3rd International Conference Eco-Innovation in Science, Engineering, and Technology
Volume 2022
http://dx.doi.org/10.11594/nstp.2022.2723

NST Proceedings
OPEN ACCESS

Conference Paper

Application of Biotechnology in the Production of Derivatives of Dairy Products: A Review

Rahmawati Rahmawati*, Larestananda Asmaul Husna Hizaumi Putri, Lutfiah Ayu Khoirunisa, Milenia Fitria Cholifah

Department of Food Technology, Faculty of Engineering, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia

*Corresponding author:
E-mail:
rahmawati.tp@upnjatim.ac.id

ABSTRACT

The increasing population has led the food industry to be more adaptable in their business, especially in production aspects. This is due to the need for improving the quantity and quality aspects of end products, including dairy food products. To meet consumer expectations the food industries are urged to produce and/or modify the methods of production to ensure the quality of the food and production cost. This review focuses on the application of biotechnological aspects during the production of cheese, kefir, and yogurt, to obtain certain characteristics, especially its sensory attributes such as color, texture, taste, and odor.

Keywords: Biotechnology, cheese, kefir, production, yogurt

Introduction

Generally, food is one of the most fundamental needs for everyone, which must be safe and nutritious for human consumption. Besides, there has been a development with regards to the production process to improve the quantity and quality aspects while it must meet consumers' expectation. As a result, the food industry must adapt by planning and executing some improvements in the production line, one of which is by using a biotechnological approach. The use of biotechnology not only increases yield and efficiency during production but is also environmentally friendly. These benefits are crucial in achieving food security, especially during the globalization era with the growing population.

According to Gupta et al. (2017) biotechnology is related to the application of engineering and technological advancements in biological cells and organisms and/or systems to produce or modify the products with desired parameters. The principles of biotechnology have been used in various fields, such as medicine, marine, environment, and food. Regarding food biotechnology, it has been widely known for centuries that the first biotechnology principles were used conventionally in the production of beer, wine, cheese, and bread. Besides, Pratiwi and Rina (2015) indicated that hat biological processing which uses live microorganisms or the compounds they produce to create useful products provides the possibility for the manufacture of new food products and materials. One of the strategies to improve food quality is by delaying or slowing down the ripening process, therefore it does not deteriorate easily during the distribution and storage process. In addition, food quality can also be improved by enriching the nutritional content of food. The nutritional quality and storability of agricultural products can also be improved, hence it can provide significant economic benefits.

One of the food commodities which is highly potential for biotechnology implementation is dairy products which are made of milk as the main raw material. The production and consumption of milk have been increased considerably and the selling price is highly stable. Besides, milk is a

How to cite:
Rahmawati, R., Putri, L. A. H. H., Khoirunisa, L. A., & Cholifah, M. F. (2022). Application of biotechnology in the production of derivatives of dairy products: A review. *3rd International Conference Eco-Innovation in Science, Engineering, and Technology*. NST Proceedings. pages 143-151. doi: 10.11594/ nstp.2022.2723

healthy food product that has high nutrition and the presence of high protein milk is increasing in the market. Whereas previously such products were only in demand by people who focused on sports and bodybuilding, now these products have become attractive to ordinary consumers, including the elderly (Gavrilova et al., 2019). Furthermore, the current change in milk composition by intervening in the nutritional and genetic composition aimed at developing dairy products for certain health benefits has a high appeal in the field of applying biotechnology into the process.

Hence, the biotechnological advancement applied during the production of dairy products would support the main goal of food industries to meet consumers' demands for highly safe, nutritious, and diverse food dairy products and their derivatives, including cheese, butter, kefir, and yogurt. This article would discuss the application of biotechnology during the production of cheese, yogurt, and kefir, along with their sensory attributes.

Material and Methods

This review was prepared by searching and screening relevant articles with regards to the Application of Biotechnology in the Production of Derivatives of Dairy Products, which consists of several main keywords, including "biotechnology", "milk", "dairy products", "cheese", "yogurt", and "kefir". All the articles published in trusted national and international journals and book publishers within the range of 20 years were used to finalize the manuscript.

Results and Discussion

Cheese

Cheese is a food product produced by separating solids in milk using a thickening or coagulation process, which is assisted by bacteria or certain enzymes (rennet). The principle of cheese making is clumping or curd formation. To create a curd or clotting condition, two types of the process can be used, namely by adding a starter bacterial culture from the lactic acid bacteria group or by direct acidification (Budiman et al., 2017). Besides, the variety of cheese in terms of its sensory attributes, such as color, aroma, texture, taste, and hardness, is influenced by the production technology, milk source, moisture content, and aging time (Santiago et al., 2018).

