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Influence of seasonal variation on the food safety and shelf-life of homemade dog food from fresh markets in Mueang District, Sakon Nakhon Province

Jakkapat Prachachit [a], Kanokwan Bootyothee [a*], Titinan Hemadhulin [b], Benjamabhorn Pumhirunroj [a] and Apisit Sorin [a]

[a] *Department of Animal Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000, Thailand*

[b] *Department of Food Science and Technology, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000, Thailand*

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Abstract

Home-cooked dog food sold in fresh markets is an alternative for dog owners seeking to avoid commercial dried or canned pet food. Although it is typically boiled and subjected to high heat during preparation, improper storage may lead to spoilage. This study aimed to assess the levels of bacteria, and to determine whether these foods remain microbiologically safe for canine consumption after refrigerated storage, especially when purchased in large quantities, across different seasons and from three fresh market vendors. The study found that all three vendors used leftovers and animal by-products from human consumption, such as the lungs, trachea, bones, blood, and chicken carcasses. During the first day of purchase, the physical characteristics and microbiological quality of the food were acceptable for canine consumption. However, by days 3 and 7 of refrigerated storage, food quality such as smell and texture had declined. Notably, in the rainy and hot seasons, when ambient temperatures were higher, the microbial quality deteriorated significantly, making food unsuitable for dogs. From Day 3 the total microbial counts exceeded the acceptable limit of 1×10^6 CFU g^{-1} for 2 of 3 vendors (6.05±0.03 and 7.12±0.01) in the summer and all vendors in the rainy season (7.32±0.03, 6.25±0.01 and 7.12±0.01), which posed health risks and might lead to gastrointestinal infections. The pathogenic bacteria which found in this study are *Escherichia coli* and coliform bacteria. Health risks are not limited to dogs; pet owners who prepare and handle this food directly may also be exposed to potential pathogens. This study recommends that dog owners purchase only a daily portion of this type of food to reduce spoilage risks, particularly given Thailand's predominantly warm climate.

*** Corresponding author** : kanokwan.b@snru.ac.th
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1. Introduction

The population of companion animals is currently increasing worldwide, resulting in the growth of the pet food market [1]. Dogs are among the oldest raised animals [2]. They are also popular companion pets worldwide, with an estimated 700 million dogs [3]. Despite the wide variety of ready-to-eat food available to dog owners, and the significant expansion of the pet food market [1], some owners still prioritize feeding their animals with home-cooked meals. This preference stems from the belief that home-cooked food offers nutrients that owners can control, a distrust of pet food companies, and the perception that such ingredients are closer to nature than the processed foods produced by animal feed companies [4]. Home-cooked dog foods have gained popularity in the United States, and this trend is on the rise in several other countries, including Australia [5]. There was an increase in the group fed raw animal products compared to the group fed a conventional diet such as heat-processing and commercial diets [6], even though homemade dog food is often more expensive, time-consuming to cook and prepare, and less convenient than commercial dog food [4]. Numerous home-cooked dog food recipes have been subjected to analysis and research [7]. However, it has also been discovered that some of these recipes may present nutritional imbalances from macrominerals, fat-soluble vitamins, including antioxidants, and trace minerals, potassium, copper and zinc were not met the standard of Association of American Feed Control Officials (AAFCO) [8]. Despite this, many pet owners continue to choose this type of food for their dogs, attracted by the freshness and natural quality of the ingredients [9].

Thai markets offer home-cooked dog food for sale, which provides convenience to pet owners at a more affordable price. The main ingredients are blood and animal organs, boiled, and sold as ready-to-eat dog foods. Owners often purchase these foods in bulk to take advantage of discounted prices, store them in a refrigerator, and reheat them for their dogs to eat later. However, the problems associated with homemade dog food arise because Thailand's climate is tropical with various seasons. The food quality is affected by the season. This can cause spoilage of dog foods, which can lead to the transmission of pathogens to humans through dog food [10].

