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Determination of Chlorine Levels in Commercial Wheat Flour from Bekasi Market Using UV–Vis Spectrophotometry

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Abstract: Wheat flour is a widely consumed food ingredient produced from the processing of wheat (*Triticum* spp.) and commonly used as a raw material for various food products. In some processing practices, chlorine may be used as a bleaching agent to produce a whiter flour appearance. However, excessive chlorine levels in food may pose potential health risks. This study aimed to determine the presence and concentration of chlorine levels in wheat flour circulating in traditional markets in Bekasi and to evaluate whether the detected levels comply with the maximum residue limit established in BPOM Regulation No. 28 of 2019 concerning processing aids in food processing, which sets the maximum chlorine residue at 1 mg/kg. A total of nine wheat flour samples were analyzed using qualitative and quantitative methods. The samples were first extracted using chlorine-free water, followed by qualitative analysis based on color changes and quantitative determination using a UV–Vis spectrophotometer with N,N Diethyl-p-phenylenediamine (DPD) reagent. The qualitative results showed that seven out of nine samples were positive for chlorine levels. Quantitative analysis indicated that seven samples exceeded the permitted limit, with the highest level of 9.44 mg/kg found in sample A2. These findings highlight the importance of monitoring chlorine levels in wheat flour to ensure food safety and consumer protection.

Keywords: Chlorine; DPD (*N,N Diethyl-p-phenylendiamine*); UV-Vis spectrophotometry; Wheat flour

Introduction

Food is a fundamental human necessity that must be fulfilled in both quality and quantity to maintain health and well-being (Stehl et al., 2025). One of the widely consumed food ingredients is wheat flour, which has become an important staple in many countries due to its relatively affordable price, high protein content, and practical use in food processing (Poole et al., 2024). Wheat flour is commonly used as a substitute for rice and can be processed into a variety of nutritious food products such as bread, noodles, and other processed foods (Muko, 2013). The high level of consumption of wheat flour products makes the quality and safety of this food commodity an important issue that needs continuous monitoring (Nadimi et al., 2023). In Indonesia, wheat flour is widely utilized in various food products (Marta et al., 2023). Although the government has promoted the development of alternative flours derived from local starch-producing plants such as rice, corn, sorghum, and cassava to reduce dependence on wheat-based products (Nirmalawaty et al., 2023).

Naturally, wheat flour has a slightly yellowish color due to the presence of pigment compounds known as xanthophylls, particularly lutein (Oduro-Obeng et al., 2024). During the natural aging process, these pigments gradually undergo oxidation, resulting in a lighter color and improved baking characteristics (Shi et al., 2024). This process generally requires approximately four to six weeks of storage to achieve optimal flour quality (Anggraeni & Ariestika, 2019). However, such a method is considered inefficient for industrial production because it requires a relatively long processing time (Acar et al., 2025). Consequently, certain chemical agents are sometimes used to accelerate the whitening process of wheat flour (He et al., 2024).

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One substance that may be used in this process is chlorine (Hou et al., 2025). Chlorine functions as a strong oxidizing agent capable of oxidizing natural pigments in wheat flour, thereby producing a whiter appearance (Cheng et al., 2021). In addition to affecting pigment compounds, chlorine can also influence the characteristics of gluten proteins, which may alter the functional properties of flour during food processing (László et al., 2008). Chlorine is widely used in various processing practices because it is effective even at low concentrations, relatively inexpensive, and capable of forming residual chlorine when applied in sufficient amounts (Situmorang, 2025). As a result, wheat flour circulating in the market is often dominated by products with a very bright white color, which tends to be perceived by consumers as having better quality and more attractive appearance (Dziki et al., 2024).

