



BIOCHEMICAL CHANGES IN BEEF PATTIES BOILED WITH ETHANOL EXTRACT OF THE ROOT OF *Glycyrrhiza glabra* LINN

*¹Ugbeni, C. O., ²Idiakheua, D. O. and ¹Ejovwokoghene, U. O.

¹Department of Biochemistry, University of Benin, Benin City, Edo State Nigeria.
²Department of Biochemistry, Ambrose Alli University, Ekpoma, Edo State Nigeria.

Corresponding Author's Email: osezele.uqbeni@uniben.edu.ng

ABSTRACT

The use of spices in boiling of meat is a common phenomenon. The root of *Glycyrrhiza glabra* is used locally as spice for meat. This work was designed to determine the effect of ethanol extract of *Glycyrrhiza glabra* on malondialdehyde content, percentage Metmyoglobin content, acid value, Heme-iron content and peroxide value of beef stored at 4°C for a storage time of 0 to 18 hours was evaluated. Samples were taken at interval of 6 hours for analysis of the biochemical parameters under study. The statistical difference observed in Acid value, Malondialdehyde content, Heme iron content and peroxide value showed no significant difference, all having $P \geq 0.05$ in beef treated with ethanol extract of *Glycyrrhiza glabra* when compared to the control. On the other hand, the statistical difference in percentage metmyoglobin content when compared to the control at 0 hour interval was significant having $P \leq 0.05$, then at other distinct time interval showed no significant difference having $P \geq 0.05$. Thus, this study revealed that the extract of *Glycyrrhiza glabra* have a decreased antioxidant property against rancidity in beef samples and peroxide formation, although the stability of antioxidant, antimicrobial properties of *Glycyrrhiza glabra* decreases with storage time resulting in the variation of percentage Metmyoglobin values, Malondialdehyde values and Heme iron values, Acid value and peroxide value of beef, which indicates it may not be a good spice for beef.

Keywords: Spices; *Glycyrrhiza glabra*; malondialdehyde; metmyoglobin; acid value.

INTRODUCTION

Plant products commonly referred to as spices are used as for flavouring, colouring or preservative agents. They could be seeds, fruit, root, bark or other parts of the plant. Their use dates back to the beginning of the history of mankind. Around the world several plants are used as spices. Varieties of spices that are produced throughout the world with some predominating in some regions eg Asia is well known for the cultivation of spices such as cinnamon, pepper, *Glycyrrhiza glabra* (liquorice), nutmeg, cloves and ginger, while white basil, bay lethyme, watercress are common in Europe. In America, pepper, nutmeg, ginger, all spice and sesame seed are mainly produced (Prasad *et al.*, 2011). Spices are also useful in the medicine and for the production of perfumes and cosmetics. Tajkarimi *et al.*, (2010) reported that essential compounds isolated from spices have antimicrobial activity against some common micro-organisms that affect food quality and shelf life. The introduction of spices through meals have several effects that are beneficial such as stimulating the secretion of saliva, promote digestion, prevent cold and influenza, reduce nausea and vomiting (Ravindran, 2002;

Sultana *et al.*, 2000). Gupta *et al.*, (2013), also reported that Spices help in the maintenance of balance of body humor. Depending on the nature of spice, its addition to food can change the taste and colour of food with many health benefits. The work of Sung *et al.*, (2012) revealed that spices have more of seasoning function than nutritional in food. Spices are also reported to help in the preservation of meat which is susceptible to disease infections (Thomas *et al.*, 2012). *Glycyrrhiza glabra* Linn is a plant used in traditional medicine across the world for its ethnopharmacological value. Ethanol and methanol extracts of *G. glabra* L roots were reported to have in vitro antioxidants activity and contains phytochemical such as phenols, flavonoids and Proanthocyanidin (Ugbeni *et al.*, 2020). Despite the usefulness of spices as reported, data are sketching on the effect of these spices on the quality of beef. This work was therefore set out to determine the effect of the ethanol extract of *Glycyrrhiza glabra* Linn of the biochemical changes in meat.

