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Chemical and Microbiological Contamination in Ground Species from Traditional Markets in Yogyakarta

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ABSTRACT

Food safety is a critical component of food quality and requiries stringent measures to prevent biological, chemical, and other contaminants that pose risks to human health. This study assessed chemical and microbiological contamination in freshly ground red chili and turmeric sourced from traditional markets in Yogyakarta City. The investigation focused on microbiological contaminants, including *Salmonella sp*., *Escherichia coli* (*E. coli*), and yeast-and-mold counts, as well as chemical contaminants such as Rhodamine B, Methanyl Yellow, and formaldehyde. The findings revealed that *Salmonella sp*. were absent in all samples; however, each sample exceeded the permissible limits for *E. coli* and yeast-and-mold counts established by the Indonesian National Standard (SNI) 7388 : 2009. No chemical contaminants (Rhodamine B, Methanyl yellow, or formaldehyde) were detected in any sample. Therefore, although all ground spice samples were free from hazardous chemicals, they failed to meet essential microbiological hygiene standards, highlighting significant risks associated with their consumption.

Keywords: Microbiological contamination; chemical contamination; freshly ground red and turmeric; traditional markets

INTRODUCTION

Food safety resperents a fundamental component of food quality, as it has direct impact on consumer health. While sensory attributes such as taste, aroma are valued, they become inconsequential if the food is compromised by biological, chemically, or physical contaminants. Therefore, strict hygiene during processing is essential to ensure safe consumption (Sitanggang, 2020).

Ground spice such as red chilies turmeric are integral to Indonesian cuisine, valued for both their flavor and convenience. These products are commonly used by restaurants and home cooks to facilitate efficient meal preparation (Simanjutak, 2024). However, the prevalent practice of selling these spices in open containers and utilizing shared utensils increases the risk of microbial contamination (Tondang, 2020).

Food contamination remains a persistent problem in Yogyakarta, Indonesia, despite ongoing efforts to improve food safety. In 2023, the Yogyakarta National Agency for Drug and Food Control (BPOM) reported several foodborne disease incidents classified as KLB (*Kejadian Luar Biasa* or Extraordinary Events). Sleman

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Regency recorded 850 cases of chemical contamination in food service establishments, along with 60 cases of chemical and microbiological contamination in households and seven cases linked to snacks of unknown origin. In Bantul Regency, 29 cases were associated with Home Food Industries (P-IRT) and 60 cases occurred in households, both with unidentified contamination sources. Gunungkidul Regency also reported 36 cases of microbiological contamination in food service establishments. Yogyakarta City recorded 280 cases of combined chemical and microbiological contamination.

Microbiological contamination has been emphasized in previous studies. Mirawati et al. (2013) reported that 25% of chili powder samples tested were contaminated with *Staphylococcus aureus,* and the colony counts ranged from 6.7×10^3 to 6.2×10^4 colonies per g. Furthermore, 15.625% of the samples contained fecal coliforms and *Escherichia coli*. MPN values ranged from 2 to 23 per g, and another 15.625% tested positive for *Salmonella sp.* Generally, more than 65% of chili samples were considered non-compliant due to contamination. In a separate study, Putriningtyas et al. (2017) discovered that 4.68% of ground red chili samples sold in Sleman Regency were contaminated with Rhodamine B, a harmful industrial dye.

A thorough evaluation of powdered spice safety is required to address persistent contamination issues. This study aimed to assess chemical and microbiological contamination in powdered red chili peppers and turmeric, referencing Indonesian National Standard (SNI) 7388 : 2009 as the quality benchmark. This standard stipulated that *Salmonella sp.* should be absent in a 25-g sample, *E. coli* is not expected to exceed 20 colonies per g, and yeast-as well as-mold count (YMC) need to remain below 100 colonies per g. Rapid testing methods were utilized to detect hazardous substances, including Rhodamine B, Methanyl yellow, and formaldehyde. The results are intended to inform strategies for improving the safety and quality of powdered spices in Yogyakarta and surrounding areas.

