

The Effect of Turmeric *Simplicia*'s (*Curcuma longa* L.) Washing Treatment on Total Ash, Total Plate Count, Yeast and Mold Count and Curcumin Level

Margaretha Harnanda Putri¹, Joko Kawiyo^{2*}, Puji Hartati³, Siti Nurraudah⁴, Nazir Ahmad⁵

¹Borobudur Herbal Medicine Industry Ltd., Semarang, Central Java, Indonesia

^{2,3}Politeknik Katolik Mangunwijaya, Semarang, Central Java, Indonesia

⁴Faculty of Pharmacy, Universitas Sultan Agung, Semarang, Central Java, Indonesia

⁵Department of Pharmacy, Abasyn University, Islamabad, Pakistan.

*Corresponding Author: jokoka2@gmail.com

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Abstract

Turmeric (*Curcuma longa* L.) is one of the traditional plants of Indonesia that has antibacterial activity, helps reduce fever, osteoarthritis, and digestive disorders. The washing process of *simplicia* is one of the sorting processes in post-harvest handling. This study aims to obtain the best washing method for *simplicia* with several washing treatments against the total ash content, Total Plate Count (TPC), Yeast and Mold Count (YMC), and curcumin content in turmeric *simplicia* and turmeric extracts. The study was conducted experimentally with 6 variations of *simplicia* washing treatments, namely without washing, washing with running water, washing with water at 50°C, washing with 0.2% NaOH, washing with 0.05% HCl, and washing with 2.0% acetic acid. The results showed that each variation of washing treatment gave significantly difference outcome of total ash, TPC, and curcumin level. The variation of washing with 50°C water provided the lowest TPC value test data of 6.7 CFU/g, washing with 50°C water provided the highest curcumin content test data of 9.38%. Based on several parameters of the test results, the washing process chosen was washing the using 50 °C water which showed high curcumin levels, TPC and YMC values that met the requirements, as well as the safety of the washing method.

Keywords: Ash content, ultrasonic extraxtion, turmeric (*Curcuma longa*), washing process

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Introduction

Turmeric (*Curcuma longa*) is a traditional plant found throughout Asia, including India and Indonesia. Turmeric has benefits for health including wound healing, antibacterial properties, reduced intestinal motility, elimination of body odor, and fever reduction [1]. The active compounds in turmeric, such as curcuminoids and sesquiterpenoids, have pharmacological effects that help to prevent cancer, atherosclerosis, reduce menstrual pain, osteoarthritis, and digestive disorders, and fight bacterial infections [2]. The main components of turmeric rhizomes include curcuminoids group, such as curcumin (75%), demethoxycurcumin (10-20%), and bisdemethoxycurcumin (5%) [3].

The process of separating active substances in *simplicia* requires an extraction method with optimum temperature, pressure, and polarity, either conventionally or by utilizing specific waves. One type of specific wave for the extraction process is ultrasonic waves. The ultrasonic method using ultrasonic waves with frequencies between 20 kHz and 2000 kHz to produce shock waves that cause cavitation. The cavitation process produces heating and pressure. This cavitation process does not cause damage because the burst bubbles occur quickly with a cooling rate of around 1010 K/s [4]. This condition causes the breaking of chemical bonds, thereby helping the diffusion of solvents into the plant cell walls [5].

The washing treatment of *simplicia* is one of the important steps in post-harvest handling, typically carried out together with the sorting process. According to the 2023 Food and Drug Supervisory Agency of the Republic of Indonesia Regulation and the 2011 Handbook of the Ministry of Health of the Republic of Indonesia, the recommended washing method is the use of clean running water. Several alternative washing methods have been

reported to influence the ash content and microbial load of *simplicia*. Washing with a 0.2% NaOH solution followed by neutralization using acetic acid can reduce the ash content by approximately 2.26%. Another method involving 0.05% dilute HCl and subsequent neutralization with water to achieve a neutral pH has also been shown to improve sample cleanliness. Washing with acetic acid alone effectively decreases the microbial count, while washing with water at 50 °C results in a 20.6% reduction in ash content [6].

