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Characterization on Safety Risks of Commercial Cheese in Indonesia

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Abstract— Most cheeses with water activity above 0.85 and a pH above 4.6 are classified as low-acid foods. They should be distributed and stored on a cold chain when packaged hermetically to prevent the growth of *C. botulinum spores*. This study aimed to profile cheese and characterize its risks. Data of cheeses with a registration number were obtained from The Indonesian Food and Drug Authority while data of cheeses without a registration number were obtained through a survey in supermarkets, e-commerce, and social media from November 2023 to February 2024. The number of marketed cheeses in Indonesia is 600, with 464 cheeses having a registration number. The representative 86 samples out of 600 commercial cheese products were analyzed for their water activity, pH, and moisture content. The analysis revealed that ripened cheese had the lowest water activity, moisture content, and pH among the cheeses. Research has shown that 93% of cheese samples are low-acid foods. The results of the risk category showed that 7% of cheeses were low risk, 70% were medium risk, and 23% were at high risk of *Clostridium botulinum* spore growth.

Keywords- Cheese, pH, profile, registration number, water activity

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I. INTRODUCTION

Cheese, a derivative of concentrated milk fat and protein, is acidified during the manufacturing process and has a longer shelf life than milk [1]. Cheese is made by thickening or coagulating milk, aided by rennet enzymes or specific bacteria. The resulting coagulum is further processed through drying and preservation [2]. Food stability and safety are determined by water activity (a_w) and acidity (pH) in food because they are prone to spoilage by microorganisms if they have high a_w and pH. Molds, yeasts, and bacteria can use water that is not bound to food to grow and produce toxins. pH measurement is necessary to determine the acidity level of food because microorganisms favor low-acid foods. Therefore, a_w and pH are two crucial parameters for food safety [3].

Clostridium botulinum, a gram-positive spore that generates botulinum neurotoxin, is a major concern for the food industries due to its role in foodborne Botulism. It's important to understand that pasteurization is often ineffective in removing C. botulinum spores from food due to their strong heat tolerance. However, refrigeration can effectively control C.

botulinum spores, which have a minimum growth temperature of 10°C [4].

Between 1912 and 1951, six botulism outbreaks were documented in California and New York, primarily linked to cottage cheese. One of these outbreaks happened in Albany, New York, in 1914 and killed three people. It was one of the first known epidemics caused by *C. botulinum* type B. In October 1993, eight cases were reported after eating a potato loaded with pork and commercial cheese sauce. In August 1996, an outbreak of Botulism in Italy killed eight people after they ate mascarpone cheese, either alone or as part of tiramisu [5]. In 2018, 10 cases of Botulism occurred due to nacho cheese sauce in California, USA, and caused one person to die. One case of Botulism occurred in Iran after consuming local cheese in 2019 [6].

According to Indonesian Food and Drug Authority (Indonesian FDA) Regulation Number 27 of 2021, regarding the requirements for low-acid packaged foods, foods with a water activity (a_w) value of more than 0.85 and an acidity level (pH) of more than 4.6 are classified as low-acid foods. Low-acid foods must meet the requirements of commercially sterilized foods when stored at room temperature. The products must go

through a cold chain distribution if they do not meet these requirements to prevent the growth of *C. botulinum* spores [7].

Commercially sterilized foods are those that have undergone sterilization processes as preservation technology. There are some preservation technology processes: heat sterilization process, non-heat sterilization process combination with heat or non-heat sterilization process without heat, and hurdle technology [7].

Hurdle technology is a preservation technology aimed at extending the shelf life of food and has become an alternative preservation technology process that can be applied to cheese because heat sterilization will deteriorate the cheese [8, 9]. Hurdle technology applicable to the cheese-making process includes the addition of salt, commonly referred to as the salting process; lowering the pH using acidity regulators or the addition of lactic acid bacteria; and reducing the water activity (a_w) and moisture content through ripening processes [1]. These three factors are crucial considerations in cheese profile. Research on the profile and characterization of the safety risks of marketed cheese in Indonesia has not been conducted; therefore, this research aims to map the cheese profiles and characterize the safety risks of marketed cheese products in Indonesia.

