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The Effect of Crude Trona (Kanwu) on the Antioxidant Activity of Some Ghanaian Foods

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ABSTRACT
Cowpea, okra, roselle calyces, "ayoyo" leaves, and guinea corn red sheath are often cooked with crude trona to enhance softness, sliminess, and color. This study aims to determine the effect of crude trona on the antioxidant activity of these foods, to inform cooks and food processors. The 2,2-diphenyl-picrylhydrazin (DPPH) inhibition method was used to assess antioxidant activity. "Ayoyo" leaves cooked without crude trona (control) showed higher inhibition (56.04 ± 0.009 %) than those cooked with 3g (43.477 ± 0.081 %) and 1 g (52.52 ± 4.75 %) crude trona. Similarly, guinea corn red sheath without crude trona had higher inhibition (66.107 ± 0.152 %) than with 1 g (28.461 ± 0.09 %) and 3 g (55.027 ± 0.063 %). Cowpea cooked without crude trona recorded 62.101 ± 0.009 % inhibition, compared to 3 g (30.927 ± 0.009%) and 1g (27.764 ± 0.018 %) crude trona. Okra showed higher inhibition without crude trona (61.551 ± 0.59 %) than with 3 g (39.06 ± 0.02 %) and 1 g (54.133 ± 0.02 %). However, roselle calyces cooked with 3 g crude trona exhibited slightly higher inhibition (83.017 ± 0.008 %) than those cooked without (82.2 ± 0.008 %) and with 1 g (78.553 ± 0.016 %).

The study reveals that adding 3 g crude trona significantly ($p < 0.05$) reduces antioxidant activity in "ayoyo", guinea corn red sheath, cowpea, and okra, while slightly increasing it in roselle. Therefore, cooking "ayoyo", guinea corn red sheath, cowpea, and okra with crude trona may reduce their health benefits, whereas its addition to roselle may enhance them.

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KEYWORDS: crude trona, Antioxidant activity, Ghanaian foods, DPPH assay.

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INTRODUCTION

Crude trona (kanwu) is also known as baking soda, otherwise known as sodium bicarbonate and, less commonly, saleratus. Common ones found in Ghana are sandy, rocky, and crystalline, which exist naturally as sodium sesquicarbonate, with the chemical formula ($Na_2CO_3.NaHCO_3.2H_2O$) (Ameh et al., 2009). It acts to neutralize acids and break down proteins. It is also added as a tenderizer to meat and cowpeas (especially in reducing the cooking time of cowpea from 75 to 50 min), which represents a saving of time and fuel to the cooker (Bandara and Mahendran, 2020). Many people add crude trona to food like okra and ayoyo to increase their sliminess. It is of the view that the addition of the crude trona to these foods makes them taste nicer and also enhances their flavor as compared to food that contains no crude trona (Bandara and Mahendran, 2020). Since color provides the initial impression of food quality, it is one of the most significant quality attributes influencing the consumer's acceptance of the dish. Without the addition of colorants, many convenience foods, including candy, gelatin desserts, snacks, cakes, pudding, ice cream, and drinks, would be colorless and thus unappealing (Barku et al., 2013). Accordingly, roselle calyces seem to be good and promising sources of red colorants that dissolve in water and could be used as natural food coloring (Barku et al., 2013). Hence, the addition of crude trona to bissap (roselle calyces' juice) and *Sorghum bicolor* (Guinea corn) red sheath can improve its color and make it more attractive than food cooked without it (Braca et al., 2003). This perception about crude trona may be true, but they do not consider its effect on the antioxidant activity. The foods (okro, cowpea, *Sorghum bicolor* red sheath (guinea corn red leaves), ayoyo, and bissap) have antioxidant activity (Coultate, 2008).

Antioxidants are important for the body because they may neutralize free radicals and stabilize oxidative stress (Duangmal et al., 2008). An antioxidant can be characterized as any material that considerably slows down or prevents the oxidation of an oxidizable substrate when present at a comparatively low concentration (Duangmal et al., 2008).

It is not known whether these antioxidants are reduced or increased by adding crude trona to them during preparation. Hence, this research is intended to determine the effect of the crude trona on the antioxidant activity of these foods, and the outcome will also help educate the public on the effect the crude trona will have on these antioxidant foods.

