



## HALAL CRITICAL POINT OF MICROBIAL BIOPROCESS BASED-DAIRY PRODUCTS

Kurniati<sup>1</sup>, Hafsan<sup>1\*</sup>

<sup>1</sup>Department of Biology, Faculty of Science & Technology, Universitas Islam Negeri Alauddin Makassar  
Sultan Alauddin Street No.63, Gowa, Indonesia, 92113

\*Email: [hafsan.bio@uin-alauddin.ac.id](mailto:hafsan.bio@uin-alauddin.ac.id)

**Abstract:** The dairy products that use certain enzymes and microbial cells to produce other product variants such as yoghurt and cheese are products that which must be observed for its halalness. Uses microbial and additional ingredients and auxiliary materials that are not necessarily halal. The bacteria commonly used in the processing of dairy products such as yoghurt and cheese are several species of the lactic acid bacteria group which are developed as a starter in the fermentation process. Generally, lactic acid bacteria are grown first on growth media before being used as a starter. The halal critical point is the growth media, which contain proteins to own chances of animals (such as beef extract) which can be derived from pigs. Non-halal sources of carbon and nitrogen in growth media, such as blood, can also cause bacterial cells and microbial products to have a haram status. The same thing in the process of finalizing processed dairy products with the addition of flavours, amino acids, preservatives, or gelatine. The addition of food additives has become a critical point in any case because it can be a microbial product that needs to be ascertained halal. This review reveals that the critical point in making yoghurt is in milk as its raw material, the step of adding starter and adding additives. While the critical point in cheese making is in milk as its raw material and at the coagulation stage.

**Keywords:** bioprocess, cheese, critical points of halal, yoghurt

**Abstrak:** Produk susu yang menggunakan enzim dan sel mikroba tertentu untuk menghasilkan varian produk lain seperti yoghurt dan keju merupakan produk yang harus diperhatikan kehalalannya. Menggunakan mikroba dan bahan tambahan serta bahan penolong yang belum tentu halal. Bakteri yang biasa digunakan dalam pengolahan produk susu seperti yoghurt dan keju adalah beberapa spesies dari kelompok bakteri asam laktat yang dikembangkan sebagai *starter* dalam proses fermentasi. Umumnya bakteri asam laktat ditumbuhkan terlebih dahulu pada media pertumbuhan sebelum digunakan sebagai *starter*. Titik kritis halal adalah media tumbuhnya, yang kemungkinan mengandung protein yang berasal dari hewan (seperti ekstrak daging sapi) yang dapat berasal dari babi. Sumber karbon dan nitrogen yang tidak halal dalam media tumbuh, seperti darah, juga dapat menyebabkan sel bakteri dan produk mikroba berstatus haram. Hal yang sama juga terjadi pada proses finalisasi produk olahan susu dengan penambahan perasa, asam amino, pengawet, atau gelatin. Penambahan bahan tambahan pangan menjadi titik kritis dalam hal apapun karena dapat menjadi produk mikroba yang perlu dipastikan kehalalannya. Tinjauan ini mengungkapkan bahwa titik kritis dalam pembuatan yoghurt adalah pada susu sebagai bahan bakunya, langkah penambahan *starter* dan penambahan aditif. Sedangkan titik kritis dalam pembuatan keju adalah pada susu sebagai bahan bakunya dan pada tahap koagulasi.

**Kata Kunci:** bioproses, keju, titik kritis kehalalan, yoghurt

### Introduction

Animal food technology as an effort to meet human needs currently uses various materials as food raw materials, one of which is microbial-based food engineering. Biotechnology processes are generally inseparable from the role of microbes to produce a variety of quality and nutritious food products. Certain microbes are used in animal food processing through fermentation, such as in the manufacture of milk-based yoghurt and cheese. Microbial products are also often used, namely products produced by or with the help of microbes which can be in the form of microbial cells themselves or in the form of microbial metabolism products, including proteins, enzymes, vitamins, organic acids, organic solvents and amino acids (Owusu-Kwarteng et al., 2020; Faridah & Sari, 2019; Widodo, 2002).

The widespread use of microbes and microbial products in food processing, including milk, is of particular concern because it is related to the status or perception of the halalness of the various processed products produced. This is in line with the increasing awareness of the world's Muslim population to consume halal food as a fulfilment of the demands of sharia in Islam (Nuraini, 2018;

Thabrani, 2014). Several critical points in milk processing can change the halal status of pure milk into a product that is haram so that it becomes mandatory for a milk derivative product to be certified halal (Omar et al., 2010; Nuraini, 2018; Muhamad Kurniadi & Frediansyah, 2017).