II. MATERIAL AND METHODS

A. Material

The materials used in this study were fresh cheese, ripened cheese, whey cheese, processed cheese, cheese analog, and whey protein cheese. Cheese products are obtained from supermarkets, minimarkets, and e-commerce platforms. The tools used for collecting data on cheese products are a laptop, search engine (Google Chrome), Microsoft Office (Excel and Word), and market research platform (Innova). The analytical tools used for this research were an analytical balance (Mettler Toledo MS204TS, USA), an oven (Binder FD-115, Germany), a pH meter (Eutech, Germany), a water activity meter (Aqualab TDL, USA), aluminum cups, beakers, a spatula, and a desiccator.

B. Data collection of commercial cheese products

Data collection for cheese products with a registered number was obtained from the Food Registration Division of the Indonesian Food and Drug Authority. The cheese products selected were registered by companies from November 2018 to November 2023 through communication by e-mail. The commercial cheese products requested from the Indonesian FDA are fresh, ripened, whey, processed, cheese analog, and whey protein cheese. Verification of marketed cheese products was obtained by conducting market surveys on each listed on the Indonesian FDA cheese product list. Market surveys are conducted directly in supermarkets or minimarkets, searching through e-commerce, social media platforms, and the Innova market research platform. Data on cheese products without a registration number are obtained from market surveys through e-commerce and social media. Data integration combines data from registered marketed cheese products with and without a registration number. Data about storage temperature for each sample is taken from the packaging of cheese products.

C. Determination of cheese samples

The data on marketed cheese products were further classified into six categories of cheeses based on the Indonesian FDA regulation number 13 of 2023. The number of samples taken from the data of marketed cheese products was calculated using the Slovin formula with a sampling error rate of 10% or a confidence level of 90%. The Proportionate Stratified Random Sampling technique determined the samples for each cheese category. Samples for each category were randomly taken from the population to analyze water activity, acidity level (pH), and moisture content with three replications (triplicate).

D. Parameters

Water Activity Analysis

Water activity was measured using an a_w meter (Aqualab TDL, USA). The instrument was standardized using standard solutions with water activity values of 0.760, 0.984, and 1.000. samples were placed in sample containers, and measurements were taken at a temperature of 25°C [10] (González et al., 2018).

pH Analysis

pH was measured using a pH spear (Eutech, Europe). The instrument was standardized using buffer solutions of pH 4, 7, and 10. The cheese samples were cut into square shapes, and the pH meter electrode was rinsed with distilled water, dried, and inserted into the cheese sample until the pH value stabilized [2].

Moisture Content Analysis

The wet basis moisture content was measured by weighing 2 g of cheese sample, placing it in an aluminum cup, and drying it in an oven at 105° C for 6 h [11]. The amount of water is calculated by subtracting the dry weight from the initial weight. Moisture content was calculated using the following formula:

%Moisture Content =
$$\left(\frac{The amount of water(g)}{initial sample(g)}\right) x 100\%$$

E. Data Analysis

Data analysis was conducted descriptively using Microsoft Excel. The cheese product mapping profile was created based on registration status and food categories. In contrast, the characterization of cheese product safety risks was grouped based on water activity (a_w), pH, and cheese product storage temperature. The information about storage temperature recommendations is taken from the cheese product packaging. Cheese products classified as acidic food groups (pH \leq 4.6; a_w \geq 0.85) have moderate risk when stored at room temperature and low risk when stored at refrigerator temperatures. Cheese products classified as low-acid food groups (pH>4.6; a_w>0.85) have moderate risk when stored at refrigerator temperatures and high risk when stored at room temperatures and high risk when stored at room temperatures.

III. RESULT AND DISCUSSION

A. Mapping Cheese Product Profiles

Data on marketed cheese products in Indonesia were obtained by combining the number of marketed cheese products with and without a registration number. The number of cheeses with a registration number from the Indonesian Food and Drug Authority from November 2018 to November 2023 was 830; however, based on market surveys, 34% of cheese products were no longer marketed (**Figure 1**.).