MATERIALS AND METHODS

Study Area and Design

The study was conducted in Cape Coast. Cape Coast is the capital of the Central Region of Ghana, which is bounded on the south by the Gulf of Guinea, and its Geographical coordinates are 5° 6' 0" North, 1° 15' 0" West. Samples were bought from the Kotokuraba market in the Cape Coast Metropolis. The laboratory analysis was performed at the Research. Cape Coast is a city, fishing port, and capital city of the Cape Coast Metropolitan District. It is one of Ghana's most historic cities. Fishing is the major occupation of the inhabitants. The study was qualitative and quantitative, and sampling was carried out within a year.

Materials

Okro, cowpea, bissap, guinea corn red sheath, crude trona, and ayoyo were bought from the Kotokuraba market in the Cape Coast Metropolis. All chemicals used in the experiment were obtained from the Department of Chemistry, University of Cape Coast. Glassware, porcelain, and ceramic were soaked in nitric acid trihydrochloride for three (3) hours. This was washed with pipe-borne water, and immediately rinsed with distilled water, then dried in a hot, dry oven.

METHODS

Sampling technique and pretreatment

Okro (*Abelmoschus esculentus*), cowpea (*Vigna unguiculata*), roselle (*Hibiscus sabdariffa*), *Sorghum bicolor* (guinea corn) red sheath, and ayoyo leaves (*Corchorus tridens*) were randomly purchased from different vendors in the Kotokuraba market in the Cape Coast market, Central Region of Ghana. Each sample was put in a distinct sterile polythene bag and labeled before being taken to the Department of Laboratory Technology for analysis. Each of these samples was divided into three portions, with all the portions bearing the same mass (21 g for okra, 45 g for cowpea, 15 g for roselle calyces, 11 g for ayoyo leaves, and 7 g for guinea corn red sheath of a particular sample, respectively. The various portions of the same sample were cooked for the same period (15 min) with one portion boiled without crude trona, the second portion boiled with 1 g of crude trona, and the third portion boiled with 3 g of crude trona.

Extraction of sample for antioxidant determination

The methanol extract from the various samples was obtained as follows:

The various cooked cowpea samples (with no crude trona, with 1 g crude trona, and with 3 g crude trona) were ground into a paste using a blender into separate containers, which were labeled with the different crude trona masses. To each 2 g of the sample, 20 ml of absolute methanol was added. It remained soaked in the methanol for 72 h. Whatman No. 1 filter paper (125 mm) was used to filter the extract, after which it was evaporated at 50 °C to obtain the dry extract. This process was repeated for the other samples, namely, okra and ayoyo. With roselle calyces and guinea corn red sheath, their extract, after boiling them with the various crude trona masses, was immediately evaporated to obtain the dry extract, whose solutions were later prepared (Fausat et al., 2024).

Scavenging activity against 2, 2-diphenylhydrazyl (DPPH) assay

The plants' crude methanol extracts were tested for their ability to scavenge DPPH radicals Fukumoto and Mazza (2000) and Goselle et al. (2017) methods, with little modification, were used. 50 ml of methanol was used to dissolve 0.05 g of dry extract to form extract solutions. An aliquot of 1 ml of plant extract in methanol at different concentrations (0.1, 0.2, 0.4, and 0.8 mg/ml) and 2 ml of 0.004 % DPPH solution in methanol were combined and incubated at 25 °C for 30 min Using a spectrophotometer (T 70 UV-VIS Spectrometer, PG Instruments Ltd.), the absorbance of the test combination was measured at 517 nm in comparison to a DPPH control that included just 1 ml of methanol instead of the extract. The DPPH solution in methanol was prepared daily before the absorbance measurements. DPPH is a purple-colored stable free radical. When reduced, it becomes the yellow-colored Diphenyl picrylhydrazine. A reagent blank of 0.004 % DPPH in methanol was measured for background correction. All experiments were repeated, and the results were averaged (Guebebia et al., 2023). Percent inhibition was calculated using the following expression:

$$\% \text{ Inhibition} = [(A \text{ blank} - A \text{ sample}) \div A \text{ blank}] \times 100 \quad (1)$$

Where A blank and A sample stand for absorption of the blank sample and absorption of the tested samples, respectively.

Statistical analysis

Data were analyzed by using GraphPad Prism v9. One-way ANOVA was conducted to determine the significant differences ($P<0.05$) in the various samples for the antioxidant activity. The same test was used to compare the means between the samples and their respective control treatment. Differences of ($P<0.05$) were considered statistically significant. Tukey's test was used for multiple comparison analyses.