The main constituent of cheese is casein, and the rest consists of whey protein, fat, lactose, vitamins, and minerals. Lactic acid bacteria will affect the rate of change of lactose into lactic acid, but it will also affect the rate of decrease in pH and the concentration of acid produced in cheese. High and low levels of lactic acid, affect the cheese both chemically and biologically. Lactic acid bacteria are widely used in cheese making such as *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus acidophilus*, and *Lactobacillus plantarum* (Budiman et al., 2017). However, the use of lactic acid bacteria culture takes a relatively long time to create acidic conditions, so acid is added from local fruit extracts such as star fruit, lime, and lemon (Hanum et al., 2022). The common method in cheese production, including milking, standardization, sterilization, cooling, the addition of starter, the addition of coagulant or acid, the addition of enzymes, clumping and cutting, stirring, heating, separation whey, printing and squeezing, salting, and curing and storage (Zheng et al., 2021).

In general, cheeses produced from different regions have different characteristics. This can be caused by different regions, production methods, and available raw materials (Zheng et al., 2021). The consumer's acceptance of cheese depends on certain sensory characteristics, including taste and aroma. Yeast influences the deamination of amino acids into NH_3 and increases the pH of cheese (El Sheikha & Montet, 2014). In the ripening process of extra-hard and hard cheeses (e.g., Parmesan and cheddar), the clot is kept under conditions that can inhibit the growth of microorganisms on the surface, including the microbial enzymatic reactions, one of which is placing the cheddar cheese under the dark condition to ensure it is ripened (Krishnan et al., 2019). With regards to the parmesan cheese, the milk is left overnight during the process, so that the fat rises to the surface of the milk. Then, the milk is stirred to remove all the butter. Then, the remaining buttermilk is mixed with fresh milk and then fermented in a tank (Zheng et al., 2018).

Once formed, the Parmesan clot is heated with a temperature of 52°C, and some of the whey is taken as a starter for cheese-making the next day (Neviani et al., 2013).

The sensory parameters of the cheese, namely flavor, taste, texture, color, and hardness, is one of the crucial aspects to meet the consumers' expectations. Flavor composition in cheese is unique, and involves three main reactions: the metabolic wastes of lactose, lactate, and citrate; proteolysis; and lipolysis. The main products of lactose metabolism that have an important influence on taste are L-lactate, DL-lactate, or a racemic mixture of the two. Acetate is an important flavoring compound in many types of cheese. Besides, being metabolized from lactose by LAB, acetate can also be formed through the metabolism of lactate and citrate. The concentration of lactose in cheese may drop because of leaching or be replaced by whey; in this case, the lactose remaining in the clot is rapidly metabolized with an increase in the pH value. As a result, cheeses with low lactose residues possess a fresh and light taste, whereas lactose with a high residue possesses a strong and sharp taste due to its low pH value. Several compounds, including acetate, diacetyl, acetoin, butanediol, and carbon dioxide, are produced from citrate if some are lactate citrate-positive, such as *L. lactis* and *Leuconostoc* (Fox et al., 2017).

Lipolysis affect the taste and texture of cheese (Voigt et al., 2012). Lipase in cheese comes from milk, rennet, and exogenous enzymes. Lipolytic enzymes present in LAB can hydrolyze substrates to produce free fatty acid esters, triacylglycerides, diacylglycerides, and monoacylglycerides. During the fermentation and ripening of cheese, a series of fatty acids with medium and short carbon chains (C > 4) are formed after the degradation of milk fat. In addition, the unique texture and hardness of the cheese result from the continuous evaporation of water. Acetic acid gives the cheese a tangy taste, but too much acetic acid can make the cheese smell like vinegar. Esters in cheese are produced through an esterification reaction between short-chain fatty acids and medium-long-chain fatty acids produced during the degradation of milk fat. In addition, primary and secondary alcohols are produced during fermentation through lactose fermentation or amino acid metabolism. Esters in cheese influence the formation of a sweet, fruity, and floral aroma. However, excessive amounts of ethyl butyrate and ethyl caproate can lead to poor and overly strong flavors (Castada et al., 2019).

Yogurt

Based on the common process of yogurt production, Utami et al. (2020) stated that making yogurt includes pasteurization, cooling, inoculation, curing, and storage. Pasteurization is required to kill pathogenic microbes and spoilage microbes so that product quality and safety can be maintained. Besides, it also functions to denature milk protein by changing the protein structure to increase the gelation and density of the resulting yogurt product. The yogurt can be pasteurized at 185 F (85 °C) for 30 minutes or at 203 F (95 °C) for 10 -20 minutes. After the pasteurization process, the milk is cooled to reach the temperature of 107 - 100 F (41-43 °C) to allow lactic acid bacterial fermentation to occur. The high temperature will cause the starter bacteria to be inoculated. After reaching 41-43 °C, the starter bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were added to milk as much as 1-3%. The fermentation then occurs for 6-12 hours. During fermentation, bacteria will use lactose to produce lactic acid which will reduce the pH of the milk to below 4.6.

