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Effect of storage duration on phenolics stability in ready-to-drink coffee beverage

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ABSTRACT

Ready-to-drink (RTD) coffee is one of the innovations in coffee beverages that is widely circulated in the market and is quite popular among various groups of people. This study aimed to partially validate the Folin-Ciocalteu method for phenolic compounds analysis in ready-to-drink (RTD) coffee and determine the effect of storage time on the stability of phenolic compounds in RTD coffee. Coffee drinks were made from Robusta coffee extract added with other ingredients (sugar and potassium sorbate), pasteurized, hot filled into plastic cups, and stored at room temperature (25 – 28 °C) for 0, 2, 4, 6, and 8 weeks. Two proposed methods (Folin-Ciocalteu methods) for routine phenolic analysis (Total phenolic content (TPC) and total tannin content (TTC)) in RTD coffee were partially validated. In general, partial validation parameters of TPC gave more satisfying results, such as linearity (R^2 = 0.9991); precision (RDS < 2/3 $RSD_{Horwitz}$); recovery (109%) and sensitivity (LOD = 14.20 mg L^{-1}). Phenolic stability in RTD coffee was evaluated using the Folin-Ciocalteu method (TPC and TTC) and HPLC method (individual caffeoylquinic acids (CQAs) (3-CQA, 4-CQA, and 5-CQA)). In general, TTC values in all storage time were higher than TPC values because the TTC method had higher recovery (132%). Furthermore, the three parameters observed (TPC, TTC and CQAs) experienced significant degradation during storage. The decrease percentage of TPC and TTC during 8 weeks of storage was 11% (from 63 to 55 mg/100 mL) and 10% (160 to 143 mg/100 mL), respectively. Meanwhile, 5-CQA was the most sensitive CQAs and its degradation for 8 weeks of storage reached 19% (from 28 to 23 mg/100mL). TPC is recommended for monitoring the effect of storage duration on phenolic compound stability in RTD coffee and analysis of 5-CQA as the most unstable individual phenolic may support the monitoring.

Key words: Caffeoylquinic acids; phenolics; ready-to-drink coffee; stability; tannins.

1 INTRODUCTION

World's coffee consumption grows significantly in the last four years with the average increase of 2% (International Coffee Organization - ICO, 2023). Among coffee products, ready-to-drink (RTD) coffee shows the fastest growth due to the convenience of consumption (Jeon et al., 2019). Furthermore, RTD coffee is a shelf-stable product, so it is easily distributed in huge areas.

The stability of RTD coffee during storage and distribution is caused by high temperature treatment (thermal process, i.e. 115-121 °C) which resulted in "commercial sterility". Commercial sterility is defined as "the absence of microorganisms capable of growing in the food at normal nonrefrigerated conditions at which the food is likely to be held during manufacture, distribution and storage" (Codex Alimentarius Commission - CAC, 1993). Thermal processes commonly used to produce RTD coffee products are in container sterilization and aseptic filling (Akiyama et al., 2014; Lin et al., 2022). It is because RTD coffee products have relative low acid content (pH ≥ 4.6) (Córdoba et al., 2021; Rao; Fuller, 2018). Beside the two treatments, hot filling treatment that usually applied for high acid beverages (pH < 4.6) (Manfredi; Vignali, 2015; Skinner et al., 2015) also may be applied for low acid beverages for some reasons.

Aseptic filling and hot filling in beverages industry look similar, but the processes are different. In hot filling, beverage is pasteurized in heat exchanger, hot-filled into packaging at 88–92 °C, closed, cooled with cold water (Manfredi; Vignali, 2015; Skinner et al., 2015). The closed packaging can be inverted to give sterility effect to headspace and lid/cap area of the packaging (Skinner et al., 2015). In aseptic filling, beverages and packaging are sterilized separately. Sterile beverage is transferred into aseptic zone, then filled into sterile packaging, closed, and sealed (Manfredi; Vignali, 2015).

In Indonesian regulation released by the National Agency of Drug and Food Control (NADFC), hurdle technology must be applied for hot-filled low acid food (pH ≥ 4.6 and Aw ≥ 0.85) (Indonesian NADFC, 2021). The hurdle technology is a simultaneous or sequential application of various intervention as hurdle to inactivate microorganisms and preserve food (Aaliya et al., 2021). The combination of thermal process and preservatives is an example of a hurdle technology. The hurdle technology for hot-filled low acid food must be supported by a challenge test which prove that the hurdle can inhibit the growth of *Clostridium botulinum* or its surrogate (Indonesian NADFC, 2021). The stability evaluation of preservatives in hot-filled low acid food becomes part of this challenge test.

