensively and increasingly used in research and food processing as important processing aids/biocatalysts. They can be produced by microorganisms through fermentation and can also be extracted from animal or plant tissues. Halal status of enzymes for industrial application is vague to consumers since they may not be properly traced back to their source of origin or production method.

Scope and approach: This review focuses on Halal related issues of enzymes i.e. used during industrial processing for food, feed, pharmaceutical and other consumer goods, with a particular focus on fermentation processes that might pose risks to Halal assurance.

Key findings and conclusions: The status of enzymes including the raw materials used and the current production methods is needed to facilitate Halal food production and comply with religious demands. Enzymes derived from Haram (not allowed) animals or from raw materials obtained from Haram sources are considered to be Haram. Whereas, enzymes derived from microorganisms during fermentation are considered to be Halal if the raw materials or any other ingredients used in the growth medium and in the final product are not from Haram or doubtful sources. If genetically modified (GM) microorganisms are used for enzyme production, recombinant DNA should not be from Haram or doubtful sources.

1. Introduction

The Quranic term 'Halal' which means lawful or permissible (Bergeaud-Blackler, Fischer, & Lever, 2015; Che Man & Sazili, 2010; Munir & Regenstein, 1994) has been gaining significant attention due to the increasing demand for Halal products (Che Man & Sazili, 2010; Shariff, Akma, & Lah, 2014). Even though some aspects related to Halalness of food enzymes are mentioned in a few publications (Al-Mazeedi, Regenstein, & Riaz, 2013; Alzeer & Abou Hadeed, 2016; Khattak et al., 2011; Nakyinsige, Man, & Sazili, 2012; Riaz & Chaudry, 2004a), the information about the Halal status of enzymes used in food industry is scarce. Halal assurance covers the product's journey from farm to fork and it is crucial to ensure that the critical Halal assurance requirements are met to claim that the final product is Halal (Soon, Chandia, & Regenstein, 2017; Tieman & Che Ghazali, 2013). Food additives, food ingredients and processing aids such as enzymes added to food materials at different process stages might pose risks to Halal assurance (Al-Mazeedi et al., 2013; Khattak et al., 2011; Lubis, Mohd-Naim, Alizul, & Ahmed, 2016; Norizah Mohamad & Backhouse, 2014).

Enzymes as biological catalysts are being used as processing aids in food processing for many purposes such as coagulation, ripening, baking, brewing, cell rupture, hydrolysis and modification of molecular structure (Riaz & Chaudry, 2004a; Simpson, Rui, & Klomklao, 2012; van Oort, 2010). Enzymes help to improve the quality, shelf life, freshness, appearance, functionality, nutritional value, aroma and form desired structure in many different food products (Kirk, Borchert, & Fuglsang, 2002; Simpson et al., 2012). In some cases, enzymes are used to clarify juices or to facilitate breakdown of cell structure of oil crops to improve extraction yield (van Oort, 2010).

Common enzymes used in the food industry are either produced by extraction from plant and animal tissues or through fermentation using a variety of microorganisms (Dodge, 2010; Riaz & Chaudry, 2004a). Fermentation is the most common method used to produce food ingredients from plant sources to substitute ingredients (i.e. enzymes) from animal sources and hence plays an important role in the food industry (Al-Mazeedi et al., 2013; Khattak et al., 2011).

According to a report issued by Thomson Reuters, the global Halal food market size is predicted to be US$1.6tn by 2018, up from

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US$1.1tn in 2013 (Thomson Reuters, 2015). The global industrial enzyme market reached to sales of around US$3bn by 2011 and food and beverage enzymes accounted for around 29% of total sales (Fraatz, Rühl, & Zorn, 2014; Grahame, Bryksa, & Yada, 2015). Halal food market is predicted to contribute up to 17.4% of the world food expenditure by 2018 (Thomson Reuters, 2015). Therefore, enzyme manufacturers can have a great opportunity in profit-making business in the Halal market through producing Halal approved enzymes and finding alternatives to animal derived enzymes suitable for Halal food production (Riaz & Chaudry, 2004a; Yunus, Ariffin, & Rashid, 2014).