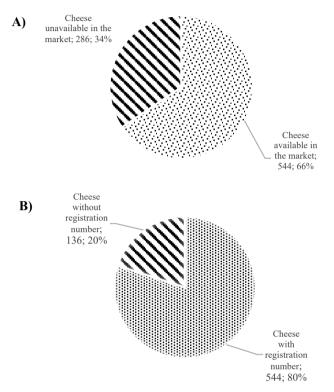


Fig. 1 Proportion of cheese distribution. (A) Distribution status; (B) registration status.

Market surveys were also conducted to collect data on cheese products without a registration number. It was found that 20% of the marketed cheese products in the market were unregistered or did not have a registration number (**Figure 1**.). Some cheese producers registered different registration numbers for different packaging sizes, resulting in 80 cheese products being removed from the data, making the total number of marketed cheeses with a registration number is 464. The number of marketed cheeses without a registration number is 136; therefore, the total number of marketed cheese products in Indonesia is 600. A total of 86 cheese samples were used for laboratory analysis based on the Slovin formula with a confidence level of 90%. There were 80 cheese samples with a registration number and six without a registration number.

Regulation of The Indonesian Food and Drug Authority number 13 of 2023 on food category categorizes cheese into six categories: fresh cheese, ripened cheese, whey cheese, processed cheese, cheese analog, and whey protein cheese [12]. Fresh cheese is produced by milk coagulation. Coagulated milk forms curd and whey. The curd is taken and subjected to a salting or brining process, resulting in ripened cheese; if fresh cheese continues the ripening process, ripened cheese will be produced [7]. Processed cheese is made from fresh or ripened cheese that is melted and emulsified with or without other food additives. The mixture was stirred until homogeneous, packaged, and cooled to 4°C [13]. A cheese analog replaces some or all of the fat with vegetable oil. The whey cheese concentration process produces whey cheese. Whey protein cheese is made from whey protein using the coagulation principle through a heating process [9,11].

Figure 2. shows that ripened cheese is Indonesia's most commonly marketed cheese product. The number of samples for each type of cheese is determined using the proportionate stratified random sampling technique, resulting in the number of each category of cheese taken for sampling as follows: 21 samples of fresh cheese, 38 samples of ripened cheese, 25 samples of processed cheese, 1 sample of whey protein cheese, and 1 sample of cheese analog.

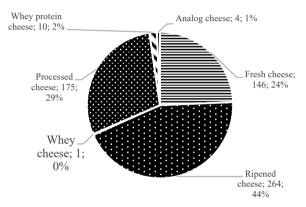


Fig. 2 Marketed cheese products in Indonesia based on the Indonesian FDA categories.

TABLE 1
DISTRIBUTION OF PH, WATER CONTENT, AND WATER ACTIVITY PROFILES
BASED ON CHEESE REGISTRATION STATUS

Pro	file	Total cheese samples (n=86)	Registered Cheeses (n=80)	Unregistered cheeses (n=6)
рН	Average	5.26	5.27	5.11
	Median	5.27	5.28	5.04
	Modus	5.21	4.99	N/A
	Range	4.13-6.38	4.13-6.38	4.86-5.49
Moisture content (%)	Average	45.85	46.54	36.56
	Median	47.01	47.17	38.01
	Modus	N/A	N/A	N/A
	Range	15.07- 76.53	15.07-76.53	20.80-46.99

Water activity	Average	0.961	0.963	0.938
	Median	0.966	0.967	0.956
	Modus	N/A	N/A	N/A
	Range	0.728-	0.728-0.998	0.868-0.969
	c	0.998		

Table 1 shows that the total marketed cheese in Indonesia has an average pH of 5.26, with a pH range of 4.13-6.38. Cheese with a pH value of 4.13 was found in the ripened cheese group, while the highest pH value of cheese was found in the fresh cheese group (**Table 2**). Cheese undergoes physical, chemical, and microbiological changes as it ripens. During the ripening process, the texture of the cheese smooths out, the consistency solidifies, the flavor improves, and the probiotic metabolite Lactic Acid Bacteria (LAB) is optimally produced. Those modifications are the result of LAB interaction in the cheese matrix. The pH of the product tends to drop due to the metabolic activity of probiotic LAB. Lactic acid bacteria break down lactose in cheese and produce lactic acid. Lactic acid resulting from lactose breakdown can lower the pH of the cheese [15]. Lactic acid bacteria also break down proteins and peptides into amino acids during ripening. These amino acids also lower the pH of cheese [16]