Preservatives used in hot-filled RTD beverages may be intentionally added as food additive or naturally exist in raw materials. Tea phenolics, catechins are well knowns natural preservatives in tea beverages (Hara; Watanabe, 1989; Hara-Kudo et al., 2005). An effective concentration of catechins to inhibit *Clostridium botulinum* growth is about 500 ppm (Hara-Kudo et al., 2005). Coffee beans also contain phenolics which have antimicrobial activity (Le et al., 2022; Wu et al., 2020). In the case of RTD coffee, the antimicrobial activities of phenolics may be expected if they are stable during storage. The stability of phenolics in RTD tea in short storage has been investigated in previous studies (Baek et al., 2021; Urías-Orona; Niño-Medina, 2019). However, based on our knowledge, the stability of phenolics in hot-filled RTD coffee during storage has not been investigated.

Investigation of phenolic compounds stability in RTD coffee during storage may be conducted by monitoring the concentration of the compounds (Total phenolic content (TPC), Total Tannin Content (TTC) and individual phenolic acids). TPC and TTC analysis using Folin-Ciocalteu methods (spectrophotometric method) are simpler and more affordable for routine analysis compared to analysis of individual phenolic acid (HPLC method). However, the method may have disadvantages because Folin-Ciocalteureagent may react with any reducing compound other than phenolic. Therefore, validity of TPC and TTC analysis for coffee sample should be conducted and the stability study of the TPC and TTC should be compared with individual phenolic compounds found in coffee sample such as 5-,4- and 3- caffeoylquinic acids (CQA) (Jeszka-Skowron et al., 2016). Based on those explanations, the objectives of this study were to partially validate Folin-Ciocalteu method (TPC and TTC analysis) for RTD coffee and to evaluate the stability of phenolics (TPC, TTC, and individual CQAs) in RTD coffee beverage. This study is expected to give recommendations about more feasible analysis for monitoring of the stability of phenolics as natural preservative for RTD coffee to support challenge test study.

2 MATERIALS AND METHOD

2.1 Materials

Medium to dark roasted robusta coffee beans as the main ingredient in this study were obtained from Kemenady Industri Mandiri company, Bogor, Indonesia. Chemicals such as 3-O-caffeoylquinic acid (3-CQA), 4-CQA, and 5-CQA were purchased from Sigma-Aldrich (St. Louis, USA). Other supporting chemicals such as gallic acid, tannic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), ascorbic acid, methanol Pro Analysis (PA), formic acid, methanol for Liquid Chromatography (LC), and water for Liquid Chromatography (LC) were purchased from Merck (Darmstadt, Germany).

2.2 Preparation of ready-to-drink coffee beverages

The RTD coffee formula and production steps followed the common procedure of commercial RTD. Before extraction, the roasted coffee beans were initially ground using a coffee grinder (Latina Electric Coffee Grinder 600N; Taiwan) until they reached the size of table salt (Fine level with griding size 2 in the grinder). 50 g of coffee powder was dissolved in 1000 mL of boiling water (90 °C) for 1 minute with constant stirring. Coffee extract was then obtained by filtering the cooled coffee solution with an 80 mesh sieve and then filtered again using a vacuum pump (VALUE Vacuum Pump FY-2C-N) with the help of a flask funnel and filter paper (Advantec Filter Paper No.2, Diameter: 90 mm, Thickness: 0.26 mm).

Coffee extract was added by 250 mg L^{-1} sorbic acid or equivalent to 337.5 mg L^{-1} potassium sorbate and sugar 60 g L^{-1}. The coffee beverages was then pasteurized at 80-85 °C and and then quickly filled into Polypropylene (PP) opaque cups (4.5 x 7 x 9 cm) of 180 mL with hot filling at 80 °C. The packages were then sealed and turned upside down, allowed to stand for 3 minutes and then put into a pot of room temperature water. The coffee beverages was then removed from the pot and cooled at room temperature. The production of RTD beverage samples was carried out in two batches with an interval of one week. Each batch produced 14 cups, the total samples produced from 2 batches were 28 cups.

