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Original Article

Effect of Basil leaf powder on oxidative stability of meat patties

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Abstract
The growing trend towards production of processed meat has made the control of oxidation important in meat industry. Utilization of medicinal plants like basil leaves, which are high in antioxidant properties can enhance the oxidative stability of meat and meat products during cold storage. The objective of this study was to examine the antioxidant content of basil leaf powder (BLP) and its impact on lipid oxidation in beef patties during cold storage. Three types of beef patties were prepared: Control (beef patties without additives), BLP 0.2 (beef patties + 0.2% Basil leaf powder), and BLP 0.4 (beef patties + 0.4% Basil leaf powder) and stored at 4°C for seven days. Results showed that BLP contain high phenol (279.29mg/g), flavonoid (104.14 mg (QE)/g) and vitamin C (66.27mg/g) contents. On the antioxidant activities BLP exhibited high DPPH (81.062%) and ABTS (98.41%) free radical scavenging ability. The incorporation of BLP at a concentration of 0.2 and 0.4% in the beef patties numerically reduced ($P > 0.05$) the level of lipid oxidation compared to control group during the cold storage. In conclusion, this study suggested that BLP holds promise as a natural preservative in beef patties to inhibit lipid oxidation during cold storage.

INTRODUCTION

Globally, the application of antioxidants as preservatives has played a pivotal role in enhancing the quality and prolonging the shelf life of muscle foods, particularly during processing and storage (Metsovas, 2013). This is essential because meat and meat products are highly prone to oxidation and microbial contamination due to their chemical makeup and the rapid depletion of endogenous antioxidants postmortem (Xiao *et al*., 2013, Falowo *et al*., 2014).

Naturally, muscle tissue contains a significant number of endogenous antioxidants, such as alpha-tocopherol, histidine-containing dipeptides, ubiquinone and glutathione, which possess the ability to scavenge free radicals and interrupt oxidative processes in vivo (Williams, 2007; Xiao *et al*., 2013). However, as the postmortem period lengthens, the effectiveness of these endogenous antioxidants diminishes (Xiao et al., 2013; Kumar et al., 2015), thereby exposing the lipid components of muscle to rapid deterioration. In order to boost meat antioxidant content, different antioxidants are employed in the meat industry. These antioxidants are readily available from a

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diverse array of natural sources, including fruits, herbs, spices, seeds, leaves, and roots (Kumar *et al*., 2015).

Sweet basil (*Ocimum basilicum L*.), a commonly utilized culinary herb belonging to the Lamiaceae family, is known to possess strong antioxidant and antimicrobial activities due to its phenolic acids and aromatic compounds (Marwat *et al*., 2011; Muhlisin *et al*., 2016). The plant is also famous for its therapeutic potential and preservation effects (Nadeem *et al*., 2022). According to Marwat *et al*. (2011), basil exhibits moderate macro and micronutrient profiles, including protein (3.15g/100 g), fat (0.64 g/100 g), energy (23 Kcal), Vitamin C (18 mg/100 g), Vitamin E (0.80 mg/100 g), Vitamin A (5275 IU), Vitamin K (414.8 mcg), Calcium (177 mg/100 g), Iron (3.17 mg/100g), Potassium (295 mg/100 g), Magnesium (64 mg/100g), and Sodium (4 mg/100 g). Numerous authors have reported the effectiveness of basil leaf extract in reducing microbial population and oxidative deterioration and also improving the sensory quality of meat products during cold storage (Falowo *et al*., 2019; Uzun and Oz, 2021). However, there have been no reports so far on the use of *O. basilicum L* leaf powder on lipid oxidation of beef patties. Thus, this study was designed to evaluate the antioxidant content of basil leaf powder and its effect on the oxidative stability of beef patties during cold storage.