The common practice of purchasing homemade dog food in bulk for refrigerated storage, coupled with Thailand's varied seasonal climate, creates potential risks for food spoilage and the growth of harmful bacteria. Therefore, this study was conducted to investigate the quality and determine the viable shelf-life of this dog food, which is sold in the fresh markets of Mueang District, Sakon Nakhon Province. By assessing food safety across different storage durations and seasons, this study aimed to establish clear guidelines on how long it can be preserved before becoming unsafe for dogs to eat.

2. Materials and Methods

Sample collection

Samples of liquid-base homemade dog food in fresh markets in Mueang District, Sakon Nakhon Province, were randomly selected in each vendor. These foods, which may include meat and offal ingredients or not, were sourced from three different vendors, with three repetitions each, weighing between 500 and 700 g, across three seasons. All samples were packaged in a clear, hot-food-safe plastic bag and secured with a rubber band, similar to the packaging used for ready-to-eat cooked food sold in Thai markets. The three seasons were defined as summer (February-May 2023), rainy (June-October 2023), and winter (November 2023-February 2024) [11]. Samples were collected at the same time, at 6:00 p.m., and same vendors, the environmental temperature and basic physical characteristic data consisting of smell, visual examination, weight and pH were recorded. Sensory evaluation was conducted based on odor and visual appearance. Observations were performed and recorded by three assessors using the same data recording format across all three seasons. The samples were subsequently stored in a refrigerator at 4 ± 2 °C until the date of analysis.

Study on the quality of the preservation of homemade dog food

Over a seven-day period, samples of homemade dog food from each vendor were tested for quality. Evaluations of all parameters were conducted on days 0, 3 and 7 of each season. These parameters encompassed chemical analysis (pH) and microbial analysis, which included the total microbial count, contamination by *Escherichia coli* (*E. coli*) and coliform bacteria, yeast and mold contamination, and *Listeria* spp. contamination. Chemical analysis (pH evaluation) was determined based on the pH values. A benchtop pH meter (Toledo®, Switzerland) was used to measure the pH by immersing the electrode into a 20-ml soup sample. The total microbial count [12] was determined using the pour-plate technique. A 25 g portion of each sample was weighed and homogenized with 225 mL of peptone water. The samples were diluted to a dilution level of 10^{-1}–10^{-6}. The test was performed using PCA agar medium and the sample was incubated at 37 °C for 24-48 h. Results were recorded as log CFU g^{-1}. *E. coli* and coliform bacteria were identified using the most probable number (MPN) method. The initial step involved testing for coliform bacteria using the gas production method, in Durham tubes. The number of gas-positive tubes was counted and compared with the MPN table, and the results were reported in units of MPN g^{-1}. Following the coliform test, a confirmatory test was performed to determine the presence of *E. coli* in the food samples.

Gas-positive tubes from the MPN test were streaked onto EMB agar plates and incubated at 35-37 °C for 24±2 h. Colonies showing positive results appeared as dark purple to red with a metallic sheen, and the presence of *E. coli* was recorded accordingly [13]. Yeast and mold were tested using the pour plate technique. A 25 g of each sample were weighed and mixed with 225 mL of peptone water solution. The sample was diluted to a dilution level of 10^{-1}– 10^{-6}. The test was performed using Potato Dextrose Agar (PDA) medium, and the sample was incubated at 25 °C for 48-72 h. The total number of colonies formed was counted, recorded and reported in units of log CFU g^{-1}. [14]. *Listeria* spp. was determined using the spread plate technique. Each sample (25 g) was aseptically weighed and mixed with 225 mL of peptone water solution. The sample was diluted to a dilution level of 10^{-1}– 10^{-6}. The test was performed using the Oxford agar medium. The samples were incubated at 37 °C for 24 - 48 h. Oxford agar (OXA) (18) (M118): After 24 h incubation typical *Listeria* spp. colonies are approximately 1 mm diameter, gray to black colonies surrounded by a black halo. Following 48 h incubation typical *Listeria* spp. colonies are approximately 2-3 mm diameter, black with a black halo and sunken center [15].