In addition to pure milk, milk circulating in the market today is in the form of processed or formula milk. This means that the milk has been mixed with other ingredients according to the desired formula, complete with different tastes and aromas. There are added chocolate flavor, strawberry flavor, vanilla flavor, and so on. There are also additional preservatives so that the milk does not spoil quickly, the addition of vitamins and amino acids. This processing and addition process makes milk which was originally halal become a product that must be observed for its halalness because it uses additional ingredients and auxiliary materials that are not necessarily halal. Likewise with dairy products that utilize certain enzymes and microbial cells to produce other product variants such as cheese and yoghurt. (Faridah & Sari, 2019; Widodo, 2002).

Microbes commonly used in dairy products such as yoghurt and cheese, are generally from the lactic acid bacteria group which was developed as a starter/inoculant in the fermentation process. Lactic acid bacteria were first grown on the growth media before being used as a starter. The critical point is the growth medium, which has the potential to contain animal protein (such as beef extract) which could have come from pigs. Non-halal sources of carbon and nitrogen in growth media, such as blood, can also cause bacterial cells and microbial products to be illegal. Likewise in the process of finalizing processed dairy products by adding flavor, amino acids, preservatives, or gelatine. So these additives are also a critical point because they can be microbial products that need to be confirmed as halal. *The main critical points determining halal perception on microbial bioprocess-based products include (1) microbial sources, (2) microbial isolates, (3) growth substrates, (4) metabolic products (5) production sites and (6) matrices or other added materials for a specific purpose* (Faridah & Sari, 2019; Atma et al., 2018; Muhammad Kurniadi & Frediansyah, 2016).

This review is a scientific rationalization to open an understanding of the critical point of halal animal food products based on microbial agents (microbes and their products) in encouraging product diversification efforts in an increasingly advanced civilization. Food that is produced at a low cost, beneficial (healthy), and easy to apply but still meets the halal standards as prescribed by Islam as '*din*' as well as '*tamaddun*' for humans, especially for Muslims (Muhammad Kurniadi & Frediansyah, 2016; Thabrani, 2014).

## 1. Dairy Milk and Its Features

Etymologically, milk means a white liquid that comes from the mammary gland which has a high nutritional content without the addition or subtraction of the original components. Milk is a food product of animal origin that has a liquid consistency, all of which are synthesized in the udder glands. This gland is specifically owned by mammals in the form of a collection of alveolar cells that develop on the ventral part (along the lower body), namely on the *ventro cauda abdominalis* (Truchet & Honvo-Houéto, 2017; Neville et al., 2003).

Milk biosynthesis is a mechanism that involves various organ system functions of livestock. The organ systems involved include the hormonal, nervous, digestive, transport, and lymphatic systems. The hormonal and nervous systems together carry out metabolic control that occurs in the udder cells. Milk biosynthesis begins when cattle give birth and the release of the hormone prolactin from the pituitary gland. The hormone prolactin together with estrogen and progesterone initiates metabolism in epithelial cells to synthesize components of milk. Milk that has been formed will then be directed to the cisterna to let down. This process occurs due to the influence of the oxytocin and vasopressin hormones. The nervous system works as a connector that transcribes various types of stimuli, then it will be forwarded to the hormonal system to release certain hormones from certain places to support milk biosynthesis. After entering the lumen, the nutrients carried by the blood have become milk, and are completely separated from the blood (Ofteidal, 2020; Truchet & Honvo-Houéto, 2017; Bauman et al., 2006; McManaman & Neville, 2003; Neville et al., 2003).

Milk is an important source of animal protein for humans. Milk has a variety of nutritional content with high quality and bioavailability. This is due to the consistency of milk in the form of a liquid. The components of milk are not bound together into a strong matrix complex, making it easier to digest compared to eggs or meat (Berge & Baars, 2020; Zwierzchowski & Ametaj, 2019; Popovic-Vranjes et al., 2015; Guetouache et al., 2014;).

The digestible value of milk reaches 95%, meaning that the whole milk component can be digested quickly without much remaining adding that, milk in each livestock species generally has a similar macronutrient content (Berge & Baars, 2020; Zwierzchowski & Ametaj, 2019; Popovic-Vranjes et al., 2015; Guetouache et al., 2014). Milk in general contains lactose, protein, fat, vitamins, and minerals. Nutrients in milk are complete enough to meet human nutritional needs, both for protein and energy sources. Milk in each livestock species has a diversity of nutritional content, due to genetic and environmental influences (Guetouache et al., 2014).

