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Title: Identification of 9,10-anthraquinone contamination during black and green tea processing in Indonesia

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Abstract: Black and green tea are popular owing to their unique flavors and health benefits. However, these teas can be contaminated with anthraquinones, which are associated with health risks in humans. Accordingly, in this study, we evaluated the presence of anthraquinones at each step of the manufacturing process for green and black tea using gas chromatography-mass spectrometry. For green tea, anthraquinone was present beginning at the withering process and increased in concentration until the first drying step. Additionally, for black tea, the first drying step resulted in a significant increase in anthraquinone content, whereas the concentration decreased during the final drying step. For black tea, anthraquinone was also detected during withering, although the concentration continued to increase throughout the entire procedure, particularly during drying. We concluded that the presence of anthraquinone in these teas was influenced by the smoke emitted by the wood fires used to drive the processing machinery.

Cover Letter

17 March 2020

Amin Ismail, Ph.D
Food Chemistry

Dear Editor:

I wish to resubmit our manuscript, titled "**Identification of 9,10-anthraquinone contamination during black and green tea processing in Indonesia**" (manuscript ID: FOODCHEM-D-19-07183, for consideration for publication in *Food Chemistry*.

We have carefully considered the comments from the editors and reviewers and have made appropriate changes to our manuscript according to the comments. We feel that the manuscript has been greatly improved based on these changes. Our responses to the reviewers' comments are given in a point-by-point format below.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,
Tuty Anggraini
Faculty of Agricultural Technology, Andalas University, Padang 2516, Indonesia
Tel.: 0751-72772
E-mail address: tuty@ae.unand.ac.id

***Response to Reviewers**

Reviewer #1 Comments

No	Reviwer comment	Changes
1.	Table. 1 is not clear, no units, no notes.	I already complete the table with notes and units in File : Table (Line 1-3)
2.	Figures are also not clear, no figure legends and the scale must be the same. I could NOT understand the figures	I already add figures caption, figure legends and make the same scale for all figures (In Figure file ; line 1-65)
3.	The GC-MS method for the determination of anthraquinones is developed by the authors or not? If yes, some important analytical merits such as detection limits and linear range should be offered. If not, some references should be offered.	I already add the citation of the method (Line 124) and I also suplement the analytical merits in Figures caption (Figure file, line 1-18)
4.	The authors conclude that the main source of AQ contamination was smoke from the processing equipment, which was woodfired. However, no information could be included in the text	I already add about the woodfire (Line 191-193, and 207-213). And the information also show in line 181-183, and 219-221.
5.	Research highlights, Title, Abstract and Conclusions should be reorganized for more informative descriptions.	I already reaorganized the highlights, title, abstract and conclusion.

Reviewer 2 #comments

No	Reviewer comment	Changes
1.	Some descriptions of English in this manuscript presents a lack in the proper use of English language, English native speaker revision is required	I already send the manuscript to elsevier Service Editing after revised.
2.	The abbreviation words in this manuscript, such as BT (Line 123), TSD (Line 123), PSA (Line 138), GCB (Line 138), PTFE (Line 142), PAHs (Line 232) appeared firstly should give full name.	I alredy give the abreviation of BT (line 96), TSD (line 111) I already mention PSA, PTFE and PAHs (already delete)
3.	Line 67-68: "…reviews from (Lakshmi, Balusamy, & Parameswari, 2017)" should be revised to "…reviews from Lakshmi, Balusamy, & Parameswari (2017)"	I already revised (Line 46)
4.	Line 65-67: "However, recently another chemical, the contaminant 9,10-anthraquinone (AQ), has been found in teas,	I already shifted to the end of paragraph in introduction section (Line 49-51)

	and this chemical may have some negative health effects on consumers of the beverage.", this sentence should be shifted to the end of first paragraph in Introduction section.	
5.	Line 107: "Fresh tea leaves (Camellia sinensis) were collected…, please supplement the specific cultivar of tea, the specific harvesting maturity and harvesting maturity parameters of fresh tea leaves.	I already supplement the specific cultivar of tea and harvesting maturity of fresh tea leaves (line 88-90).
6.	Line 110-115, 2.2 Green tea processing: please supplement the detailed processing parameters during green tea processing.	I already supplement the detailed green tea processing (line 93-99) and Supplementary Figure 1.
7.	Line 117-124, 2.3 Black tea processing: please supplement the detailed processing parameters during black tea processing.	I already supplement the detailed black tea processing (line 106-112) and supplementary figure 2.
8.	Samples from each stage of the process were dried to 5 % moisture content before the analysis (Line 114-115, Line 120-121), please supplement the detail drying method.	I already supplement the detail drying method in line 97-99 and 111-112.
9.	Line 128-129: "To create a calibration curve, a standard working solution of AQ…", please supplement the detail information of AQ, for example, purity? Grade? And you purchase AQ from which company?	I alredy supplement the detail information of AQ in line 126-127.
10.	rpm (Line 136, Line 139) is not SI unit, please revise	I already revised in line 134 and 137.
11.	Please supplement the number of repeated measurements.	I already supplement the number of repeated measurements in line 153 and line 164-165.
12.	Statistical analyzing method should be included in the section materials and methods.	I already add the statistical analyzing method in line 163-167.
13.	Check the Y-axis units of Fig. 1, Fig. 2, Fig. 4, Fig. 5, the unit of"mg/kg" should be revised to "mg/kg DW", because the moisture contents in tea samples from different processing stages are different, and the tea samples from each stage of the process were dried to 5 % moisture content before the analysis. In general, the tea with 5 % moisture content is regarded as dry tea. Thus, DW for dry weight should be added materials and methods section, and the unit	I already improved the figure (Figure file) and also revised the unit to mg/kg DW (line 185 and 204)