The quantities of bacterial contamination detected in food samples are evaluated according to the Microbiological Quality Standards for Food and Food Contact Surfaces 3rd edition (2017) [16], the guideline of microbiological standards in food (1993) of the Department of Medical Science, Ministry of Public Health, Thailand, as there are no established criteria for pet food to date [17]. The bacterial colony counts using maximum acceptable levels were: bacterial colony count (BCC) (≤ 10^6 CFU g^{-1}), MPN of coliforms (≤500 CFU g^{-1}), MPN of *E. coli* (≤ 3 CFU g^{-1}), and *S. aureus* (≤10^2 CFU g^{-1}), and the absence of pathogenic bacteria [18].

Statistical analysis

The statistical analysis of the collected data involved two primary methods: Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DMRT). ANOVA was employed to determine whether there were statistically significant differences among the group means, providing insights into the overall impact of the experimental factors. Following ANOVA, DMRT was applied to compare individual group means, allowing for a more detailed understanding of the specific differences between the groups. Both analyses were conducted at a 95 percent confidence level to ensure a high degree of statistical reliability of the results.

Fig. 1 Home-made dog food from each vendor.

Table 1 The results of the acidity-alkaline (pH) analysis for each vendor. (mean ± SD of three replicates.)

Vendor	Season	pH		
		Day 0	Day 3	Day 7
1	Summer	6.06 ± 0.05^{b}	6.46 ± 0.12^{d}	6.51 ± 0.01^{d}
	Rainy	5.75 ± 0.04^{a}	6.19 ± 0.12^{bc}	6.17 ± 0.07^{bc}
	Winter	5.76 ± 0.05^{a}	6.12 ± 0.07^{b}	6.27 ± 0.03^{c}
2	Summer	6.64 ± 0.03^{a}	6.77 ± 0.32^{a}	7.14 ± 0.04^{b}
	Rainy	6.62 ± 0.03^{a}	6.73 ± 0.02^{a}	7.09 ± 0.04^{b}
	Winter	6.61 ± 0.03^{a}	6.82 ± 0.01^{a}	7.08 ± 0.15^{b}
3	Summer	6.44 ± 0.03^{abc}	6.67 ± 0.19^{d}	6.91 ± 0.08^{e}
	Rainy	6.35 ± 0.03^{a}	6.43 ± 0.03^{abc}	6.55 ± 0.03^{cd}
	Winter	6.40 ± 0.03^{ab}	6.54 ± 0.03^{bc}	6.54 ± 0.03^{bc}
P-value		< 0.0001	< 0.0001	< 0.0001

Noted: The characters a, b, c, d, e, ab, bc, cd, and abc that differ both vertically and horizontally show statistically significant differences.

3. Results and Discussion

This study investigated the quality and safety of homemade dog food sold in fresh markets, focusing on post-purchase storage in refrigerators during different seasons. People usually purchase cooked food in large quantities to get a lower price and then keep it in refrigerators to slow down bacterial growth [19]. The total bacterial counts were compared within the same vendor across seasons, revealing that the winter season consistently exhibited the lowest bacterial counts among all vendors. When examining the bacterial counts from the day of purchase and during refrigerated storage at 4–6 °C, a significant increase in bacterial counts was observed in all vendors over prolonged storage periods.

This type of dog food is available in fresh markets in Thailand, but its characteristics and ingredients vary from area to area, which is similar to the cooked food available in Thailand, but the ingredients and seasonings are different. Moreover, dog owners in Thailand are familiar with feeding their dogs this type of food, which is popular because it is similar to cooked food sold to humans. In some countries, there are restrictions on the production of dog food for sale, as production standards and permits are required [20]. Therefore, this type of food may not be available in other countries. It is important to note that the homemade dog food studied here may not represent the more commonly available forms of dog food, as this preparation method reflects a regional culinary tradition unique to certain areas. However, it has been observed that dog owners are increasingly preparing homemade meals for their pets [21 - 24]. Therefore, this type of dog food has gained interest.

Environmental data

Environmental temperature data were recorded on the day of food purchase (D0) for each season as follows: summer, with an average temperature of 30.5±1.60 °C; rainy season, with an average temperature of 28.97±0.37 °C; and winter, with an average temperature of 23.85±1.42 °C.