2.3 Product Storage

Product storage was done by labeling the samples according to the production time. The product was stored in room conditions (25 – 28 °C) for 8 weeks, with common light condition in the laboratory. Observations were made every two weeks to determine the content of phenolic compounds, while caffeoylquinic acids were observed every four weeks to determine their amount and stability. Determination of phenolic compounds were carried out using four samples and two repetitions resulting in eight test data obtained. While, caffeoylquinic acids test were carried out using four samples resulting in four test data obtained for each week.

2.4 Partial validation of Total Phenolic and Total Tannin Content analysis method

Total phenolic content (TPC) and Total tannin content (TTC) were partially validated according to (Association of Official Agricultural Chemists - AOAC, 2023) guidelines. The four parameters were evaluated in terms of linearity, sensitivity (limit of detection (LOD) and limit of quantification (LOQ)), precision, and accuracy. Linearity was conducted 7 replicates using 7 points of standard curves with range of 30 – 90 mg L^{-1} of gallic acid for TPC and 60 – 180 mg L^{-1} of tannic acid for TTC. LoD and LoQ were estimated from the calibration curve with Equation 1 and 2, respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOD = \frac{10\sigma}{S} \quad (2)$$

Where:
σ = Standard deviation of the lowest concentration of standard curve
S = Slope of standard curve

The precision was estimated by analyzing the RTD coffee sample with 7 replications. The relative standard deviation (RSD) analysis was compared with 2/3 RSD Horwitz's. The accuracy was determined triplicates by spiking the RTD coffee sample with 30 mg L^{-1} of gallic acid and 60 mg L^{-1} of tannic acid.

2.5 Determination of Total Phenolic Content (TPC)

Total phenol content (TPC) was determined using the Folin-Ciocalteu method according to (Kc et al., 2020), with slight modifications. Briefly, 0.1 mL of sample was taken and then added with 0.4 mL of methanol. The mixture was then added with 2.5 mL of 10% Folin-Ciocalteu reagent followed by 2.5 mL of 7.5% Na_2CO_3 into each tube. The assay mixture was vortexed until fully mixed and incubated at 45°C for 45 minutes. The absorbance was then measured using a uv-vis spectrophotometer at a wavelength of 765 nm. Standard solution as a reference was made using gallic acid with seven different concentration (30, 40, 50, 60, 70, 80, and 90 mg L^{-1}). A mixture of methanol (0.5 mL), 10% Folin-Ciocalteu reagent (2.5 mL) and 7.5% Na_2CO_3 (2.5 mL) was used as a blank. The determination of TPC was carried out using four samples and two repetitions resulting in eight test data obtained. The absorbance data was then plotted into a standard curve to analyze the concentration and TPC was expressed as mg GAE/100 mL

2.6 Determination of Total Tannin Content (TTC)

The tannin content was determined using the Folin-Ciocalteu method according to (Haile; Kang, 2019), with slight modifications. Briefly, 0.1 mL of sample was taken and then added with 8.4 mL of distiled water (total amount of 8.5 mL). The mixture was then added with 0.5 mL of 100% Folin-Ciocalteu reagent followed by 1 mL of 35% Na_2CO_3 into each tube. The assay mixture was vortexed until fully mixed and incubated at room temperature in a dark room for 30 minutes. The absorbance was then measured using a Uv-Vis Spectrophotometer at a wavelength of 700 nm. Standard solution as a reference was made using tannic acid with seven different concentration (60, 80, 100, 120, 140, 160, and 180 mg L^{-1}). A mixture of distiled water (8.5 mL), 100% F-C reagent (0.5 mL), and 35% Na_2CO_3 (1 mL) was used as a blank. Similar to TPC, the determination of tannin content was carried out using four samples and two repetitions resulting in eight test data obtained. The absorbance data was then plotted into a standard curve to analyze the concentration and tannin content was expressed as mg TAE/100 mL

2.7 Determination of caffeoylquinic acids (CQAs)

Determination of CQAs content was carried out using an HPLC following Herawati et al. (2022), without any modifications. Briefly, a total of four coffee samples (two from each batch) were prepared and then diluted five times with distilled water. All samples were further filtered using a 0.45 µm PTFE filter membrane and then 20 µL of the coffee sample was injected into HPLC (LC-20AD system equipped with UV-Vis detector; SHIMADZU Corp, Kyoto, Japan). Concentration of CQAs was expressed in mg/100 mL.