MATERIALS AND METHODS

Plant materials: The root of *Glycyrrhiza glabra*. L. was purchased from a local market at Lagos Street in Benin City, Edo State, Nigeria. The plant leaves were identified and authenticated at the department of plant biology and biotechnology of the University of Benin, Benin City. It was assigned a Voucher number of UBHG 394.

Sample preparation: The root of *Glycyrrhiza glabra*. L. were obtained, cut into pieces and sun dried, after which, it was ground to powdered form. 16g of the dried powdered roots of *Glycyrrhiza glabra* was weighed and soaked in 800ml of absolute ethanol (95 % v/v) for 72 hours at room temperature after which it was filtered using a muslin cloth into a clean conical flask to yield a crude ethanol extract which was freeze dried into powdered form and stored in a sealed tube at 4°C until required for use (Abdou-Bouba *et al.*, 2010). Percentage yield of 3.9 % was obtained. The beef was purchased fresh from an abattoir and immediately transported in ice pack to the laboratory.

Processing of beef sample: 202g of the beef was treated with ethanol extract of *Glycyrrhiza glabra* and boiled with distilled water at 100°C for 10mins. The boiled meat was then wrapped in aluminum foil and kept in a refrigerator at 4°C. For the control, 202g of the beef was steamed in distilled water only for 10mins at a temp of 100°C. The boiled meat was then wrapped in aluminum foil paper and kept in a refrigerator at 4°C. The effects of ethanol extracts and storage time were analyzed and determined in minced meat every 6 hours (0, 6, 12, and 18).

Determination of percentage metmyoglobin content: The analysis of metmyoglobin content was performed as described by Krzywicki (1982). Metmyoglobin is the oxidized form of oxygen carrying some protein myoglobin. Metmyoglobin is the cause of the characteristics brown colouration of meat that occurs due to cooking. The chemistry of beef colour is due to the presence of the pigment myoglobin. Meat undergoes oxygenation when exposed to air to form oxymyoglobin. Myoglobin and oxymyoglobin have the capacity to lose an electron which turns the pigment to a brown colour called metmyoglobin.

Determination of malondialdehyde content of beef: Malondialdehyde content of beef was determined using Buege

and Aust method (1978). This assay is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming a MDA-TBA₂ adducts that absorbs strongly at 535nm. In the presence of heat and acid, MDA reacts with TBA to produce a coloured end product. The intensity of the colour at 535nm corresponds to the level of lipid peroxidation in the sample.

Determination of heme iron contents: Haem iron was determined by means of acidified acetone extraction followed by spectrophotometry (Clark *et al.* 1997). The minced beef patties sample (2 g) was transferred into a 50 ml centrifuge tube and 9 ml of acid acetone mixture (90% acetone, 8% deionised water, and 2% HCl) was added. The mixture was macerated using a glass rod and allowed to stand for 1 h at room temperature. The extract was centrifuged at 2200 g for 10 minutes. The supernatant was filtered through Whatman #42 filter paper, and the absorbance was read at 640 nm against the acid acetone blank. The total pigments were calculated as haematin using the following formula (Lee *et al.* 1999): Total pigment (mg/kg) = $A_{640} \times 680$ and haem iron was calculated as follows (Clark *et al.* 1997): Haem iron (mg/kg) = total pigment (mg/kg) $\times 8.82/100$

Determination of peroxide value (PV): it was determined by the method as described by AOACS (1999): The peroxides value determines the concentration of hydroperoxide, the primary oxidation product. The principle involves peroxidases liberating iodine from potassium iodide. The amount of ROOH was determined by measuring the amount of iodine formed which is done by titration with sodium Thiosulphate and using a starch indicator. The amount of peroxidase was calculated by the amount of sodium Thiosulphate (Na₂S₄O₆) consumed.

Determination of acid value: The analysis of acid value was performed as described by AOCs (1999): The acid value is also a measure of the amount of fatty acids, which have been liberated by hydrolysis from the glycerides. The acid value is determined by directly titrating the fat in an alcoholic medium against a standard potassium hydroxide/sodium hydroxide solution.