METHODS

Materials

This study was conducted at the Food Technology Laboratory of Ahmad Dahlan University in Yogyakarta. The main samples analyzed were fresh-ground red chili and turmeric. The sources were five traditional markets in Yogyakarta, namely Kranggan, Beringharjo, Ngasem, Prawirotaman, and Giwangan. Using the Slovin formula with a 95% confidence level, a sample size of 10 vendors was considered sufficient. The selection ensured broad representation of the market's diversity. This is because the vendors operated across a variety of market environments and cater to different customer bases, effectively reflecting local practice. Approximately 100 g of fresh-ground red chili and turmeric were collected from each of the markers. This generated 20 samples, including 10 fresh-ground red chilies and 10 fresh-ground turmeric.

The process commenced with field observations to document the number of vendors and the sales locations. Subsequently, a comprehensive literature review informed the selection of study methodology. An orientation phase was conducted to finalize the sample collection process, determine sample size, and establish the specific analyses to be performed. Freshly ground red chili and turmeric were collected from the markets and transported to the laboratory for further analysis. High-quality spice samples were identified by bright color, fresh appearance, and strong, pleasant aroma. Conversely, low-quality spices typically featured a dull color and an unpleasant or rancid odor.

For microbiological testing, various media were used, including *Salmonella-Shigella Agar (SSA; Oxoid), Lactose Borth LB (Merck 500), Eosin-Methylene Blue Agar (EMBA;* HIMEDIA), and *Potato Dextrose Agar* (PDA; Merck 500). Rapid test kits manufactured by LABTEST (ET) detected potential contaminants, such as formaldehyde, Rhodamine B, and Methanyl yellow.

Salmonella sp.

Testing for the presence of *Salmonella sp*. started with weighing 25 g of sample, and put into 220 mL of distilled water and homogenized for 1-2 minutes. Bacterial isolation was conducted by serial dilution method up to 10^{-5}. A total of 100 mL of the solution was inoculated by quadrant scratch using an Ose needle on *Salmonella Shigella Agar* (SSA) medium. Petri dishes were then incubated at ± 28 °C for 18-24 hours. The following formula was used for calculating the number of colonies per milliliter (Equation 1).

$$\text{CFU/mL} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume plated (mL)}} \quad (1)$$

After incubation, initial characterization was performed based on colony color. The growing bacterial colonies were observed to determine the morphological characteristics on SSA medium. Gram staining was then performed to determine the morphological characteristics of bacterial cells. Bacterial colonies incubated for 18 hours are stained with Gram stain. Finally, observation was performed under a light microscope to identify cell shape, cell arrangement, and Gram staining reactions.

Escherichia coli

To estimate *coliform bacteria*, sterile *Lactose Broth* (LB) medium was prepared by dispensing 9 mL of LB into each test tube, followed by the addition of 1 mL of the sample. This process was repeated three times, leading to a total of nine test tubes per sample, which were incubated at 35 °C for 48 hours. Following incubation, the presence of gas bubbles was assessed to reflect a positive result, necessitating further testing.

To confirm the presence of *E. coli*, bacterial isolation was conducted using a serial dilution method up to 10^{-5}. A total of 100 mL of the solution was inoculated onto *Eosin Methylene Blue Agar* (EMBA) plates by streaking quadrants with a loop needle. Petri dishes were then incubated at 35 to 37 °C for 24 hours.

Following incubation, *E. coli* colonies were enumerated using a colony counter. This enabled the quantification of *E. coli* levels in the samples. Results were reported as Standard Plate Counts (SPC) and presented in tabular format for clarity. SPC method determined the number of microorganisms in a sample, typically in the range of 30 to 300 colony forming units (CFU) per milliliter, to minimize analytical and statistical errors. The following formula was used for calculating the number of colonies per milliliter (Equation 2).

$$\text{CFU/mL} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume plated (mL)}} \quad (2)$$

After incubation, initial characterization was conducted by assessing colony morphology on EMBA media. Colonies were examined to identify the characteristic coloration associated with *E. coli*.

Physiological characterization was performed by culturing bacterial isolates on *Triple Sugar Iron Agar* (TSIA) medium at a concentration of 5.5 g/L. TSIA test evaluated the ability of bacteria to ferment *glucose*, *lactose,* and *sucrose*, as well as to produce *hydrogen sulfide* (H_2S). A positive result was signified by a color change in the medium to red-black for H_2S production or red-yellow for sugar fermentation.