Extract quality assurance can be performed by testing non-specific and specific parameters. Non-specific parameter testing includes the general profile of the extract in the form of water content, total ash content, acid-insoluble ash content, loss on drying, total plate count, and mold and yeast count, while specific parameters include the levels of specific compounds in the extract. The ash content parameter describes the inorganic components in the *simplicia* or in the extract. The Total Plate Count (TPC) parameter describes the number of bacteria that grow during the incubation period. Meanwhile, the Yeast Mold Count (YMC) parameter is a description of the number of molds and yeasts that grow during the incubation period [7].

Ash content reflects the inorganic components of a sample, including minerals and impurities. These impurities can be minimized by washing the medicinal plant material prior to extraction. Various washing methods for medicinal plants have been investigated, and several standards have been established. According to the Indonesian Food and Drug Monitoring Agency (BPOM) and the Center for Research and Development of Medicinal Plants of the Ministry of Health of the Republic of Indonesia, the recommended washing procedure involves the use of clean running water [8].

Microbial contamination parameters based on total plate count (TPC) and yeast and mold count (YMC) are considered non-specific quality indicators for both extracts and *simplicia*. The TPC is an important parameter influencing the stability and overall quality of herbal preparations [9]. According to the BPOM Regulation No. 29 of 2023, the acceptable limits for microbial contamination in *simplicia* are $\leq 5 \times 10^7$ CFU/g for bacteria and $\leq 5 \times 10^5$ CFU/g for molds and yeasts.

The use of ultrasonic extraction methods offers several advantages, particularly in reducing extraction time compared to conventional techniques. Sugiandi *et al.* (2021) reported that turmeric extraction using 95% ethanol and ultrasonic waves for 5 minutes yielded a curcumin content of 47.41% [10]. Another study found that ultrasonic-assisted extraction for 5 minutes produced a curcumin content of 32.85% [11].

The novelty of the current research lies in the washing treatment of *simplicia* using several variations of washing methods and their effects on extraction outcomes obtained through ultrasonic-assisted extraction. This study also evaluates both non-specific and specific quality parameters. To date, no studies have correlated the washing treatment with both non-specific parameters (total ash and microbial counts) and specific parameters (marker compound levels) when using the ultrasonic extraction method. Therefore, this study aims to analyze the effects of different washing methods on the total ash content, TPC, YMC, and curcumin levels in both medicinal plant materials and turmeric extracts.

Materials and Methods

Materials

The materials used in this study include turmeric *simplicia* from Semarang, Ethanol (Merck, Darmstadt, Germany), NaOH (Merck, Darmstadt, Germany), HCl (Merck, Darmstadt, Germany), Aquadest (Merck Darmstadt, Germany), Acetic Acid (Merck Darmstadt, Germany).

Methods

Preparation and particle size reduction of turmeric simplicia

Approximately 1 kg of turmeric *simplicia* was divided into several treatment groups as follows: (1) unwashed (control), (2) washed with running water for 10 minutes, (3) washed with water at 50 °C for 15 minutes, (4) washed with a 0.2% NaOH solution, (5) washed with a 0.05% HCl solution, and (6) washed with a 2% acetic acid solution. Each *simplicia* sample subjected to washing treatments (2)–(6) was subsequently dried at 50 °C until the moisture content was below 10%. All samples were then pulverized using a blender (VitaMix 5200, Canada) and sieved through a 25-mesh screen.

Extraction of turmeric according to each treatment

Turmeric *simplicia* from each previously sieved treatment was weighed (50 g) using an analytical digital balance (AND GH-252, Tokyo, Japan). The samples were extracted with 500 mL of 70% ethanol using an ultrasonic bath (TDR Force, Fuzhou, China) for 5 minutes. The resulting extract was then concentrated using a rotary evaporator (Steroglass Strike 300, San Martino, Italy) and subsequently subjected to ultrasonic treatment (TDR Force, Fuzhou, China) at 50 °C until the water content reached 30–40%.