RESULTS AND DISCUSSION

Effect of crude trona on ayoyo (*Corchorus tridens*)

From Figure 1 below, Ayoyo (*Corchorus tridens*) cooked without crude trona (control) gave a higher inhibition (%), which increased with an increase in the concentration, as compared to that which was cooked with 3 g crude trona.

From the results, there was a significant difference between the inhibitions of the samples cooked with crude trona as compared to those that which was cooked without crude trona ($P< 0.05$). These percentage inhibitions increased with an increasing concentration (10, 20, 40, 80) % of the samples, respectively. Ayoyo has antioxidant activity with a significant α-tocopherol equivalent to vitamin E, beta-carotene, and vitamin C (Coultate, 2008). These antioxidants scavenge free radicals and inhibit oxidation (Guo et al., 2024). However, after the addition of crude trona, the inhibition properties of the antioxidant reduced drastically. Dehydro ascorbic acid (DHAA), a still-useful vitamin, is produced when ascorbic acid in green leafy vegetables like "ayoyo" is oxidized by high temperatures or the enzyme ascorbic acid oxidase. Crude trona, which reacted with the boiling water at a high temperature, caused the ascorbate oxidase in the food to undergo oxidation. This oxidation converted dehydro ascorbic acid (DHAA) inactive form, to diketogulonic acid (DKGA), which is a degraded vitamin C (Hall, 1952).

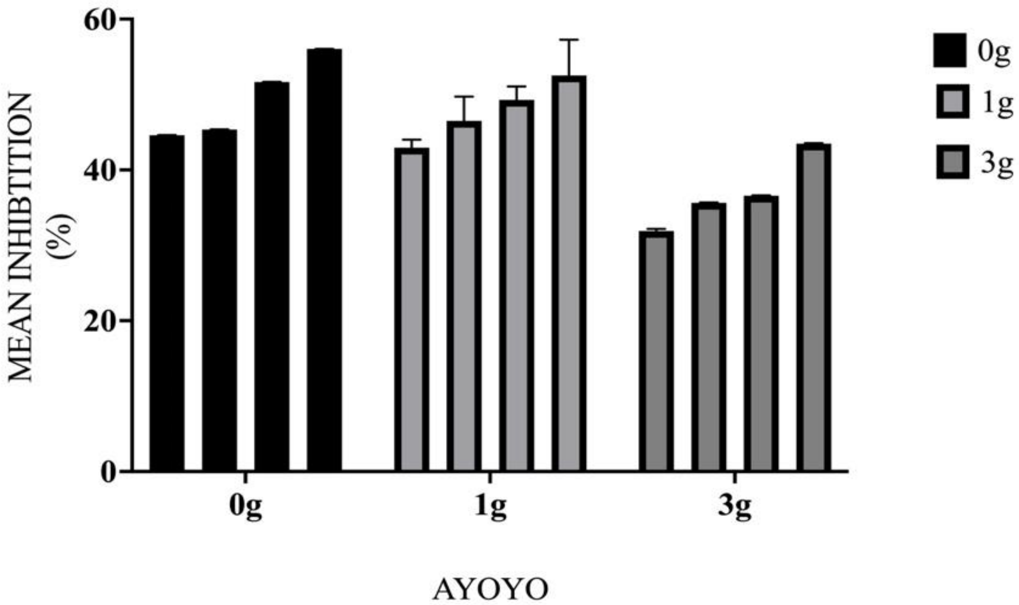

Figure 1. Inhibition (%) of ayoyo cooked with and without crude trona.

Effect of crude trona on *Sorghum bicolor* (Guinea corn) red sheath

According to Figure 2, *Sorghum bicolor* red sheath cooked without crude trona showed a higher inhibition with an increasing concentration as compared to that which was cooked with 3 g crude trona. The percentage inhibition of the *Sorghum bicolor* red sheath cooked with 3 g crude trona was significant as compared to the inhibition of the millet sheath cooked with the control ($P< 0.05$). The higher the inhibition, the higher the antioxidant activity, hence its ability to scavenge free radicals. Crude trona is basic and reacts with the acids in the food to produce a neutral color, just as in the case of an acid-base reaction (Hall, 1952). This was seen as a shift in the original color (reddish brown) of the Sorghum bicolor red sheath to dark brown color with the addition of crude trona. The color intensified when 3 g crude trona was added.

The higher the color intensity, the more the solution becomes neutral, and hence the more the destruction or dilution of the antioxidant (Kayahan and Saloglu, 2021).