Considering the type and source of enzymes found in products are not mentioned on product label, manufacturers and certifying/auditing bodies should make sure that Halal assurance is not compromised (Al-Mazeedi et al., 2013; Riaz & Chaudry, 2004a). Mixing, adulteration, cross-contamination and mislabeling of Halal food products with non-Halal substances such as porcine are big obstacles in Halal food industry (Fadzlillah, Che Man, Jamaludin, Rahman, & Al-Kahtani, 2011; Soon et al., 2017). Enzymes may cause deterioration in Halal status of the product if they are obtained from non-Halal sources or produced using non-Halal or doubtful materials even if a company is a Halal certified producer (Fischer, 2015). Therefore, investigation of the source and the production method of enzymes used in the food industry is crucial to assure Halal status of the final product. There are currently regional and global Halal standards such as Malaysian Halal food standard (MS1500:2009, 2009) which the Halal food producers need to follow (Hayati, Khairul Anuar, & Khairur Rijal, 2008). However those standards do not specifically address the enzymes with regard to Halalness of the product. The official international standards body Standards and Metrology Institute for the Islamic Countries (SMIIC), the affiliated institution to the Organization of Islamic Cooperation (OIC), have released General Guidelines on Halal Food (OIC/SMIIC, 2011) with the reference of Codex Alimentarius Commission's (CAC) *General Guidelines for the use of the term "Halal"* (CAC/GL 24-1997) (CAC, 2016) to establish a strong Halal certification system in the world and to provide the worldwide recognition of accreditation certificates issued on OIC/SMIIC General Guidelines on Halal Food (Dag & ErbasiGonc, 2013). SMIIC's General Guidelines on Halal Food highlights enzymes and their labeling, which indicates a step forward in enzyme regulation in Halal assurance. It states that enzymes used as raw material, as processing aid or used in final product must be derived from Halal sources and must be declared on product label (OIC/SMIIC, 2011).

The aim of this review is to make further understanding of the enzymes used in food industry and their effects on Halal assurance to make sure that the Halal status of final product is not compromised.

2. Enzymes for food processing

The enzymes used in food industry are diverse due to their roles and abilities in the production of food and beverage products (Grahame et al., 2015). Based on the type of reactions they catalyse, enzymes are classified into 6 main categories. These are Oxidoreductases (dehydrogenases, reductases, or oxidases), Transferases, Hydrolases, Lyases, Isomerases (racemases, epimerases, *cis*-transisomerases, isomerases, tautomerases, mutases, cycloisomerases) and Ligases (synthases) (Ako & Nip, 2015; Fernandes, 2010; Fraatz et al., 2014; Grahame et al., 2015; James, Simpson, & Marshall, 1996; Olsen, 2000).

The activity of an enzyme depends on several factors such as temperature, pH, amount of substrate and type of substrate. A mathematical model was created to quantify the enzyme activity by Michaelis and Menten (Grahame et al., 2015). Eq. (1) defines the center of this model.

$$E + S \leftrightarrows ES \leftrightarrows E + P \quad (1)$$

where E is enzyme, S is substrate, ES is enzyme-substrate complex and P is product.

Depending on the processing conditions (i.e pH and temperature), enzymes could have a higher efficiency even at low concentrations and could considerably speed up production processes compared to other catalysts (Fraatz et al., 2014; Simpson et al., 2012; van Oort, 2010). Their efficiency is high even at low concentrations which is around 0.1% or less of the product (Ray, Sunita, & Bera, 2016) under mild conditions such as at low temperatures and could be used several times faster than other catalysts (James et al., 1996). The ability to immobilize them on to stationary support materials and reuse of enzymes can help to reduce processing costs in food processing (James et al., 1996; Simpson et al., 2012; van Oort, 2010). Recent developments in industrial biotechnology such as recombinant DNA entails the introduction of genes into microorganisms from traditional sources that encode them into special vectors to produce enzyme with a higher degree of purity and yields at reduced costs (James et al., 1996). Recent developments in fermentation technologies provide novel tailor-made enzymes to suit specific food processing applications. Therefore enzymes produced using these technologies have superiority to traditionally produced enzymes in functioning under harsh processing conditions such as extreme pH and temperature (James et al., 1996; van Oort, 2010). Because of these and some other advantages, enzymes are used in the food industry for numerous applications including: production of beverages, baking, milling as well as manufacturing of dairy, egg, fish, meat, cereal and confectionery products (Aguilar et al., 2008; Fraatz et al., 2014; Grahame et al., 2015; Simpson et al., 2012).