Gram staining was performed to observe the morphological characteristics of bacterial cells. The colonies were incubated for 18 hours and stained using Gram stain method. Finally, observation was conducted under a light microscope to identify cell shape, cell arrangement, and Gram stain reaction.

Yeast and Mold

In yeast and mold analysis, 1 mL of sample was diluted in 9 mL of distilled water to obtain a 10^{-1} dilution, then further diluted to 10^{-6}. The final three dilutions were transferred to petri dishes containing *Potato Dextrose Agar* (PDA) in triplicate. The samples were incubated at 20-25 °C for 5 days, and colonies were counted to determine yeast and mold levels. Microbial count was calculated using the following formula (Equation 3).

$$\text{Yeast and Mold Count (MYC)} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume plated (mL)}} \quad (3)$$

Rhodamine B

The presence of Rhodamine B in the samples was determined using the respective test kit. First, 5 g of a freshly ground red chili pepper sample were weighed and placed in a 100 mL Erlenmeyer flask, before adding 20 mL of distilled water was added. A total of 1–3 mL of the prepared sample solution was transferred to a test tube, where the reagents were added. The reagents were applied in two steps, namely 1 drop of Rhodamine-1 (reagent A) and 3 drops of Rhodamine-2 (reagent B). The test tube was then shaken to ensure even mixing and left for 5–15 minutes to observe the color changes. A positive result for Rhodamine B was signified by a brick-red coloration of the sample. This change occurred due to the formation of a brick-red complex between Rhodamine B and an antimony salt, which was soluble in organic solvents. The resulting reaction was consistent with the results of Masthura (2019).

Methanyl Yellow

Analysis of Methanyl yellow in freshly ground turmeric was performed using the respective test kit. Initially, 5 g of turmeric was weighed and placed in a 100 mL Erlenmeyer flask, then 20 mL of sterile distilled water was added. After mixing, 1–3 mL of the solution was transferred to a test tube, and the reagent was added. The reagent consisted of 3 drops of Methanyl yellow-1 which was considered as (reagent A). The reaction mixture was shaken until the reagent was completely dissolved, and the test tube was left for 5–15 minutes to observe the color change. A positive result for Methanyl yellow was signified by the appearance of a purplish-brown or pink color, reflecting the presence of a synthetic yellow dye. This change was attributed to the interaction between Methanyl yellow and the acid in the reagent, as explained by Masthura (2019).

Formaldehyde

Formaldehyde content in the samples was tested using a formalin test kit from LABTETS. For the initial analysis, 5 g of each sample (freshly ground red chili or turmeric) was weighed and placed into a 100 mL Erlenmeyer flask, then 20 mL of sterile distilled water was added. After mixing, 1-3 mL of the solution was

Table 1. Experimental design

Sample	Sample code	Repetition		
		1	2	3
Fresh-ground red chili	C1	C1 1	C1 2	C1 3
	C2	C2 1	C2 2	C2 3
	C3	C3 1	C3 2	C3 3
	C4	C4 1	C4 2	C4 3
	C5	C5 1	C5 2	C5 3
	C6	C6 1	C6 2	C6 3
	C7	C7 1	C7 2	C7 3
	C8	C8 1	C8 2	C8 3
	C9	C9 1	C9 2	C9 3
	C10	C10 1	C10 2	C10 3
Fresh-ground turmeric	K1	K1 1	K12	K13
	K2	K2 1	K2 2	K2 3
	K3	K3 1	K3 2	K3 3
	K4	K4 1	K4 2	K4 3
	K5	K5 1	K5 2	K5 3
	K6	K6 1	K6 2	K6 3
	K7	K7 1	K7 2	K7 3
	K8	K8 1	K8 2	K8 3
	K9	K9 1	K9 2	K9 3
	K10	K10 1	K10 2	K10 3

Notes: C = Freshly ground red chili; K = Freshly ground turmeric

transferred into a test tube, followed by the addition of reagents. Specifically, 1 drop of formalin-1 reagent A and 3 drops of formalin-2 reagent B were added. The test tube was shaken to ensure even mixing and left for 5-15 minutes for the reaction to occur. A positive result for formaldehyde was signified by a purple color change. The intensity of color was directly proportional to the concentration of formaldehyde in the sample. The experimental design is outlined in Table 1, which shows the repetition of samples for each analysis.