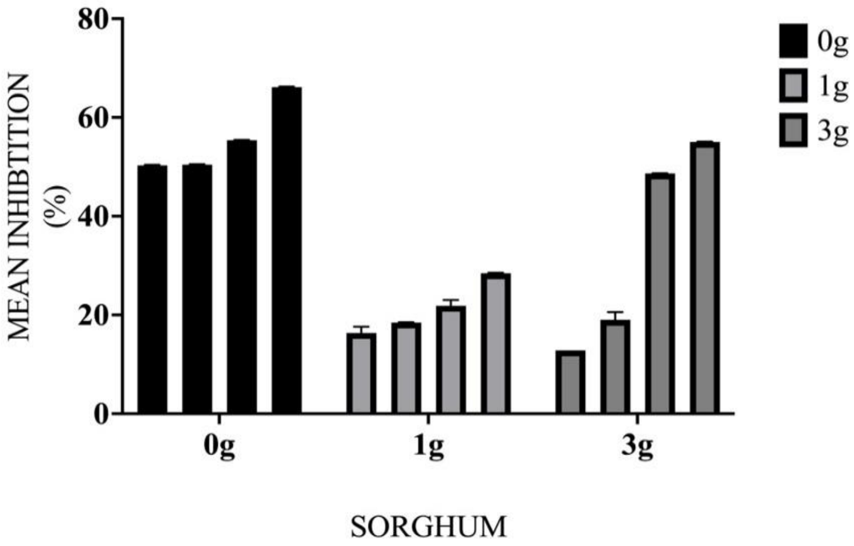

Figure 2. Inhibition of *Sorghum bicolor* red sheath cooked with and without crude trona

Effect of crude trona on cowpea (*Vigna unguiculata*)

The inhibition of the cowpea cooked without crude trona gave the highest yield, followed by that which was cooked with 1 g crude trona, with the cowpea cooked with 3 g crude trona having the least inhibition, as shown in Figure 3 below. The inhibitions (%) increased with an increasing concentration (mg/ml) of the sample. Although cowpeas cooked without crude trona had the same mass (45 g) as those that which was cooked with the various masses of crude trona (1 g and 3 g), from Figure 3, the inhibitions of the cowpea cooked without crude trona and those that were cooked with 1 g and 3 g were significant ($P < 0.05$). These inhibitions increased with increasing concentration. Crude trona was observed to have a negative effect on the antioxidant activity of cowpeas due to a decrease in the inhibition with the addition of crude trona. Cowpea extract exhibited higher antioxidant activity on β-carotene as well as vitamin C. Addition of crude trona to cowpea tenderizes it and also enhances its flavor; it also converts ascorbic acid present in it to an inactive form of diketogulonic acid in the presence of water since it is basic (Kayahan and Saloglu, 2021).

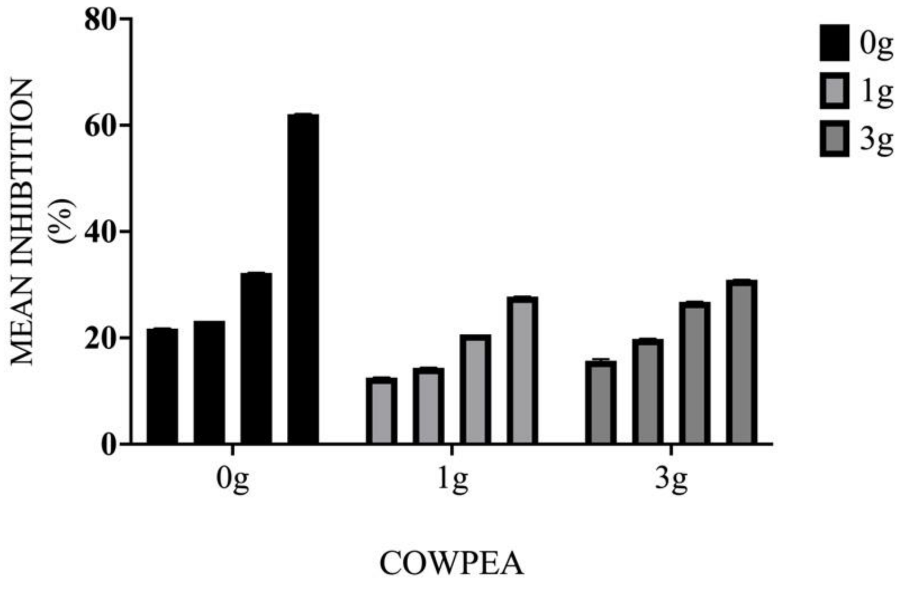

Figure 3. Inhibition of cowpea (*Vigna unguiculata*) cooked with and without crude trona