Strategies to develop the quality of yogurt products are by producing yogurt that has low lactose content (lactose-free products). The production of lactose-free yogurt products is expected to expand the consumer market so that dairy products made from animal milk can still be consumed even by consumers with lactose intolerance. According to Deng et al. (2015), lactose intolerance is generally associated with lactase deficiency, either primary or secondary. In addition, it should be noted that the cause of lactose intolerance is due to not only the expression/reaction caused by lactase, but also the levels, intestinal microflora, motility of the digestive tract, and the sensitivity of the digestive tract to produce gas. Therefore, based on these things, the treatment of lactose intolerance can be done by consuming lactose-reduced products and enzyme replacement.

Enzyme replacement that can be done in the yogurt production process is the addition of enzymes that can produce lactase activity. An example of an enzyme that produces lactase activity is the enzyme β-galactosidase. The same thing is also mentioned by Amiri et al. (2021) who observed that one form of milk yield modification through biotechnology applications is to modify the lactose component. The lactose component can be broken down using the enzyme lactase or often called the enzyme lactase-phlorizin hydrolase (LPH), which is classified as one type of β-galactosidase enzyme. This enzyme converts the lactose into monosaccharides, such as glucose and galactose, hence the lactose can easily be absorbed into the body. The β-galactosidase enzyme is classified as one type of cold-active enzyme. According to Kuddus (2018), cold-active enzymes are generally produced by microorganisms and fish that live in the arctic region. Schmidt et al. (2016) stated that the galactosidase enzyme can be isolated from several microorganisms, including *Kluyveromyces lactis, Kluyveromyces fragilis*, *Aspergillus niger* and *Aspergillus oryzae*.

The use of enzymes obtained from these microorganisms is classified as one of the production process innovations that can be used to reduce production costs. According to Kuddus and Ramteke (2012), the application of cold-active enzymes would be beneficial to reduce energy use. The β -galactosidase enzyme obtained from psychrophilic microorganisms can be active and break down lactose components even though they are in a low-temperature environment, which from this can be said that the galactosidase enzyme can still work during the distribution process to consumer storage. Moreover, the galactosidase belongs to glycosyl hydrolases enzymes which have the principle to hydrolyze glycosidic bonds between carbohydrates and half of the certain non-carbohydrate components or between two or more carbohydrate components. Hydrolysis generally occurs using only two types of amino acids function as a proton donor and a nucleophile/base, resulting in retention or inversion of the anomeric configuration of the resulting carbohydrate (Coker, 2016; Pawlak et al., 2014).

In addition to considering the health benefits that can be offered by a product, product development by applying new methods such as the application of biotechnology must also consider the organoleptic aspects produced after the production process has been completed. The organoleptic aspect as we know is one of the factors that can influence consumers in the aspect of product selection. According to several studies reviewed based on the color aspect, it is known that lactose-free products will give a lighter color when compared to the color of yogurt in general. Giving lactase enzyme will cause a decrease in the color aspect of luminosity (L*) and color chromatic parameters (a* and b*) (Skryplonek et al., 2017; Pawlos et al., 2020). The comparison of yogurt color between treatments according to Capcanari et al. (2021) can be seen in the Figure 1.

Figure 1. The color of yogurt based on a different source of milk; (a) control, (b) lactose-free yogurt from cow’s milk, (c) lactose-free yogurt from goat’s milk

Based on the color comparison in Figure 1, the control yogurt will have a more yellow color when compared to lactose-free yogurt (goat’s milk). The whiter color in the sample of lactose-free yogurt (goat’s milk) is because goat's milk does not contain β-carotene and only has a smaller proportion of globules compared to cow's milk. The color difference in each sample is related to the difference in gel opacity, where the high and low gel opacity produced is related to the casein content contained in the milk and the ability to clot the type of milk used (Pawlos et al., 2020).

Furthermore, the taste produced in lactose-free products will produce a sweeter taste when compared to control yogurt. When the lactose has been hydrolyzed before fermentation by 50-70%, the yogurt will have a better flavor (Ibarra et al., 2012). The product given the lactase enzyme will have the ability to break down lactose so that the lactose that is broken down will indirectly produce a more soluble monosaccharide component in larger quantities when compared to the control yogurt. This causes lactose-free products to possess a sweeter taste compared to products that are not treated with the lactase enzyme (Schmidt et al., 2016; Capcanari et al., 2021). Skryplonek et al. (2017) found that lactose-free frozen yogurt had a higher sensory score compared to control yogurt, including aspects of taste, flavor, and mouthfeel. Furthermore, the lactase enzymes affect syneresis. According to Pawlos et al. (2020), the low-lactose yogurt had a lower syneresis score index (50.72%) compared to the control yogurt (64.32%). Similar results were also found by Kárnyáczki and Csanádi (2017) who observed the syneresis of control yogurt was higher than lactose-free yogurt (21.47% > 14.63%).