Despite its functional role during processing, the use of chlorine in food products must be carefully controlled due to its potential health risks (Pakdel et al., 2023). According to the Regulation of the Minister of Health of the Republic of Indonesia No. 472/Menkes/Per/V/1996, chlorine is categorized as a hazardous and toxic substance that may cause irritation when exposure occurs in excessive amounts (Wally et al., 2024). Furthermore, the Regulation of the Minister of Health No. 033 of 2012 states that chlorine is not included in the category of Food Additives, particularly within the group of bleaching and flour-maturing agents (Bunyanis et al., 2021). However, the use of chlorine in food processing is addressed in the Regulation of the Indonesian Food and Drug Authority (BPOM) No. 28 of 2019 concerning Processing Aids, which establishes a maximum residue limit of 1 mg/kg in the final food product. This regulation indicates that chlorine may be used during processing but should not remain in excessive amounts in the final product (Petri et al., 2021).

Excessive chlorine levels in food may pose potential health risks if consumed continuously over a long period (Crinnion, 2009). Exposure to chlorine in high concentrations may cause irritation of the digestive tract and potentially contribute to gastric disorders such as gastritis (Aminah et al., 2019). In addition, prolonged exposure to chlorine levels may lead to toxic effects that could appear after long-term accumulation in the body (Novitasari & Khotimah, 2020).

Previous studies have reported the presence of chlorine levels in flour products circulating in the market. Research conducted by Apriani & Latifani (2020) on several branded and non-branded wheat flour samples indicated that all tested samples were positive for chlorine, with the highest levels detected in branded wheat flour products. Similarly, a study by H & AW (2016) involving six wheat flour samples both branded and non-branded. Also reported the presence of chlorine levels, although the detected levels were still below the maximum limits set by the Food and Drug Administration (FDA). These findings suggest that chlorine may still be present in wheat flour products distributed in the market, making further monitoring necessary.

Considering that wheat flour is widely used as a raw material for various food products consumed by the public, ensuring its safety is essential (Feng & Archila, 2021). Therefore, it is important to evaluate the presence and concentration of chlorine levels in wheat flour circulating in local markets. Based on these considerations, this study aims to determine the presence of chlorine and to measure its concentration in wheat flour samples obtained from markets in Bekasi using the UV–Vis spectrophotometric method. The results of this study are expected to provide scientific information regarding the safety of wheat flour products and support efforts in monitoring food quality in accordance with applicable food safety regulations.

Method

Research Instruments and Materials

Instruments

The instruments used in this study included a dropper pipette, filter paper, funnel, measuring pipette, stirring rod, PA 224 Ohaus analytical balance, measuring cylinder, volumetric pipette, volumetric flask, UV-Vis 1900 spectrophotometer, and micropipette. The materials used in this study are wheat flour obtained in the Bekasi market, especially suspected wheat flour, chlorine, chlorine-free aqua, N,N Diethyl-p-phenylendiamine (DPD) Reagents, Phosphate Buffers.

Research Procedure

Sample Collection

The sample collection for the analysis of chlorine content was conducted using a purposive sampling method, which is a technique in which samples are selected based on specific considerations determined by the researcher so that they are relevant to the objectives of the study. In this research, wheat flour was chosen as the sample with several predetermined criteria. The inclusion criteria referred to wheat flour suspected of containing chlorine, which was indicated by a strikingly bright white color, and wheat flour circulating in Pasar Baru Bekasi. Meanwhile, the exclusion criteria included wheat flour that did not exhibit a striking color and wheat flour distributed outside the Pasar Baru Bekasi area.

Sample Preparation

Each wheat flour sample was weighed 10 g using an analytical balance and placed into a beaker glass.

Subsequently, 50 mL of chlorine-free distilled water was added to the sample. After mixing, the solution reached a total volume of approximately 60 mL, which was confirmed using a measuring cylinder.

Figure 1. Total volume 60 ml

The mixture was then homogenized to ensure that chlorine levels potentially present in the flour were dissolved into the aqueous phase. After homogenization, the mixture was filtered using filter paper to separate the insoluble particles from the liquid phase. The resulting filtrate was collected and used as the test solution for qualitative and quantitative analyses (Wongkar et al., 2014).

