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 leethylene, 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.

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

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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 design 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≥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≤0.05, then at other distinct time interval showed no significant difference having P≥0.05. Thus, this study revealed that the extract of *Glycyrrhiza glabra* has 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.
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 UBH0 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 aluminium 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) = A640 × 680 and haem iron was calculated as follows (Clark et al. 1997): Haem iron (mg/kg) = total pigment (mg/kg) × 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 (Na2S2O3) 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.
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 been reported to be reduced in meats subjected to thermal processing (Lombardi-Boccia et al., 2002; Kongkachuitchai 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 various degrees 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≤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).
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**


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