RESULTS AND DISCUSSION

Microbial contamination in processed foods presented a substantial risk to human health. Microorganisms may enter food through contaminated animal products, food handlers, cross-contamination from other foods, or exposure to environments conducive to bacterial and fungal growth. Bacteria and fungi were the most prevalent microorganisms. Among bacterial species causing foodborne infectious diseases, *Salmonella* sp. and *E. coli* were particularly significant (Jenie, 2022).

Salmonella Sp.

Salmonella sp. referred to a group of bacteria capable of causing gastrointestinal diseases, including diarrhea and infections of the human digestive system. These bacteria, members of Enterobacteriaceae family, were approximately 2–4 micrometers in length and 0.5–0.8 in width. *Salmonella* was pathogenic to both humans and animals when ingested, typically through contaminated food, and caused enteritis, systemic infections, and typhoid fever (Andari & Yudhayanti, 2022). Table 2 presents the results of *Salmonella sp*. analysis in this study.

Table 2. *Salmonella* sp. contamination levels in freshly ground red chili

No.	Sample	*Salmonella sp.* microbial count (NA/g)	Maximum Limit for *Salmonella sp.* (BPOM, 2019) (NA/25g)
1.	C1	Negative	Compliant
2.	C2	Negative	Compliant
3.	C3	Negative	Compliant
4.	C4	Negative	Compliant
5.	C5	Negative	Compliant
6.	C6	Negative	Compliant
7.	C7	Negative	Compliant
8.	C8	Negative	Compliant
9.	C9	Negative	Compliant
10.	C10	Negative	Compliant

By: Food and Drug Supervisory Agency Regulation Number 13, 2019

Table 3. *Salmonella* sp. contamination levels in freshly ground turmeric

No.	Sample	*Salmonella sp.* microbial count (NA/g)	Maximum Limit for *Salmonella sp.* (BPOM & SNI) (NA/25g)
1.	K1	Negative	Compliant
2.	K2	Negative	Compliant
3.	K3	Negative	Compliant
4.	K4	Negative	Compliant
5.	K5	Negative	Compliant
6.	K6	Negative	Compliant
7.	K7	Negative	Compliant
8.	K8	Negative	Compliant
9.	K9	Negative	Compliant
10.	K10	Negative	Compliant

By: Food and Drug Supervisory Agancy Regulation Number 13, 2019.

Analysis of all freshly ground turmeric samples showed no contamination with *Salmonella* sp., as detailed in Table 3.

The standards for *Salmonella* sp. contamination in freshly ground red chili and turmeric were regulated by BPOM Regulation Number 13 (BPOM, 2019) and SNI 7388 : 2009 (SNI,2009). According to the regulations, the maximum allowable limit for this group of bacteria contamination in spices and ready-to-use condiments was negative per 25 g. Additionally, the maximum microbial contamination limits for spices, including freshly ground red chili and turmeric, were *Salmonella* sp. negative per 25 g, *Staphylococcus aureus* ≤ $1x10^2$ colonies/g, and MPN Coliform ≤ 100/g.

Analysis of 10 freshly ground red chilies and turmeric obtained from traders at traditional markets in Yogyakarta City showed that all samples met SNI and BPOM standards. The absence of *Salmonella* sp. in the spices was consistent with the results, where the bacteria typically thrives in foods of animal origin. Furthermore, it was discovered in air, water, soil, feces, as well as in humans and animals (Melli, 2020). The primary sources include poultry (e.g., chicken, goose, duck, and turkey), pork, seafood, eggs, and milk. The infections were more common in warmer months, as the bacteria proliferate at temperatures between 35 °C and 37 °C, particularly in protein-rich foods (Melli, 2020). *Salmonella sp.* broke down carbohydrates into acids and gases, utilized citrate as its sole carbon source, produced *hydrogen sulphide* (H_2S), and decarboxylated lysine as well as ornithine into cadaverine and putrescine. Additionally, it was oxidase-negative and catalase-positive (Ilahi et al., 2021).