Milk for humans not only acts as a food complement, but also has health effects for those who consume it. Bauman et al. (2006) explained that milk is a potential food product that can have a heterogeneous effect on the body as well as a therapeutic effect (healthy or healing). The heterogeneous effect in question is that certain nutrient components in milk will have a certain effect on the human body. For example, lactose and fat, both provide a supply and reserve of energy for all body tissues, while protein can be utilized in the process of regenerating body cells. The therapeutic effect of milk is supported by its complete nutrient content and high digestible value. This allows milk to speed up the body's recovery process after illness (Popovic-Vranjes et al., 2015).

Milk contains several essential compounds that cannot be found in other food products, because they can only be synthesized in the udder, for example, casein, lactose, and several types of amino acids and fatty acids. In addition, there are also some milk content that can be affected by the feed eaten by the livestock. Most of the animal feed comes from various types of plants. Plants are natural foods that contain potential bioactive compounds that can enter milk. Bioactive substances of plant origin can be carried along with other nutrients in the blood to the udder glands and filtered into the milk. Bioactive compounds from plants such as antioxidants, carotenes, flavonoids, polyphenols, alkaloids, terpenoids, and so on can be filtered through transitocytes, apical and paracellular membrane transport. Filtration is the entry route for certain components from the blood into the milk without changing (synthesis). Substances that are filtered generally have low molecular weights, so they easily seep (diffuse) through the epithelial cell membrane (Truchet & Honvo-Houéto, 2017; German & Dillard, 2010; Bauman et al., 2006; McManaman & Neville, 2003; Neville et al., 2003; Clegg et al., 2001).

Bauman et al. (2006) added that the main bioactive compounds commonly found in milk are fatty acids and amino acids. One of the fatty acids known to provide health effects is conjugated linoleic acid (CLA). This compound is the result of biohydrogenation of fatty acids in the rumen which is proven to be able to provide health effects as antimutagen, anticarcinogenic, and anti-cancer. In addition to CLA, there are also many compounds that have health effects such as casein, minerals, vitamins, biopeptides and so on (Guetouache et al., 2014).

By consuming a minimum of a glass of milk every day, it will get the most milk for the body, among others:

- a. The potassium content can move the walls of blood vessels so as to keep them stable. So you are far from high blood pressure and heart disease.
- b. The content of iodine, zinc and leticine can drastically increase the efficiency of the work of the cerebrum.
- c. Iron, copper and vitamin A in milk have a function for beauty, which can keep the skin glowing.
- d. The content of tyrosine in milk can encourage the hormone of joy and make a person sleep more soundly.
- e. Milk calcium can increase bone strength, prevent bone shrinkage and fractures.
- f. The magnesium content in milk can make the heart and nervous system resistant to fatigue.
- g. The zinc content in milk can heal wounds quickly.

- h. The content of vitamin B2 in milk can improve visual acuity (Berge & Baars, 2020; Sozańska, 2019; Popovic-Vranjes et al., 2015; German & Dillard, 2010; Singh & Bennett, 2005).

## 2. Microbial Bioprocess based-Dairy Products

Milk is a fat-in-water emulsion containing several dissolved compounds. So that the fat and water in milk are not easily separated, the milk protein acts as an emulsifier (emulsifier). The water content in milk is very high, which is about 87.5%, with milk sugar (lactose) content of about 5%, protein of about 3.5%, and fat of about 3-4%. Milk is also an excellent source of calcium, phosphorus, and vitamins A, B, D and E. The quality of milk protein is commensurate with the value of meat and egg protein, and especially it is very rich in lysine, which is one of the essential amino acids that the body needs (Sozańska, 2019; Popovic-Vranjes et al., 2015; Guetouache et al., 2014).

Although the nutritional value of milk is so perfect, not everyone can enjoy milk without problems. For some people, milk can cause intolerance, either in the form of lactose intolerance or protein intolerance. Lactose intolerance is a condition where there is no or insufficient amount of the enzyme lactase in a person's body. Lactase enzyme is an enzyme whose job is to break down the sugar lactose into simpler sugars, namely glucose and galactose. Compared to lactose, which is a disaccharide, glucose and galactose are monosaccharides that can be digested and absorbed by the intestines for metabolic processes. The absence of the enzyme lactase is what causes symptoms of diarrhea, heartburn, or nausea sometime after drinking milk (Widodo, 2002).