Qualitative Analysis of Test Samples

Qualitative analysis was carried out using a color reaction method with the reagent N,N-diethyl-p-phenylenediamine (DPD). A total of 10 mL of the sample filtrate obtained from the filtration step was transferred into a test tube. Subsequently, the DPD reagent was added to the solution and the mixture was homogenized.

After homogenization, the solution was allowed to stand for 1 minute to observe any color change. The presence of chlorine was indicated by the formation of a pink coloration, while the absence of color change indicated that chlorine was not detected in the sample (Feladita et al., 2017).

Quantitative Analysis of Test Samples

Quantitative analysis was performed using a UV–Vis spectrophotometer to determine the concentration of chlorine in the wheat flour samples. The quantitative procedure consisted of several stages, including the determination of the maximum wavelength, operating time, calibration curve, and determination of chlorine content (Al-Jabari et al., 2022).

Determination of Maximum Wavelength

The maximum wavelength was determined using a UV–Vis spectrophotometer. A chlorine stock solution of 100 ppm was prepared by weighing 10 mg of calciumhypochlorite (kaporit) and dissolving it in a 100 mL volumetric flask, followed by dilution with chlorine-free distilled water up to the mark. A working solution with a concentration of 10 ppm was prepared by pipetting 1 mL of the stock solution into a 10 mL volumetric flask. Subsequently, 0.5 mL of phosphate buffer and DPD reagent (N,N-diethyl-p-phenylenediamine) were added, and the solution was diluted to the mark using chlorine-free distilled water.

The absorbance of the solution was then measured using a UV–Vis spectrophotometer within a wavelength range of 490–530 nm in order to determine the maximum wavelength (Feladita et al., 2017).

Operating Time

The operating time was determined using a 10 ppm chlorine standard solution prepared from the 100 ppm stock solution. The stock solution was prepared by dissolving 10 mg of calcium hypochlorite in a 100 mL volumetric flask and diluting it with chlorine-free distilled water to the mark. A 10 ppm working solution was prepared by pipetting 1 mL of the stock solution into a 10 mL volumetric flask. Subsequently, 0.5 mL of phosphate buffer solution and DPD reagent (N,N-diethyl-p-phenylenediamine) were added, and the solution was diluted to the mark with chlorine-free distilled water.

The absorbance of the solution was then measured using a UV–Vis spectrophotometer at a wavelength of 511 nm. The absorbance values were recorded at several time intervals for up to 60 minutes in order to determine the time required for the reaction between chlorine and the DPD reagent to reach a stable absorbance value.

Preparation of Calibration Curve

The calibration curve was constructed using five chlorine standard concentrations, namely 3 ppm, 5 ppm, 7 ppm, 9 ppm, and 11 ppm. Each standard solution was placed in a 10 mL volumetric flask, followed by the addition of 0.5 mL phosphate buffer and DPD reagent (N,N-diethyl-p-phenylenediamine). The solution was then diluted with chlorine-free distilled water up to the mark. The absorbance of each solution was measured using a UV–Vis spectrophotometer, and the calibration curve was constructed by plotting absorbance against concentration. The linear regression equation was calculated using the equation:

$$y = bx + a \quad (1)$$

Where y represents absorbance and x represents the chlorine concentration (Drochioiu et al., 2016).

Determination of Chlorine Levels

For the determination of chlorine content, 10 mL of filtrate from each test sample was transferred into a reaction tube. Subsequently, 0.5 mL of phosphate buffer solution and DPD reagent (N,N-diethyl-p-phenylenediamine) were added to the solution. The total volume of the solution obtained was 10.6 mL. The mixture was then incubated for 16–20 minutes to allow the reaction to reach stability. After incubation, the absorbance of the solution was measured using a UV–Vis spectrophotometer at the predetermined wavelength (Abdollahi et al., 2019).