Kefir

Kefir is a fermented beverage that is low in alcohol content, and has a sour and fizzy taste because of the fermentation process. John and Deeseenthum (2013) stated that kefir has benefits for the body, which possesses anti-microbial and anti-carcinogenic properties, inhibits tumor growth, lowers cholesterol levels, increases lactose tolerance, accelerates wound healing, as well as containing probiotics and prebiotics. Kefir can be made from milk or non-dairy with a kefir grain starter. However, kefir is different from other fermented products. The difference is in the starter, which is in the form of seeds or grains. Kefir grains have a size of less than 4 cm in the form of small grains and have a clear white to yellowish-white color (Kesenkas et al., 2017). Microbes that are often found in kefir grains consist of lactic acid bacteria, yeast, and acetic acid bacteria, which includes *Lactobacillus kefiranofaciens*, *Lacticaseibacillus paracasei* (basonym *Lactobacillus paracasei*), *Lactiplantibacillus plantarum* (basonym *Lactobacillus plantarum*), *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii* subsp. bulgaricus. On the other hand, *Saccharomyces cerevisiae*, *S. unisporus*, *Candida kefyr*, and *Kluyveromyces marxianus* ssp.

Microflora composition and nutritional content of kefir can vary based on the type of solution used for fermentation as well as the culture treatment, such as the length of fermentation time, temperature, stirring time, and the ratio of the size of the kefir grains (Talib et al., 2019). Kefir generally contains 80-90% water content, 0.2% fat, 3.0% protein, 6.0% sugar, 0.7% ash, and 1.0% lactic acid and alcohol (Sarkar, 2007). Kefir fermentation also contains metabolic products such as ketone, esters and acetates, alcohols, hexanoic acid, butanoic acid, and octanoic acid (Garofalo et al., 2020). Kefir is a functional food because it has a consistent sensory appearance like yogurt because of its lactic acid bacteria content and has a slight alcohol aroma due to its yeast starter. Kefir can be made from dairy products such as cow's milk, goat's milk, or sheep's milk, however, kefir can also be made with soy milk and other vegetable milk because of its nutritional content and similar physical properties to animal milk (Rossi et al., 2016).

There are two types of kefir-making processes, namely traditional and modern methods which can be seen in Figure 2. In the industrial process, raw milk is homogenized first and then the milk is heated to a temperature of 95°C for 15 minutes in both stages of the process to kill microbes. Then, the milk is cooled this cooling process aims to cool the milk so that when added to the starter, the starter does not turn off due to high temperatures. Subsequently, the kefir starter is added and the fermentation is started for 18 - 28 hours. After fermenting the kefir grains as a starter, they are separated from the kefir solution to be used in the next fermentation process. In the kefir industrial process, maturation is carried out, this is useful to add aroma and taste to make it sharper. Finally, the kefir solution is then put in a packaged container and cooled at the temperature of 4°C to maintain its quality (Guzel-Seydim et al., 2021).

Kefir fermented milk that is often used in Indonesia is goat's milk. Goat's milk is a little less popular because it has a characteristic unpleasant stinging aroma. This *prengus* aroma is produced by the short-chain fatty acids contained in goat's milk, such as caproic acid, caprylic acid, and capric acid (Balia et al., 2011; Kinteki et al., 2018). The production of kefir made of goat's milk can be one of the potential solutions to increase the consumption of goat's milk.

There has been a sensory assessment of goat's milk kefir, based on sensory attributes, including color, odor, taste, and texture. Color is one aspect of panelists' assessment of the quality of a food product to determine its acceptability to consumers. Goat's milk kefir has a white color that tends to be yellowish. The color of goat's milk kefir is influenced by the fat and solid content in the milk is by research by Mandang et al. (2016). Martharini and Indratiningsih (2017) suggested that the higher content of *Lactobacillus acidophilus* FNCC 0051 decreased the fat contained in milk due to the development of lactic acid bacteria during the fermentation process which produces lipase enzymes to break down fat. Kinteki et al. (2018) observed that the longer the fermentation, the higher the color aspect assessment from the panelists for goat milk kefir. Moreover, the physicochemical characteristics, microbiology, and sensory of goat-milk kefir were affected by the dose of kefir grain (Sulmiyati et al., 2019). With regards to the sensory aspect, a study conducted by Wulansari et al., (2022) indicated that the addition of 16% oat milk and 4% of *Lacticaseibacillus casei* AP resulted in improved sensory aspects of kefir products.