2.8 Statistical Analysis

Data on phenolic compound stability during storage was analyzed using Analysis of Variance (ANOVA) to determine significant differences between means of the sample stored in different durations (weeks). Significant differences were then analyzed using Duncan's test at the 0.05 level ($P<0.05$). Both ANOVA and Duncan tests were conducted using IBM SPSS 26 software.

3 RESULTS

3.1 Method quality of total phenolic content and total tannin content analysis in ready-to-drink coffee

The results showed that the overall test parameters (R^2, precision, accuracy, and sensitivity) gave satisfactory results as presented in Table 1. The coefficient of determination (R^2) of the regression curve for TPC and TTC were >0.99. Those coefficients of determination are recommended as evident for the goodness of fit. The precision test was carried out using repeatability by considering the RSD value. Overall, the RSD value obtained in the repeatability test for TPC and TTC is in accordance with the acceptance criteria (RSD of analysis < 2/3 RSD Horwitz), which indicates that the precision test data is acceptable. The accuracy test (recovery) results in Table 1 show a satisfactory value in the TPC test of 108.67%, which is in accordance with the acceptance limits. On the other hand, the accuracy test for TTC determination gave unfavorable tes results of 132% which exceeds the standard limit. For the sensitivity test, the LoD and LoQ test results for TPC were 14.20 and 43.03 mg L^{-1} and TTC were 9.02 and 27.33 mg L^{-1}, respectively.

Table 1: Partial validation of total phenolic content (TPC) and total tannin content (TTC) analysis for RTD coffee.

Parameter		TPC	TTC
Calibration	Regression equation	y = 0.01x + 0.0031	y = 0.0045x – 0.0118
	Coefficient of determination (R^2)	0.9991	0.9989
LOD (mg L^{-1})		14.20	9.02
LOQ (mg L^{-1})		43.03	27.33
Repeatability	RSD (%)	1.71	1.62
	2/3 RSD Horwitz (%)	2.84	2.49
Recovery (%)		108.67	132

Calibration curves were conducted using 7 replicates. LoD and LoQ were estimated using the data of 7 standard curves. Repeatability was conducted 7 replicates. Recovery was conducted using 3 replicates.

3.2 Stability of total phenolic content and total tannin content in ready-to-drink (RTD) coffee during storage

Evaluated TPC and TTC method were used to analyze the stability of the compound during storage. TPC content of RTD coffee during storage is presented in Figure 1. The initial TPC content in the beverage before storage was 63.02 mg GAE/100 mL. It decreased to 54.61 mg GAE/100 mL at the end of the storage period (8 weeks). The decrease percentage in TPC was known to be 11%. Statistical tests showed a significant decrease ($P< 0.05$) only occurred at 2-4 week, while in other weeks no significant changes were found. The downward trend is also known to occur in the TTC (Figure 2). The TTC recorded at the beginning was 160.42 mg TAE/100 mL and this result then decreased to 142.98 mg TAE/100 mL at the end of the storage period. The percentage decrease in total tannins measured was 11%. Statistical tests showed a significant decrease ($P< 0.05$) only occurred at0-4 weeks and then tended to decrease slowly after 4 weeks of storage (nosignificantdifferences).

3.3 Stability of caffeoylquinic acids in ready-to-drink (RTD) coffee during storage

The individual CQA analysis was also performed to have more comprehensive data about the alteration of phenolic compound composition during RTD coffee storage. At the beginning of storage, the total caffeoylquinic acids (CQAs) content in RTD coffee beverage samples was 46.10 mg/100 mL. During storage, the total chlorogenic acid content slowly decreased along with the storage time but then decreased rapidly in the week 8. The total CQAs content recorded at the end period of storage was 40.50 mg/100 mL (Figure 3). The percentage decrease in total CQAs compounds in RTD coffee beverage samples was found to be 12%.

The 3-CQA compound did not experience a decrease but an increase in concentration. This increase occurred significantly ($P< 0.05$) in the first week and tended to stabilize in the following week. In the 4-CQA compound, the stability of the compound decreased slowly which was relatively low in the first week and tended to be significant ($P< 0.05$) in the eighth week of storage. The 5-CQA compound as the largest contributor compound in total CQA also experienced a slow decline in the initial week and tended to be significant in the week 8. These results indicate that storage can affect the stability properties of 5-CQA compounds in RTD coffee beverage samples.