	of AQ content was mg/kg based on the dry weight basis of tea, accordingly, improve Fig. 1, Fig. 2, Fig. 4, Fig. 5. In addition, the data of the Y-axis in Fig. 1, Fig. 2, Fig. 4, Fig. 5 is error, please check. For example, the data of the Y-axis in Fig. 1 is "0、0.02、0.04、0.06、0.08、0.10、0.12", NOT "0、0,02、0,04、0,06、0,08、0,1、0,12", please improve Fig. 1, Fig. 2, Fig. 4, Fig. 5.	
14.	In Results and discussion section, Line 167~192: 3.1 Green and black tea processing, this section should be shifted to the section of Materials and methods.	I already shifted the green and black tea processing to the section of materials and methods (line 92-121).
15.	In Results and discussion section, Line 195~199:"Green tea liquor produced……and heavy metals (Yang, Peng, Thompson, & Wang, 2019) ", the above descriptions have nothing to do with the topic of 9,10-Anthraquinone contamination during processing of black and green tea in Indonesia, thus, the above descriptions should be deleted.	I already deleted the sentences.
16.	Line 211, "located in the black tea processing room", this section belongs to 3.2 AQ content in green tea processing, please check the black tea processing room?	I already change the black tea processing room with green tea processing room (line 181).
17.	In Results and discussion section, Line 194~225: 3.2 AQ content in green tea processing. In this section, please supplement the significant difference analyses of AQ content among different processing stages of green tea, and add the letters to mark significant differences in Fig. 1.	I already supplement the significat different analyses of AQ content (line 176-177) and add letter to mark significant different in Fig.1
18.	In Results and discussion section, Line 227~239: 3.3 AQ content in black tea processing. In this section, please supplement the significant difference analyses of AQ content among different processing stages of black tea, and add the letters to mark significant differences in Fig. 2.	I already supplement the significant difference analyses of AQ content among different processing stages of black tea (line 200-201), and add the letters to mark significant differences in Fig. 2.

19.	Line 231~237: "AQ has long been known to be a direct combustion product…increase the airborne AQ content that the tea is exposed to", the explanation and discussion of this section are not convincing, thus, please delete this section and Fig. 3.	I already delete the sentences and also Fig 3. And add more citation about AQ (line 205-206 and 207-213).
20.	In Results and discussion section, Line 241~253: 3.4 AQ content of processed green tea and black tea on heating. In this section, please supplement the significant difference analyses of AQ content among heating at 70 °C, 80 °C and 90 °C of black tea, and add the letters to mark significant differences in Fig. 5.	I already supplement the significant difference analyses of AQ content among heating at 70 °C, 80 °C and 90 °C of black tea (line 216-219), and add the letters to mark significant differences in Fig. 5.
21.	In Results and discussion section, Line 255~265: 3.5 AQ content in green tea and black tea after steeping. In this section, please supplement the significant difference analyses of AQ content among steeping at 70 °C, 80 °C and 90 °C of black tea or green tea, and add the letters to mark significant differences in Table 1.	I already please supplement the significant difference analyses of AQ content among steeping at 70 °C, 80 °C and 90 °C of black tea or green tea (line 230-233), and add the letters to mark significant differences in Table 1
22.	Please add the unit of AQ content in Table 1.	I already add the unit of AQ content in Table 1.
23.	Cao dan Zho, 2011, there is not reference in this manuscript, please check.	I already add the citation (line 266)

Highlights

- We evaluated AQ contents during green and black tea manufacturing.
- AQ contents increased during green and black tea production.
- AQ content was influenced by the smoke emitted from wood fires during processing.

Identification of 9,10-anthraquinone contamination during black and green tea processing in Indonesia

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ABSTRACT

Black and green tea are popular owing to their unique flavors and health benefits. However, these teas can be contaminated with anthraquinones, which are associated with health risks in humans. Accordingly, in this study, we evaluated the presence of anthraquinones at each step of the manufacturing process for green and black tea using gas chromatography-mass spectrometry. For green tea, anthraquinone was present beginning at the withering process and increased in concentration until the first drying step. Additionally, for black tea, the first drying step resulted in a significant increase in anthraquinone content, whereas the concentration decreased during the final drying step. For black tea, anthraquinone was also detected during withering, although the concentration continued to increase throughout the entire procedure, particularly during drying. We concluded that the presence of anthraquinone in these teas was influenced by the smoke emitted by the wood fires used to drive the processing machinery.