Data Analysis

The data obtained from the analysis were presented descriptively in the form of tables, narratives, and discussion. The chlorine (Hasan, 2006).

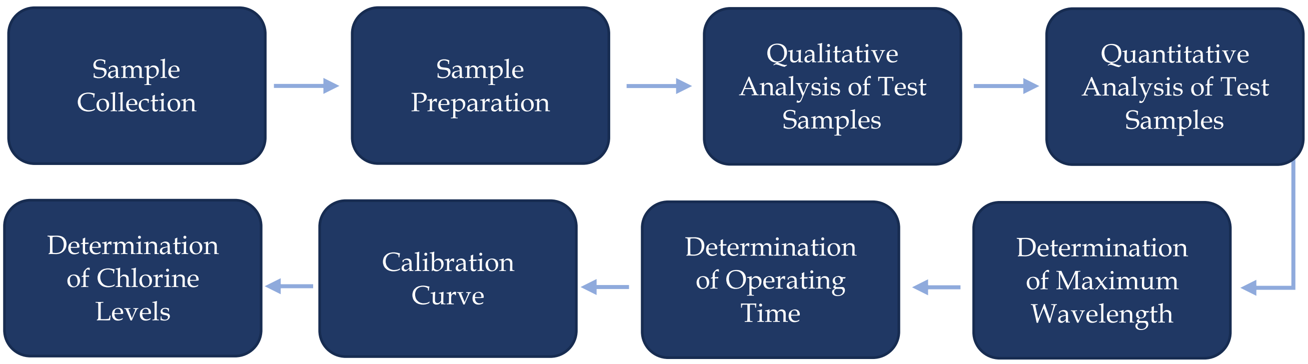

Figure 2. Research procedure

Result and Discussion

Nine wheat flour samples suspected of containing chlorine were analyzed in this study. The samples were selected based on visual characteristics, particularly the presence of a strikingly white color, which is commonly associated with the use of bleaching agents. The samples were coded A1, A2, A3, A4, A5, A6, A7, A8, and A9. All samples were collected from Pasar Baru Bekasi.

Prior to analysis, each wheat flour sample was prepared by weighing 10 g of sample, followed by extraction with 50 mL of chlorine-free distilled water. The mixture was homogenized and filtered using filter paper to obtain a clear filtrate. Filtration was performed to remove insoluble particles that could interfere with spectrophotometric measurements. An aliquot of 10 mL of the filtrate was then used for further qualitative and quantitative analyses.

Qualitative Test

The qualitative analysis was carried out to determine the presence or absence of chlorine levels in wheat flour samples. The test used N,N-diethyl-p-phenylenediamine (DPD) reagent, which is commonly applied in chlorine detection due to its ability to produce a distinctive color reaction when oxidized by chlorine. Before analyzing the samples, positive and negative controls were prepared to ensure the reliability of the qualitative test. The positive control consisted of chlorine-free distilled water mixed with calcium hypochlorite and DPD reagent, while the negative control consisted of chlorine-free distilled water mixed only with DPD reagent. The positive control produced a pink coloration, indicating the presence of chlorine, whereas the negative control remained clear, confirming the absence of chlorine in the control solution (Maharjan et al., 2017).

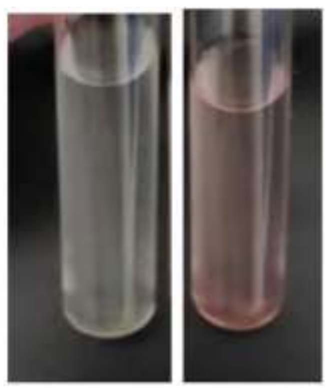

Figure 3. Positive and negative controls of the color reaction test

Table 1. Qualitative test on wheat flour samples

Sample	Code	Qualitative Results	Test Description
Wheat Flour	A1	Positive	Pink
	A2	Positive	Pink
	A3	Negative	Clear
	A4	Positive	Pink
	A5	Positive	Pink
	A6	Negative	Clear
	A7	Positive	Pink
	A8	Positive	Pink
	A9	Positive	Pink

The appearance of the pink color occurs because chlorine acts as a strong oxidizing agent that oxidizes the amine group present in the DPD reagent. This reaction forms a compound known as Würster dye, which produces a pink to magenta coloration in neutral conditions. The intensity of the color is generally proportional to the amount of chlorine present in the solution.