Statistical analysis: The statistical significance was evaluated by one-way ANOVA using Graph prism 6 followed by post – hoc LSD and Tukey tests for individual comparisons. In all the results, the P-value of 0.05 was used to check for the level of significance.

RESULTS AND DISCUSSIONS

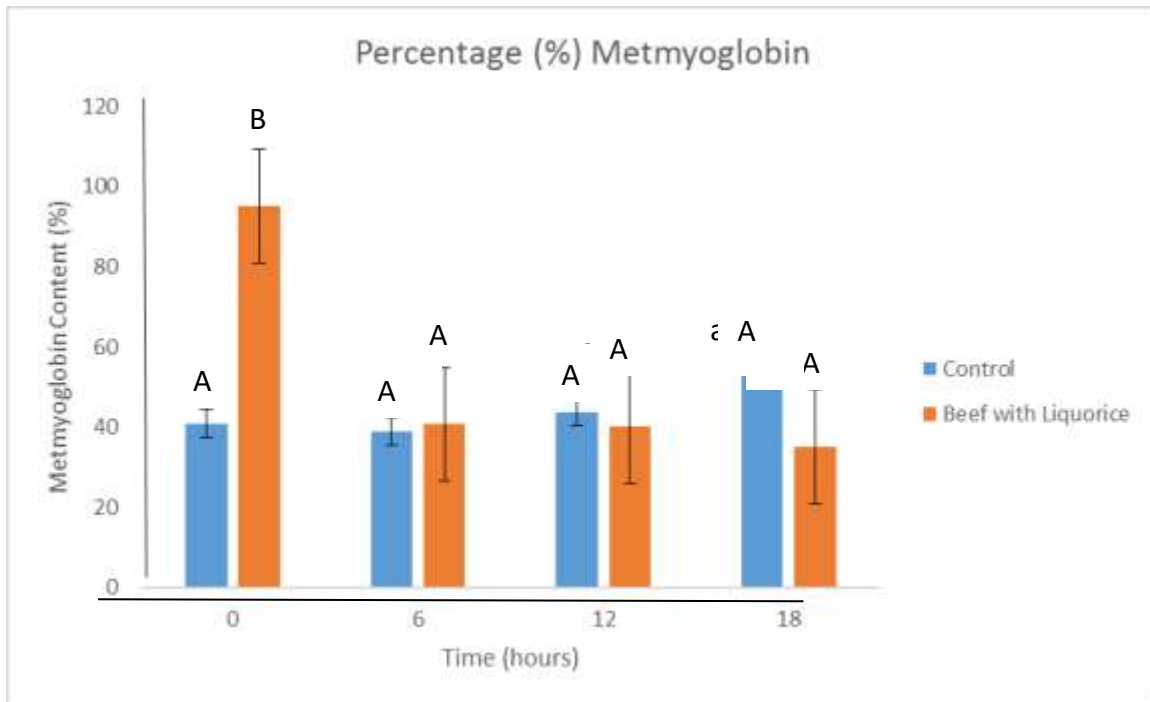


Figure 1: Effect of ethanol extract of the root of *Glycyrrhiza glabra* on Percentage metmyoglobin (MetMb) content of beef. (Values were obtained from three determinations)

Beef boiled with ethanol extract of *Glycyrrhiza glabra* at 0 hour showed a significant increase ($p < 0.05$) in Metmyoglobin when compared with control at 0 hour. However, meat with *Glycyrrhiza glabra* at other distinct time (6, 12, 18 hours respectively) with 6 hours interval showed no significant difference ($p > 0.05$) in metmyoglobin. Formation of brown MetMb results from the oxidation of the three ferrous forms to a ferric state and is associated with meat discoloration. Heat-induced denaturation of MetMb results in denatured globin hemichrome (ferrihemochrome), which is responsible for the dull-brown appearance of cooked meats (Surendranath and Poulson, 2013). *Glycyrrhiza glabra* was not effective in the prevention of autooxidation associated with meat discoloration and therefore cannot be used to improve meat colour.