*Evaluation of turmeric *simplicia* and extract under different washing treatments*

Total ash content determination

Approximately 1 g of sample powder was weighed and placed into a silica crucible that had been previously heated at 105 °C in an oven until a constant weight was achieved. Each sample was then incinerated in a muffle furnace (Nabertherm L09, Lilienthal, Germany) at 600 °C for 5 hours. The crucible and residue were reweighed until a constant weight was obtained [7].

Total plate count (TPC) and yeast and mold count (YMC) determination

The TPC was determined using plate count agar (PCA) medium with an incubation period of 24 hours at 37 °C. The YMC was determined using potato dextrose agar (PDA) medium with an incubation period of 5 days at 25 °C. According to BPOM standards, the acceptable TPC limits are $\leq 5.0 \times 10^7$ CFU/g for *simplicia* and $\leq 1.0 \times 10^5$ CFU/g for extracts, while YMC limits are $\leq 5.0 \times 10^5$ CFU/g for *simplicia* and $\leq 1.0 \times 10^3$ CFU/g for extracts [8].

Determination of curcumin content

Curcumin content was analyzed using the thin-layer chromatography (TLC) densitometry method with a TLC Scanner 4 (CAMAG, Muttenz, Switzerland). The mobile phase consisted of chloroform–methanol (95:5, v/v). The detection was performed at a wavelength of 420 nm using a standard series ranging from 50 to 250 ppm. The spotting volume was 2 µL [12].

Data analysis

The analyzed data included the percentage of total ash content, total plate count (TPC), yeast and mold count (YMC), and curcumin content of each *simplicia* and extract under different washing treatments. Data were tested for normality and homogeneity, followed by variance analysis using two-way ANOVA. Paired sample differences were analyzed using paired t-tests, while non-parametric tests (Kruskal–Wallis, Mann–Whitney, and Wilcoxon) were applied for non-normally distributed data. Statistical analysis was conducted using SPSS software version 23.0, and results were expressed as mean values.

Results and Discussion

Turmeric preparation and extraction

Curcumin, the major active compound in turmeric (*Curcuma longa*), can be isolated through various extraction techniques. Extraction efficiency is influenced by several factors, including temperature, pressure, extraction duration, and the method used. In this study, ultrasonic-assisted extraction was employed for 5 minutes. According to Wakte *et al.* (2011), ultrasonic extraction of turmeric for 5 minutes produced a curcumin yield of up to 32.85% [13], whereas Gopal *et al.* (2015) reported a yield of 55.7% [14]. Ultrasonic extraction has been shown to produce nanocurcumin, enhancing its solubility in solvents [15]. This method utilizes high-frequency sound waves that disrupt plant tissues and cause cell wall deformation, thereby increasing the release of active constituents. Consequently, ultrasonic extraction is more efficient than conventional methods such as maceration, which typically requires several days [16].

Previous comparative research between conventional maceration (three days of immersion) and ultrasonic extraction demonstrated extraction yields of 7.67% and 9.81%, respectively, indicating that ultrasonic extraction is more effective. Prior to extraction, turmeric *simplicia* from each washing treatment was ground and sieved to pass through a 25-mesh screen. Particle size reduction facilitates solvent penetration into plant tissues, thereby accelerating the diffusion of the active compound from the turmeric rhizome [17]. Smaller particle sizes increase the surface contact area between the solvent and *simplicia*, resulting in faster and more efficient extraction of curcumin. Moreover, the grinding and sieving processes help remove larger impurities, reducing the amount of inorganic material in the *simplicia* [18].

Washing treatments and ash content determination

The first treatment (unwashed sample) served as a control for comparison with other washing treatments. The second treatment, washing with running water, followed the procedures stated in the Good Manufacturing Practices for Traditional Medicines (CPOTB, BPOM, 2021) and the Post-Harvest Guidelines for Medicinal Plants issued by the Ministry of Health, and was therefore used as the regulatory standard washing method. The third treatment involved washing with water at 50 °C, can reduce ash content by 20.6%. The fourth treatment used a 0.2% NaOH solution for washing *simplicia*. The fifth treatment, washing with 0.05% HCl reported a reduction in ash content from 5.04% to 1.94%. The sixth treatment used 2% acetic acid (vinegar), a weak acid commonly used in food processing and regarded as a safe washing solution; it was included as an acidic derivative of the fifth treatment [6].