4 DISCUSSION

Performance evaluation of the Folin-Ciocalteu method was carried out using four parameters which are linearity, precision, recovery and sensitivity. The linearity (R^2) test in this study gave good results (TPC = 0.9991 and TTC = 0.9989) in accordance with the literature (AOAC, 2023). The linearity results are linear if the coefficient of determination (R^2) value is ≥ 0.99 or closes to 1 (Martins et al., 2021).

The RSD values obtained in the repeatability test for TPC (1.71%) and TTC (1.62%) were in accordance with the acceptance criteria by (AOAC, 2016) and was less than 2/3 RSD Horwitz (TPC = 2.84% and TTC = 2.49%), which indicated that the precision test data was acceptable (Rivera; Rodríguez, 2011). In the accuracy parameter, TPC (108%) was known to provide a percentage recovery that matches the (AOAC, 2016) with acceptance range of 80-110%. Meanwhile TTC analysis (132%) did not fit with the accuracy criteria. In the sensitivity test, the LOD test values (TPC = 14.20 mg L^{-1} and TTC = 9.02 mg L^{-1}) were lower than the lowest concentration of standard curves (TPC = 30 mg L^{-1} and TTC = 60 mg L^{-1}). It indicated the ability of the Folin method to determine the substance in its lowest concentration within standard curve range (Ferreira et al., 2020).

By using the partially validated method, this study can monitor the stability of the TPC and TTC in

RTD coffee during storage. Previous study reported that TPC degradation during thermal processing of the extract of *Bixa orellana* L. occurred following the first-order kinetic, which depends on temperature, pH, and soluble solids (Zapata et al., 2022). Furthermore, the extract with pH 5.5 and soluble solid Brix 8 which was thermally processed at 70, 80, and 90°C had t½ of TPC of 3505.19, 3219.50, 2099.32 min, respectively. Supporting factors such as light, pH, and storage time are known contribute to accelerating the rate of degradation of phenolic compounds through various mechanisms such as oxidation, isomerization, hydrolysis, and molecular structures breakdown (Ali et al., 2018; Legowo et al., 2021; Urías-Orona; Niño-Medina, 2019).

Studies on the stability of phenolic compounds and total tannin in coffee beverages at room temperature storage are still quite rare. For this reason, studies with similar samples are used as comparative data. Similar study was reported by Urías-Orona and Niño-Medina (2019), where degradation of TPC in RTD tea beverage samples stored under room temperature (22-25 °C) for 9 days was 44.67%. This result is known to be much higher when compared to the results obtained in our study. Legowo et al. (2021) conducted an experiment measuring TPC and TTC in coffee leaves tea stored at room temperature (25°C) and low temperature (10 °C) for 24 days. The degradation of TPC and TTC in room temperature was more significant than in the low temperature.

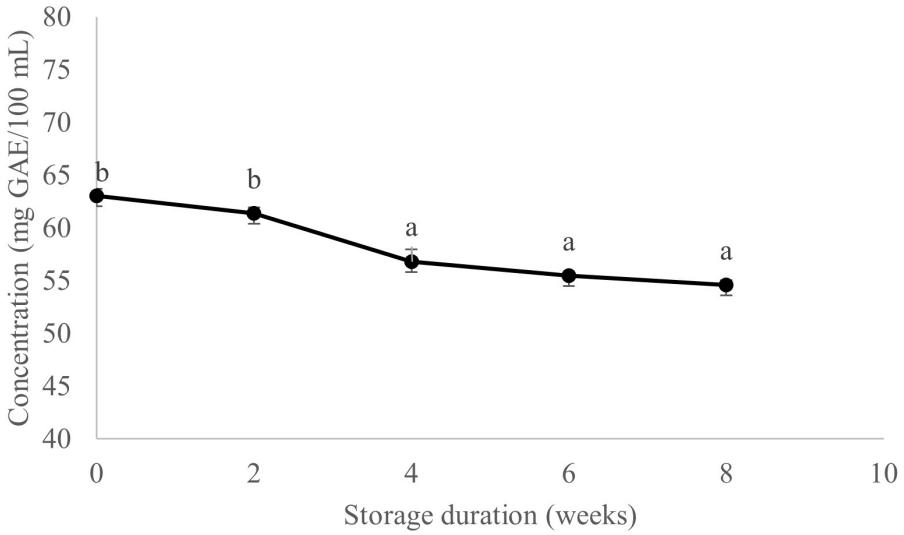

Figure 1: Stability of total phenolic content in ready-to-drink coffee beverage at room temperature (25 - 28°C). Average of eight data samples and standard error. Values with different superscripts are significantly different (*P*< 0.05).