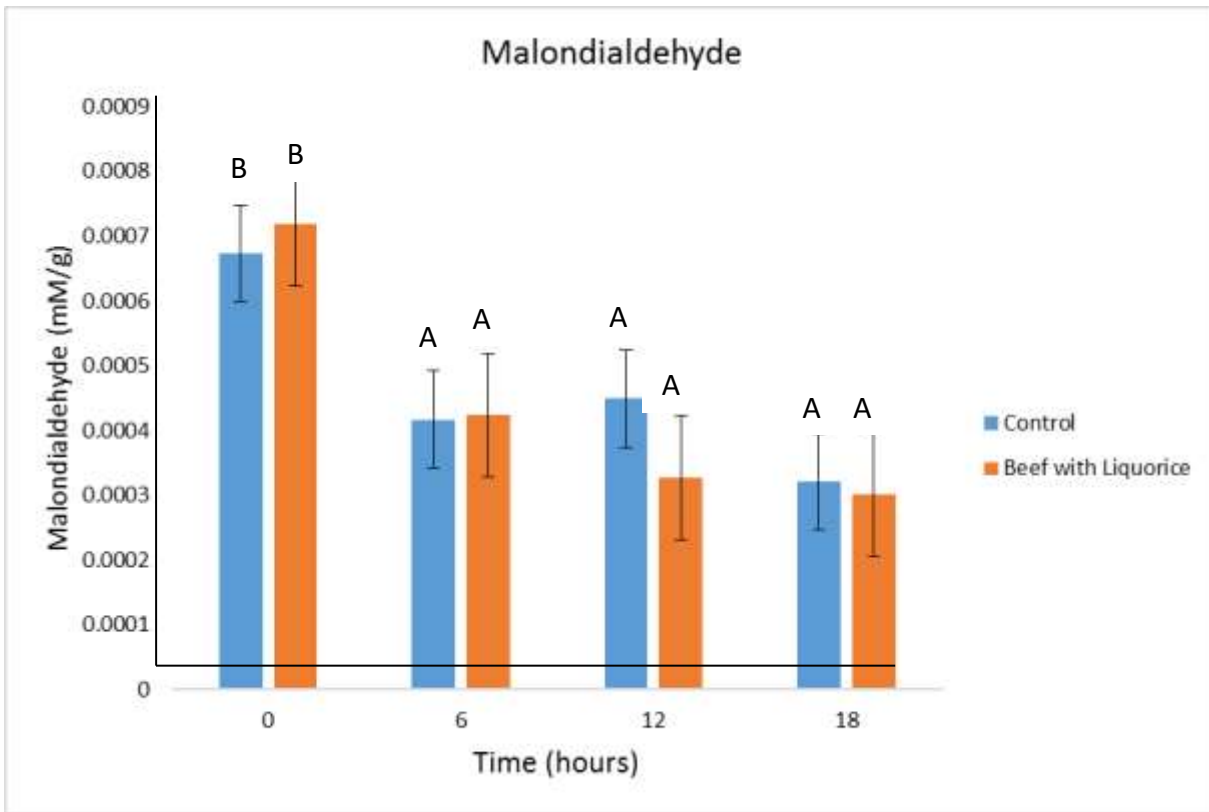


Fig. 2: Effect of ethanol extract of the root of *Glycyrrhiza glabra* on Malondialdehyde content of beef. (Values were obtained from three determinations)

The result from MDA values presented in Figure 2 showed that the lipid peroxidation associated with beef significantly reduced from 0-6 hrs when subjected to thermal treatment. However, treatment with the ethanol extract of *Glycyrrhiza glabra* showed no significant difference ($P > 0.05$) when compared to the control at the same time. Malondialdehyde (MDA) is used to monitor the spoilage of beef due to lipid peroxidation by microorganisms. Meat patties are susceptible to lipid oxidation during cooking (Esterbauer, 1993). It was previously demonstrated that a mixture of some such as ginger, black pepper, turmeric etc and meat during cooking decreased malondialdehyde (MDA) in the meat (Li *et al.* 2010, 2013). Unlike these spices, the ethanol extract of *Glycyrrhiza glabra* did not demonstrate compelling properties to be used to prevent lipid peroxidation in beef.