According to Fraatz et al. (2014), approximately 260 different enzymes are available in the EU with around 91% obtained through fermentation (58% from fungi, 5% from yeasts and 27% from bacteria) and 9% through extraction (3% from plant and 6% from animal) (Fraatz et al., 2014). Ako and Nip (2015) have reported recommended names, systematic names, and enzyme codes (EC) for some common food enzymes. According to EU enzyme database, there are currently around 15 different enzymes and their mixtures extracted from animal sources widely used in the food industry including catalase, thrombin, trypsin, chymotrypsin, elastase, carboxipeptidase, lactoperoxidase, lysozyme, pancreatin, phospholipase, chymosin (rennet), pepsin and triacylglycerol lipase (EC, 2016; Ray et al., 2016; Simpson et al., 2012; van Oort, 2010). Enzymes derived from animal sources like catalase, chymotrypsin, trypsin, peptidase, protease, pepsin, lipases, peroxidase, chymosin could also be alternatively produced through fermentation (EC, 2016). The main process steps of enzyme production by conventional fermentation process are illustrated in Fig. 1.

3. Evaluation of the Halal assurance risks in enzyme production for food processing

Enzymes used in food industry have been traditionally derived from plant, animal, and microbial sources using mainly extraction and fermentation techniques (Fraatz et al., 2014; James et al., 1996; Ray et al., 2016; Søndergaard, Grunert, & Scholderer, 2005). Since some of the enzymes are extracted from various animal sources such as stomachs (Al-Mazeedi et al., 2013; Lubis et al., 2016; Rahman, Khatun, Hasibur Rahman, & Ansary, 2014; Riaz & Chaudry, 2004a), an argument may occur which can potentially lead to major dissatisfaction among Muslims if the source is Haram or doubtful. The Halal and Haram are clear and in between of two of

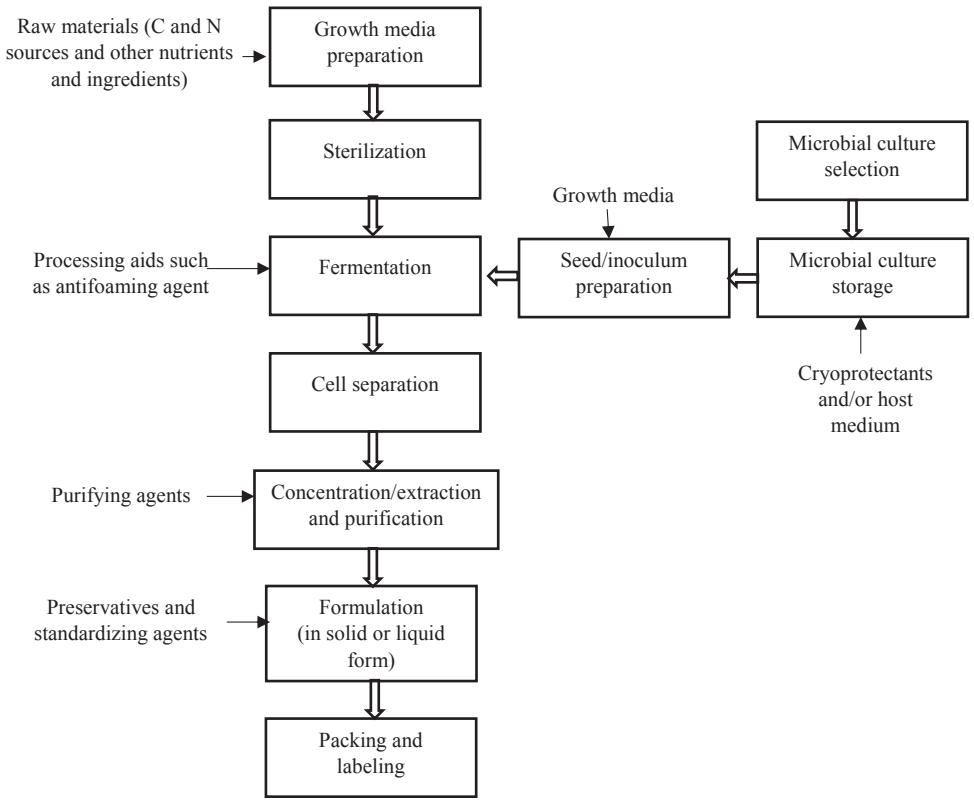

Fig. 1. General overview of production steps of enzyme production by conventional fermentation. C: carbon, N: nitrogen [Adapted from Dodge (2010) and Riaz and Chaudry (2004a)].

them are doubtful (Jahangir et al., 2016). Bearing in mind the gray areas and the complexity in enzyme production, it is important to identify the status of enzymes used in food manufacturing and to better understand their Halal status. If the enzyme to be used in a certain product is Haram or obtained from Haram source, the product should not be certified as Halal by certifying bodies (Khattak, 2007; Riaz & Chaudry, 2004a) and if the enzyme is allowed, it should be mentioned on the product label as stated in OIC/SMIIC's General Guidelines on Halal Food (OIC/SMIIC, 2011).