Physical analysis

A survey of seasonal dog food vendors revealed that all three vendors had slightly different ingredients. The main ingredients of vendors No.1 and No.2 were cow lungs without a bad smell. The total weight was 613.22±27.98 and 647.22±17.77 g, respectively. Vendor No.2 also added some herbs, such as lemongrass, to the soup. The byproducts from chicken grills, such as chicken bone and skin, were the main materials used by vendor No.3. The total weight of the food from this vendor was 568.56±17.53 g. The ingredients used in each season were not significantly different. The soup weight was higher than the content weight in all samples. The appearance of each product is shown in Fig. 1.

Chemical analysis (pH evaluation)

The results of the chemical analysis are shown in Table 1. The results indicated that the pH of the food increased with prolonged storage, which was associated with a rise in the number of detected microorganisms. This increase in pH may be attributed to the activity of various bacteria, including members of the Enterobacteriaceae, which can produce alkaline compounds such as ammonia. The accumulation of these compounds contributes to the progressive alkalinization of stored muscle tissues, resulting in an elevated pH [25]. Additionally, protein breakdown was observed, resulting in the production of foul odors due to the release of hydrogen sulfide gas, which is commonly referred to as a "rotten egg" smell [26]. An increase in pH during the spoilage of meat-based soup is commonly observed as a result of microbial and biochemical processes involved in protein degradation [27].

Sensory analysis

The sensory analysis focused on appearance and odor through visual and olfactory observation. Vendor 1 primarily used bovine offal as the main ingredient. On the first day of purchase, the food remained warm, with a normal appearance and no detectable off-odor. However, on days 3 and 7 of refrigerated storage, changes were observed; solidified fat appeared in the liquid components. This occurred because the fat naturally presents in the food solidified at cold temperatures, aligning with the physical properties of fat. Despite these changes, no off-odors or spoilage smells were detected throughout the storage period across all seasons.

Vendor 2 also used mostly bovine offal, similar to Vendor 1, but added lemongrass to introduce an herbal aroma. On the first day of purchase in all seasons, lemongrass scent was still noticeable. However, from days 3 to 7, the herbal aroma disappeared. In terms of odor, food stored during the summer developed a fishy or meaty off-smell, whereas those stored during the rainy and winter seasons did not exhibit such odors.

Vendor 3 primarily used chicken-based ingredients. Across all seasons, a consistent pattern was observed: the food had a noticeable off-smell, even on the first day of purchase, which intensified over time and eventually resembled a strong putrid odor indicative of spoilage. In both the summer and winter, the broth appeared cloudy. Additionally, package swelling was observed during the summer. The surviving microorganisms likely multiplied during storage, leading to protein decomposition and the production of hydrogen sulfide gas, contributing to the foul and rotten odors. Moreover, it was also clearly observed that there was a change in the shape of the food packaging bag, which was swollen due to gas generation inside the bag [28] as depicted in Fig. 2.

Fig. 2 The shape of the food packaging bag from vendor 3 was swollen on Day 7.

Microbiological analysis

To determine if homemade dog food was safe for consumption, its microbial load was measured against the safety threshold set by the Microbiological Quality Standards for Food and Food Contact Surfaces, 3rd edition (2017) [16] due to there being no strict regulations on maximum limits of particular bacterial and fungal contaminations for pet foods [17]. According to the guidelines, the total microbial count in ready-to-eat food must remain below 1×10^6 CFU g^{-1} to be considered safe. According to our results, in the summer season, on the day of purchase, all three vendors met the standard. However, after storage for 3 and 7 days, vendors 1 and 3 showed increased total microbial counts exceeding the standard, whereas vendor 2 failed to meet the standard only on day 7. In the rainy season, all three stores met the standards on the first day of purchase. However, after 3 and 7 days of storage, none of the stores met the standards. In the winter season, all three vendors complied with the standard from days 1 through 7 of storage. These findings highlight seasonal influences on microbial safety, with winter conditions being the most favorable for maintaining microbial counts within acceptable limits during refrigerated storage. This may be attributed to the initial production process during the winter season, where lower ambient temperatures result in fewer microorganisms contaminating the raw materials. Additionally, homemade dog food is typically boiled until it reaches the boiling point, which serves as a partial sterilization step to reduce the microbial load. When stored under refrigeration for 7 days, this combination helped maintain lower microbial counts compared to other seasons.