Based on the results obtained, two samples (A3 and A6) showed no color change and were therefore considered negative for chlorine. Meanwhile, seven samples (A1, A2, A4, A5, A7, A8, and A9) showed a color change to pink, indicating the presence of chlorine. The color formation occurs because chlorine oxidizes the amine group present in the DPD reagent. Under neutral pH conditions, this reaction forms Würster dye, which produces a pink coloration that serves as an indicator of chlorine in the tested solution.

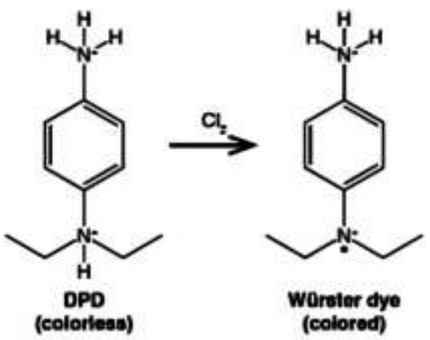

Figure 4. Reaction of DPD with chlorine

Quantitative Test

In the quantitative test of chlorine, the purpose was to determine the chlorine content present in the flour using a UV-Vis spectrophotometer. The samples used in this quantitative test were 7 samples that were positive for containing chlorine. In the quantitative test, the determination of maximum wavelength, calibration curve, and concentration determination were carried out. In this test, a standard calcium hypochlorite solution was used, in which the reaction formed in calcium hypochlorite is as follows.

$$Ca(OCl)^2 + 2(H^2O) \rightarrow 2HOCl + Ca(OH)^2$$

Hypochlorous acid in water will dissociate (Said, 2018), as shown in the following equation.

$$HOCl \leftrightarrow H^+ + OCl^-$$

The hypochlorite ion formed (OCl^-) is a strong oxidizing agent that acts on carotenoid pigments, namely the yellow pigments present in wheat (Panasenko et al., 1997). Carotenoid pigments belong to the lipid fraction; therefore, the hypochlorite ion can reduce carotenoid pigments, thereby making the flour appear whiter (Gómez-Cisneros et al., 2025).

Maximum Wavelength

The maximum wavelength was determined using a chlorine standard solution prepared from calcium hypochlorite with a concentration of 10 ppm. The standard solution was mixed with phosphate buffer and DPD reagent to produce the colored complex, which was then scanned using a UV–Vis spectrophotometer within a wavelength range of 490–530 nm.

The scanning results indicated that the highest absorbance value was observed at a wavelength of 511 nm. This wavelength corresponds to the maximum absorption of the colored complex formed between chlorine and the DPD reagent. Therefore, 511 nm was selected as the analytical wavelength for all subsequent measurements.

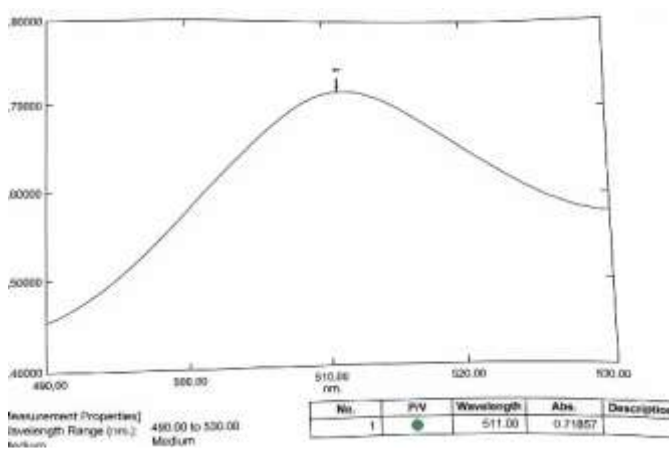

Figure 5. Maximum wavelength of chlorine

The use of the maximum wavelength is essential in spectrophotometric analysis because it provides the highest sensitivity for detecting the analyte and minimizes measurement errors caused by small wavelength shifts.