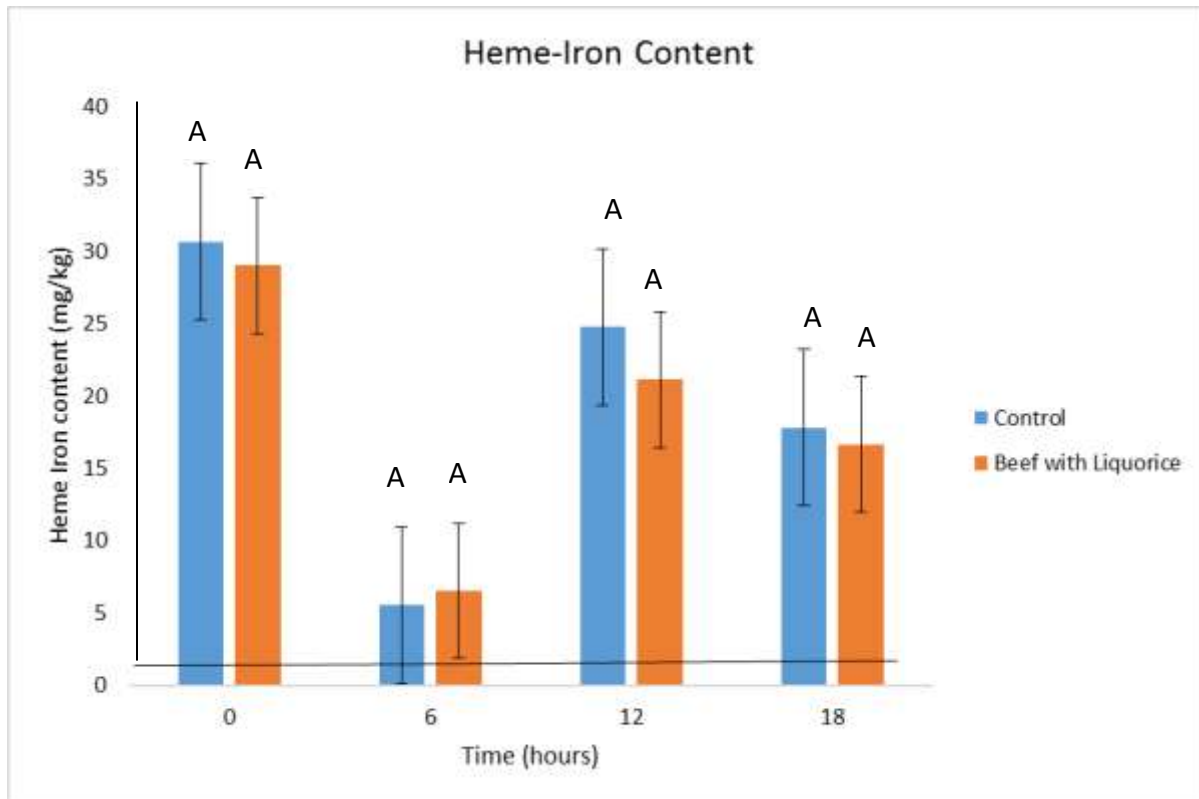


Fig. 3: Effect of ethanol extract of the root of *Glycyrrhiza glabra* on Heme-Iron content of beef. (Values were obtained from three determinations)

The result of heme iron content is presented in Figure 3. As can be seen from the graph, meat with *Glycyrrhiza glabra* after every time interval (0, 6, 12 and 18 hours) showed no significant difference ($p > 0.05$) in heme-iron content when compared with control at the same time. However, a significant reduction was observed in both samples 6 hrs, an increase after 12 hrs and a further reduction after 18hrs. Heme iron concentration have be reported be reduced in meats submitted to thermal processing (Lombardi-Boccia *et al.*, 2002; Kongkachuichai *et al.*, 2002; and Turhan *et al.*, 2004). This may be attributed to the oxidation of the porphyrin ring and iron liberation (Garcia *et al.*, 1996). The heat may have also contributed to breakage of the bonds associated with the varies degree of protein structure formation (Urbain, 1986). The increase observed after 12 hrs may be unconnected to the realignment of the weakly dissociated bonds.