Escherichia coli

E. coli was widely recognized as an indicator bacterium for sanitation and cleanliness. Its presence in food products signals inadequate sanitation practices during processing. *E. coli* was typically discovered in the intestines of humans and animals. The detection of this bacterium in food or air suggested fecal contamination during handling. Common food sources often associated with *E. coli* contamination include raw milk, raw beef, and unwashed fruits as well as vegetables (Rahayu et al., 2018).

Analysis of samples collected from traditional markets in Yogyakarta City showed that none met the established safety standards. Both freshly ground red chili and turmeric featured *E. coli* levels surpassing the maximum limits specified by SNI (SNI, 2009). The results for freshly ground red chili and tumeric are presented in Table 4 and 5, respectively.

SNI 7388 :2009 on the Maximum Limit of Microbial Contamination in Food stipulated that the maximum limit of *E coli* in wet spices was <3 MPN/g. Analysis of levels of this bacteria in fresh-ground red chili and fresh-ground turmeric in this study showed <10 CFU/g. These values suggested that the ground spices were unsafe for consumption and exceed the maximum limits set by SNI. The presence of *E. coli* in food can be influenced by various factors, including the cleanliness of the processing facility, the method of serving, and the cleaning process of the equipment used (Halimatussadiyah et al., 2024). This result is in line with the study of Rejeki et al. (2024), who reported that in traditional markets, wet spices were generally sold openly in bulk without hygienic packaging, and were vulnerable to cross-contamination.

The crowded, humid, and less-clean market environment increased the risk of microbial contamination of food products. Pathogenic bacteria *E. coli* grew at 7 to 44 °C with the optimal condition observed at 37 °C. The optimum *pH* was 7 to 7.5, with a minimum of 4 and a maximum of 9. Furthermore, *E. coli* habited in humid environments, remained heat-sensitive, and died during pasteurization or food-cooking processes at high temperatures (Kurniati et al., 2020; Tangahu, 2014). When the seasoning was heated to high temperatures, the bacteria possibly died, making it safe to consume. *E. coli* tended to flourish in animal products rather than in plant-based products (Melli, 2020; Rizaldi & Zelpina, 2023). Previous studies emphasize antimicrobial effects of turmeric extract, which has been shown to inhibit the growth of *E. coli* and *Salmonella* (Gul & Bakht, 2015; Thinwang et al., 2023). This effect was related to

Table 4. Results of *E. coli* counts in freshly ground red chili

No.	Sample	*E. coli* Count (CFU/g)	SNI Maximum *E. coli* Limit (<3/g)
1.	C1	< 10	Non-compliant
2.	C2	< 10	Non-compliant
3.	C3	< 10	Non-compliant
4.	C4	< 10	Non-compliant
5.	C5	< 10	Non-compliant
6.	C6	< 10	Non-compliant
7.	C7	< 10	Non-compliant
8.	C8	< 10	Non-compliant
9.	C9	< 10	Non-compliant
10.	C10	< 10	Non-compliant

(SNI 7388 : 2009)

Table 5. Results of *E. coli* counts in freshly ground turmeric

No.	Sample	*E. coli* count (CFU/g)	SNI maximum *E. coli* Limit (<3/g)
1.	K1	< 10	Non-compliant
2.	K2	< 10	Non-compliant
3.	K3	< 10	Non-compliant
4.	K4	< 10	Non-compliant
5.	K5	< 10	Non-compliant
6.	K6	< 10	Non-compliant
7.	K7	< 10	Non-compliant
8.	K8	< 10	Non-compliant
9.	K9	< 10	Non-compliant
10.	K10	< 10	Non-compliant

(SNI 7388 : 2009)

turmeric ability to interfere with the processes, such as the type 3 secretion system in *Salmonella*, a mechanism crucial for bacterial invasion and infection (Thinwang et al., 2023).

Various species of chili peppers were reported to possess antibacterial activity, effectively inhibiting the growth of pathogens such as *E. coli* and *Salmonella* strains (Ayariga et al., 2022; Dorantes et al., 2000;

Lee et al., 2018; Periferakis et al., 2023). The results corroborated previous studies that emphasized the potential of turmeric and chili as natural protectors against microbial contamination, with promising implications for food safety and preservation strategies.