Keywords: anthraquinone, black tea, green tea, processing

1. Introduction

Tea from *Camellia sinensis* is a popular beverage worldwide owing to its attractive flavor and the presence of functional compounds that confer health benefits (Carloni et al., 2013). Green tea is made from young leaves without enzyme oxidation, whereas black tea is processed with enzyme oxidation (Jiang et al., 2019). This difference results in the different colors and taste characteristics of the two teas. Green tea is green, and black tea leaves produce a tea with a red-brown color. Both tea types are rich in catechin, epicatechin, epicatechin gallate, epigallocatechin (EGC), and EGC gallate (Singh, Shankar, & Srivastava, 2011; Reygaert, 2018; Abdolmaleki, 2016). In black tea, enzyme oxidation leads to the synthesis of theaflavins from epicatechin and EGC; theaflavins-3 gallate is synthesized from epicatechin and EGC gallate, theaflavin-3' gallate is synthesized from epicatechin gallate and EGC, and theaflavin-3,3' gallate is synthesized from epicatechin gallate and EGC gallate (Hilal & Engelhardt, 2007). Thearubigin is the final product of enzyme oxidation, contributing to the color of black tea (Melkadze, 2015). The consumption of tea is associated with improved human health owing to the presence of these bioactive compounds, some of which are antioxidants that have been shown to have anticancer, anti-cardiovascular disease, and antibacterial effects (Zhang, Qi, & Mine, 2019). A review by Lakshmi, Balusamy, and Parameswari (2017) showed that green tea contains epicatechin, EGC, epicatechin gallate, and EGC gallate and has the ability to fight cancer, reduce the risk of heart disease, inhibit diabetes, improve skin health, act as an antioxidant, and nourish the skin. However, the contaminant 9,10-anthraquinone (AQ) has recently been found in teas, and this chemical may have some negative health effects on consumers.

AQs are secondary metabolite compounds found in plants (such rhubarb), insects, fungi, and other microorganisms. AQs are natural pigments (Dave & Ledwani, 2012), and some derivatives of AQs are used in Chinese herbal medicines. Indeed, AQ derivatives, including aloe-emodin, emodin, physcion, chrysophanol, and rhein, have been found to have antioxidant,

anticancer, and antifungal properties (Hu, Qiao, Deng, Wu, & Liu, 2017). However, research has shown that AQ derivatives from noni fruit seed (i.e., ucidin, alizarin, and rubiadin) can be toxic (Bussmann et al., 2013). AQs are water-insoluble and are used as textile dyes (Epolito et al., 2005) and pesticides, particularly bird repellents (Werner et al., 2014). Owing to the potential dangers of this contaminant and the necessity for reducing their concentrations in teas for export, it is important to determine the sources of AQ in the tea processing industry. AQs have been shown to be produced by microorganism activity during the fermentation of Fuzhuan brick tea (Mo et al., 2008). However, the sources of AQ in Indonesian-grown black and green teas have not been identified.

West Sumatra, Indonesia produces both green tea and black tea for local and international markets. For exportation to many developed countries, the tea must fulfill a requirement recently established by the European Union, limiting the AQ content to 0.02 mg/kg. This regulation has negatively affected the export of tea produced in Indonesia.

AQ can be detected using gas chromatography (GC) coupled with mass spectrometry (MS) and by employing a micro electron capture detector (ECD) method along with GC (Pitoi, Ariyani, Koesmawati, & Yusiasih, 2019). QuECHhERS, a solid-phase extraction method using GC-MS/MS, has also been found to be effective for AQ analysis of teas (Harmoko et al., 2016). Moreover, AQ has been detected in slimming tea using capillary zone electrophoresis (Wang, Su, Liang, & Sun, 2016). Despite these approaches, the source of AQ remains unclear. Some researchers have proposed that AQ originates from the environment in plantations or from the fertilizers used during plant growth (Wang et al., 2018). Additionally, the processing procedure, including the machinery used in manufacturing, can also affect the generation of AQ.

The processing of green tea consists of five steps: plucking; withering (inactivation of the enzyme); cutting, tearing, and curling; and two drying steps. The processing of black tea is similar; however, withering does not inactivate the enzyme, and the cutting, tearing, and curling step is followed by wet grading, enzyme oxidation, and one drying step. In this study, we hypothesized

that AQ content may be affected by processing steps during the production of green and black teas. Therefore, we examined the AQ contents of black and green teas grown and processed in West Sumatra, Indonesia at different processing steps.

2. Materials and Methods

2.1 Plant material

One bud and three tender leaves tea (*Camellia sinensis cv Assamica*) were collected and processed into green tea and black tea on the Mitra Kerinci tea plantation in West Sumatra, Indonesia in June 2019. The harvesting maturity was 25 days.

2.2 Green tea processing

Leaves were picked and withered on a rotary panner (RP) at 90–100°C for 5 min until the moisture content was reduced to 60–70%. Leaves were then rolled in an open top roller (OTR) for 15 min and dried in an endless chain pressure (ECP) dryer at 110–135°C for 25 min until the moisture content was 30%. A second drying process used a ball tea (BT) machine set at approximately 80°C, reducing the moisture content to 5%. This process is illustrated in Figure S1. The output samples from RP, OTR, and ECP were then dried to 5% moisture content in an oven at 80°C before analysis. Withering with RP activated the enzymes, reduced the water content, and made the leaves limp, thus facilitating the rolling process. The OTR reduced the tea leaves to smaller sizes by curling them and removed intracellular fluids, including pectin. The first drying occurred in the ECP, which concentrated the remaining intracellular fluids and reduced the water content to 30%. The BT dried the tea and curled the leaf fragments further (Anggraini, 2018).

2.3 Black tea processing

For black tea, the shoots were picked, withered in a withering trough (WT) for 15 h, and rolled using the OTR. Then, the material was wet sorted with a double Indian ballbreaker net sorter

(DIBN), in which wet fermentation occurred, and the size of the leaf fragments was made uniform to allow for even enzymatic oxidation. This occurred over 90–110 min and oxidized catechin to theaflavin and thearubigin. Finally, the leaves were dried to a moisture content of 5% in a two-stage dryer (TSD). This processing is illustrated in Figure S2. The outputs of WT, OTR, wet sorted, and enzyme oxidation processes were dried to 5% moisture content in an oven at 80°C before analysis. Withering of black tea was performed at a lower temperature (ambient temperature) than withering of green tea because there was no destruction of enzymes, and then enzyme had to be kept active to metabolize the catechins and derivatives. For black tea, withering only concentrated the intracellular fluids and made the leaves limp to facilitate further processing. The function of the OTR was the same for both black and green teas.