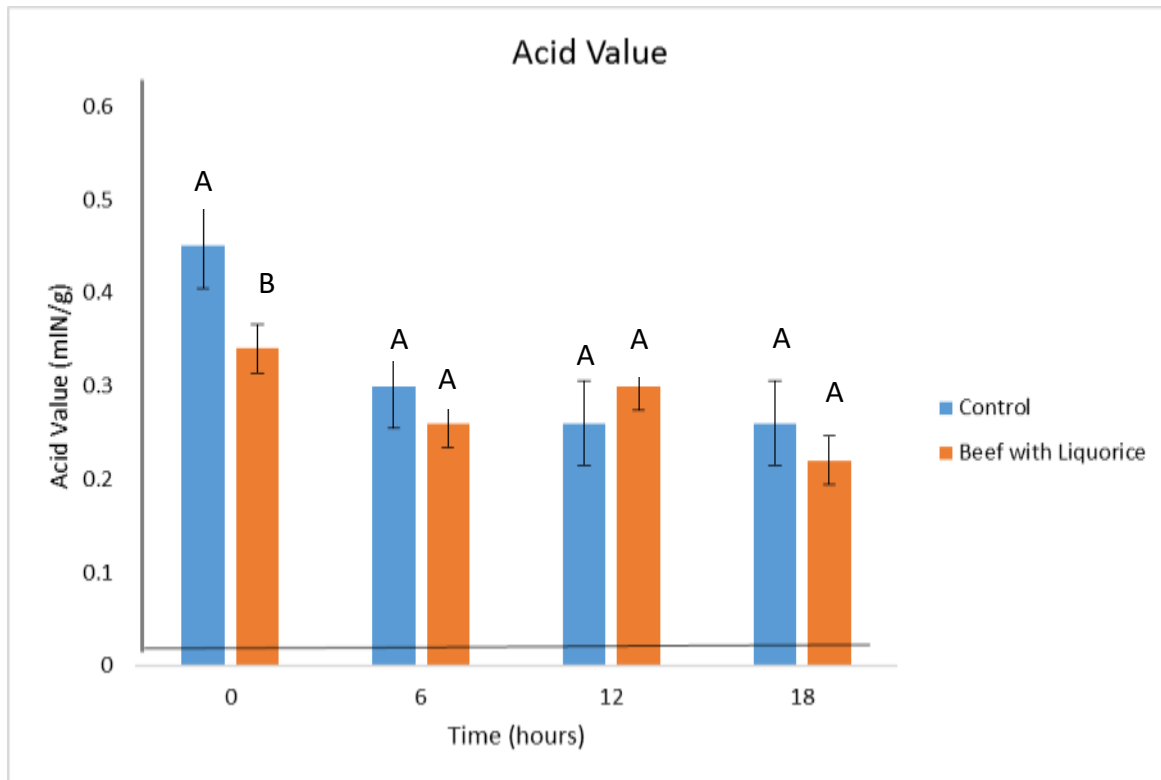


Fig 4: Effect of ethanol extract of the root of *Glycyrrhiza glabra* on Acid value of beef. (Values were obtained from three determinations)

The result of the acid value of beef treated with extract of *Glycyrrhiza glabra* shows that the acid value was significantly lower ($p \leq 0.05$) at 0 hour when compared with the control. However, at time interval (6, 12 and 18 hours) there was no significant difference ($p > 0.05$) in acid value when compared with control. This indicates that the preservative effect of ethanol extract of *Glycyrrhiza glabra* decreases with time. This is in line with the previous work of Fuzzat (2004) which confirmed that the antimicrobial properties of spices in general decrease when storage time increases. Similar findings revealed that addition of garlic extract did not cause any significance difference ($P > 0.05$) in the pH value of chicken meat Sallam *et al.*, (2004).