Based on the findings from vendor 1, it was found that during the winter season, home-cooked dog food had the lowest level of surviving microorganisms ($P \leq 0.05$). On the first day of purchase, the microbial count was lowest at 1 log CFU g^{-1}. In contrast, during the summer and rainy seasons, the microbial counts on the first day were higher, with summer showing a greater count than the rainy season at 4.88 and 5.90 log CFU g^{-1}, respectively. After storage for 3 and 7 days, the total microbial count increased significantly ($P \leq 0.05$). Notably, during the rainy season, the microbial count was higher than that in both the summer and winter seasons, reaching as high as 8.05 log CFU g^{-1} on day 7.

Similar patterns to those observed for vendor 1 were seen for vendor 2. During the winter season, the total microbial count on the first day of purchase was the lowest (1.00 log CFU g^{-1}), and increased with extended storage time, reaching 4.58 log CFU/g on day 7. In the rainy season, the initial microbial counts were lower than those in the summer, at 3.51 and 3.79 log CFU g^{-1}, respectively. However, with prolonged storage, the microbial count in the rainy season surpassed that of other seasons, reaching 6.25 and 6.71 log CFU g^{-1} on days 3 and 7, respectively.

The results from vendor 3 revealed that on the first day of purchase, microbial contamination was high across all seasons. Due to the vendor 3, the ingredients used were chicken carcasses, which were scraps left over from grilling chicken in each day. This was evident from the fact that some pieces of chicken were burnt from grilling, whereas the first and second vendors which used leftover ingredients from butchering cows, thus making their ingredients fresher. The lowest level was found in the rainy season at approximately 3.54 log CFU g^{-1}, whereas the levels in winter and summer were approximately 3.88 and 4.32 log CFU g^{-1}, respectively ($P \leq 0.05$). As the storage duration increased, the microbial counts also increased. Overall, on days 3 and 7, winter samples tended to have a lower total microbial count than those of the rainy and summer seasons, with values ranging from approximately 4.70 to 5.33 log CFU g^{-1} ($P \leq 0.05$). These values were significantly different from those in the rainy and summer seasons, which ranged from approximately 7.12 to 7.50 log CFU g^{-1}. No statistically significant differences were observed between the rainy and summer seasons ($P > 0.05$) as showed in Table 2.

In contrast, during the summer, all three vendors exhibited the highest initial microbial counts. However, after 3 and 7 days of storage, microbial growth was even greater in the rainy season samples. This finding is consistent with the study of Ruangsombat *et al*. [29], which reported that bacterial contamination in water sources increases during the rainy season, particularly when rainfall is high. This could be due to the presence of microbial species during the rainy season that are more capable of thriving at lower temperatures. Consequently, even when homemade dog food is refrigerated, spoilage can occur more rapidly, resulting in higher microbial loads than those observed during the summer.

***Escherichia coli* and coliform bacteria**

According to the MPN method analysis for the identification of *E. coli* and coliform bacteria (Table 3), the findings indicated that all three vendors exhibited similar patterns. During the winter season, the levels of coliform and fecal coliform bacteria were lower than those during the summer and rainy seasons. Both coliform and fecal coliform bacteria were detected during the summer and rainy seasons. On the first day of examination, bacterial counts were low, but increased as the duration of storage of homemade dog food increased, with the highest recorded level exceeding 1100 MPN g^{-1}.

All three vendors were affected by *E. coli* contamination during the summer and rainy seasons. In alignment with the standards, which stipulate that *E. coli* levels must be below 3 MPN. The results showed that homemade dog food produced during the winter season at all three stores met the standard and tested negative for *E. coli*. However, during the summer and rainy seasons, the products did not meet the standard and tested positive for *E. coli*. *E. coli* is one of the most significant pathogens in this context, as it can infect both humans and animals [30]. In dogs, several studies have reported gastrointestinal infections caused by *E. coli*, leading to enteritis, a condition that can range in severity and may even result in death [30 - 33].

