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Effects of Indigenous Reed (*Typha latifolia*) Salt and Iodized Commercial Salt on Total Phenolic and Total Flavonoid Contents and Antioxidant Activity of Garlic (*Allium sativum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TA, PW and AK designed the study. Author TA collected the samples and performed laboratory analyses. Authors PW and AK supervised the work and provided technical support. Authors TA, PW and AK performed literature search and analyzed the collected data. Author TA wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The use of natural food additives such as garlic, ginger, turmeric and indigenous reed salts is increasing over synthetic ones due to their availability, affordability and the mental picture that ''natural is safe''. Food is usually cooked in the presence of other additives such as salt. It has been established that food additives (such as salt) and their degradation products can interact with

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other food additives and food constituents (additive-additive, additive-micronutrient) to form an array of products and these can have positive or negative effects on the antioxidant activity of the other additives and the food. The aim of this study was to investigate the effects of indigenous reed salt (*Typha latifolia*) and iodized commercial salt (Kensalt) used in Kenyan culinary recipes on the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of garlic.

Place and Duration of the Study: Garlic bulbs and iodized commercial salt (Kensalt) were purchased from Khethia supermarket and Food Plus mall of Eldoret town (Kenya), respectively. Indigenous reed salt was purchased from vendors in Busia County of Western Kenya. The samples were analyzed at Directorate of Government Analytical Laboratory, Kampala (Uganda) between August 2019 and January 2020.

Methodology: The TPC of fresh and salted garlic extracts were determined using the Folin-Ciocalteu assay, TFC by aluminum chloride colorimetric method and antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay.

Results: The TPC, TFC and antioxidant activity of fresh aqueous garlic extract were 303.07 ± 6.58 mg gallic acid equivalent per 100 g, 109.68 ± 6.78 mg quercetin equivalent per 100 g and 56.60 ± 0.05%, respectively. Salt had a significant effect on TPC ($P = .03$) and antioxidant activity ($P < 0.05$). However, the mean differences were insignificant for the effect of salt on TFC ($P = .66$). Changes in salt concentrations did not significantly affect the TPC, TFC and antioxidant activity of garlic.

Conclusion: Cooking garlic with salt enhances the extraction of its phytochemicals, thereby increasing its antioxidant potential.

Keywords: Allium sativum; allicin; condiment; flavor enhancer; culinary recipes.

1. INTRODUCTION

Food additives may be defined as natural or synthetic substances added to food to improve their quality and safety, increase shelf life, modify the taste, smell and appearance [1]. There are different functional classes of food additives namely, sweeteners, colorants, preservatives, antioxidants, emulsifiers, stabilizers, flavorings and flavor enhancers [1]. All these are used during processing, packaging and storage to retain the original form or further improve the value of the food [2]. For many years, people have used food additives for medicinal purposes because they were aware of the existence of their biologically active constituents in them. However, of recent, there has been a renewed interest in the use of plant-derived food additives due to their medicinal values, availability, low cost, less side effects, and the general belief that "natural is safe" [3,4].

Garlic (*Allium sativum* L.) is among the popular culinary herbs used worldwide due to its pleasant aroma and therapeutic properties [5,6]. Plant-based food additives are well known for providing protection against degenerative and microbial diseases which are attributed to the presence of antioxidants in them [5]. Direct consumption of raw food additives is limited due to their taste and aroma when uncooked. As such, they are added to food and cooked as whole spices, chopped, powder or extracts. Normally, when cooking, salt is added to almost all dishes due to its ability to enhance flavor. Salt is also used in preservation of foods due to its antimicrobial effect which is as a result of its ability to reduce water activity.

It has been established that food additives (such as salt) and their degradation products can interact with other food additives and food constituents (additive-additive, additive-micronutrient) to form an array of products and these can have positive or negative effects on the antioxidant activity of the other additives and the food [7]. Information on the effects of indigenous reed salt (*Typha latifolia*) and Kensalt (an iodized commercial salt) commonly used in Kenyan culinary recipes is lacking yet this may affect the antioxidant properties of the other food additives or the food itself if they are added to food. This study therefore was undertaken to investigate the effects of indigenous reed salt and iodized commercial Kensalt on the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant (2,2-diphenyl-1-picrylhydrazyl radical scavenging) activity of garlic.