 TABLE 2

 DISTRIBUTION OF PH, WATER CONTENT, AND WATER ACTIVITY PROFILES BASED ON CHEESE CATEGORY

Р	Profile	Ripened cheeses (n=38)	Fresh cheeses (n=21)	Processed cheeses (n=25)	Whey protein cheese (n=1)	Cheese analog (n=1)
pН	Average	5.10	5.13	5.56	5.65	5.93
	Median	5.20	5.17	5.56	N/A	N/A
	Modus	5.21	N/A	N/A	N/A	N/A
	Range	4.13-6.13	4.65-6.38	5.22-6.05	N/A	N/A
Moisture content (%)	Average	39.73	52.94	47.55	76.53	0.975
	Median	38.97	49.37	47.03	N/A	N/A
	Modus	N/A	N/A	N/A	N/A	N/A
	Range	15.07-55.71	45.30-69.98	39.61-58.53	N/A	N/A
Water activity	Average	0.945	0.984	0.964	0.994	55.92
	Median	0.956	0.984	0.963	N/A	N/A
	Modus	N/A	N/A	N/A	N/A	N/A
	Range	0.728-0.988	0.973-0,998	0.943 - 0.988	N/A	N/A

The moisture content of the cheese samples varied from 15.07% to 76.53% (**Table 1**). Ripened cheese had the lowest moisture content (15.07 %) because the moisture content decreased during the ripening process (**Table 2**). The longer the ripening process, the lower the moisture content of the cheese [16]. The whey protein cheese had the highest moisture content. The whey protein cheese used in this study was ricotta cheese. **Table 2** shows that ricotta cheese has a high moisture content of 76.53%, making it a soft-textured cheese [17]. Ricotta cheese is produced from whey cheese. Whey was heated to a temperature of $60-70^{\circ}$ C, and milk was added at a ratio of 10%

Adding milk increases the casein content in cheese, making the cheese texture more stable and less prone to crumbling. Heating continues until it reaches a temperature of 85-90°C, after which it is cooled to the coagulation temperature. Subsequently, an acid solution was added to the mixture of whey and milk that had been heated to form curds. The curd was removed, drained overnight, and stored in a refrigerator. Making ricotta cheese does not involve stretching or pressing, resulting in high moisture content [6,10].

The water activity values of the cheese samples varied from 0.728 to 0.998 (Table 1). The cheese with the lowest water activity value was ripened cheese, while the highest was for fresh cheese (Table 2). The water activity of ripened cheese may decrease because the ripening process can reduce the water activity. The longer the ripening time, the lower the water activity value. Ripened cheese also undergoes salting or is soaked in salt. Salting can lower the water activity value because salt can bind free water still present in cheese [19]. Table 1 shows that the pH, moisture content, and water activity values of cheeses without a registration number were lower than those of cheeses with registration numbers because cheeses without a registration number are limited to artisanal cheeses. Artisanal cheeses in Indonesia are still predominantly ripened cheese, while cheeses with a registration number are of various categories, including ripened, fresh, processed, whey protein, and cheese analog. Each type of cheese has different pH, moisture content, and water activity profiles; therefore, in the registered cheese group, the pH, moisture content, and water activity profiles have a more extended range than cheeses without a registration number.