Operating Time

The operating time refers to the time required for the reaction between chlorine and the DPD reagent to reach a stable absorbance value. This parameter is important to ensure that measurements are taken when the reaction has reached equilibrium. To determine the operating time, a 10 ppm chlorine standard solution was measured at the previously determined maximum wavelength of 511 nm. The absorbance was recorded at regular time intervals to observe changes in absorbance over time.

The results showed that the absorbance values gradually increased during the early stages of the reaction and eventually reached a stable value between 16 and 20 minutes. This indicates that the reaction between chlorine and the DPD reagent had reached equilibrium within this time range. Therefore, the incubation time used for sample measurements was set at 16–20 minutes.

Calibration Curve

The calibration curve was constructed to establish the relationship between chlorine concentration and absorbance. Standard chlorine solutions with concentrations of 3, 5, 7, 9, and 11 ppm were prepared by dilution of the stock solution. Each standard solution was reacted with phosphate buffer and DPD reagent and allowed to stand for the predetermined operating time before measurement.

The absorbance values obtained from the spectrophotometric measurements were plotted against the corresponding concentrations to produce a calibration curve. The linear regression equation obtained from the curve was:

$$y = 0.0513x + 0.2051 \quad (2)$$

with a correlation coefficient (r) = 0.9988, indicating a strong linear relationship between chlorine concentration and absorbance.

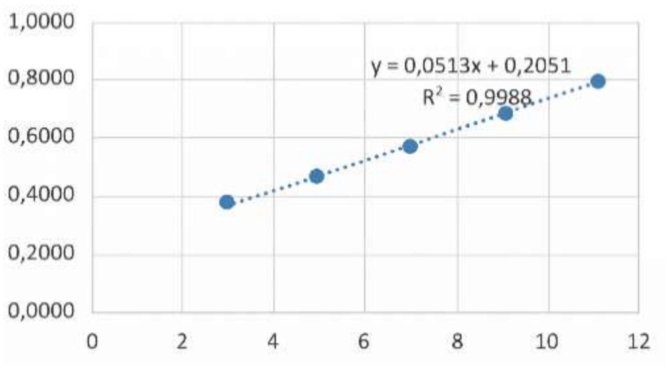

Figure 6. Chlorine calibration curve

The correlation coefficient value close to 1 indicates a strong linear relationship between chlorine concentration and absorbance. This suggests that the analytical method has good linearity and is suitable for quantitative determination of chlorine within the tested concentration range.

Determination of Concentration

Quantitative determination of chlorine was performed on the seven wheat flour samples that previously tested positive in the qualitative analysis. The filtrate obtained from sample preparation was reacted with phosphate buffer and DPD reagent, allowed to stand for 16–20 minutes, and then measured using a UV–Vis spectrophotometer at 511 nm. The absorbance values obtained from the spectrophotometric measurements were substituted into the linear regression equation derived from the calibration curve to determine the chlorine concentration in the test solution. The concentration calculated from the regression equation represents the chlorine concentration in the liquid test solution (ppm or mg/L).

However, this value does not directly represent the chlorine content in the wheat flour sample. Therefore, the concentration must be converted by considering the dilution factor used during sample preparation. In this study, 10 g of wheat flour was extracted with a final volume of 60 mL, resulting in a dilution factor calculated as follows:

$$Dilution\ Factor = \frac{60\ mL}{10\ g} = 6$$

Thus, the chlorine content in the wheat flour samples was calculated using the following equation:

$$\text{Chlorine Content}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{Concentration}(\text{ppm}) \times 6 \quad (3)$$

The results of the quantitative determination are presented in Table 2.