2. MATERIALS AND METHODS

2.1 Sample Collection

Random sampling technique was used for sample collection. Garlic bulbs (Chinese origin) and commercial iodized salt (Kensalt) were purchased from Khethia supermarket and Food Plus mall in Eldoret town (Kenya), respectively. Indigenous reed salt from *Typha latifolia* [8] was purchased from vendors in Busia County of Western Kenya [9].

2.2 Effect of Indigenous Reed Salt and Iodized Kensalt

Measured 100 g of chopped garlic was used. A stainless garlic chopper was used to cut garlic bulbs into pieces measuring 2 mm by 2 mm. The garlic samples were cooked for 60 minutes at 150 ℃ in 400 mL of water. The choice of these conditions followed our previous study [10] which indicated that aqueous extracts of garlic cooked for 60 minutes at 150°C in 400 mL of water recorded the highest TPC and TFC.

The sample solutions obtained above were cooled and then filtered through Whatman No.1 filter paper. Fresh (uncooked) garlic extract and the filtrate obtained were then analyzed for TPC, TFC and antioxidant activity as described in Section 2.3. To investigate the effect of the salts on TPC, TFC and antioxidant activity of garlic, garlic extracts were prepared from garlic chopped with the two salts added at different concentrations of 0.00, 0.05, 0.10, 0.20 and 0.40 M. This gave a total of 9 samples: with no salt (1), with indigenous reed salt (4) and with Kensalt (4) at the mentioned concentrations.

2.3 Determination of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity

2.3.1 Total phenolic content

The TPC of garlic extracts were determined using the Folin-Ciocalteu assay as described by Sengul et al. [11] with slight modifications. Folin-Ciocalteu reagent (1.50 mL, 10 times dilution) and sodium carbonate (1.20 mL, 7.5% w/v) were introduced into test tubes containing 0.20 mL of the extracts. The mixtures were incubated for 30 minutes in the dark. After, their absorbances were measured at 760 nm using UV-1900 UV-Vis spectrophotometer (Shimadzu Corporation, Japan). The TPC was expressed as gallic acid equivalent (GAE) in mg/100 g [12]. TPC was calculated from a calibration curve that was obtained using gallic acid as the standard [13].

2.3.2 Total flavonoid content

Aluminum chloride colorimetric method was used for the determination of TFC of the extracts [14]. Quercetin was used to make a calibration curve. Stock quercetin solution was made by dissolving 5.0 mg in 1 mL of ethanol, and then the standard solutions of quercetin were prepared by serial dilutions using ethanol (5-200 mg/mL). Measured 1 ml of the extracts were separately mixed with 1 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 minutes at room temperature. The absorbance of the reaction mixture was measured against a blank at 420 nm using UV-1900 UV-Vis spectrophotometer (Shimadzu Corporation, Japan). The TFC of the test samples was calculated from the calibration plot and expressed as g quercetin equivalent (QE)/g.

2.3.3 Antioxidant activity

The antioxidant activity of the garlic extracts were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method of Aksoy et al. [15] with some modifications. Measured 1 mL of the garlic extract was mixed with 1.20 mL of 0.003% DPPH in ethanol. The reducing ability of the antioxidants towards DPPH was evaluated by monitoring the absorbance at 517 nm using UV-1900 UV-Vis spectrophotometer. The percentage of DPPH inhibition was calculated using Equation 1.

$$\text{Inhibition (\%)} = \frac{Ac-As}{Ac} \times 100 \quad (1)$$

From which A_s is the absorbance of the sample and Ac is the absorbance of the control.

2.4 Statistical Analysis

All quantitative data were reported as means ± standard deviations of triplicates. Data were subjected to one-way ANOVA at *P = .05* using SPSS for Windows (version 20). The differences in the effects of the two salts on the TPC, TFC and antioxidant activity of garlic extracts were established using independent sample *t*-test.

3. RESULTS AND DISCUSSION

For TPC, a calibration curve was prepared for its quantitative analysis in the extracts using gallic acid as a standard and the linearity was established from the range of 1 to 300 mg/ml which was fitted on the line $y = 0.0065x$. Similarly, a calibration curve (prepared using quercetin as a standard) for TFC quantification was generated. Linearity for the standard was established from the range of 5 to 200 mg/ml which was fitted on a straight line that gave the equation $y = 0.0023x - 0.0012$. The mean TPC, TFC and antioxidant activity of the aqueous extract of fresh garlic were 303.07 ± 6.58 GAE/100 g, 109.68 ± 6.78 mg QE/100 g and 56.60 ± 0.05%, respectively.