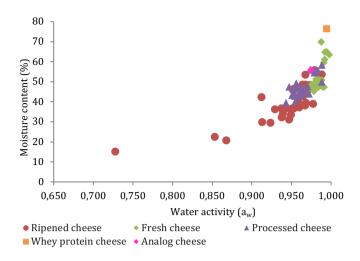


Fig. 3 Mapping of water activity and moisture content of marketed cheese in Indonesia

The distribution pattern of moisture content and water activity of ripened cheese has a wide range because it is influenced by ripening time (Figure 3.). The longer the ripening time, the lower the cheese's moisture content and water activity values [8,11]. Fresh cheese has high water activity and moisture content values because it does not undergo ripening; therefore, it is located in the upper right corner area. Processed cheese is located between ripened and fresh cheese because water and emulsifying salt are added to natural cheese (ripened or fresh cheese) when making processed cheese. These ingredients were stirred, heated at a temperature above 70°C for at least 1 min, put into aluminum foil, pressed, and cooled at 4°C. Adding emulsifying salt to the processed cheese process can reduce the water activity value, so the area of processed cheese is between ripened and fresh cheese [5,12]. The cheese analog in the samples was made from a mixture of cream cheese (fresh cheese) and vegetable oil emulsified with emulsifying salt. The cream cheese, vegetable oil, and emulsifying salt mixture were stirred, heated, and packaged in glass bottles through a hot filling process. Adding emulsifying salt to the cheese analog reduces water activity like processed cheese does. Emulsifying salt can increase the capacity to bind free water so that the water activity value can be reduced and smaller than that of fresh cheese [21]. Whey protein cheese, or ricotta cheese, has high moisture content and water activity values because the process of making ricotta cheese does not go through the ripening process or the addition of emulsifying salt [14].

B. Safety risk of cheese

Regulation of the Indonesian Food and Drug Authority number 27 of 2021 regulates that low-acid processed foods (a_w above 0.85 and pH above 4.6) packaged hermetically should be stored at refrigerator temperatures below 5°C or at room temperature if they meet the requirements of commercial sterile foods to prevent the growth of *Clostridium botulinum* spores. Commercial sterilization processes must undergo process

adequacy validation or pass challenge tests to ensure that the reduction level of *Clostridium botulinum* spores has reached at least 12 log cycles. A challenge test is a microbiological test involving the inoculation of microorganisms into food materials, followed by monitoring of their growth during processing and/or storage [7]. *Clostridium botulinum* spores originate in the environment and can contaminate raw food materials. These bacterial spores grow and produce toxins under conditions of $a_w \ge 0.93$ and pH ≥ 4.6 and resist pasteurization temperatures. The toxin produced is botulinum neurotoxin, which can cause fatal paralysis. *Clostridium botulinum* is a safety indicator in food because these spores are resistant to high temperatures; however, their growth and toxin production can be prevented at refrigerator temperatures [14,15].

A total of 93% of the total samples belong to the low-acid cheese group and are hermetically packed, so it is highly recommended that they undergo cold chain distribution and storage at the refrigerator temperature to reduce the potential growth of C. botulinum. The 75% of the low-acid cheese samples had a moderate risk because they were instructed to be stored at refrigerator temperatures, whereas 25% of low-acid cheese samples were instructed to be stored at room temperature, thus potentially having a high risk of C. botulinum spore growth (Figure 4.). When viewed from the 86 cheese samples or the total cheese samples, 23% of the cheese samples had high-risk potential, 70% had moderate risk potential, and 7% had low-risk potential (Figure 4.). Low-risk cheeses are acidic foods and stored at room temperature conditions. This risk is ideal for inhibiting the germination and growth of C. botulinum.

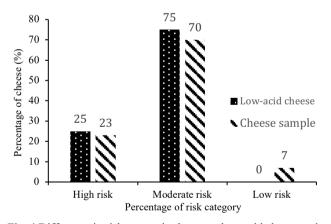


Fig. 4 Difference in risk categories between low-acid cheese and total cheese samples.

Table 3 shows that the acidic food group is only present in the ripened cheese category because the ripened process in cheese can lower the pH value because of the formation of lactic acid and amino acids [15, 16]. Storing low-acid foods at refrigerator temperatures can control germination and growth of *C. botulinum* [22]. Ripened cheese samples included in the low-

acid food category were stored at refrigerator temperatures, thus reducing the risk to moderate levels. The risks of low-acid food fresh and whey protein cheese are the same, with a potential moderate risk due to storage at refrigerator temperatures. The risk of low-acid food processed cheese is divided into high and moderate risks because not all cheeses are stored at refrigerator temperatures. The safety risk of cheese analogs is potentially high because they are stored at room temperature (**Table 3**).