In certain people, drinking milk can also cause allergies. This is known as protein intolerance. One type of protein in milk is lactoglobulin, which in the body of certain people can act as a very strong antigen that can cause allergies. Another problem with fresh milk is that it is very perishable. Fresh milk is a very high nutritional food, so it is not only beneficial for humans but also for spoilage microorganisms. Bacterial contamination can grow very quickly so that the milk becomes damaged and unfit for consumption. To extend the usability, shelf life, and to increase the economic value of milk, handling and processing techniques are needed. One of the most prospective milk processing efforts is through milk fermentation (Owusu-Kwarteng et al., 2020; Rahman et al., 2012; Singh & Bennett, 2005; Widodo, 2002).

### a) Yoghurt as a dairy product

Yoghurt is made from fresh milk, starter bacteria, flavoring and the addition of skim milk as a thickener. The bacteria used in making yoghurt are a group of lactic acid bacteria (Hendarto et al., 2019; Syainah et al., 2014; Purwoko et al., 2006; Widodo, 2002). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the most widely used lactic acid bacteria in the food industry, especially in starter cultures for the dairy industry, mixed with other microbes (Widodo, 2002). In this modern era, yoghurt can be made by utilizing a combination of *Lactobacillus acidophilus* with yoghurt starter *L. bulgaricus* and *S. thermophilus* (Khalili et al., 2020; Hassan et al., 2020; Sunaryanto, 2017; Hidayat, 2017; Chowdhury, 2012; Estrada et al., 2005; Widodo, 2002). Often in its manufacture also added other beneficial and useful probiotic bacteria to help increase metabolic processes.

Making yoghurt begins with the growth of *S. thermophilus* which ferment lactose into CO<sub>2</sub> and lactic acid, causing the atmosphere to become acidic. This condition can stimulate the growth of inoculant bacteria, *L. bulgaricus* and *L. acidophilus* and inhibit the growth of pathogenic bacteria that cannot survive in an acidic environment. *L. acidophilus* can utilize lactose and sucrose for metabolic activities. *L. bulgaricus* is responsible for producing a distinctive aroma, while *S. thermophilus* is responsible for producing the taste of yoghurt (Widodo, 2002).

In general, the process of making yoghurt begins with determining the total solids of milk, then pasteurization (heating) so that the milk is sterile from other bacteria, cooling, and then adding a bacterial starter. To determine the halalness of food, it is necessary to observe critical points at all stages of the process, starting from the selection of raw materials (input), processing, until the final product is ready for consumption (Nuraini, 2018; Ali, 2016; Kurniadi & Frediansyah, 2016; Rosele et al., 2013).

### b) Cheese as a dairy product

Cheese is the general name for a group of fermented dairy-based food products (milk protein coagulation) produced worldwide with a variety of flavors, textures and forms. Cheese is one of the dairy products obtained due to the formation of coagulation of milk by rennet (digestive enzyme from the stomach of mammals). The part of the liquid milk that undergoes the coagulation process will form a solid gel-like substance called curd and a large amount of water and some dissolved substances will separate from the curd called whey. The raw material for cheese produced in the world comes from cow's milk, but milk from other animals has also been widely used.

Cheese products are made through fermentation with the help of several types of microbes such as *S. lactis*, *S. cremoris*, *L. casei*, *L. plantarum*, *L. brevis*, *S. thermophilus*, *L. lactis* dan *Leuconostoc mesenteroides* (Estikomah, 2020; Faridah & Sari, 2019; Widodo, 2002). Cheese making follows hygienic procedures and uses proper equipment. What needs to be done is to see what processes are involved and what equipment is used. The principle of cheese making is basically to remove water, lactose and some minerals from milk to produce a dense mass of protein and fat. The ingredients used for making cheese are milk, rennet, bacterial culture, and salt. Rennet causes clumping of milk protein to change from a liquid to a gel, if the gel is cut into smaller pieces, it will cause the whey (water and lactose) to separate from the curd (solid consisting of casein). This curd will be produced into cheese according to the desired type of cheese. A culture of lactic acid bacteria is added to assist in the curd formation process and will determine the texture, aroma and moisture content of the cheese (Johnson, 2017; Singh & Bennett, 2005).

## Discussion

Halal dairy products in this review can be traced and identified through the ingredients and processing processes. As a product based on microbial bioprocesses, it is known that several stages that are generally likely to be the cause of the illegitimacy (critical point) of a product are: 1) the source of the inoculant; 2) inoculant properties; 3) inoculant growth media; 4) metabolic products; 5) place of production; and 6) matrices or other materials added for a specific purpose (Faridah & Sari, 2019; Kurniadi & Frediansyah, 2016).