The TSD used for black tea and the RP and ECP used in processing green tea in this study all used wood as fuel. The BT used electric elements.

To gauge the heat stability of AQ in the processed teas, samples of the green tea from BT and the black tea from TSD were heated for 30 min at 80°C, 90°C, or 100°C in an oven.

2.4 Analysis of AQ content in dried tea

Analysis of AQ was performed according to the methods described by Harmoko et al. (2016). One gram of tea from each stage of processing was weighed in a 50-mL polypropylene centrifuge tube. To create a calibration curve, a standard working solution of AQ (purity: 99.5%; Supelco, Belletonte, PA, USA) was added to the tea sample at concentrations of 10, 20, 50, 100, and 200 µg/kg and left alone for 30 min to allow AQ to interact with the tea before extraction. Then, 5 mL cold water was added, and the mixture was vortexed. After 30 min, the hydrated sample had settled to the bottom of the tube. Acetonitrile (10 mL) containing 1% acetic acid was added, and the mixture was vortexed or shaken by hand for 1 min. The mixture was placed in a laboratory rotator for 30 min. Then, 4 g anhydrous $MgSO_4$, 1 g anhydrous CH_3COONa, and 1 g anhydrous NaCl were added, and the mixture was shaken immediately by hand for 1 min. The mixture was then

placed in the laboratory rotator for 5 min and centrifuged at 5000 × *g* for 5 min. Subsequently, 5 mL of the supernatant was placed in a 15-mL polypropylene centrifuge tube containing 150 mg primary secondary amine, 150 mg graphitized carbon black, and 900 mg $CaCl_2$; the sample was vortexed for 5 min and then centrifuged at 5000 × *g* for 5 min. The supernatant (2.5 mL) was transferred to a 15-mL glass tube and dried by evaporation using a nitrogen evaporator at 40°C. The resulting product was dissolved in 1 mL acetone, placed in an ultrasonic bath for 1 min, and vortexed for 1 min. Finally, the mixture was filtered with a 0.45-μm polytetrafluoroethylene syringe filter, and the filtrate was placed in a 1.5-mL vial for analysis by GC-MS/MS.

2.5 Steeping the tea

Five grams of dried tea was placed in a laboratory tea tasting cup (equipped with a lid and a row of small holes for straining the tea). Then, 150 mL hot water (80°C, 90°C, or 100°C) was added, and the cup covered and left for 5 min. The leaves were then strained out, and the tea cooled before AQ analysis.

2.6 AQ analysis using the prepared tea

Ten milliliters of the steeped tea was placed in a 50-mL polypropylene centrifuge tube. For preparation of a standard calibration curve, AQ was prepared at concentrations of 0.1, 0.5, and 2.5 μg/L. These standards were then prepared in the same way as the previous samples for the calibration curve. The analysis was carried out in triplicate.

2.7 GC-MS/MS analysis

A Shimadzu GCMS-TQ8030 was used for GC-MS/MS. The inlet temperature was 270°C. Splitless injection was used into an HP-1MS column with a 1-min sampling time. Helium carrier gas was used as the mobile phase. The oven was held at 150°C for 1 min and then heated at a rate of 15°C/min to 300°C and held for 2 min. The MS interface was heated to 280°C, and the ion source

was heated to 250°C. MS was conducted in electron ionization (EI) mode using an acceleration voltage of 70 eV and was operated in multiple reaction monitoring mode using argon at 200 kPa.

2.8 Statistical analysis

The AQ content was evaluated at each step of green tea and black tea processing four times, and that of processed green tea and black tea after heating and steeping was evaluated three times. One-way analysis of variance was used for data analysis. Results with *P* values of less than 0.05 were considered significant, and all analyses were carried out using SPSS software.

3. Results and Discussion

3.1 AQ content during green tea processing

Teas contain AQ derivatives as contaminants (Shang et al., 2019). AQ is a phenolic compound and a member of the quinone family (Fouillaud et al., 2018). The manufacturing process of green tea uses an RP for enzyme inactivation; an OTR to roll, cut, and tear the leaves; an ECP for the first drying; and a BT for the final drying and shaping of the green tea leaves. The AQ content after each step is shown in Figure 1. The AQ contents of dried samples were measured after each step of green tea processing and found to increase during processing. Significant differences in AQ content were observed for green tea at the OTR, ECP, and BT steps. Notably, no AQ was found in fresh shoots; however, after withering, the contaminant was present in small amounts, and the amount of AQ continued to increase after rolling using the OTR. The OTR did not use heating itself; however, when the tea was applied to the RP, the shoots of tea were moved to the OTR using a conveyor located in the green tea processing room. This room contains abundant amounts of smoke because the RP engine used firewood as a heat source; this smoke was thought to be the source of the contaminant.

As processing proceeded, the tea that had been dried using the ECP exhibited the highest AQ content (greater than 0.05 mg/kg dry weight [DW]). However, after the second drying using the

188 BT, the AQ content decreased. The BT used for this step was a closed dryer, which isolated the tea
189 from the smoke in the processing room. Based on the lack of AQ in fresh leaves, we assumed that
190 the source of the contaminant was not the growing process. There was no evidence that AQ
191 originated from the soil, fertilizer, or other agricultural chemicals, nor was the contaminant related
192 to atmospheric pollution in the plantation area.