This discrepancy may be due to the fact that, in winter, the raw materials were either free from or had minimal contamination with coliform bacteria, allowing the heat treatment during food preparation to effectively eliminate these bacteria. Coliform bacteria are a group of gram-negative, rod-shaped, non-spore-forming bacteria [34, 35]. They are facultative anaerobes, meaning that they can grow in both the presence and absence of oxygen [34]. Coliforms are capable of fermenting lactose to produce acids and gases within 48 h at 35–37°C. They are heat-sensitive and can be easily destroyed by pasteurization. Fecal coliforms are a subset of coliform bacteria found specifically in the feces of warm-blooded animals, as they inhabit the large intestine. This group includes bacteria such as *Escherichia* spp. [36]. Conversely, in the summer and rainy seasons, higher ambient humidity and temperatures may have contributed to increased coliform contamination and provided more favorable conditions for bacterial growth. The rapid proliferation of these bacteria, combined with potentially insufficient sterilization during the homemade dog-food production process, allowed some coliform bacteria to survive. The experimental results showed that coliform and fecal coliform bacteria were detected from the first day during the summer and rainy seasons. This indicated that the distribution of these bacteria was more prevalent from the outset during the rainy and summer seasons.

Yeast and mold

The yeast and mold count throughout the 7-day storage period showed that vendor 2 consistently had yeast levels below 1 log CFU g^{-1} across all three seasons (Table 4). Vendors 1 and 3 exhibited similar patterns; during the summer and rainy seasons, yeast levels were also below 1 log CFU g^{-1}. However, in winter, a different trend was observed; no yeast or mold growth was detected on the first day, but growth appeared on days 3 and 7. Specifically, vendor 1 had yeast and mold counts ranging from approximately 3.07 to 3.74 log CFU g^{-1}, while vendor 3 showed counts between 2.83 and 2.93 log CFU g^{-1}.

Table 2. Total microorganisms vendor 1, throughout the 7-day shelf life of each shop. (mean ± SD of three replicates.)

Vendor	Season	Total microorganism (log CFU g^{-1}.)			Coliform/ Fecal Coliform (MPN g-1)		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
1	Summer	5.90±0.10^{e}	6.05±0.03^{f}	7.32±0.03^{g}	240/93	>1,100/>1,100	>1,100/1,100
	Rainy	4.88±0.09^{d}	7.32±0.03^{g}	8.05±0.02^{h}	240/93	1,100/210	>1,100/1,100
	Winter	1.00±0.00^{a}	3.54±0.07^{b}	4.18±0.04^{c}	<3/<3	<3/<3	<3/<3

Noted: The characters a, b, c, d, e, ab, bc, cd, and abc that differ both vertically and horizontally show statistically significant differences.

Table 3. Total microorganisms vendor 2, throughout the 7-day shelf life of each shop. (mean ± SD of three replicates.)

Vendor	Season	Total microorganism (log CFU g^{-1}.)			Coliform/ Fecal Coliform (MPN g-1)		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
2	Summer	3.79±0.08^{c}	4.73±0.06^{e}	6.26±0.02^{f}	28/<3	>1,100/>1,100	>1,100/1,100
	Rainy	3.51±0.04^{b}	6.25±0.01^{f}	6.71±0.04^{g}	28/28	>1,100/93	>1,100/>1,100
	Winter	1.00±0.00^{a}	3.53±0.01^{b}	4.58±0.10^{d}	<3/<3	<3/<3	<3/<3

Noted: The characters a, b, c, d, e, ab, bc, cd, and abc that differ both vertically and horizontally show statistically significant differences.

Table 4. Total microorganisms vendor 3, throughout the 7-day shelf life of each shop. (mean ± SD of three replicates.)