The ash content was determined gravimetrically. This method involves heating the sample at high temperatures until all organic components and their derivatives are decomposed and volatilized, leaving only inorganic and mineral residues. The inorganic fraction may consist of intrinsic minerals of the *simplicia* or external impurities attached to it [19]. To minimize impurities, the samples were sieved during preparation. Washing treatments were expected to affect the measured ash content, particularly the proportion derived from impurities.

The mineral composition of turmeric includes calcium, iron, copper, sodium, potassium, phosphorus, and zinc, with a total mineral content of 2.85 g per 100 g of turmeric. The Indonesian Herbal Pharmacopoeia specifies that the total ash content should not exceed 8.2% for turmeric *simplicia* and 0.4% for turmeric extract [2]. The 0.4% ash limit for the extract depends on the extraction method. In this study, ultrasonic extraction with a short extraction duration was employed, which differs from the conventional extraction method referenced in the pharmacopoeia. The results of total ash determination for turmeric *simplicia* and extract under different washing treatments are summarized in Table 1.

Table 1. Total ash of turmeric *simplicia* and extract

Sample	Replication	Total ash	Mean	SD
<i>Simplicia</i>	1	7,85%	7,32%	0,005439
	2	7,53%		
	3	6,57%		
	1	6,88%	6,36%	0,004057
	2	6,31%		
	3	5,89%		
	1	5,23%	5,26%	0,000648
	2	5,35%		
	3	5,20%		
	1	6,77%	6,88%	0,000573
	2	6,85%		
	3	6,91%		
	1	5,01%	5,04%	0,000478
	2	4,99%		
	3	5,10%		
Extract	1	4,73%	4,73%	0,000653
	2	4,65%		
	3	4,81%		
	1	4,26%	4,08%	0,003552
	2	4,39%		
	3	3,58%		
	1	2,78%	2,76%	0,000262
	2	2,73%		
	3	2,79%		
	1	4,34%	4,05%	0,00226
	2	4,01%		
	3	3,79%		
	1	4,47%	4,04%	0,00427
	2	4,20%		
	3	3,46%		
	1	1,64%	1,57%	0,000806
	2	1,62%		
	3	1,46%		
	1	1,89%	1,81%	0,00091
	2	1,85%		
	3	1,68%		

The results of ash content analysis showed that washing treatments affected the total ash content of turmeric *simplicia*. Six washing variations produced differences in ash reduction ranging from 1.09% to 3.83% between *simplicia* and extract samples. The decrease in ash content of the turmeric extract was also influenced by drying conditions, including temperature and drying method. Extraction temperature and duration could further affect both the curcumin and ash content of the resulting extract. Variations in washing effectiveness were associated with each

method's ability to dissolve or remove water-soluble compounds and inorganic impurities that contribute to total ash content (residual inorganic minerals after combustion of organic matter) [20].

In the unwashed sample, all contaminants and non-organic impurities remained, producing the highest initial ash content (7.69%). After extraction, many inorganic substances remained insoluble, resulting in a substantial decrease in ash content to 4.32% due to precipitation and filtration through the mesh. In samples washed with running water, surface dust and water-soluble inorganic impurities were removed, reducing the ash content to 6.59% in *simplicia* and 2.76% in the extract. Washing with water at 50 °C produced only a slight difference (1.09%) between *simplicia* and extract, likely because some inorganic molecules remained undissolved and could not be separated during extraction.

Washing with 0.2% NaOH reduced total ash to 6.88%, but the difference was not statistically significant compared to the unwashed control. NaOH, as a strong base, can dissolve certain organic components such as lignin and cellulose, but is less effective than acid in breaking down inorganic residues. Consequently, only small insoluble compounds became soluble, causing limited ash reduction in the extract.