193 Because the AQ content was roughly proportional to the time the tea was exposed to the
194 smoke in the processing room, we concluded that the source of contamination was likely the smoke
195 from the wood fires driving the machinery. During green tea processing, including RP for withering
196 and ECP for first drying using firewood, the production space was filled with smoke because there
197 was no air flow to remove the smoke produced from wood burning. The AQ content decreased
198 after the second drying probably because AQ is not heat stable and the BT operated at a high
199 temperature for 12 h, leading to the breakdown of this chemical.

200

201 *3.2 AQ content during black tea processing*

202 The AQ content during black tea processing is shown in Figure 2. Significant differences were
203 observed in AQ contents during the different processing steps of black tea. The smoke in the
204 processing room may also have been the source of AQ in black tea. Indeed, the drying process of
205 black tea with the TSD used firewood as a heat source. After enzymatic oxidation, the AQ content
206 increased to 0.08 mg/kg DW, which was four times the limit established by the European Union
207 (0.02 mg/kg). Both green and black teas were processed in a smoky environment, and both showed
208 AQ contamination levels above the allowed standard.

209 Naeher et al. (2007) showed that biomass combustion causes the production of many
210 pollutants, including AQ. During combustion, the pyrolysis reaction breaks the polymers,
211 producing a variety of substances. Moreover, Rohr et al. (2015) showed that oxygenation of
212 organics, such as AQ, occurs as a result of combustion. AQ has also been detected in barbecue
213 samples during smoking, and the content increases following exposure to high smoke densities

(Zastrow et al., 2019). *Cinnamomum* sp., which is also used as a source of firewood for processing machinery, has also been reported to produce AQ (Kumar & Kumari, 2019).

3.3 AQ contents in processed green tea and black tea after heating

The influence of heating to 70°C, 80°C, and 90°C on AQ contents in green and black teas was then evaluated (Figures 3 and 4). Importantly, heating had no effect on the AQ contents of green tea, potentially because the heating duration may have been insufficient for breaking down AQ. However, in black tea, heating significantly affected AQ content. Indeed, heating at 90°C reduced the AQ content of black tea by 25%. Thus, AQ reacted differently to changes in temperature in the two teas. It is possible that the interaction between smoke and the different compounds in green and black teas could produce AQs with different degrees of heat stability. Previous studies have shown that the heat stability of phenolic compounds, including AQ, is low and that these compounds can be degraded after only 10 min at 70°C, 80°C, 100°C, or 180°C (Mcdougall et al., 2010; Yen & Chung, 1999); dimeric AQ derivatives are decomposed to monomeric anthrone glycosides. Further studies are required to elucidate the reason for these differences among green and black teas.

3.4 AQ content in green tea and black tea after steeping

AQ could be ingested by drinking tea. Therefore, we next evaluated the AQ content of teas steeped from leaves for 5 min. The AQ contents in both green and black tea are shown in Table 1.

Both teas showed very low AQ contents. However, significant differences were observed for different steeping temperatures in both green and black teas. Thus, the steeping process appeared to have diluted the AQ content owing to the large amount of water used, and AQ did not seem to dissolve well in the water. Indeed, AQ is not as soluble in water as it is in chloroform, glycerol, and sulfuric acid (20%) (Cao dan Zhao, 2011). Because Indonesians generally drink their tea very sweet and often do not strain out the leaves, it would be interesting to see whether the addition of sugar during the steeping process or allowing the leaves to remain in the tea longer could increase AQ contents.

4. Conclusion

The AQ found in black and green teas was found to be generated during processing in tea manufacturing plants. In green tea, AQ started accumulating during the drying process using an RP and then increased continuously throughout the process until the first drying; AQ content finally decreased slightly during the second drying process. In black tea, the increase continued throughout the entire process. A simple subsequent heating step slightly reduced AQ levels in the processed black tea but not in the green tea. Thus, our findings supported that the main source of AQ contamination was smoke from the processing equipment. Although measurements of AQ contents in beverages produced from the two products suggested that the AQ levels present in the drink were unlikely to pose any health risks, tea processing will need to be modified for the export market. Reducing smoke levels in the rooms used for processing could be the simplest step and could be achieved either by improving ventilation or exhaust systems or by the use of cleaner heat sources to drive the machinery. This remediation could also have a positive impact on the workers who spend long hours in tea factory buildings.

Acknowledgements

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit author statement

Tuty Anggraini : Conceptualization, Methodology, Writing- Original draft preparation, Neswati : statistic, Ririn Fatma Nanda : Statistic, Daimon Syukri : Method

Table 1. AQ contents in green and black tea (final processing step) brew at different temperatures.

	70°C ± SD	80°C ± SD	90°C ± SD
Green tea (mg/L)	$0.002 \pm 6.49 \times 10^{-7}$a	$0.003 \pm 6.36 \times 10^{-5}$a	$0.003 \pm 3.83 \times 10^{-5}$b
Black tea (mg/L)	$0.002 \pm 3.34 \times 10^{-5}$a	$0.003 \pm 3.53 \times 10^{-5}$a	$0.005 \pm 5.06 \times 10^{-5}$b

Note: The reporting limit was 0.0001 mg/L AQ, and the linear range of the standard was 0.1–2.5 ppb. SD: standard deviation.

Figure captions

Fig. 1 AQ contents during different green tea processing steps (FL: fresh leaves, RP: rotary panner [output], OTR: open top roller [output], ECP: endless chain pressure [output], BT: ball tea [output]). The method limit of quantification for the residue analysis of each green tea processing step was 0.01 mg/Kg DW AQ, and the linear range of the standard was 10–200 ppb.