MATERIALS AND METHODS

Sample Collection and extract preparation

Basil leaf powder (*O. basilicum* L*)* was procured from supermarket, in Akure, Ondo state, Nigeria and stored in a cool dry place until the time of use. For extract preparation, five grams (5 g) of plant sample was placed within separate glass thimbles and subjected to extraction using 200 ml of distilled water as the extraction solvent. The mixture was heated on a hot plate at 30-40ºC and continuously stirred for 20 minutes. After extraction, the resulting extract was filtered through Whatman qualitative filter paper No. 1 to remove any solid particles. Subsequently, the filtered extracts were concentrated to dryness using a rotary evaporator. The concentrated extracts were then stored in a refrigerator set at 4°C, awaiting further analysis. During analysis, the dried extracts were used for determination of the antioxidant activity at concentration of 1 mg/mL

Determination of antioxidant content and activity of the plant extract

The concentration of total phenolic compounds in the basil leave extracts was determined spectrometrically by the Folin–Ciocalteu method (Athanasiadis *et al.,* 2022) using gallic acid as a standard to prepare a calibration curve. The total flavonoid content of the extract was determined using a colorimeter assay as described by Bao (2005). The vitamin C content of the leaf was determined using the method of Benderitter et al. (1998). The antioxidant activities of the DPPH (1, 1-diphenyl-2-picryhydrazyl) and ABTS- 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) were determined using the method described by Gyamfi *et al*. (1999) and Re *et al*. (1999), respectively.

Meat sample Preparation and Analysis

Three kilograms of boneless beef was purchased at a local Abattoir in Akungba Akoko, Ondo State, Nigeria, a few hours after post mortem. Visible connective tissues, subcutaneous, and intramuscular

fat were meticulously removed through trimming under hygienic conditions using a sharp knife. The beef samples was then diced into small cubes and minced using a meat grinder (Panasonic food machine, El-classico, Japan). A portion (200 g) of the ground beef was randomly allocated to one of the following three treatments: B0 (control, beef patties without additives); B0.2 (beef patties with 0.2% basil leaf powder); and B0.4 (beef patties with 0.4% basil leaf powder). Following the addition of the leaf powder, the ground beef samples were immediately aerobically packed in polyethylene bags and stored in a refrigerator at 4°C for 7 days for analysis of lipid oxidation. The lipid oxidation of the meat samples was assessed (at 0, 3 and 7 days during cold storage at 4°C) by analysis of Thiobarbituric acid reaction (TBARS) based on the method described by Xia *et al*. (2012).

Statistical Analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) Software (version 20.0, IBM SPSS, Armonk, NY, USA). Differences between means were considered statistically significant at $p < 0.05$.

RESULTS

The results of the anti-oxidant contents (Phenol, Vitamin C and Flavonoid content) of BLP are shown in Figure 1 while Figure 2 shows the antioxidant activities (DPPH and ABTS) of basil leaf powder. The results revealed that BLP contained 279.29mgGAE/g phenol content, 66.27mg/g Vitamin C content and 104.14 mg (QE)/g flavonoid content. The result of the antioxidant activities showed that BLP possessed 81.062%, 2-Diphenyl-2-picrylhydrazyl (DPPH) free radical