Yeast and Mold

Analysis of YMC in freshly ground red chili pepper showed that all samples exceeded the maximum limits set by BPOM and SNI regulations, as detailed in Table 6. This result suggested that none of the samples met the standards, raising concerns about the safety for consumption.

None of the freshly ground turmeric samples complied with BPOM and SNI standards, as detailed in Table 7.

The results in Tables 6 and 7 showed that none of the samples complied with food safety regulations. Yeast and mold contamination were primarily influenced by environmental conditions and raw material quality. Mold growth on freshly ground chili and turmeric was most possibly due to poor raw material quality and improper storage practices. Observations showed that vendors sold not only ground chili and turmeric but also other fresh ingredients, and the open displays contributed to airborne contaminants exposure. Ground spices were often stored in plastic containers without lids, with measuring spoons used for both turmeric and others, increasing the risk of cross-contamination (Rejeki et al., 2024; Winandari et al., 2022).

Previous studies showed that industrially processed Capsicum products were contaminated with *aflatoxins* (AFs), *ochratoxin A* (OTA), *fumonisins* (FBs), *zearalenone* (ZEN), *trichothecenes* (TCTs), and *patulin* (PAT) (Santos et al., 2011; Costa et al., 2019). *Aflatoxins had* oncogenic and immunosuppressive properties as well as induced infections in humans contaminated with this substance (Alhousen & Gurbuz, 2016). Mycotoxins triggered a number of acute and chronic diseases and, in more serious cases, led to fatal consequences (e.g., most recently in Tanzania in 2016). Meanwhile, the daily intake of mycotoxin-contaminated pepper was subject to the accumulation of the toxic metabolite in the body. This possibly led to acute intoxication and being translated into different kinds of mycotoxin-related diseases. Several studies also suggested that microbiota present in red pepper may be a risk factor for gallbladder cancer (GBC) (Costa et al., 2019; Serra et al., 2002; López-Carnllo et al., 1994).

Traditional markets, which often had humid, poorly lit conditions, provide an ideal environment for mold growth. These conditions, alongside the low *pH*

Table 6. Results of YMC in freshly ground red chili

No.	Sample	Yeast-and-mold count (CFU/g)	BPOM maximum limit (10^2 colony/g)
1.	C1	1,7 x 10^6	Non-compliant
2.	C2	3,9 x 10^4	Non-compliant
3.	C3	2,9 x 10^7	Non-compliant
4.	C4	8,7 x 10^6	Non-compliant
5.	C5	9,3 x 10^5	Non-compliant
6.	C6	3,8 x 10^5	Non-compliant
7.	C7	4,1 x 10^5	Non-compliant
8.	C8	3,2 x 10^5	Non-compliant
9.	C9	1,9 x 10^6	Non-compliant
10.	C10	1,1 x 10^6	Non-compliant

By: Food and Drug Supervisory Agency Regulation Number 13, 2019

Table 7. Results of YMC in freshly ground turmeric

No.	Sample	Yeast-and-mold count (CFU/g)	BPOM maximum limit (10^2 colony/g)
1.	K1	2,6 x 10^6	Non-compliant
2.	K2	2,3 x 10^7	Non-compliant
3.	K3	2,3 x 10^7	Non-compliant
4.	K4	4,2 x 10^6	Non-compliant
5.	K5	4,4 x 10^6	Non-compliant
6.	K6	1,5 x 10^6	Non-compliant
7.	K7	1,7 x 10^4	Non-compliant
8.	K8	3,4 x 10^6	Non-compliant
9.	K9	3,1 x 10^5	Non-compliant
10.	K10	5,2 x 10^3	Non-compliant

By: Food and Drug Supervisory Agency Regulation Number 13, 2019 of ground spices, promoted mold growth. *Aspergillus niger, Aspergillus flavus,* and *Aspergillus candidus,* common fungi discovered in spices such as turmeric, red chili, black pepper, and white pepper (Esmeralda et al., 2020; (Winandari et al., 2022), was a particular concern. Mold and yeast spores are generally killed during cooking (Rima & Saidi, 2020). However, since mold spores survived high temperatures for extended periods, proper handling and storage were essential to ensure food safety.