Washing with 0.05% HCl significantly decreased total ash from 5.04% in *simplicia* to 1.63% in the extract. HCl, a strong acid, dissolves mineral salts, inorganic impurities, and heavy metals through dissociation reactions that release protons (H^+). These protons react with metal ions or inorganic compounds such as silica, forming soluble salts and thus markedly reducing ash content [21]. Similarly, washing with 2% acetic acid also significantly decreased ash levels (4.73% in *simplicia* to 1.87% in extract). Although weaker than HCl, acetic acid remains effective in dissolving metal and mineral compounds, producing a notable ash reduction though less pronounced than that caused by HCl.

Statistical analysis using SPSS version 23.0 showed that the ash content data were normally distributed but not homogeneous. Therefore, two-way ANOVA and paired t-tests were applied. ANOVA results indicated that washing with running water, water at 50 °C, HCl, and acetic acid produced significant differences compared to the unwashed *simplicia*, while NaOH did not. This indicates that washing with NaOH had no significant effect on total ash content. Among the treatments, washing with acetic acid yielded the lowest ash content in *simplicia*.

For extracts, ANOVA results showed significant differences for washing with running water, HCl, and acetic acid, while washing with NaOH and water at 50 °C did not differ significantly from the unwashed extract. The lowest ash content in the extract was observed in the HCl-treated sample. Overall, both ANOVA and paired t-tests confirmed significant differences between the ash content of *simplicia* and their corresponding extracts. The greatest reduction in ash content occurred in samples washed with HCl and acetic acid, likely due to the ability of acids to dissolve mineral salts—the main inorganic constituents of turmeric. The hydrogen cations (H^+) in acids react with mineral anions to form soluble, stable compounds, thereby reducing residual inorganic matter [22,23].

Total plate count (TPC) and yeast and mold count (YMC)

Microbial quality was evaluated based on total plate count (TPC) and yeast and mold count (YMC). The TPC test was performed to determine the total bacterial population, while the YMC test measured the presence of yeast and mold contamination in each sample. Testing was carried out on 12 turmeric samples, each analyzed in triplicate. According to BPOM Regulation No. 29 of 2023, the acceptable microbial limits are $\leq 5 \times 10^7$ CFU/g for *simplicia* and $\leq 5 \times 10^5$ CFU/g for extracts. The *simplicia* samples that did not meet the TPC requirements were the unwashed sample, the sample washed with running water, and the sample washed with HCl. Among the extracts, only the sample washed with HCl exceeded the TPC limit. For yeast and mold contamination, all *simplicia* and extract samples met the regulatory limits of $\leq 5 \times 10^5$ CFU/g for *simplicia* and $\leq 5 \times 10^3$ CFU/g for extracts.

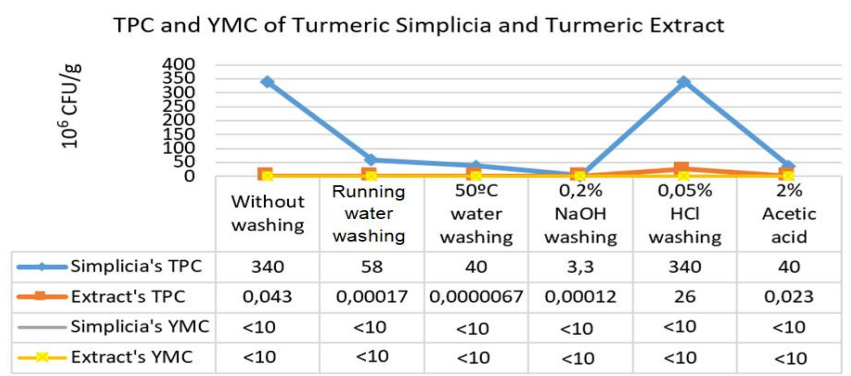


Figure 2. Total plate count (TPC) and yeast and mold count (YMC) of turmeric *simplicia* and extracts under different washing treatments.