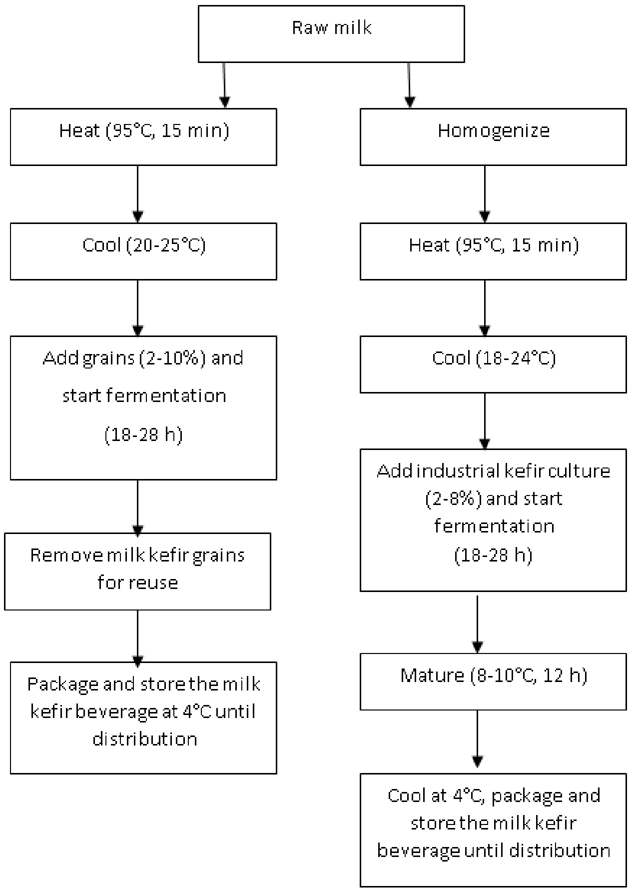

Figure 2. The process of kefir-making

Next is the assessment of the aroma aspect of goat milk kefir. This aspect assessment determines the panelists' acceptance of goat milk kefir. The aroma of goat's milk kefir is influenced by the aroma of goat's milk and yeast contained in kefir grains. The yeast activity in kefir grains during the fermentation process generates acetaldehyde, diacetyl, acetic acid, and other acids that can affect the aroma of kefir (Rahman et al., 1992; Harun et al., 2013). In the research of Kinteki et al. (2018) on the effect of fermentation time on goat's milk kefir, the panelists most liked kefir with 36 hours of fermentation and did not like the aroma of kefir with 48 hours of fermentation. The longer the fermentation, the more yeast activity in goat's milk, resulting in a sour aroma. Then the research on the temperature and storage time of kefir by Setyawardani et al. (2017) showed that storage time and storage temperature did not affect the aroma of kefir. The highest assessment was in the 30-day storage treatment which had a characteristic sour aroma and no musty or goat smell. While in the study of Wulansari et al. (2022), the combination of adding wheat milk (8,12, and 16%) and the addition of *L. casei* AP (2% and 4%) did not affect the aroma of goat's milk kefir.

Regarding the taste, Kinteki et al. (2018) found that the duration of fermentation of goat's milk kefir shows that the length of fermentation affects the panelist's assessment of the taste of kefir. In this aspect, kefir with 36 hours of fermentation time was favored by panelists because of its sour taste. The sour taste in kefir is produced by organic acids during the fermentation process. Storage of goat kefir for 20 days also did not significantly affect the taste of goat kefir, goat kefir has a distinctive yeast sour taste (Setyawardani et al., 2017). While the addition of kefir grains in the research of Sulmiyati et al. (2018) the addition of 2% kefir grains produces a non-acidic aroma, the addition of 4% kefir grains produces a slightly sour aroma, and the addition of 6% kefir grains produces an acidic aroma.

The last aspect is the texture of the goat's milk kefir, particularly the thickness. The viscosity of milk kefir is influenced by the viscosity of the kefir solution itself. Research conducted by Kinteki et al. (2018) indicated that the length of fermentation significantly affects the viscosity of the kefir produced. The highest viscosity preferred by the panelists was in the 24-hour fermentation treatment. The research of Setyawardani et al. (2017) observed that the storage of kefir for 20 days is the most preferred treatment by panelists and produces kefir with a smooth texture. In the study of Wulansari et al. (2022) with the addition of wheat milk and starter *L. casei* AP, the structure of goat milk kefir increased. In this study, it was proven that the addition of wheat milk caused the addition of total solids to goat milk kefir. Furthermore, in Rusdhi et al. (2021) research, the hedonic texture score of goat milk kefir increased with the length of the fermentation process (48-, 60-, and 72 hours). Lactic acid bacteria in starters also affect the composition, texture, and composition of kefir such as *Lactobacillus Bulgaricus* and *Streptococcus thermophilus* (Irigoyen et al., 2003; Rusdhi et al., 2021).