All samples exceeded the maximum limits established by BPOM and SNI regulations. The results suggested that ground spices from traditional markets in Yogyakarta City were unsafe for consumption. This underscored the urgent need for vendors to enhance hygiene and storage practices to mitigate contamination risks and protect public health. Maintaining the hygiene of ground chili required the use of fresh, uncontaminated raw materials, regular handwashing, and thorough cleaning of work surfaces as well as equipment, including chili grinders, spoons, and containers (Simanjutak, 2024).

Rhodamine B

Rhodamine B test was conducted using a LABTEST test kit. The results showed that none of the samples (0%) tested positive, as detailed in Table 8. This evidence was consistent with the 2023 BPOM annual report for Yogyakarta, where out of 21 food samples tested, 20 were negative for Rhodamine B and only one contained the dye.

Rhodamine B was classified as a hazardous substance under Indonesian Minister of Health Regulation No. 239/Men.Kes/Per/V/85, which addresses certain dyes considered harmful in food. It was also classified as a probable carcinogen by the International Agency for Research on Cancer (IARC) and was correlated to liver cancer and dysfunction (Cheng & Tsai, 2017). Additionally, the dye caused respiratory irritation and other health problems (Wulandari et al., 2023). The absence of these substances in the analyzed samples suggested that spice powder traders in Yogyakarta were generally aware of the risks and had taken measures to ensure compliance with safety standards.

Methanyl Yellow

Metanyl Yellow is a synthetic dye derived from methanol and phenylamine, both of which are toxic. This dye was commonly used to color wool, nylon, leather, paper, aluminum paint, detergents, wood, fur, and cosmetics (Zein et al., 2019). Although primarily used in textile industries, Methanyl Yellow was occasionally applied inappropriately as a food coloring (Nath et al., 2015).

Methanyl Yellow was classified as a hazardous substance under Indonesian Minister of Health Regulation No 239/Men.Kes/Per/V/85. The substance potentially caused liver and kidney damage (Sharma et al., 2019; (Saxena & Sharma, 2014). This dye had also been correlated to bladder cancer risk (Zuraida et al., 2017).

Test results obtained using LABTEST kit showed that none of freshly ground turmeric samples tested positive for Methanyl Yellow, as detailed in Table 9. This result is consistent with the 2023 BPOM annual report for Yogyakarta, where out of 13 food product samples tested, 12 were negative for Methanyl Yellow and only

Table 8. Results of Rhodamine B testing in freshly ground red chili

No.	Sample	Test results	Rhodamine B content	Remarks
1.	C1	Negative (-)	Absent	Compliant
2.	C2	Negative (-)	Absent	Compliant
3.	C3	Negative (-)	Absent	Compliant
4.	C4	Negative (-)	Absent	Compliant
5.	C5	Negative (-)	Absent	Compliant
6.	C6	Negative (-)	Absent	Compliant
7.	C7	Negative (-)	Absent	Compliant
8.	C8	Negative (-)	Absent	Compliant
9.	C9	Negative (-)	Absent	Compliant
10.	C10	Negative (-)	Absent	Compliant

Table 9. Methanyl yellow test results in freshly ground turmeric

No.	Sample	Test results	Methanyl Yellow content	Remarks
1.	K1	Negative (-)	Absent	Compliant
2.	K2	Negative (-)	Absent	Compliant
3.	K3	Negative (-)	Absent	Compliant
4.	K4	Negative (-)	Absent	Compliant
5.	K5	Negative (-)	Absent	Compliant
6.	K6	Negative (-)	Absent	Compliant
7.	K7	Negative (-)	Absent	Compliant
8.	K8	Negative (-)	Absent	Compliant
9.	K9	Negative (-)	Absent	Compliant
10.	K10	Negative (-)	Absent	Compliant

Table 10. Formaldehyde test results for freshly ground red chili

No.	Sample	Test results	Formaldehyde content	Remarks
1.	C1	Negative (-)	Absent	Compliant
2.	C2	Negative (-)	Absent	Compliant
3.	C3	Negative (-)	Absent	Compliant
4.	C4	Negative (-)	Absent	Compliant
5.	C5	Negative (-)	Absent	Compliant
6.	C6	Negative (-)	Absent	Compliant
7.	C7	Negative (-)	Absent	Compliant
8.	C8	Negative (-)	Absent	Compliant
9.	C9	Negative (-)	Absent	Compliant
10.	C10	Negative (-)	Absent	Compliant