Effect of crude trona on okro (*Abelmoschus esculentus*)

Okro cooked without crude trona gave a higher inhibition than that which was cooked with 1 g of crude trona. The cooked okro with 1 g Crude trona also gave an inhibition higher than that which was cooked with 3 g crude trona. In all these instances, the percentage inhibition increased with an increasing concentration of the sample. From Figure 4 below, samples of the okro cooked with (1 g and 3 g) crude trona and those that which was cooked without crude trona gave a significant difference ($p < 0.05$) of inhibition, which increased with an increasing concentration (10, 20, 40, and 80) % respectively. Green leaves and vegetables contain chlorophyll as well as vitamin C, which is an antioxidant. The addition of crude trona removed hydrogen ions, saved the chlorophyll (the green color) of the okro, and promoted the destruction of vitamin C (Mazza and Miniati, 1993). This increased its sliminess and also deepened the color of the okro from a light green to a deep green. The color intensified as the mass of the crude trona increased. However, the destruction of vitamin C reduced the antioxidant activity when crude trona was added. Okro also has high phenolic and flavonoid content, which are antioxidants (Onibi et al., 2009). Studies of the structure-activity link of flavonoids have revealed that the following order is where the dissociation of hydroxyl function takes place: 4-OH > 5-OH > 7-OH. Effective free radical scavenging activity requires the O-hydroxyl structure in the B ring, the 2, 3-double bond in conjugation in the C ring, and the 3- and 5-OH groups with the 4-oxo function in the A and C rings. (Pasaribu et al., 2018). A reduction in the antioxidant activity can also be attributed to an alteration in the structure of the O-hydroxyl structure in the B ring, the 2, 3 - 3-double bond conjugation in the C ring, as well as the 3- and 5-OH groups with the 4-oxo function in A and C rings by the crude trona (Pasaribu et al., 2018).

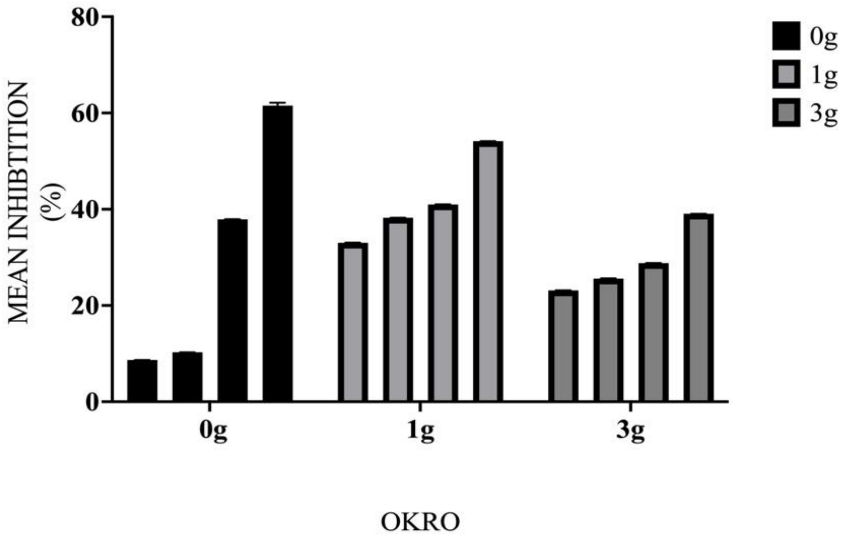

Figure 4. Inhibition of okro (*Abelmoschus esculentus*) cooked with and without crude trona

Effect of crude trona on Bissap (*Hibiscus sabdariffa*)

From Figure 5, the roselle calyces cooked with 3 g crude trona gave a higher inhibition than those cooked with 1 g crude trona. The inhibition of the bissap cooked with 1 g crude trona was also greater than that cooked without Crude trona.