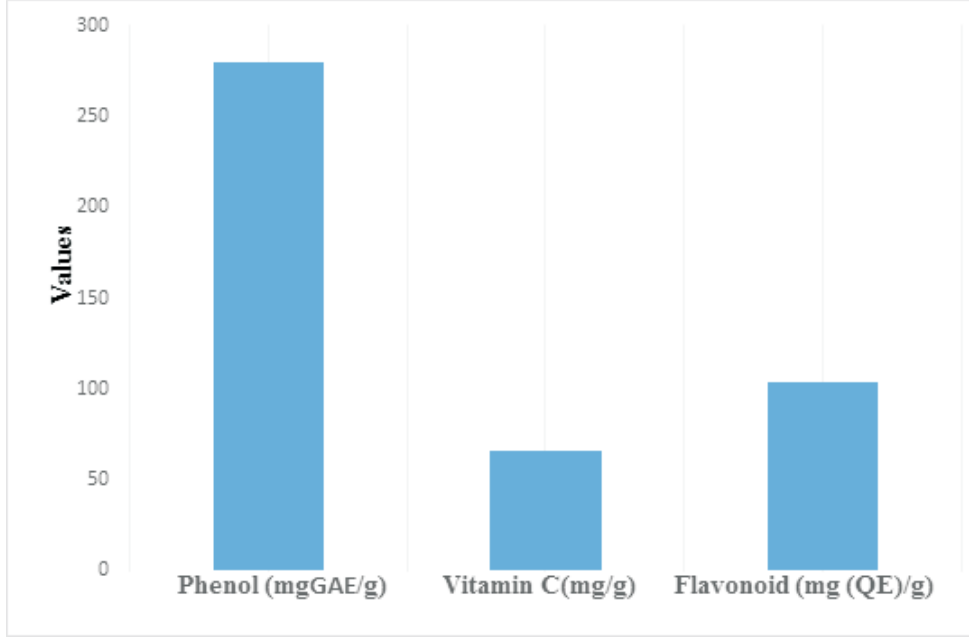

Figure 1: Phenol, Vitamin C and Flavonoid content of Basil leaf powder.

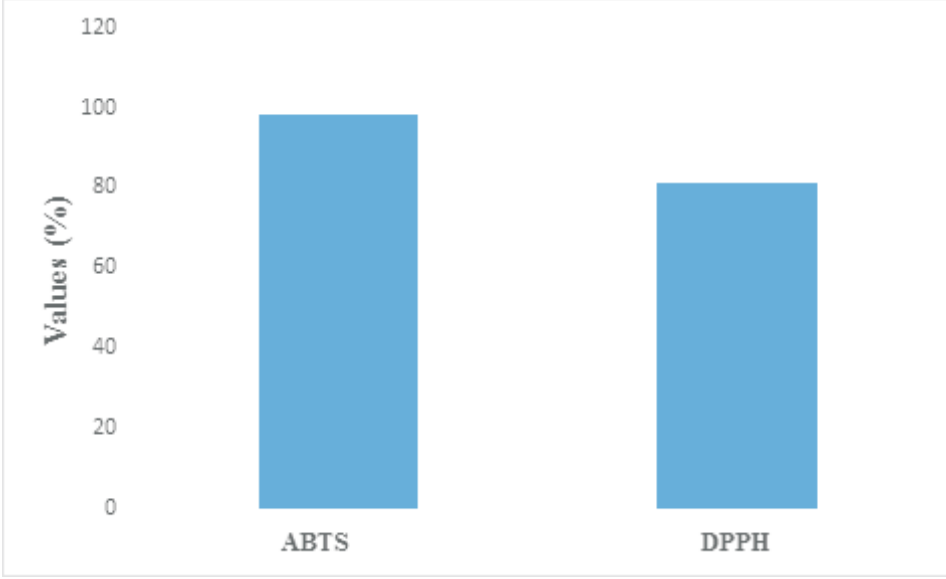

Figure 2: Antioxidant activities (ABTS and DPPH) of Basil leaf powder
ABTS- 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
DPPH- 2-Diphenyl-2-picrylhydrazyl free radical scavenging ability

scavenging ability, and 98.41%, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical. Table 1 shows the effect of basil leave powder (BLP) on lipid oxidation of meat patties during cold storage. The addition of BLP to beef patties did not significantly influence ($p>0.05$) the production on lipid oxidation across the storage days. However, the inclusions of BLP at 0.2 and 0.4% numerically reduced the production of lipid oxidation in beef patties compared to control group across the storage days. In fact, at day 7 the control group had higher lipid oxidation content (1.40 mg MDA/kg) compared to BLP treated beef patties at 0.2% (1.15 mg MDA/kg) and 0.4% (1.01mg MDA/kg) levels.