### 1. Halal Critical Point-Risk Identification of Yoghurt

Yoghurt is a fermented milk product using lactic acid bacteria to obtain a distinctive texture and taste. Bacteria that are widely used in making yoghurt are *S. thermophilus* and *L. bulgaricus*. Yoghurt can also be added with probiotic bacteria. The initial stage of the yoghurt making process is pasteurization or heating, then cooling. If the milk that is processed into yoghurt has cooled after pasteurization, the bacteria starter is added, then incubation is carried out. During the incubation process additives were added. The final stage of making yoghurt is packaging yoghurt before it reaches consumers.

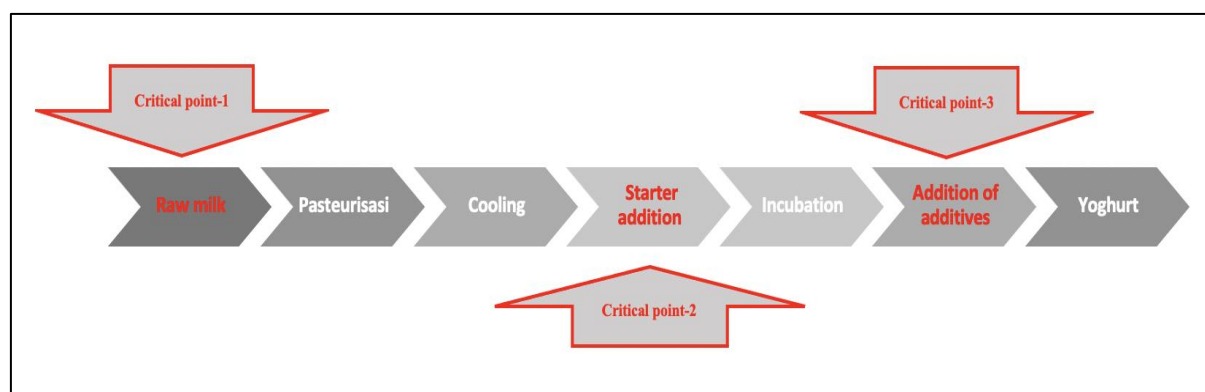


Figure 1. Halal critical point and yoghurt-making process flow

There are three critical point risks in the stages of the yoghurt making process. *The first critical point* is milk, which is the raw material, because milk-producing animals can come from non-halal animals. When the raw material (milk) comes from animals that are halal for consumption such as cows, buffalo or goats, then the dairy

products from these animals are also halal. Meanwhile, if the milk comes from non-halal animals, then the milk products are also confirmed to be non-halal (Nuraini, 2018).

*The second critical point* in the yoghurt making process is the addition of a bacterial starter. Basically, microbes are halal as long as they are not harmful and are not mixed with unclean and haram goods. The source, nature, growth media and metabolites produced by bacterial isolates used as starters/inoculants are factors that determine the halal nature of the products fermented by these isolates. Starter bacteria are usually propagated on a medium. The risk of not being halal will occur if the composition of the bacterial growth media contains ingredients that are haram. When bacteria are grown on holy media, the bacteria that are propagated are also holy. If bacteria are grown on unclean media, such as Blood Agar Plate (BAP) media derived from blood, then the bacteria can remain lawful as long as they can be separated between the bacteria and the media mixed with the unclean object. Then the bacteria must be cleaned. However, when bacteria are grown on media that is mixed with haram objects, such as pigs, then the law is haram based on MUI Fatwa No. 01 of 2010 concerning the use of microbial products in food products (Hartina et al., 2020; Nuraini, 2018; Ali, 2016; Thabrani, 2014). The risk of non-halal can also come from the bacteria used. Today many bacteria are genetically modified. Gene transfer can be done between bacteria and animals. Genes derived from unclean animals can be a risk for non-halal products. The starter inoculant used must be ensured that it is free from haram substances, is non-toxic and uses growth media that is free of non-halal substances and produces non-haram metabolites in the form of lactic acid (Atma et al., 2018; Kurniadi & Frediansyah, 2016; Rosele et al., 2013; Regenstein et al., 2003).