Vendor	Season	Total microorganism (log CFU g^{-1}.)			Coliform/ Fecal Coliform (MPN g-1)		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
3	Summer	4.32±0.02^{c}	7.12±0.01^{f}	7.48±0.03^{g}	240/15	240/150	1100/150
	Rainy	3.54±0.06^{a}	7.12±0.01^{f}	7.50±0.02^{g}	93/15	240/150	1100/150
	Winter	3.88±0.06^{b}	4.70±0.05^{d}	5.33±0.01^{e}	<3/<3	<3/<3	<3/<3

Noted: The characters a, b, c, d, e, ab, bc, cd, and abc that differ both vertically and horizontally show statistically significant differences.

Table 5 Yeast and mold count, throughout the 7-day shelf life of each shop. (mean ± SD of three replicates.)

Vendor	Season	Total microorganism (log CFU g^{-1}.)		
		Day 0	Day 3	Day 7
1	Summer	<1.00±0.0^{a}	<1.00±0.0^{a}	<1.00±0.00
	Rainy	<1.00±0.0^{a}	<1.00±0.0^{a}	<1.00±0.0^{a}
	Winter	<1.00±0.0^{a}	3.07±0.05^{b}	3.74±0.06^{c}
2	Summer	<1.00±0.0^{ns}	<1.00±0.0^{ns}	<1.00±0.0^{ns}
	Rainy	<1.00±0.0^{ns}	<1.00±0.0^{ns}	<1.00±0.0^{ns}
	Winter	<1.00±0.0^{ns}	<1.00±0.0^{ns}	<1.00±0.0^{ns}
3	Summer	<1.00±0.0^{a}	<1.00±0.0^{a}	<1.00±0.0^{a}
	Rainy	<1.00±0.0^{a}	<1.00±0.0^{a}	<1.00±0.0^{a}
	Winter	<1.00±0.0^{a}	2.83±0.03^{b}	3.93±0.01^{c}

Noted: The characters a, b, c, d, e, ab, bc, cd, and abc that differ both vertically and horizontally show statistically significant differences.

Typically, yeast and mold grow optimally at low pH values of 3.5–3.8. Considering the pH values of homemade dog food (Table 1), which were approximately six or higher, the environment was generally unfavorable for yeast and mold growth. However, when factoring in the ambient temperatures of each season, the winter season had the lowest temperatures (23.85±1.42 °C), which may be more conducive for the growth of these microorganisms. Additionally, incomplete sterilization during the production process could allow some yeasts and molds to survive, enabling their growth during refrigerated storage on days 3 and 7. Elevated pH levels create an environment conducive to bacterial growth and prolonged storage further enhances these conditions. This increase in pH was attributable to microbial decomposition.

Listeria **spp.**

Listeria spp. was not detected in any of the three vendors across all seasons. This was likely because *Listeria* spp. can be effectively destroyed by heat treatment at 65 °C or higher, although contamination may occur after heating [37]. An examination of the homemade dog food production processes at each vendor revealed similar practices, specifically boiling the food until it reached a rolling boil, which likely ensured complete eradication of *Listeria* spp. Furthermore, even after storing the food for seven days under refrigerated conditions, no colonies of *Listeria* spp. were observed. Although *Listeria* spp. can grow at low temperatures, seasonal variations did not appear to influence the growth of this bacterium in the tested samples.

Microbial contamination of food poses significant health risks to both humans and animals [38, 39], especially in the case of homemade dog food sold in fresh markets, in which food is normally left on the shelf until customers buy it [18]. Although heat is applied during preparation, multiple stages, including raw material sourcing, preparation methods, storage before sale, packaging, cooking, and sterilization, can introduce contamination. Moreover, the fresh market in Thailand is an open area which has multiple sources of contamination such as rodents, insects, sewage and water [40]. Another possible cause is the absence of standardized production controls for this type of food were enforced. Or the insufficient heat, duration during the cooking process and lack of reheat process for food waiting to be sold which may have failed to eliminate all microbial contaminants [41]. When the bacteria are not completely removed, the contaminated bacteria will multiply rapidly, resulting in food spoilage, as in vendor 3. A limitation of this study was the relatively small sample size due to the limited number of vendors available in the specified areas. Therefore, the comparison was restricted to these particular vendors across different sampling days according to the season. Furthermore, commercially available dog food of this type may be specific to a particular region and therefore may not be comparable to standards of other areas, such as European Pet Food Industry Federation (FEDIAF) or Association of American Feed Control Officials (AAFCO) guidelines.