3.1 Effect of Different Types of Salts on Phytochemicals and Antioxidant Activity of Garlic Extracts

The effects of indigenous reed salt and iodized Kensalt on the phytochemical content of the garlic extracts were tested at different concentrations. Fig. 1 summarizes the effects of the salts on TPC and TFC of garlic.

Generally, TPC and TFC increased with the addition of indigenous reed salt more than with addition of iodized Kensalt with TPC > TFC in each case. However, without addition of the indigenous reed salt or Kensalt, it was observed from the experiment that there were reduction in TPC and TFC of the extracts. TPC and TFC were on the lower side than where salt was added/present regardless of the salt type. Further, the effect of different types of salts on TPC and TFC was verified using independent sample *t*-test under no equal variance assumption. Based on the outputs, it was confirmed that these differences were significant ($P = .003$) for TPC. However, the mean differences were insignificant for the effect of salt types on TFC ($P = .66$). A plausible explanation could be that for TFC, flavonoids are usually present in small quantities than phenolic compounds hence the differences that the presence/absence of the different types of salts brought about could be very small. The increase in TPC after the addition of salt regardless of the type could be explained by the fact that salt is a tenderizer [16] and as such, it softens tissues thereby enhancing the extraction of phytochemicals. By visual inspection, residues of garlic that were cooked with salt were much softer than those cooked without salt. A similar study [17] aimed at determining the effect of soaking in salt solutions on water absorption, pH, and cooking time of African yam bean (*Sphenostylis stenocarpa* Hochst ex A Rich Harms) seeds found that salt had tenderizing effects. In addition, reduction in cooking time was much higher in the beans soaked in indigenous akanwu salt unlike sodium chloride salt, signifying that the former salt is a better tenderizer than the latter [17]. Table 1 and Table 2 gives the detailed account of the effects of indigenous reed salt and Kensalt on TPC and TFC of the garlic extracts.

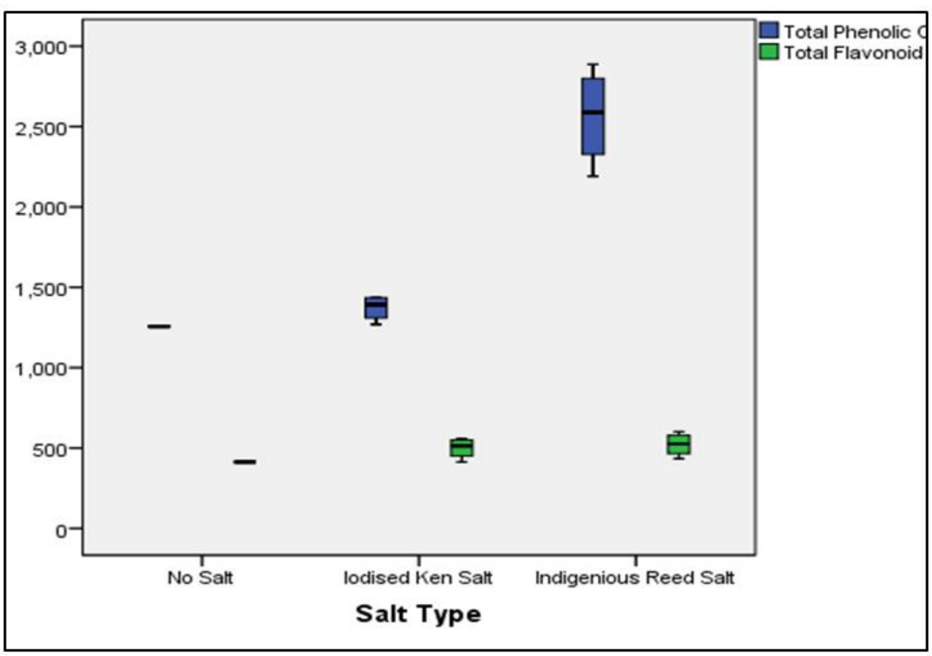

Fig. 1. Effect of the different salts on total phenolic and total flavonoid contents of aqueous garlic extracts

Table 1. Effect of salt and its concentration on TPC of the garlic extracts

Concentration (M)	Iodized Kensalt	Indigenous reed salt
0.00	1255.52 ± 5.87[a]	1255.52 ± 5.87[a]
0.05	1268.75 ± 17.27[b]	2190.29 ± 17.50[c]
0.10	1348.63 ± 12.08[b]	2464.62 ± 8.83[c]
0.20	1435.33 ± 6.48[b]	2710.21 ± 6.50[c]
0.40	1434.06 ± 8.64[b]	2887.68 ± 15.03[c]