Fig. 2 AQ contents during different black tea processing steps (FL: fresh leaves, WT: withering through [output], OTR: open top roller [output], DIBN: double Indian breaker netsortieder [output], EO: enzyme oxidation [output], TSD: two stage dryer [output]). The method limit of quantification for the residue analysis of each black tea processing step was 0.01 mg/Kg DW AQ, and the linear range of the standard was 10–200 ppb.

Fig. 3 AQ contents after additional heating of green tea (BT output). The method limit of quantification was 0.01 mg/Kg DW AQ, and the linear range of the standard was 10–200 ppb.

Fig. 4 AQ contents after additional heating of black tea (TSD output). The method limit of quantification was 0.01 mg/Kg DW AQ, and the linear range of the standard was 10–200 ppb.

FIGURE 1

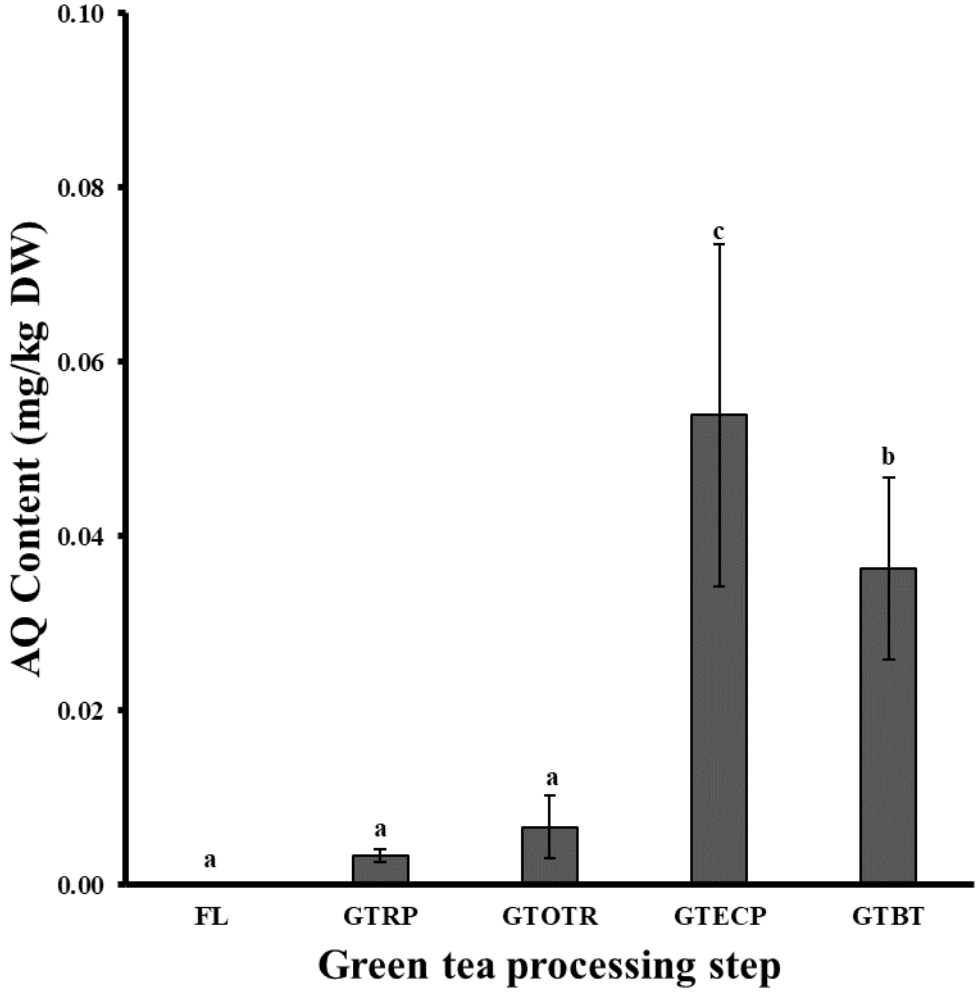

0.10
0.08
0.06
0.04
0.02
0.00
AQ Content (mg/kg DW)
a
a
a
c
b
FL
GTRP
GTOTR
GTECP
GTBT
Green tea processing step