In our study, we found that purchasing large quantities of homemade dog food for extended refrigeration and reheating for later consumption was not advisable. Instead, we suggest buying only the amount that can be consumed within the same day to minimize microbial growth and reduce the risk of foodborne pathogens. Both dogs and their owners are affected by this contamination, as owners come into contact with the food before feeding their pets [42]. This recommendation is especially crucial during seasons with higher ambient temperatures, such as the summer and rainy seasons, when microbial proliferation occurs more readily than in winter.

This issue is not limited to homemade dog food; studies surveying ready-to-eat foods sold in fresh markets for human consumption have also reported contamination with coliform bacteria and *Staphylococcus aureus* [18, 40, 43, 44]. The presence of these pathogenic microorganisms can cause gastrointestinal inflammation in dogs, leading to symptoms such as diarrhea and loss of appetite [33, 45 - 47]. In this study, the focus is on bacterial contamination; however, previous research highlighted that veterinarians primarily educated pet owners about nutritional imbalances, particularly in relation to this type of food, as a strategy to promote pet health and wellness [48].

At present, zoonotic diseases have become a major focus of study, largely because of the growing trend of pet ownership, with pets increasingly regarded as integral members of the family. In particular, dogs have long been one of the most popular companion animals. The close physical and emotional proximity between humans and dogs [49] have raised concerns about the potential for dogs to act as important carriers of infectious diseases that are transmissible to humans. This concern has contributed to the emergence of the "One Health" concept, which emphasizes the interconnected health of humans, animals and the environment [50, 51].

4. Conclusion

Home-cooked food for dogs is an alternative dietary option that often utilizes animal offal, which is less commonly consumed by humans. When properly prepared with sufficient heat and adequate cooking time, this type of food can be appropriate and safe for canine consumption, in terms of reducing the risk of pathogenic microbial contamination. However, purchasing pre-cooked dog food in large quantities for cold storage at typical refrigeration temperatures may not be ideal. Some types of bacteria are capable of surviving the initial heat treatment and can continue to grow during refrigerated storage, leading to spoilage and rendering food unsuitable for consumption. To ensure the safety of both the dog and the owner who handles the food, it is recommended that pet owners reheat the food thoroughly before serving. Ideally, food should be consumed entirely on the same day it is purchased. This practice helps reduce the risk of microbial contamination and supports the maintenance of dog food quality and safety, in alignment with the One Health approach.

5. Suggestions

In future studies, the quality of raw materials and production processes, including packaging methods, of this type of food product should be investigated. Such research would be beneficial for improving formulations with extended shelf life. In addition, recommendations regarding ingredient selection may be provided, particularly advising against the use of bones, which may pose risks as foreign bodies and cause obstruction in the gastrointestinal tract of animals.

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7. Declaration of Generative AI in Scientific Writing

The authors acknowledge that generative AI tools were used to improve language clarity, grammar, and formatting in this manuscript. The graphical abstract was created with the assistance of ChatGPT. No content, data interpretation, or results were generated or altered by AI.

8. CRediT Author Statement

Jakkapat Prachachit: Conceptualization, Methodology, Data analysis, Writing Original Draft

Kanokwan Bootyothee: Conceptualization, Methodology, Data analysis, Writing Original Draft, Review, Editing.

Titinan Hemadhulin: Methodology, Data analysis.

Benjamabhorn Pumhirunroj: Methodology, Data analysis.

Apisit Sorin: Methodology, Data analysis.

9. Research Involving Human and Animals Rights

This study did not involve any research on human participants or animals and therefore does not pertain to research involving human or animal rights.

10. Ethics Approval and Consent to Participate

Not applicable

11. Declaration of Competing Interest

The authors declare that they have no conflict of interest.

12. References

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