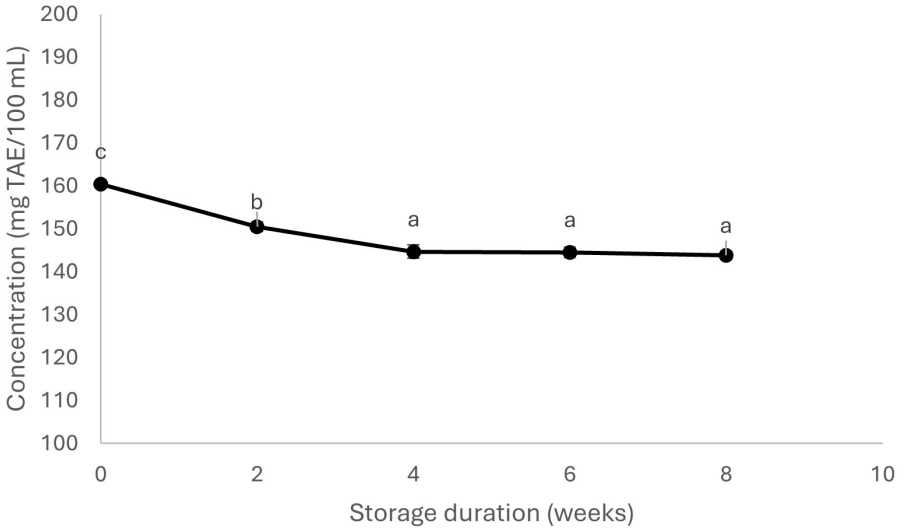

Figure 2: Stability of tannin in ready-to-drink coffee beverage at room temperature (25 - 28°C). Average of eight data samples and standard error. Values with different superscripts are significantly different (*P*< 0.05).