FIGURE 2

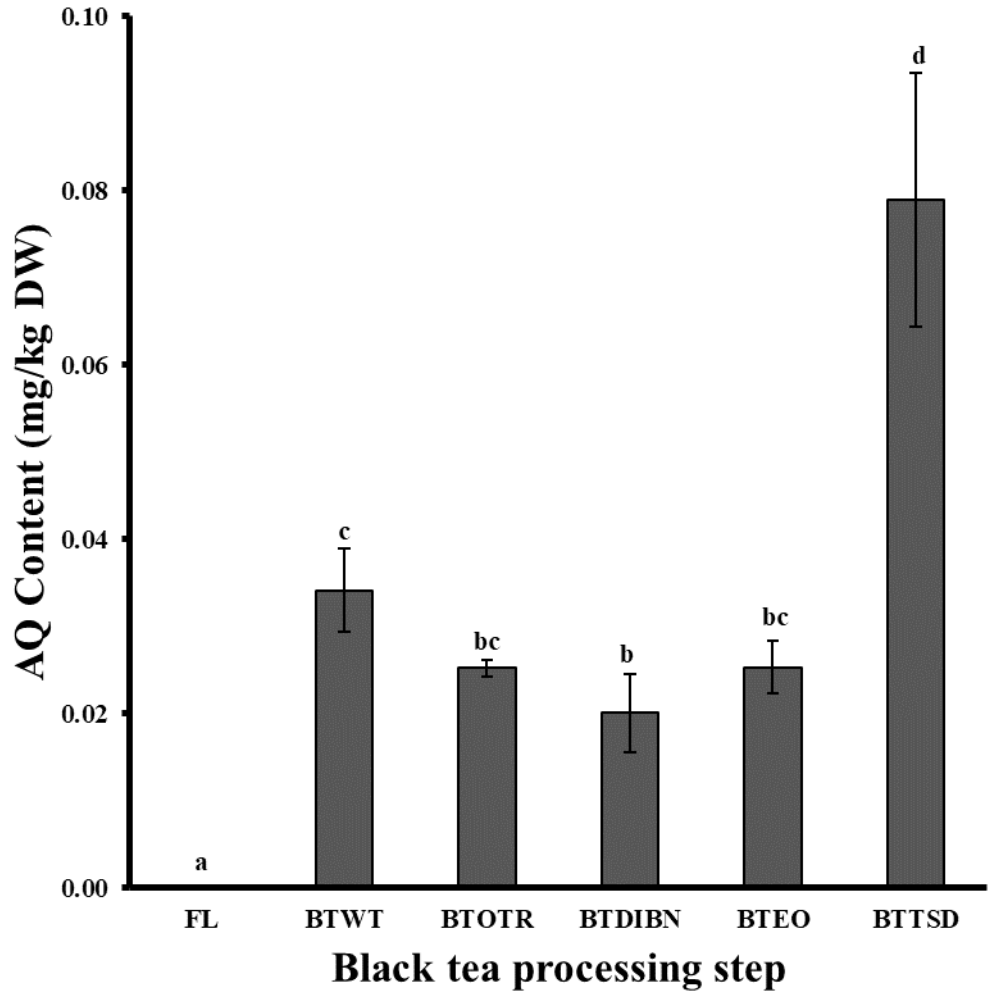

0.10
0.08
0.06
0.04
0.02
0.00
AQ Content (mg/kg DW)
a
c
bc
b
bc
d
FL
BTWT
BTOTR
BTDIBN
BTEO
BTTSD
Black tea processing step

FIGURE 3

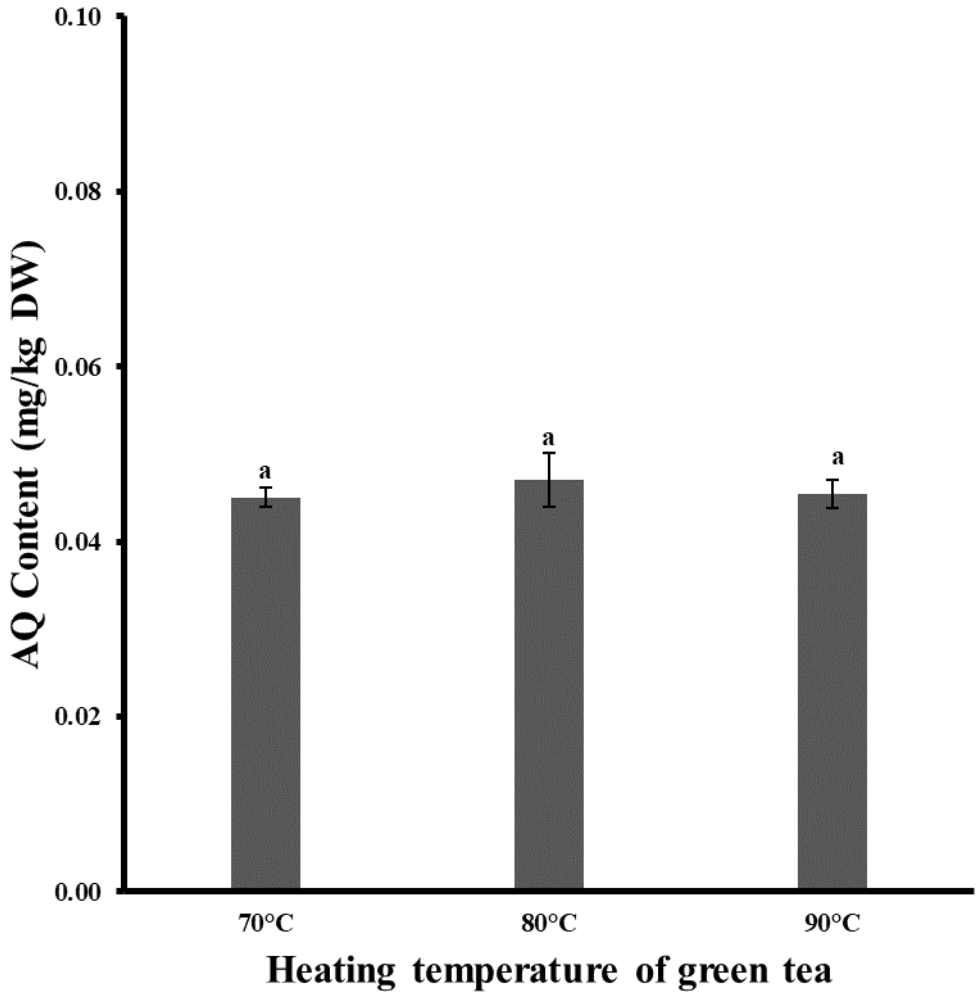
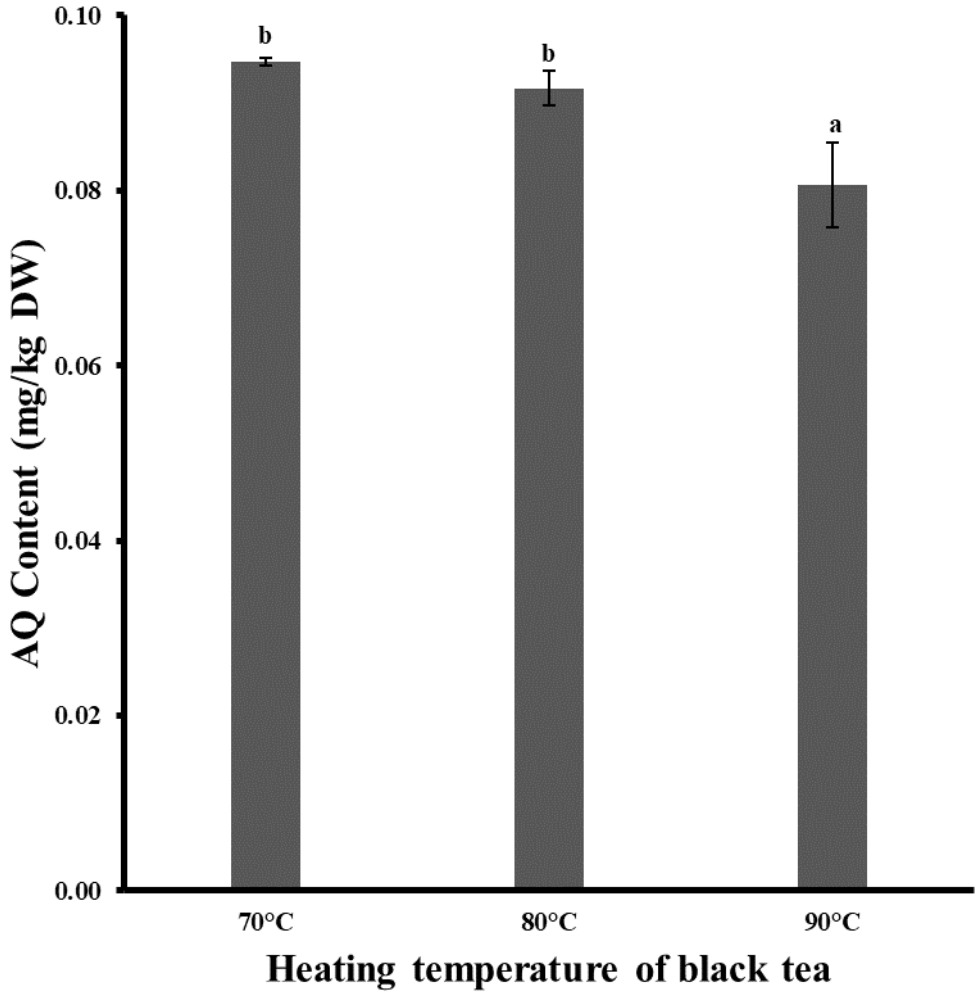