The quantitative analysis results showed that chlorine levels were detected in all seven samples that tested positive in the qualitative analysis. The average chlorine concentrations ranged from 5.13 mg/kg to 9.44 mg/kg. Among the analyzed samples, sample A2 showed the highest chlorine concentration, while sample A4 showed the lowest concentration among the positive samples.

When compared with the Regulation of the Food and Drug Supervisory Agency (BPOM) No. 28 of 2019, which specifies a maximum residue limit of 1 mg/kg for chlorine used as a processing aid in food processing, the chlorine concentrations detected in several samples exceeded the permitted limit. These findings suggest that chlorine levels were still present in certain wheat flour products circulating in the market.

The presence of chlorine levels in wheat flour may be associated with the use of chlorine-based bleaching agents during flour processing. Chlorine is known to oxidize natural pigments in flour, which contributes to a whiter color that is often preferred by consumers. However, excessive use of chlorine or improper processing conditions may cause residual chlorine to remain in the final product.

Continuous consumption of food products containing chlorine levels above the permitted limit may pose potential health risks (Kettlitz et al., 2016). Chlorine compounds can act as strong oxidizing agents that may cause irritation to the digestive tract and may lead to adverse health effects if consumed over a long period. Therefore, monitoring chlorine levels in food products is essential to ensure food safety and consumer protection.

Table 2. Quantitative test on wheat flour samples

Sample code	Absorbance	Concentration (ppm)	Sample content (mg/kg)	Average sample content (mg/kg)	Standard deviation
	0.24	0.73	4.69		
A1	0.24	0.80	5.13	5.14	0.25
	0.25	0.87	5.59		
	0.28	1.48	9.43		
A2	0.28	1.47	9.37	9.44	0.03
	0.28	1.48	9.42		
	0.25	0.87	5.57	5.13	
A4	0.24	0.04	5.14		0.44
	0.24	0.73	4.69		
	0.24	0.82	5.22		
A5	0.25	0.93	5.94	6.31	1.30
	0.26	1.22	7.76		
	0.25	1.02	6.49		
A7	0.24	0.80	5.13	6.37	1.18
	0.26	1.17	7,50		
	0.25	0.91	5.84		
A8	0.25	0.92	5.90	5.86	0.03
	0.25	0.91	5.86		
	0.28	1.47	9.37		
A9	0.27	1.40	8.95	9.16	0.21
	0.27	1.43	9.15		

Conclusion

Based on the results of qualitative and quantitative analyses, chlorine levels were detected in several wheat flour samples circulating in traditional markets in Bekasi. The qualitative test using the DPD reagent showed that seven out of nine samples changed to a pink color, indicating the presence of chlorine levels in the tested wheat flour. Quantitative analysis using UV–Vis spectrophotometry further showed that all seven samples that tested positive contained chlorine residue levels exceeding the maximum limit of 1 mg/kg established in BPOM Regulation No. 28 of 2019 concerning processing aids in food processing. These results indicate that 100% of the samples that were positive for chlorine did not meet the regulatory requirements. Considering the widespread use of wheat flour as a raw material for various food products, monitoring chlorine levels in flour circulating in the market remains important to ensure food safety. Further studies are recommended to include a wider sampling area and a larger number of samples in order to obtain a more comprehensive evaluation of chlorine levels in wheat flour products.

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Author Contributions
Conceptualization, S.D. and F.; methodology design and validation of analytical procedures, critical review and editing of the manuscript, supervision of the research process, F.; preparation of standard and sample solutions, qualitative and quantitative laboratory analysis using UV-Vis spectrophotometry, data analysis and interpretation, statistical analysis and calibration curve evaluation, manuscript drafting, overall project coordination, S.D. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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