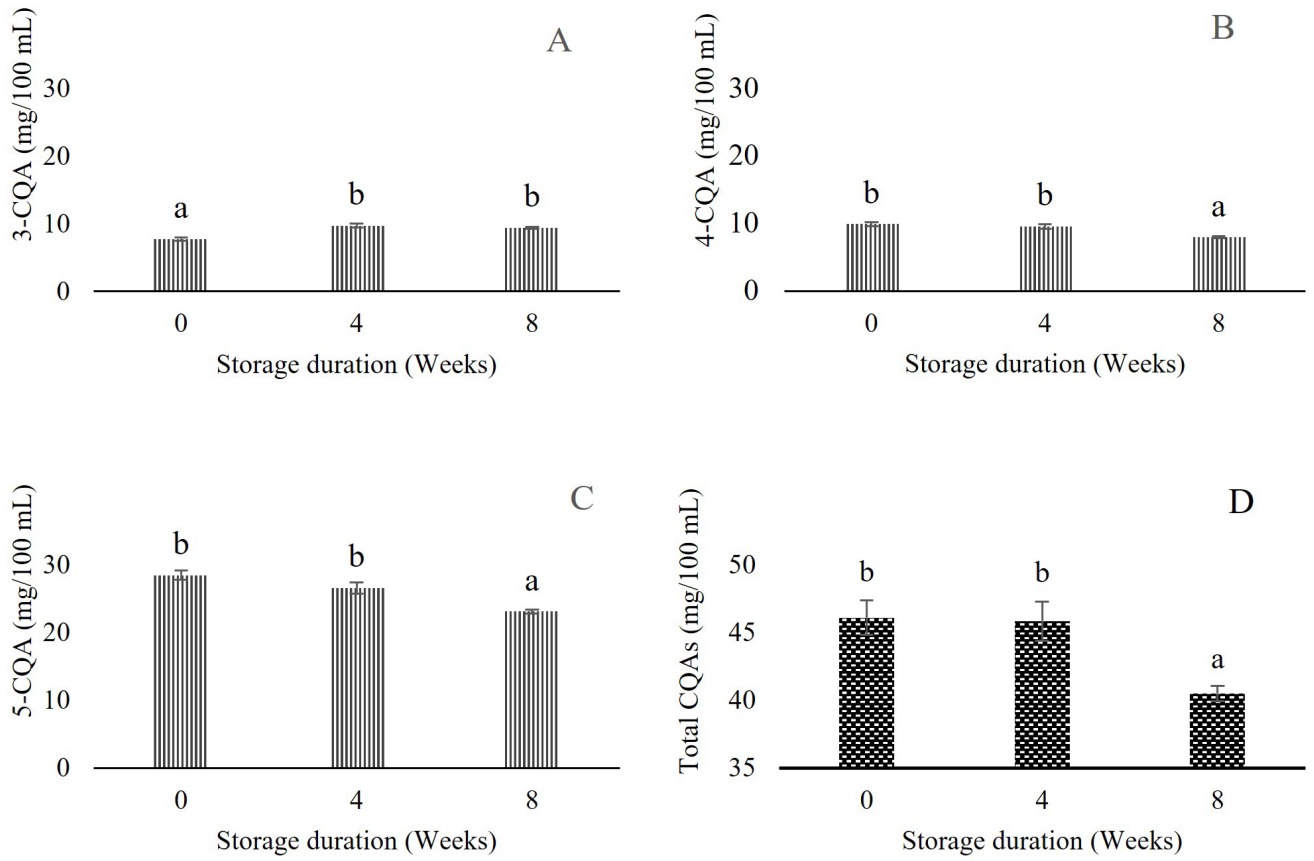

Figure 3: The stability properties of caffeoylquinic acid (CQAs) in ready-to-drink coffee beverage at room temperature (25-28°C): 3-CQA (A), 4-CQA (B), 5-CQA (C) and total CQAs (D). Values were average of four data samples and standard error. Values with different superscripts were significantly different (P< 0.05).

Caffeoylquinic acids (Chlorogenic acids) are the main contributors to phenolic compounds in coffee beverages. The stability of chlorogenic acid in general can be influenced by several factors such as light, temperature, oxygen, moisture content, and storage time (Tripetch; Borompichaichartkul, 2019; Wianowska; Gil, 2019; Zarebska et al., 2022).The decrease in chlorogenic acid (5-CQA) contentcan berelated to its role as an antioxidant in counteracting free radicals (ROS) which then form quinones. Furthemore, CQA compounds may have been transformed during storage into other compounds as a result of enzymatic and non-enzymatic oxidation (Tripetch; Borompichaichartkul, 2019; Zarebska et al., 2022). In addtion, it was found in this study that the 3-CQA compound increased significantly along with the increase in storage time. We predict that 5-CQA compound was isomerized to 3-CQA during storage because the degradation of 5-CQA followed by the formation of 3-CQA. This isomerization also occurred during coffee bean roasting(Herawati et al., 2022).

5 CONCLUSIONS

Total phenolic content (TPC) analysis demonstrated satisfactory performance for partially method validation (linearity (R^2 = 0.9991), sensitivity (LoD = 14.20 mg L^{-1}), repeatability (RSD analysis = 1.71% and RSD Horwitz = 2.84), and accuracy (recovery =109%)). TPC was a better parameter for monitoring the phenolic stability of ready-to-drink (RTD) coffee during storage compared to total tannin content (TTC) which had recovery 132%. TPC degradation during RTD coffee storage at room temperature (25 - 28°C) for 8 weeks reached 11%. Meanwhile, total caffeoylquinic acids (CQAs) and 5-CQA degradation for 8 weeks of storage at same temperature were 11% and 19%, respectively. From this study, we conclude that the storage of RTD coffee beverages in room conditions can indeed affect the stability of the phenolic compounds contained within. TPC analysis for monitoring of phenolic stability in RTD coffee during storage may be combined with analysis of 5-CQA as the most unstable CQAs. Further research needs to be conducted specifically on CQA compounds to find out the specific reasons for the increase of 3-CQA compound in the sample.

6 AUTHORS' CONTRIBUTION

Conceptualization idea: Herawati, D.; Davin, C.; Methodology design: Herawati, D.; Davin, C.; Yuliana, N. D.;

Data collection: Herawati, D.; Davin, C.; Data analysis and interpretation: Davin, C.; Yulianti, and Writing and editing: Herawati, D.; Davin, C.; Yuliana, N. D.; Yulianti.

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