0.10
0.08
0.06
0.04
0.02
0.00
AQ Content (mg/kg DW)
a
a
a
70°C
80°C
90°C
Heating temperature of green tea

FIGURE 4


0.10
0.08
0.06
0.04
0.02
0.00
AQ Content (mg/kg DW)
b
b
a
70°C
80°C
90°C
Heating temperature of black tea

Supplementary Material

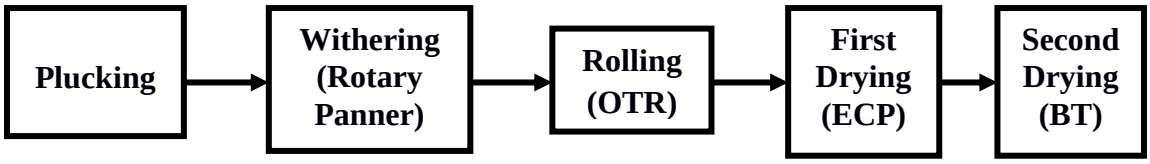


Fig. S1. Green tea processing.

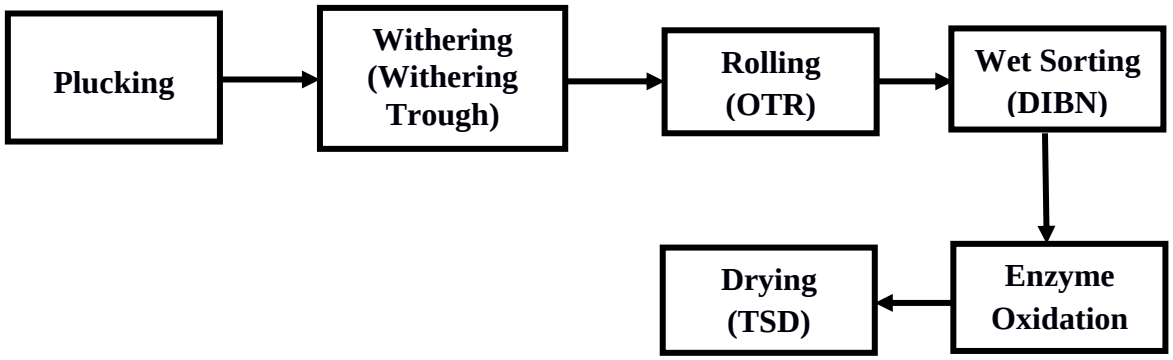


Fig. S2. Black tea processing.