With the advance in technology, the debate on the Halal status of various ingredients gets increasingly complicated (Satiawihardja, 2012). To reach the right conclusion, it is crucial that every step in enzyme production should be debated by both 'scientists' and 'ulama (the guardians, transmitters and interpreters of religious knowledge, of Islamic doctrine and law (Gilliot et al., 2012))'. In analyzing the Halal status of the enzymes, three main aspects should be taken into consideration. These are: (i) whether the substances used as raw material (and their derivatives) or processing aid in enzyme manufacturing are forbidden or doubtful by Islamic law, (ii) if the enzymes are derived from haram animals and (iii) if the enzymes are derived from animals slaughtered not following the Islamic requirements (Satiawihardja, 2012; Shafii & Wan Siti Khadijah, 2012).

The substances which are stated forbidden in Qur'an and Hadith are (i) carrion (the dead animal by itself, without cause of human effort), (ii) animals which died due to strangulation, or hit, or dropped (iii) animals which are not slaughtered prior to their death under the terms of religion (except fish and grasshopper), (iv) meat from an animal while the animal is still alive, (v) blood, (vi) pig (including all the parts), (vii) disgusting (extremely unpleasant) substances, (viii) fanged beasts, (ix) animals that live on land and in water, (x) hunted animals not complying with Islamic rules, (xi) intoxicants (all kind of alcoholic drinks) and (xii) narcotics (Awan, 1988; Che Man & Sazili, 2010; Munir & Regenstein, 1994; Satiawihardja, 2012; Shafii & Wan Siti Khadijah, 2012). In addition to these, OIC/SMIIC's General Guidelines on Halal Food (OIC/SMIIC, 2011) detailed the Halal and Haram status of different food sources including animals. Due to increasing amount of enzymes produced using microorganisms, special attention should be given to fermentation process in terms of the raw materials and processing aids used to produce enzymes, to make sure that the enzymes meet the Halal requirements. The potential risks to Halal status of enzymes which may occur during fermentation is discussed by Riaz and Chaudry (2004a). These risks are outlined in Table 1.

Before the fermentation process, proper cleansing of equipment is crucial especially if a non-Halal product was produced using the same equipment prior to Halal production. Determination of possible cross-contamination risks with any kind of non-Halal substances during production, proper personnel hygiene, waste and pest management also need to be properly conducted (Al-Mazeedi et al., 2013; Che Man & Sazili, 2010; Riaz & Chaudry, 2004a). In addition, it is crucial that any kind of processing aids and ingredients used during enzyme production would have a confirmed Halal status (Riaz & Chaudry, 2004a; Satiawihardja, 2012).

In producing enzymes through fermentation process, the important factors need to be considered are the growth media used, type and source of microorganism and the substances added to produce enzyme preparations such as diluents, preservatives, and stabilizers (Olempska-Beer, Merker, Ditto, & DiNovi, 2006). If the culture is in lyophilized form, the cryoprotectants (glycerol,

Table 1
Potential Halal assurance risks (HARs) in enzyme production by conventional fermentation process.

	Process stages	Potential risks	Actions to be taken
HAR1	Preparation of growth media	Raw materials from non-Halal sources or using non-Halal enzymes and/or ingredients to prepare raw materials (i.e. enzymatic hydrolysis of meat proteins)	Raw materials need to be Halal approved
HAR2	Selecting microorganisms as culture	Isolation of microorganisms from non-Halal sources, genetic materials from non-Halal sources in case of using GM technology	Microorgansims need to be Halal approved
HAR4	Seed/inoculum preparation	Ingredients of growth media and other substances from non-Halal sources	Ingredients and growth media need to be Halal approved
HAR3	Adding processing aids	Emulsifiers and antifoaming agents from non-Halal sources	Processing aids and ingredients need to be Halal approved
HAR5	Standardization of enzyme produced by microorganisms	Preservatives, emulsifiers, and other standardizing materials from non-Halal sources, using alcohol as preservative	Alcohol is generally acceptable if below 0.5% by volume in the final enzyme preparation
HAR6	Packaging and labeling	Stearates, waxes and coatings applied to packaging materials from non-Halal sources, packaging materials contaminated with animal fats from non-Halal sources	Cross-contamination during packaging should be avoided; enzymes should be packed in acceptable and Halal approved containers; enzymes should be written on the label; reused packages must be properly cleaned