Different letters in the same row/column indicate significant differences (P = .05) as established by independent sample t-test

Table 2. Effect of salt and its concentration on TFC of the garlic extracts

Concentration (M)	Iodized Kensalt	Indigenous reed salt
0.00	397.68 ± 8.78[a]	397.68 ± 8.78[a]
0.05	413.17 ± 3.69[a]	433.91 ± 0.88[a]
0.10	487.75 ± 0.00[a]	497.25 ± 16.60[a]
0.20	538.74 ± 0.87[a]	555.08 ± 6.27[a]
0.40	558.87 ± 20.25[a]	601.87 ± 1.54[a]

Different letters in the same row and column indicate significant differences (P = .05) as determined by independent sample t-test

Table 3. Effect of salt and its concentration on the antioxidant activity of the garlic extracts

Concentration (M)	Iodized Kensalt	Indigenous reed salt
0.00	73.65 ± 0.43[a]	73.65 ± 0.43[a]
0.05	73.48 ± 0.75[b]	86.66 ± 0.42[c]
0.10	76.53 ± 0.33[b]	90.32 ± 0.38[c]
0.20	76.71 ± 0.82[b]	95.03 ± 0.20[c]
0.40	80.12 ± 0.08[b]	97.03 ± 0.20[c]

Different letters in the same row/column indicate significant differences (P = .05) as established by independent sample t-test

The results showed that there were significant differences between the amounts of phytochemicals in the presence of salts with antioxidant activity ($P < 0.05$) at higher salt concentrations and hence it can be concluded that the salts had an effect on the antioxidant activity of garlic and that the indigenous reed salt also have antioxidant properties since it is a salt derived from a plant. This is supported by a research done by Om and Jeong [18] which reported that bamboo salt used for prevention and treatment of various diseases in Korea has anti-oxidative effect more than vitamin E.

Table 3 above shows the effects of salt and its concentration on the antioxidant activity of the garlic extracts.

Different concentrations of the two types of salts were measured for their different effects on TPC and TFC. TPC increased with increase in concentration of both iodized Kensalt and indigenous reed salt but the differences were more for the latter. However, the differences were not significant ($P = .20$). These results are in complete agreement with a study by Abacan et al. [19] which looked at the effect of cooking time, temperature and salt concentration on phenolic content and antioxidant activity of selected edible mushrooms which found that salt concentration did not significantly affect the levels of phenols and antioxidant activity.

However, a different result was reported by Burg and Oshrat [20] which demonstrated that salt composition and concentration affected the antioxidant activity of red microalgal polysaccharides and thus it was postulated that the salt interfered with the polysaccharide chains hence exposing the antioxidant sites. In another study [12], the effect of different methods of processing (blanching, microwave, freezing, brining and prickling) on the antioxidant properties of selected herbs and vegetables (garlic, ginger, chili fruit, onion, mushroom cap and baby corn) were studied. The authors found that brining with salt (salt is used as a preservative and softening agent) led to decline in antioxidant properties of vegetables and it was concluded that brining reduces the nutritional value of preserved vegetables because of the elimination of water-soluble vitamins and minerals. These differences could be as a result of the different concentration ranges used.

4. CONCLUSION

Though commercial iodized Kensalt is common in Kenya, indigenous reed salt increased the antioxidant properties of garlic. Therefore, the use of indigenous reed salt should be encouraged. Further studies have to be done on the additive-additive and/or additive-matrix effect on phytochemicals and antioxidant activity of food additives and/or vegetables since there is limited literature on this. There is also a need to determine the composition of the indigenous reed salt and establish the chemical(s) responsible for increasing its phenolic and flavonoid contents when the salts were added to garlic.