*The third critical point* in the yoghurt making process is the addition of food additives. Additives that can be added in the process of making yoghurt include gelatine as a stabilizer, flavoring, coloring and emulsifier. These additives may come from non-halal ingredients (Faridah & Sari, 2019), for example in the clotting process. When the yoghurt settles and separates from the water, it is often necessary to add a stabilizer such as gelatin to increase the water holding capacity so as to prevent separation and improve the appearance quality of the yoghurt. The choice of gelatin material needs to be watched out for because there is gelatin from non-halal animals such as pork. It is known that 41% of gelatin production comes from pork skin and bones (Khattak et al., 2011). If the additional ingredients used come from non-halal animals, it can cause the resulting yoghurt to be non-halal as well (Atma et al., 2018). However, when the stabilizer comes from vegetable ingredients such as pectin derived from plant extracts, it will not change the halalness of the product (Atma et al., 2018; Faridah & Sari, 2019).

## 2. Halal Critical Point-Risk Identification of Cheese

Cheese is a solid and sour food made from milk with the addition of rennet to coagulate the milk casein. The stages of cheese making are, in succession, starting from milk preparation, coagulation, pressing and separation as well as curd handling, and ripening. The first halal critical point of cheese comes from the raw material of cheese. If it comes from an animal that is halal for consumption such as cow, goat, buffalo, camel, or sheep milk, then the animal's milk product can remain halal and cheese become not halal when it is produced from non-halal animal milk. The flow of the cheese-making process and the critical point of its halalness described in Figure 2.

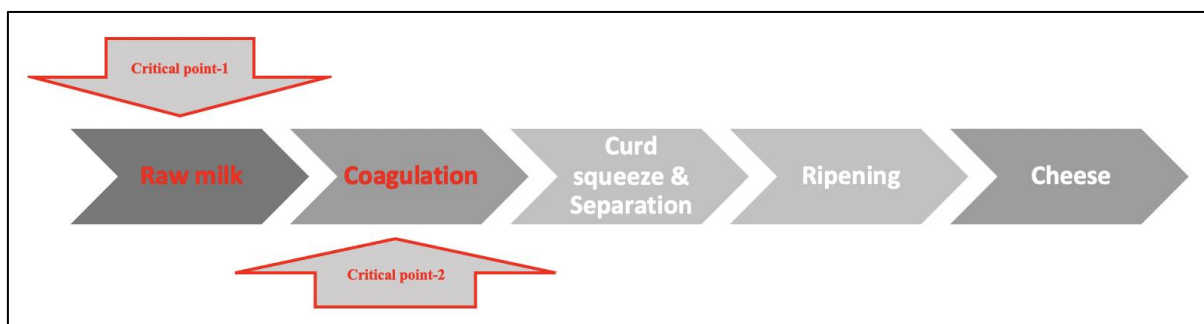


Figure 2. Halal critical point and cheese-making process flow

The second critical point is in the cheese-making process at the coagulation stage. Coagulation is the stage of adding material to the clumping process. There are two methods of coagulation, namely the enzymatic method and the microbiological method. The enzymatic method is carried out using the enzyme rennin (rennet) to coagulate milk. Rennet is an extract of calf abomasum that has not been

weaned or can also be derived from other mammals. This becomes a risk because the rennet-producing animals can also be from non-halal animals. Furthermore, even if it comes from halal animals, it will be a risk if the method of slaughtering rennet-producing animals is not carried out according to Islamic law. Rennet is categorized as halal, if the rennet comes from halal animals and the slaughter process is in accordance with Islamic law. However, if it does not meet these two conditions, it cannot be categorized as halal. (Farouk et al., 2014; Rosele et al., 2013; Omar & Jaafar, 2011). The second method is the microbiological method. Microbiological methods usually use lactic acid bacteria. The use of microbiological methods is actually halal, but cheese products are at risk of becoming non-halal if the lactic acid bacteria used for cheese production are grown on media mixed with haram ingredients. The media used to grow inoculant bacteria may contain yeast extract, where yeast extract can be at risk of coming from a by-product of beer processing (Atma et al., 2018).

## Conclusion

Milk as one of the products of mammals has a high fermentable carbohydrate content, making it possible to become a growing medium for lactic acid-producing microbes through fermentation, which produces sour milk (yoghurt) with a longer shelf life and is functional. It is also known that milk casein can coagulate which is then the product is known as cheese. The two dairy products are known as foods with high economic value. The production process of yoghurt and cheese requires controlling the risk of non-halal dairy products because they are products based on microbial bioprocesses. This review reveals that the critical point in making yoghurt is found in milk as the raw material, the starter addition stage and the additive addition stage. While the critical point in cheese making is in milk as its raw material and at the coagulation stage. This information becomes the basis for ensuring the halalness of the two products produced.

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