The highest TPC value for turmeric *simplicia* was observed in samples washed with running water, whereas the lowest was found in samples washed with 0.2% NaOH. Interestingly, the unwashed samples exhibited lower TPC values than those washed with running water, water at 50 °C, or acetic acid. One possible explanation is that washing with running water may increase the moisture content and humidity of the *simplicia*, which can promote microbial growth. Although drying was performed to achieve a moisture content below 10%, uneven drying could have occurred, creating localized areas favorable for microbial proliferation. Moisture content above 10% can increase humidity and subsequently stimulate microbial growth; therefore, both drying temperature and uniformity should be carefully monitored [24].

For turmeric extracts, the highest TPC value was recorded in samples washed with HCl, while the lowest was found in extracts washed with water at 50 °C. Strong acids such as HCl have low pKa values, meaning that they remain mostly dissociated in solution. As a result, acid stress occurs primarily outside the bacterial cell membrane, preventing effective intracellular acidification, which allows some bacteria to survive. In contrast, weak acids with higher pKa values exist in both dissociated and undissociated forms, enabling them to penetrate bacterial membranes and disrupt cell wall integrity. The microbial response to acid washing depends on the type of microorganisms present, as certain bacterial species are capable of surviving acidic environments [25].

Statistical analysis using SPSS version 23.0 revealed that the TPC data were not normally distributed and not homogeneous; therefore, non-parametric tests (Kruskal–Wallis followed by Mann–Whitney) were applied. The results showed that nearly all treatments significantly affected TPC values (asymptotic significance < 0.05), except for *simplicia* washed with 0.05% HCl and extracts washed with acetic acid. The highest TPC values were observed in unwashed samples, whereas the lowest occurred in *simplicia* washed with 0.2% NaOH and extracts washed with water at 50 °C. Comparison between *simplicia* and extracts under identical washing treatments also revealed significant differences. The YMC values in all samples were below 10 CFU/g, indicating that all treatments met the microbial quality requirements.

Curcumin content in turmeric

Curcumin content in turmeric varies depending on the extraction method, washing treatment, and raw material quality. According to the Indonesian Herbal Pharmacopoeia, 2nd Edition (2017), the curcumin content should not be less than 3.82% in turmeric crude drug and not less than 11.17% in turmeric extract. However, the results of this study indicated that both turmeric *simplicia* and extracts did not meet these pharmacopoeia standards.

Variations in curcumin content among washing treatments were associated with differences in curcumin stability, solubility, and potential loss during washing. The percentage of curcumin content in turmeric *simplicia* and extracts under different washing treatments is presented in Figure 3.

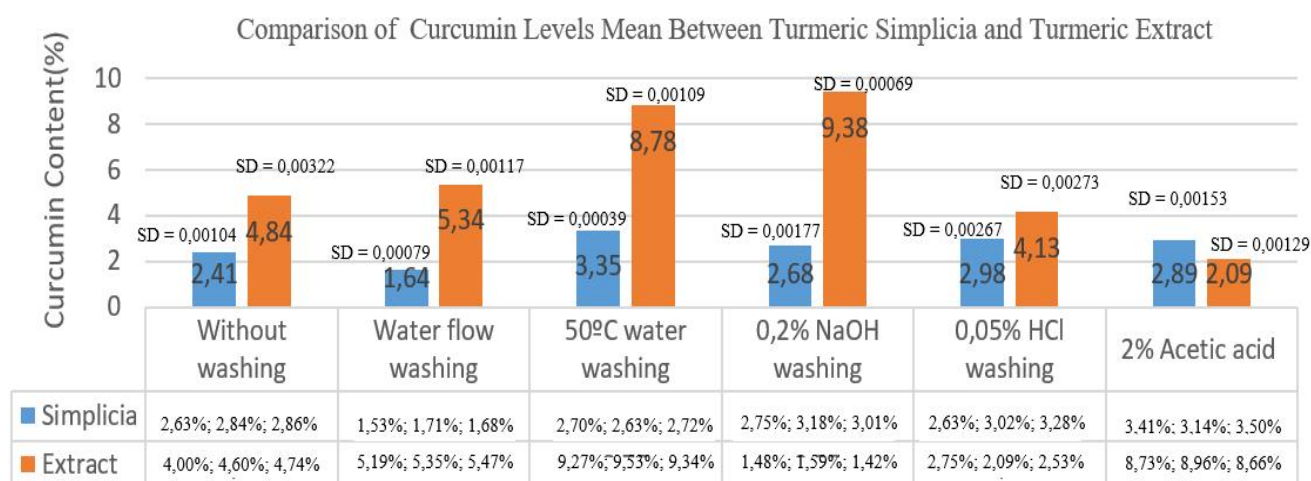


Figure 3. The percentage of curcumin content between turmeric *simplicia* and extract varied according to the washing treatment.