TABLE 3 DISTRIBUTION OF PH, WATER CONTENT, AND WATER ACTIVITY PROFILES BASED ON CHEESE TYPE

Profile	Ripened cheese (n=38)	Fresh cheese (n=21)	Processed cheese (n=25)	Whey protein cheese (n=2)	Cheese analog (n=1)
Acid food	6	0	0	0	0
Low-acid food	32	21	25	1	1
High risk	0	0	19	0	1
Moderate risk	32	21	6	1	0
Low risk	6	0	0	0	0

Besides storage at refrigerator temperatures, one hurdle that can prevent the growth of C. botulinum is the addition of preservatives. Organic acid salts, such as sorbic acid and its salts (potassium and calcium), are highly effective due to their antimicrobial properties, serving as food preservatives in the food industry. These salts inhibit bacterial and mold growth, thus extending the shelf life of food [24]. Organic acids lower pH and create unfavorable conditions for the growth of pathogenic microorganisms. Additionally, the non-ionized form of organic acids can penetrate the cell membrane of the target organism, ionize inside the cell, and lower cytoplasmic pH, thereby preventing microorganisms from surviving [25]. In Indonesia, potassium sorbate and calcium sorbate are permitted for use as food preservatives, but there are maximum limits for their addition that must be followed. Potassium sorbate can be added to fresh cheese and ripened cheese with a maximum addition limit of 1000 mg/kg or 0.1%, while in processed cheese, the maximum addition limit is 3000 mg/kg or 0.3%. Calcium sorbate can only be added to processed cheese with a maximum usage limit of 3000 mg/kg or 0.3% [26]. The organic acid salt that can be added to cheese to inhibit C. botulinum growth is potassium sorbate at a concentration of 2 - 6% [22].

As shown in **Table 3**, there are 20 cheese samples, including 19 processed cheeses and one cheese analog, which have a potentially high risk due to storage at room temperature. Of the 20 cheeses in the high-risk category, 17 processed cheeses used potassium sorbate as a preservative. Based on the Indonesian FDA regulation number 11 of 2019 on food additives, the maximum limit of potassium sorbate preservatives allowed in processed cheese products is 3000 mg/kg or 0.3%. Adding potassium sorbate to these 17 processed cheese samples is still

considered not effective enough because the Indonesian FDA's permissible limit of preservative addition is still below the minimum concentration limit for inhibiting the growth of *C. botulinum* [22, 26]. Several studies show that processed cheese spreads can undergo commercial sterilization at temperatures of 120-122°C for 20-40 minutes after hermetic packaging to enable storage at room temperature.

Commercial sterilization processes are limited to liquid or melted cheese forms such as processed cheese spreads, thus not applicable to block-shaped processed cheeses. After sterilization, processed cheese spreads become firmer, compromised cheese flavor and aroma and darker color due to Maillard reactions and lipid oxidation during commercial sterilization processes [9, 27]. Due to the preservatives added to processed cheeses not being able to suppress the growth of *C. botulinum* and the difficulty of applying commercial sterilization processes, processed cheeses must undergo a cold chain and be stored refrigerated. These ensure the reduction of potential risks and prevent the growth of *C. botulinum*, thereby maintaining the product's safety.

IV. CONCLUSION

The cheese still marketed in Indonesia from December 2023 to January 2024 was 600 products, 464 with a registration number, and 146 without a registration number. Ripened cheese is the most widely marketed cheese in Indonesia and has the lowest water activity, pH, and moisture content profiles compared to other types of cheese. Research has shown that 93% of cheese samples are low-acid foods. Based on activity water, pH, and storage instruction, 7% of cheese samples have the potential for low risk, 70% of cheese samples have the potential for moderate risk, and 23% of cheese samples have the potential for high risk. High-risk cheese samples are processed cheese and cheese analogs marketed in Indonesia.

CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

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