Source: Riaz and Chaudry (2004a).

lactose, skim milk powder etc) should be from Halal source and Halal approved. Both plant and animal materials could be used as a fermentation medium. Materials derived from animals need to be Halal approved. Plant materials modified or processed by using porcine or other non-Halal enzymes should not be used and animal materials (e.g. meat extract, meat peptone, blood, gelatin, collagen, glycerol, fat, hydrolyzed protein, enzymes for hydrolization of starch or protein and antifoaming agents such as fat derivatives and blood serum) need to meet food safety requirements and to conform to Halal requirements (Che Man & Sazili, 2010; Grahame et al., 2015; Riaz & Chaudry, 2004a; Satiawihardja, 2012). The microorganism to be used in fermentation should be isolated from Halal sources. Isolation from Haram sources such as pig's gut may lead to doubtful situation and therefore need to be further discussed. It is crucial that the media in which the fermenting microorganisms are grown in has to be derived from Halal sources (Munir & Regenstein, 1994; Riaz & Chaudry, 2004a). Further discussions are also necessary with regards to other metabolites from the microorganisms during fermentation and residues of raw materials/purifying agents (i.e. active carbon) used (Olempska-Beer et al., 2006; Satiawihardja, 2012). The ingredients, standardization agents and/or preservatives used to prepare final enzyme product as well as the packaging material need to meet Halal requirements (Mohamed Syazwan Ab & Mohd Remie Mohd, 2012).

Recombinant DNA technology helped to advance in the manufacturing of novel enzymes suitable for specific food-processing conditions, to increase the yield and to lower the production costs (James et al., 1996; Olempska-Beer et al., 2006). A large number of microorganisms used to produce enzymes are genetically modified (EC, 2016). Even though there has been intensive public debate about GMO foods regarding with ethical and religious issues, the use of this technology in the production of enzymes is not well known by consumers and need to be elucidated carefully not to threaten Halal assurance (Khattak et al., 2011; Søndergaard et al., 2005). According to OIC/SMIIC's General Guidelines on Halal Food (OIC/SMIIC, 2011), GMO organisms, GMO containing ingredients or final products should not be produced using non-Halal genetic material. Therefore, it is important that genetically modified microorganisms in the inoculums would not contain genetic materials from Haram sources (e.g. pig genes) (OIC/SMIIC, 2011; Riaz & Chaudry, 2004b). However GMO produced enzymes are allowed in case the genetic material is derived from Halal sources (Chaudry & Riaz, 2014; OIC/SMIIC, 2011).

4. Conclusion

Enzymes have been used in various food products for numerous benefits such as improving quality while decreasing processing time and production costs. With the increase in the use of enzymes derived from different sources, it is crucial to keep tract of the possible substances, which might threaten Halal assurance. As far as many Muslim consumers are concerned about the food additives and enzymes, Halal authorities have been trying to clarify the Halal status of enzymes, their sources and labelling. OIC/SMIIC's General Guidelines on Halal Food had been released in 2011 and it states that enzymes used as raw material, as processing aid or used in final product must be derived from Halal sources and must be declared on product label. Around 260 enzymes are used in the European region with about 91% obtained through fermentation and 9% through extraction from plant and animal sources. Due to recent developments in biotechnology and numerous advantages of microbial enzymes, fermentation is becoming the main production method and therefore it is important to study the growth media, substrates, ingredients and their treatment to determine possible non-compliances with Halal assurance. In addition, majority of enzymes have been produced by using genetically modified microorganisms and since using non-Halal animal genes pose risks to Halal assurance, further research is needed to identify their use by Halal food industry.

As the Muslim population has been growing and the demand for Halal products has been increasing rapidly, the Halal food industry is set to expand, further emphasizing the importance of proper Halal certification of food products. As part of Halal assurance, Halal situation of food additives and processing aids need to be clarified to ensure Halal requirements are met. Obviously, there is great demand for clarification of doubtful cases and gray areas in food production that can assist Muslims with the religious obligation of ensuring that the food they consume is Halal. Using raw materials and processing aids from Halal approved sources and using suitable technology and technique will ensure that the product is Halal and generate trust amongst consumers.

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