The curcumin level in unwashed turmeric was used as the baseline for comparison with other washing treatments. However, contaminants may still be present, resulting in non-optimal curcumin yield due to the presence of impurities. Washing with running water effectively removed dirt and dust without damaging the curcumin structure, producing a purer extract with minimal curcumin loss. Consequently, the curcumin content in these samples increased compared to the unwashed extract.

Table 2. Comparison of curcumin content between turmeric *simplicia* and extract

<i>Simplicia</i>	Extract	p-value	Meaning
Without washing	Without washing	0,000	Significantly different
Running water washing	Running water washing	0,000	Significantly different
50°C water washing	50°C water washing	0,000	Significantly different
0,2% NaOH washing	0,2% NaOH washing	0,000	Significantly different
0,05% HCl washing	0,05% HCl washing	0,000	Significantly different
2% Acetic acid washing	2% Acetic acid washing	0,007	Significantly different

The use of NaOH, a strong base, facilitated cell wall disruption and enhanced curcumin extraction efficiency, indicating that alkaline treatment improved curcumin availability. Conversely, the curcumin content in samples washed with 0.05% HCl was markedly lower. Strong acids such as HCl can degrade or denature curcumin molecules, as curcumin is sensitive to acidic conditions. Washing with 2% acetic acid, a weak acid, resulted in a milder reduction; degradation still occurred, possibly due to partial dissolution of curcumin or minor structural alterations during washing.

Washing with warm water (50 °C) increased curcumin content because moderate heat softens plant tissue, enhances solvent penetration, and accelerates curcumin release during extraction [26]. Although curcumin is reported by the Food and Agriculture Organization (FAO) to be more stable under acidic conditions than basic ones, the present study found that acid-washed samples contained lower curcumin levels than those washed with water or NaOH. This suggests that during acid washing, curcumin may have been leached into the washing solution, leading to a lower remaining content.

Among all treatments, washing with NaOH and water at 50 °C produced the highest curcumin levels, likely because these conditions enhanced cell permeability without substantial curcumin loss. In contrast, washing with HCl and acetic acid reduced curcumin concentration due to the acid-induced degradation of curcuminoids. Curcumin degradation may occur at temperatures above 55 °C, where curcuminoids can transform into diketene curcumin, 4-vinylguaiaicol, and ferulic acid. Therefore, limiting the washing temperature to 50 °C helps prevent degradation of these compounds.

The curcumin data were normally distributed and homogeneous, allowing for two-way ANOVA and paired t-test analyses. As shown in Table 2, the curcumin content differed significantly ($p < 0.05$) between turmeric *simplicia* and extract across all washing treatments. The results showed that *simplicia* washed with running water and water at 50 °C differed significantly ($p < 0.05$) from the unwashed control. The highest curcumin content was obtained in *simplicia* washed with water at 50 °C, while the lowest was found in samples washed with running water. Heating at 50 °C likely initiated partial blanching, which enhances solvent absorption into plant cells and facilitates the release of active compounds during extraction [27].

Conclusion

This study demonstrated that washing treatments had a significant effect on the total ash content, total plate count (TPC), and curcumin content of turmeric *simplicia* and extracts. Among all treatments, washing with water at 50 °C produced the best results, yielding the highest curcumin levels (3.35% in *simplicia* and 8.78% in extract), the lowest microbial counts (TPC 6.7 CFU/g and YMC < 10 CFU/g), and a reduction in total ash from 5.26% to 4.17%.

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- Ethics Declaration : This study did not require ethical approval from an institutional research ethics committee because it did not involve human participants or animal experimentation.

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