Conclusion

According to the above-mentioned information, it can be concluded that the application of biotechnology during the production of derivatives of dairy products would be a great potential to improve the sensory quality attribute for the end product. The derivatives of dairy products, especially cheese, yogurt, and kefir from which the biotechnological principles apply during the production steps indicated a better sensory attribute, hence it can be used as a potential solution to meet consumers' expectations.

Acknowledgment

This work was financially supported by the Department of Food Technology, Faculty of Engineering, University of Pembangunan Nasional "Veteran" Jawa Timur, Indonesia.

References

Amiri, S., Aghamirzaei, M., Mostashari, P., Sarbazi, M., Tizchang, S., & Madahi, H. (2021). The impact of biotechnology on dairy industry. In *Microbial biotechnology in food and health*, 53-79. https://doi.org/10.1016/B978-0-12-819813-1.00003-7

Balia, R. L., Chairunnisa, H., Rachmawan, O., & Wulandari, E. (2011). Derajat keasaman dan karakteristik organoleptik produk fermentasi susu kambing dengan penambahan sari kurmayang diinokulasikan berbagai kombinasi starter bakteri asam laktat. *Jurnal Ilmu Ternak 11*(1), 49-52. Doi : https://doi.org/10.24198/jit.v11i1.411

Budiman, S., Hadju, R., Siswosubroto, S. E., & Rmbet, G. D. G. (2017). Pemanfaatan enzim rennet dan *Lactobacillus plantarum* YN 1.3 terhadap pH, *curd*, dan total padatan keju. *ZOOTEC 37*(2), 321-328. Doi: https://doi.org/10.35792/zot.37.2.2017.16139

Capcanari, T., Chirsanova, A., Covaliov, E., & Siminiuc, R. (2021). Development of Lactose Free Yogurt Technology for Personalized Nutrition. *Food and Nutrition Sciences*, *12*(11), 1116-1135.

Castada, H. Z., Hanas, K., & Barringer, S. A. (2019). Swiss cheese flavor variability based on correlations of volatile flavor compounds, descriptive sensory attributes, and consumer preference. *Foods, 8*(2), 78. https://doi.org/10.3390/foods8020078

Coker, J. A. (2016). Extremophiles and biotechnology: current uses and prospects. *F1000 Research*, *5*, 1-5. https://doi.org/10.12688/f1000research.7432.1

Deng, Y., Misselwitz, B., Dai, N., & Fox, M. (2015). Lactose intolerance in adults: biological mechanism and dietary management. *Nutrients, 7*(9), 8020-8035. https://doi.org/10.3390/nu7095380

El Sheikha, A. F., & Montet, D. (2014). *African fermented foods: historical roots and real benefits, in Microorganisms and Fermentation of Traditional Foods*. eds. R. C. Ray and D. Montet (Boca Raton, Florida, USA: Science Publishers Inc. and CRC Press), 248–282.

Fox. P. F., Guinee. T. P., Cogan. T. M., McSweeney. P. L. H., Fox. P. F., Guinee. T. P., Cogan. T. M., & McSweeney. P. L. H. 2017. *Pathogens in Cheese and Foodborne Illnesses*. In Fundamentals of Cheese Science.

Garofalo, C., Ferrocino, I., Reale, A., Sabbatini, R., Milanovi´c, V., Alki´c-Subaši´c, M., Boscaino, F., Aquilanti, L.., Pasquini, M., Trombetta, M.F., et al. (2020). Study of kefir drinks produced by backslopping method using kefir grains from Bosnia and Herzegovina: Microbial dynamics and volatilome profile. *Food Research International, 137*, 1-15. https://doi.org/10.1016/j.foodres.2020.109369

Gavrilova, N., Chernopolskaya, N., Rebezov, M., Moisejkina,D., Dolmatova, I., Mironova, I., Peshcherov, P., Gorelik, O., & Derkho, M. (2019). Advanced biotechnology of specialized fermented milk products. *International Journal of Recent Technology and Engineering, 8*(2), 2718-2722. Doi: 10.35940/ijrte.B3158.078219

Gupta, V., Sengupta, M., Prakash, J., Tripathy, B. C. (2017). *Basic and Applied Aspects of Biotechnology*. Springer

Guzel-Seydim, Z. B., Gokırmaklı, Ç., & Greene, A. K. (2021). A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. *Trends in Food Science & Technology, 113*, 42-53. https://doi.org/10.1016/j.tifs.2021.04.041

Hanum, E. A. R., Yulistiani, R., & Sarofa, U. (2022). Utilization of fruit extract as acidulant on physicochemical and sensory characteristics of cottage cheese with addition calcium chloride. *Asian Journal of Applied Research for Community Development and Empowerment, 6(2),* 1-7. https://doi.org/ 10.29165/ajarcde.v6i2.95

Harun, N., Rahmayuni, & Y. E. Sitepu. 2013. Penambahan gula kelapa dan lama fermentasi terhadap kualitas susu fermentasi kacang merah (*Phaesolus vulgaris* L.). *SAGU, 12*(2), 9-14.