Table 1: Effect of Basil leave powder (BLP) on lipid o xidation (mg MDA/kg) of beef during cold storage

Storage days	Control	BLP 0.2%	BLP 0.4%	SEM	*P value*
Day 0	0.32	0.55	0.42	0.06	0.35
Day 3	0.79	0.79	0.52	0.07	0.21
Day 7	1.40	1.15	1.01	0.09	0.22

Means within a row with different letters are significantly different ($p < 0.05$). SEM Standard error.
BLP 0.2% = Beef patties treated with 0.2% basil leaf powder.
BLP 0.4% = Beef patties treated with 0.4% basil leaf powder.

DISCUSSION

The result of the antioxidant analysis revealed that basil leaf powder is exceptionally rich in phenol, flavonoid, and vitamin C contents. The phenol content observed in this study surpassed that reported by Kaur *et al.* (2019), who noted a relatively high phenol content of 97.71 mg/g, but is similar to those reported by Nadeem *et al.* (2022), who found that basil leaf extract contained 191.2 mg GAE/g basil leaf powder. Similarly, the result of the flavonoid content observed in this study slightly exceeded that reported by Hendrawan *et al.* (2019) and Nadeem *et al.* (2022). These variances in phenol and

flavonoid contents may be attributed to differences in leaf developmental stage, environmental conditions, harvest timing, processing and storage methods, and solvent of extraction (Guedes *et al.,* 2019; Nadeem *et al.,* 2022). Plant phenols and flavonoids are natural free radical scavengers that delay or inhibit the production of lipid oxidation, thus decreasing the formation of volatile decomposition products (Kumar and Pandey, 2013; Shahidi and Ambigaipalan, 2015).The recorded vitamin C level in this study exceeded those reported by Muráriková and Neugebauerová (2018) for various species of basil leaf, yet were consistent with findings from Kavitha and Rao (2012). Many studies have indicated that the vitamin C content of basil leaves can be influenced by a multitude of factors, ranging from external factors such as climatic conditions, growing conditions, fertilization, and agronomic methods, to internal factors such as genotype and developmental stage (Saadatian *et al.,* 2014; Fraszczak *et al.,* 2015; Muráriková and Neugebauerová, 2018).

The outcomes of DPPH and ABTS assays in this study is similar to the results reported by Prasath *et al*. (2019), that found that basil leaf extract exhibited scavenging activity of 84% and 79% against DPPH and ABTS radicals, respectively. The DPPH and ABTS radical-scavenging assays are widely employed methods for assessing the antioxidant potential of plant materials in order to provide insight into their redox-functioned proton capabilities (Lee *et al*., 2015). Furthermore, the ABTS radical scavenging activities of the extract in this study exhibited superior antioxidant efficacy compared to the DPPH radical. This difference can be ascribed to variances in the mechanisms of action and reactions of the DPPH and ABTS radicals. ABTS has been documented to be soluble in both aqueous and organic solvents, thereby enabling the assessment of both hydrophilic and lipophilic antioxidant capacities (Abegg *et al.,* 2012). This outcome aligned with the findings of Romano *et al*. (2022), who also observed that the scavenging potency of basil leaf extract against ABTS radicals surpassed that against DPPH radicals.

Lipid oxidation stands out as a principal contributor to the deterioration of food quality, leading to the emergence of undesirable odours and flavours, a decline in shelf life, alterations in texture and color, and a decrease in the nutritional value of food (Falowo *et al*., 2014; Shahidi and Ambigaipalan, 2015). The observed decrease in lipid oxidation levels in beef patties containing BLP compared to the control group can be attributed to the inherent phyto-constituents and antioxidant activities of the leaf meal. This result is in agreement with previous studies that have reported the efficacy of plant leaf powders in inhibiting lipid oxidation in meat and meat products during cold storage (Jaworska *et al.,* 2021; Mashau *et al.* 2021).

CONCLUSION

The study demonstrated that basil leaf powder possesses a high antioxidant content (phenol, flavonoid and vitamin C) and could elicit high free radical scavenging activity of DPPH and ABTS in biological system. Also, the addition of basil leaf powder at concentration of 0.4% could be effective in inhibiting the generation of lipid oxidation in meat products during cold storage.

Conflict of interest: The authors state that no commercial funding was acquired for this study that may be construed as potential conflict of interest.

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