Table 11. Formaldehyde test results for freshly ground turmeric

No.	Sample	Test Results	Formaldehyde content	Remarks
1.	K1	Negative (-)	Absent	Compliant
2.	K2	Negative (-)	Absent	Compliant
3.	K3	Negative (-)	Absent	Compliant
4.	K4	Negative (-)	Absent	Compliant
5.	K5	Negative (-)	Absent	Compliant
6.	K6	Negative (-)	Absent	Compliant
7.	K7	Negative (-)	Absent	Compliant
8.	K8	Negative (-)	Absent	Compliant
9.	K9	Negative (-)	Absent	Compliant
10.	K10	Negative (-)	Absent	Compliant

one tested positive for the dye. These results suggested that local traders in Yogyakarta's traditional markets were possibly aware of the associated risks.

Formaldehyde

Formaldehyde (HCHO) is commonly used as an antiseptic, odor neutralizer, and fumigation agent. It was also applied to protect products from microbial contamination in various industries, including textiles, leather, and cosmetics (Astuti & Tebai, 2018). The use of formaldehyde as a food preservative is considered illegal under the Regulation of the Minister of Health of Indonesia Number 033 of 2012. However, its application remained pervasive in developing countries such as Bangladesh and Indonesia (Anissah et al., 2021; Wahed et al., 2016).

Formaldehyde posed significant health risks when present in food. It was recognized as a potential human carcinogen by several health agencies, including IARC and the US Environmental Protection Agency (EPA) (Fappiano et al., 2022; Reingruber & Pontel, 2018). Formaldehyde was also associated with nasopharyngeal cancer and possibly leukemia (Tang et al., 2009). Analysis showed that none of the samples, including both ground red chili and turmeric, contained formaldehyde, as detailed in Tables 10 and 11.

The results were consistent with the 2023 BBPOM Yogyakarta annual report, where all 20 samples tested were free of formaldehyde contamination. The absence of this substance in powdered spices reflected increased awareness among traders in Yogyakarta's traditional markets regarding food safety regulations.

The absence of harmful synthetic dyes and formaldehyde in the red chili powder and turmeric samples signified increased awareness among traders regarding the risks associated with chemical additives in food products. However, the elevated *E. coli* levels in all samples, which surpass the maximum permissible limits, point to inadequate sanitation and hygiene practices during processing, handling, and storage. The significant presence of mold and yeast, including potentially aflatoxin-producing fungi such as *Aspergillus spp.*, poses a considerable health risk to consumers when effective control and oversight are lacking.

CONCLUSION

In conclusion, this study evaluated chemical and microbiological safety of freshly ground red chili peppers and turmeric sold in traditional markets in Yogyakarta City. The results showed that all samples were free of harmful contaminants, including *Salmonella sp.*, Rhodamine B, Methanyl Yellow, and formaldehyde, and complied with established regulatory standards. However, significant contamination with *E. coli*, yeast, and mold was detected, where all samples exceeded the permissible limits set by BPOM and SNI. These evidences raised concerns regarding the safety of the products and emphasized deficiencies in handling, storage, and market conditions.

The results underscored the urgent need to improve hygiene practices and storage conditions in traditional markets. Implementing measures such as using tightly sealed containers, improving ventilation, and maintaining proper sanitation, significantly reduced fungal contamination. Educating vendors about appropriate food handling and contamination risks was essential to ensure the safety of ground spices and protect public health. Government agencies, including BPOM, should adopt proactive strategies, such as educational campaigns, to inform local vendors about proper handling procedures.

This study was limited to two types of ground spices and conducted in a specific region. Future investigations should include a broader range of food products and markets across different regions to provide a more comprehensive perspective. Additionally, investigating factors contributing to mold growth, such as environmental conditions and storage practices, could help identify targeted interventions to reduce contamination risk.

CONFLICT OF INTEREST

The authors declare no competing interests.

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