ETHICAL APPROVAL

This study was approved by the Department of Chemistry and Biochemistry, Moi University, Eldoret, Kenya for Thandiwe Alide (Approval No. MSC/ACH/11/18).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Food Safety Authority of Ireland. Guidance on Food Additives. 2010;64. Available:https://www.fsai.ie/training/food_add/story_content/external_files/Food%20Additives%20Report%202010%20FINAL.pdf.
2. Nam P, Chan A. Chemical Properties and Applications of Food Additives : Preservatives, Dietary Ingredients, and Processing Aids. Handbook of Food Chemistry. Springer-Verlag Berlin Heidelberg. 2014;1-20. Available:https://dx.doi.org/10.1007/978-3-642-41609-5_37-1
3. Ilbäck NG, Busk L. Food additatives. Use, intake and safety. Food Nutr Res. 2000;44: 141-149. Available:https://doi.org/10.3402/fnr.v44i0.1778
4. Omara T, Nnankabirwa R, Nassazi W, Nakabuye BV, Musau B. Spectroscopic screening of assorted pigmented vegetables and fruits common in Metropolitan Kampala culinary recipes for anthocyanins. Int J Food Sci Nutr. 2018;3(6):282-290.
5. Prati P, Henrique CM, Souza AS, De Sônia V, Teresa M, Pacheco BP. Evaluation of allicin stability in processed garlic of different cultivars. Food Sci Technol. 2014; 34:623-628.
6. Omara T, Kiprop AK, Ramkat RC, Cherutoi J, Kagoya S, Nyangena DM, et al. Medicinal plants used in traditional management of cancer in uganda: A review of ethnobotanical surveys, phytochemistry, and anticancer studies. Evid-Based Complement Alternat Med. 2020;(3529081):1-26. Available:https://dx.doi.org/10.1155/2020/3529081
7. Scotter MJ, Castle L. Chemical interactions between additives in foodstuffs : a review. Food Addit Contam. 2004;21(2):93-124. Available:https://doi.org/10.1080/02652030310001636912
8. Wangila TP. Phytochemical analysis and antimicrobial activities of cyperus rotundus and *Typha latifolia* reeds plants from lugari region of Western Kenya. Pharm Anal Chem. 2017;3:128. Available:https://doi.org/10.4172/2471-2698.1000128
9. Wangila TP, Kinyanjui T, Nakhone LN. Investigation of the Transformation and Effect of Preparation Method, Storage Conditions and Time on Iodine and Iron (II) Present in the Reed Salt. Int J Sci Res. 2016;5(9):341-349.
10. Alide T, Wangila P, Kiprop A. Effects of cooking temperature and time on total phenolic content, total flavonoid content

and antioxidant activity of garlic. Unpublished; 2020.
11. Şengül M, Yildiz H, Kavaz A. The effect of cooking on total polyphenolic content and antioxidant activity of selected vegetables. Int J Food Prop. 2014;17:481-490.
12. Chan EWC, Tan YP, Chin SJ, Gan LY, Kang KX, Fong CH, et al. Antioxidant properties of selected fresh and processed herbs and vegetables. Free Rad Antiox. 2014;4(1):39-46.
13. Hwang IG, Shin YJ, Lee S, Lee J, Yoo SM. Effects of Different Cooking Methods on the Antioxidant Properties of Red Pepper (*Capsicum annuum* L.). Prev Nutr Food Sci. 2012;17(4):286-292. Available:https://doi.org/10.3746/pnf.2012.17.4.286
14. Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, Khan IA. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops : A comparative study. Evid-Based Complement Altern Med. 2014;2014:1-9. Available:https://doi.org/10.1155/2014/253875
15. Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. Saudi J Biol Sci. 2013;20(3):235-239. Available:https://doi.org/10.1016/j.sjbs.2013.02.003
16. Gooch RC, Guenther JJ. Google Patent US4224349A. Meat tenderizing method. Available:https://patents.google.com/patent/US4224349A/en
17. Onyeike EN, Uzogara SG. Effect of soaking in salt solutions on water absorption, PH and cooking time of African yam bean seeds (Sphenostylis stenocarpa Hochst ex A Rich Harms). Global J Pure Appl Sci. 2016;6(1):67-73. Available:https://doi.org/10.4314/gjpas.v6i1.16079
18. Om A, Jeong J. Bamboo Salts Have Antioxidant Activity and Inhibit ROS Formation in Human Astrocyte U373MG Cells. Cancer Prevent Res. 2007;12(3):225-230. Available:http://www.jcpjournal.org/journal/view.html?volume=12&number=3&spage=225
19. Abacan SF, Hurtada WA, Devanadera MAR. Effects of cooking time, temperature, and salt concentration on the phenolic content and antioxidant activity of selected edible mushrooms. Int Food Res J. 2017;24:2028-2032.
20. Burg A, Oshrat L. Salt Effect on the Antioxidant Activity of Red Microalgal Sulfated Polysaccharides in Soy-Bean Formula. Marine Drugs. 2015;13(10):6425-6439.

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