Ibarra, A., Acha, R., Calleja, M. T., Chiralt-Boix, A., & Wittig, E. (2012). Optimization and shelf life of a low-lactose yogurt with *Lactobacillus rhamnosus* HN001. *Journal of Dairy Science*, *95*(7), 3536-3548. http://dx.doi.org/10.3168/jds.2011-5050

Irigoyen, A., Arana, I., Castiella, M., Torre, P., & Ibanez, F. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry, 90*(4), 613-620. https://doi.org/10.1016/j.foodchem.2004.04.021

John, S. M., & Deeseenthum, S. (2013). Properties and benefits of kefir. A review. *Songklanakarin Journal of Science and Technology, 37*(3), 275-282.

Kárnyáczki, Z., & Csanádi, J. (2017). Texture profile properties, sensory evaluation, and susceptibility to syneresis of yoghurt prepared from lactose-free milk. *Acta Alimentaria*, *46*(4), 403-410. http://dx.doi.org/10.1556/066.2016.0018

Kesenkas, H., Gürsoy, O., & Özbas, H. (2017). *Kefir. Fermented Foods in Health and Disease Prevention*. Academic Press: Cambridge, MA, USA, pp. 339–361.

Krishnan, K., Campbell, Y. L., To, K. V., Lima, G., Byron, M. D., Zhang, X., et al. (2019). Effects of temperature, relative humidity, and protective netting on *Tyrophagus putrescentiae* (schrank) (Sarcoptiformes: Acaridae) infestation, fungal growth, and product quality of cave-aged Cheddar cheese. *J. Stored Prod. Res., 83*, 44–53. doi: 10.1016/j.jspr.2019.05.014

Kinteki G. A., Rizqiati, H., & Hintono, A. (2018). Pengaruh lama fermentasi kefir susu kambing terhadap mutu hedonik, total bakteri asam laktat (BAL), total khamir, dan pH. *Jurnal Teknologi Pangan, 3*(1), 42-50. https://doi.org/10.14710/jtp.v3i1.20685

Kuddus, M. (2018). Cold-active enzymes in food biotechnology: An updated mini review. *Journal of Applied Biology and Biotechnology*, *6*(3), 5-3.

Kuddus, M., & Ramteke, P. W. (2012). Recent developments in production and biotechnological applications of cold-active microbial proteases. *Critical Reviews in Microbiology*, *38*(4), 330-338. Doi: 10.3109/1040841X.2012.678477

Mandang, F. O., Dien, H., & Yelnetty, A. (2016). Aplikasi penambahan konsentrasi susu skim terhadap kefir susu kedelai (*Glycine Max Semen*). *Jurnal Ilmu dan Teknologi Pangan, 4*(1), 9-17.

Martharini, D., & I. Indratiningsih. 2017. Kualitas mikrobiologi dan kimiawi kefir susu kambing dengan penambahan *Lactobacillus acidophilus* FNCC 0051 dan tepung kulit pisang kepok (musa paradisiaca). *Jurnal Agritech., 37*(1), 22-29. https://doi.org/10.22146/agritech.17002

Neviani, E., Bottari, B., Lazzi, C., & Gatti, M. (2013). New developments in the study of the microbiota of raw-milk, long-ripened cheeses by molecular methods: the case of grana Padano and Parmigiano Reggiano. *Front. Microbiol., 4,* 36. doi: 10.3389/fmicb.2013.00036

Pawlak-Szukalska, A., Wanarska, M., Popinigis, A. T., & Kur, J. (2014). A novel cold-active β-d-galactosidase with transglycosylation activity from the Antarctic *Arthrobacter* sp. 32cB–Gene cloning, purification, and characterization. *Process Biochemistry*, *49*(12), 2122-2133. http://dx.doi.org/10.1016/j.procbio.2014.09.018

Pawlos, M., Znamirowska, A., Kluz, M., Szajnar, K., & Kowalczyk, M. (2021). Low-lactose fermented goat milks with *Bifidobacterium animalis* ssp. *lactis* Bb-12. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(4), 751-755. https://doi.org/10.15414/jmbfs.2020.9.4.751-755

Pratiwi & Hidayati, R. (2015). Peranan Bioteknologi dalam Mengatasi Multikrisis. *Faktor Exacta, 3*(2), 158-166.