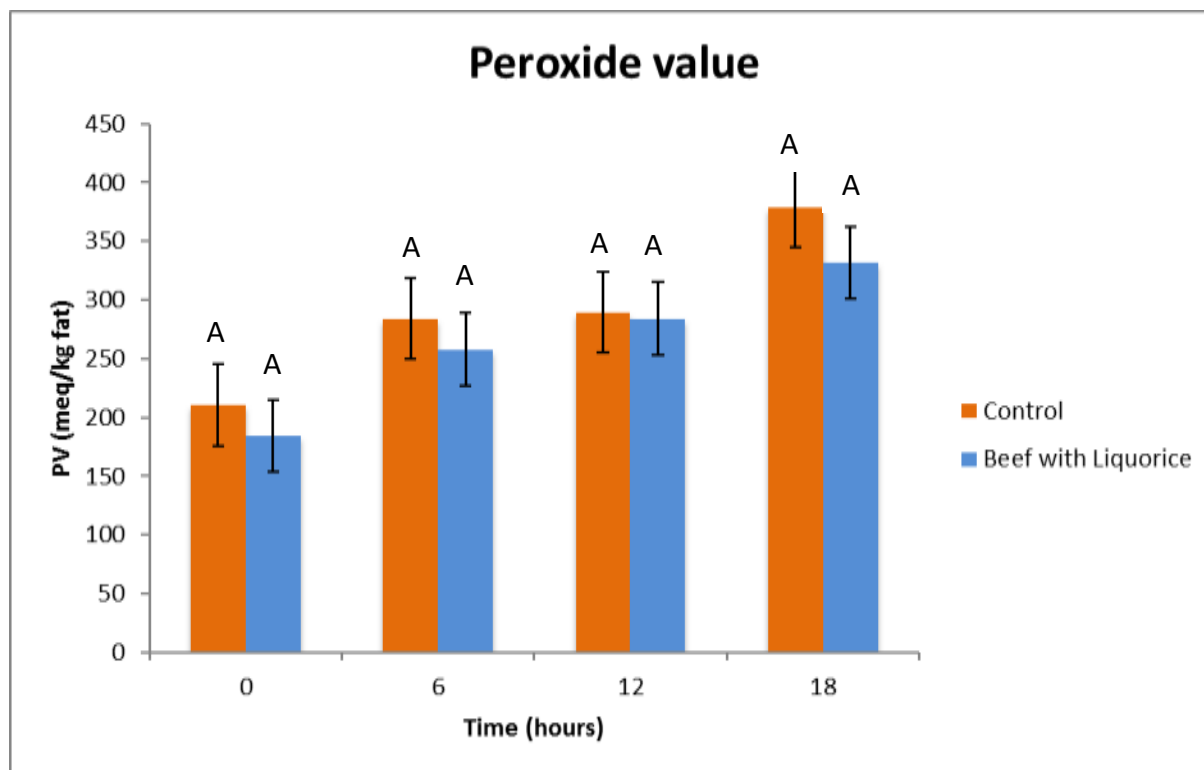


Fig 5: Effect of ethanol extract of the root of *Glycyrrhiza glabra* on Peroxide value of beef. (Values were obtained from three determinations)

The result of peroxide value of *Glycyrrhiza glabra* is shown in Figure 5. As can be seen from the graph, the Peroxide value of beef treated with ethanol extract of *Glycyrrhiza glabra* from 0 to 18 hours showed increase in value although was not significantly difference ($P>0.05$) when compared with the control at the same interval. This indicates that the ethanol extract of *Glycyrrhiza glabra* does not have effective antioxidant properties against Peroxide formation when boiled with beef. This is in contrast with the previous work done with other spices on beef. A case in question was reported by Hayam *et al.*, 2011 and Stoilova *et al.*, 2007, who showed that ethanol extract of Ginger on beef reveals the antioxidant effect of the ginger extract against Peroxide formation.

CONCLUSION

In this study, the ethanol extract of *Glycyrrhiza glabra* commonly known as Liquorice used as spice for meat have not shown scientific evidence to be used as antioxidant, antimicrobial and a preservative. This has opened more window of research to unravel the rationale behind its use as a spice.