Roselle calyces cooked without and with crude trona (1 g and 3 g), respectively, gave a significant difference in the inhibition ($P< 0.05$) as seen in Figure 5 below. The percentage inhibition increased with increasing concentration (0.1, 0.2, 0.4, and 0.8) mg/ml for the same masses of samples used (15 g). Studies demonstrated that the calyces of roselle (Rajeswara et al., 2012) are rich in phenolic compounds and water-soluble anthocyanins. It was reported that those compounds could be considered a great source of natural antioxidants. It is generally supposed that the active constituents contributing to these protective effects are antioxidant nutrients such as vitamin E, carotene, lycopene, anthocyanins, polyphenols, and other water-soluble antioxidants (Pasaribu et al., 2018). The 4-OH groups are attached to the aromatic rings, and the pigments are very soluble in water (Schaffner and Proctor, 2012). As the number of phenolic-OH -OH groups rise, so does the capacity to scavenge free radicals (Pasaribu et al., 2018). Factors including pH, temperature, ascorbic acid, oxygen content, and intermolecular co-pigmentation affect how quickly anthocyanins degrade. In juice processing and long-term product storage, the reactions are typically undesired (Sun et al., 2018).

The antioxidant activity of roselle extract is pH dependent (pH 2 to 7); the activity decreases as pH increases. However, at a constant pH (pH 7), only a relatively small decrease in antioxidant activity and total phenolic content is observed (Wu et al., 2018). Like the red cabbage, the very acidic solution turns the anthocyanin red color, neutral solutions result in a purplish color, and basic solutions appear greenish-yellow. Although crude trona is basic, its addition to the bissap deepened the red pigment (anthocyanin), thereby increasing the antioxidant activity. It is also very common practice with most sellers of bissap to artificially induce the calyces with synthetic dyes, and this may contribute to the reason for the increase in the inhibition of the antioxidant, even when crude trona was added.

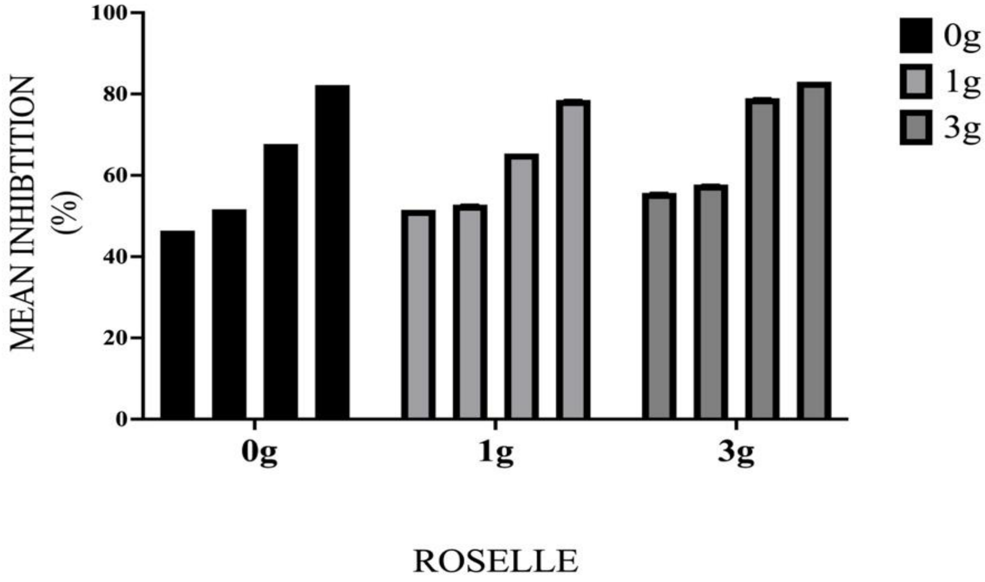

Figure 5. Inhibition of bissap (*Hibiscus sabdariffa*) cooked with and without crude trona

CONCLUSION

From the study, the addition of crude trona reduces the antioxidant activity of ayoyo (*Corchorus tridens*), *Sorghum bicolor* red sheath, cowpea (*Vigna unguiculata*), and okra (*Abelmoschus esculentus*), thereby making them unhealthy for consumption due to the risk of oxidative stress. Even though the addition of crude trona to okro tends to increase the sliminess during cooking to enhance its desirability in certain homes, crude trona inhibits the antioxidant property of okro. However, the addition of crude trona to roselle calyces (*Hibiscus sabdariffa*) increased the antioxidant activity.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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DATA AND MATERIALS AVAILABILITY

All data associated with this study are present in the paper

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AUTHOR CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author JA conceived the study, participated in the laboratory analyses, analyzed data, interpreted, drafted, and edited the manuscript. Author JN assisted in the laboratory analyses, data analysis, and editing of the manuscript. Author BA participated in the study design, collected samples, conducted laboratory analysis, data analysis, and interpreted data. Author JKO designed the study and edited the final manuscript. Author LSG assisted in the computer simulations, data analysis, and drafting of the manuscript. All authors read and approved the final manuscript

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