Rahman, A., S. Fardiaz, W.P. Rahaju, Suliantari, & Nurwitri, C. C. (1992). Bahan Pengajaran Teknologi Fermentasi Susu. Pusat Antar Universitas Pangan dan Gizi. Institut Pertanian Bogor

Rossi E, Hamzah, F., & Febriyani. (2016). Perbandingan susu kambing dan susu kedelai dalam pembuatan kefir. *Jurnal Peternakan Indonesia. 18*(1). 13-20.

Rusdhi, A., Julianti, E., & Tafsin, M. (2021). Microbiological and organoleptic test of kefir from the balance of goat milk and cow milk with different fermentation time. *International Conference on Agriculture, Earth, and Environmental Science, 782*, 1-7.

Santiago-López, L., Aguilar-Toalá, J. E., Hernández-Mendoza, A., Vallejo-Cordoba, B., Liceaga, A. M., & González-Córdova, A. F. (2018). Invited review: bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. *Journal of Dairy Science, 101*, 3742–3757. https://doi.org/10.3168/jds.2017-13465

Sarkar, S. (2007). Potential of kefir as a dietetic beverage—A review. *British Food Journal, 109*(4), 280–290. https://doi.org/10.1108/00070700710736534

Schmidt, C., Mende, S., Jaros, D., & Rohm, H. (2016). Fermented milk products: effects of lactose hydrolysis and fermentation conditions on the rheological properties. *Dairy Science & Technology*, *96*(2), 199-211. https://doi.org/10.1007/s13594-015-0259-9

Setyawardani, T., Sumarmo, J., Rahardjo, A. H. D., Sulistyowati, M., & Widayaka K. (2017). Kualitas kimia, fisik dan sensori kefir susu kambing yang disimpan pada suhu dan lama penyimpanan berbeda. *Buletin Peternakan, 41*(3), 298-306. https://doi.org/10.21059/buletinpeternak.v41i3.18266

Sulmiyati, Said, N. S., Fahrodi, D. U., Malaka, R., & Maruddin, F. (2019). The physicochemical, microbiology, and sensory characteristics of kefir goat milk with different levels of kefir grain. *Tropical Animal Science Journal. 42*(2), 152-158. https://doi.org/10.5398/tasj.2019.42.2.152

Skryplonek, K., Gomes, D., Viegas, J., Pereira, C., & Henriques, M. (2017). Lactose-free frozen yogurt: Production and characteristics. *Acta Scientiarum Polonorum Technologia Alimentaria*, *16*(2), 171-179. https://doi.org/10.17306/J.AFS.2017.0478

Talib, N., Mohamad, N. E., Yeap, S. K., Hussin, Y., Aziz, M. N. M., Masarudin, M. J., Sharifuddin, S.A., Hui, Y.W., Ho, C.L., & Alitheen, N. B. (2019). Isolation and characterization of *Lactobacillus* spp. from kefir samples in Malaysia. *Molecules,* 24 (2606), 1-18. https://doi.org/10.3390/molecules24142606

Utami, M. M. D., Pantaya, D., Subagja, H., Ningsih, N., & Dewi, A. C. (2020). Teknologi pengolahan yoghurt sebagai diversifikasi produk susu kambing pada kelompok ternak Desa Wonoasri Kecamatan Tempurejo Kabupaten Jember. *PRIMA: Journal of Community Empowering and Services*, *4*(1), 30-35. https://doi.org/10.20961/prima.v4i1.39531

Voigt, D. D., Chevalier, F., Donaghy, J. A., Patterson, M. F., Qian, M. C., & Kelly, A. L. (2012). Effect of high-pressure treatment of milk for cheese manufacture on proteolysis, lipolysis, texture, and functionality of Cheddar cheese during ripening. *Innovative Food Sci. Emerg. Technol., 13*, 23–30. Doi:10.1016/j.ifset.2011.10.004

Wulansari, P. D., Widodo, Sunarti, & Nurliyani. (2022). Incorporation of oat milk with probiotic *Lacticaseibacillus casei* AP improves the quality of kefir produced from goat milk. *Food Science Technology, 42*(1), 1-10. https://doi.org/10.1590/fst.10322

Zheng, X. J., Liu, F., Shi, X., Wang, B., Li, K., Li, B., et al. (2018). Dynamic correlations between microbiota succession and flavor development involved in the ripening of Kazak artisanal cheese. *Food Res. Int., 105*, 733–742. doi: 10.1016/j.foodres.2017.12.007

Zheng, X., Xuewei, S., & Wang, B. (2021). A review on the general cheese processing technology, flavor biochemical pathways and the influence of yeasts in cheese. *Frontiers in Microbiology 12*,703284. https://doi.org/10.3389/fmicb.2021.703284