REFERENCES

Abdou-Bouba, A., Njintang, Y.N., Schort, J & Mbofung, C.M.F. (2010). Phenolic compounds and radicals scavenging potential of twenty Cameroonian spices. *Agricultural and Biology Journal of North America*, **1**: 213-224.

AOACS Cd 3d-63 (1999). AOCS official method, Cd 3d-63: acid value. Champaign, IL(USA): American Chemist's Society

Clark, E.M., Mahoney, A.W. & Carpenter, C.E. (1997). Heme and total iron in ready to eat chickens. *Journal of Agriculture and Food Chemistry*, **45**, 124-126

Buege, J.A. & Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, **53**: 302-310.

Esterbauer H. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *America Journal of clinical Nutrition* **57**:779S-785S

Gupta, S.C., Sung, B., Kim, J.H., Prasad, S., Li, S & Aggarwal, B.B. (2013). Multitargeting by tumeric, The golden spice: from kitchen to clinic. *Molecular and Nutritional Food Research*, **57**: 1510-1528.

Hayam, M.I, Azza A.A & Ferial M.A (2011). Antioxidant and antimicrobial effects of some natural plant extracts added to lamb patties during storage. *grasas y aceites*, **62** (2) :139-148,

Kongkachuichai, R.; Napatthalung, P & Charoensiri, R.(2002). Heme and nonheme iron content of animal products commonly consumed in Thailand. *Journal of Food Composition and Analysis*, **15** (4): 389-398.

- Krzy-Wick, K. (1982). The determination of haem pigments in meat. *Meat Science*, **7**: 29-36.
- Lee B.J., Hendricks D.G & Cornforth D.P. (1999): A comparison of carnosine and ascorbic acid on colour and lipid stability in a ground beef patties model system. *Meat Science*, **51**: 245–253.
- Li Z, Henning SM, Zhang Y, Rahnama N, Zerlin A, Thames G, Tseng CH & Heber D. (2013). Decrease of postprandial endothelial dysfunction by spice mix added to high-fat hamburger meat in men with Type 2 diabetes mellitus. *Diabet Med* **30**:590–595.
- Li Z, Henning SM, Zhang Y, Zerlin A, Li L, Gao K & Lee RP (2010). Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations. *America Journal of clinical Nutrition*, **91**:1180–1184.
- Prasad, S., Gupta, S. C., & Aggarwal, B. B. (2011). Micronutrients and cancer: addspice to your life. *Nutritional Diet Cancer* **23**–48.
- Ravindran, M. K. (ed.). (2002). Cardamom: The Genus *Elettaria*. New York, NY: Taylor and Francis.
- Sallam KhI, Ishioroshi M & Samejim K. (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. *Lebensm. Wiss Technology*. **37**, 849-55.
- Stoilova I, Krastanov A, Stoyanova A, Denev P & Gargova S (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry*. **102**, 764-770
- Sultana, S., Ripa, F.A. & Hamid, K. (2010). Comparative antioxidant activity study of some commonly used spices in Bangladesh. *Pakistan Journal of Biological Science*, **13**: 340-343.
- Sung, B., Prasad, S., Yadav, V.R. and aggarwal, B.B. (2012). Cancer cell signalling pathway targeted by spice-derived nutraceuticals. *Nutritional Cancer*, **64**: 173-197.
- Surendranath, P.S & Poulson, J (2013). Myoglobin chemistry and meat colour. *Annual Review Food Science Technology*, **4**: 79-99.
- Tajkarimi, M.M., Ibrahim, S.A. & Cliver, D.O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, **21**: 1199-1218.
- Thomas, F., Daoust, S. & Raymond, M. (2012). “Can we understand modern humans without considering pathogens” *Evolutionary Applications*, **5**(4): 368-379.
- Ugbeni, O. C., Idiakheua, D. O & Ogbeiwi E. O (2020). A comparative study of the in vitro antioxidant activities and some phytochemical properties of methanol and ethanol extract of *Glycyrrhiza glabra* L. roots. *Biokemistri*, **32**(4): 285-292.
- Urbain, W. M. (1986). Food Irradiation. London: Academic Press Inc. Pp 351



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