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Oxidative Stability and Free Radical Scavenging Activity of Beef and Pork Burger Patties Treated with *Portulaca oleracea* Phytochemicals

BY

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APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for the acceptance of a research dissertation entitled**:**

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Signature of chairperson: ……  …….. Date: ……**15/10/2025**…..

DEDICATION

I honor God, the Almighty tower of strength, source of inspiration, wisdom, knowledge and insight.

ACKNOWLEDGEMENTS

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ABSTRACT

Meat quality and shelf life are compromised by lipid oxidation, which causes rancidity, discoloration, off-flavors, texture degradation, and nutritional loss. To stop spoiling and preserve oxidative stability, synthetic antioxidants like butylated hydroxytoluene and butylated hydroxy anisole have been used. However, consumer demand for natural alternatives has surged due to worries about their toxicity and carcinogenicity. Purslane, also known as *Portulaca oleracea*, is a nutrient-rich plant with potent bioactive components that have demonstrated encouraging antioxidant qualities. The potential of the plant's methanolic extract as a natural preservative for beef and pork burger patties was examined in this study. The plant was washed, rinsed, dried, ground and extracted using absolute methanol. Qualitative phytochemical profiling of the plant showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, phytosterols, and terpenoids except saponins. The total alkaloid, phenolic and flavonoid content of the extracts were found to be 65.00±1.33 mg/g, 63.12±1.21 mg GAE/g and 14.89±2.73 mg QE/g respectively. Over the course of 28 days, beef and pork burger patties fortified with different concentrations of extracts of *P. oleracea* were evaluated for their antioxidant and oxidative stability. The results obtained in this study proved that lipid oxidation was reduced in the patties as a function of concentration of plant extract. The levels of thiobarbituric acid reactive substances remained below the allowable limit, but control patties were over this limit. DPPH scavenging increased dramatically with extract content, peaking at 2.5% extract for both pork and beef patties. In beef patties the scavenging activity maximum was 39 % at day 28 at 2.5 % extract formulation while the minimum was 19 %. The maximum scavenging was 33 % on day 28 down from 35 % on day 1 in pork patties with 2.5 % plant extract. These results show that *P. oleracea* methanolic extract effectively improves the oxidative stability of meat patties, offering a natural alternative to synthetic antioxidants.

LIST OF ABBREVIATIONS

TBARS – thiobarbituric acid reactive substance

ROS - reactive oxygen species

FRAP - Ferric Reducing Antioxidant Power

MDA – malondialdehyde

DPPH - 1,1-diphenyl-2-picrylhydrazyl

ABTS - 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

ORAC - Oxygen Radical Absorption Capacity

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Chapter 1

1.1 Introduction

Meat-based food products have a tendency to lose integrity during storage as a result of production of reactive oxygen species (ROS) by bacteria or through oxidative processes (Antonino et al., 2024). Lipid oxidation causes rancidity, discoloration, degradation of texture, development of off-flavor and changes in the nutritional value of meat products (Basuny et al., 2024). Antioxidant compounds are able to reduce the rate at which these processes occur. Artificial antioxidants (butylated hydroxytoluene, butylated hydroxy-anisole, sodium benzoate) have been used to prevent spoilage and improve the oxidative stability of food (Cunha et al., 2021). Such antioxidants therefore improve the quality of meat products and extend their shelf-life. Major setbacks of artificial antioxidants include concerns relating to them being carcinogenic and toxic (Elhadef et al., 2020).

In addition, artificial antioxidant compounds increase the risk of manipulation together with elevated chemical residues found in food products and the environment. The health of consumers is at risk due to these antioxidant compounds being carcinogenic (Nieto et al., 2023). Plants are endowed with innumerable natural antioxidants present in spices, herbs and essential oils applied in meat products to enhance taste. In the recent past, natural preservatives of plant origin as shelf-life enhancers have found great application (Gutiérrez-del-Río et al., 2021). This has become a potentially promising technology because plant metabolites possess broad spectrum activity. This has led to the rise in the demand for preservatives that are of plant origin (Bolouri et al., 2022). Plant extracts that are rich in phenolic compounds have been shown to possess potential antioxidant and free radical scavenging abilities hence their use as safe and effective antioxidant and antimicrobial agents (Mostafa & El Azab, 2022). Owing to the ever-increasing concerns relating to synthetic antioxidants, consumers have turned to meat products containing natural herbs, spices or their extracts (Sabry et al., 2024). These improve sensory characteristics besides extending the shelf-life of meat products (Antonino et al., 2024).

Portulaca oleracea L. (purslane), a warm season plant with a wide range of medicinal properties (Al-Moghazy et al., 2017), contains numerous phenolic compounds known for their antioxidant capacity (Basuny et al., 2024). These compounds have the capacity to provide protection against

neurodegenerative, oncologic and cardiovascular diseases besides lipid oxidation on food (Elhadef et al., 2020). Therefore, this study explores the phytochemical profile and in vitro antioxidant activity of purslane polar extracts in preserving beef and pork burger patties under refrigeration over 2 months.

1.2 Objectives

- To evaluate the effect of *P. oleracea* extract on the lipid oxidation of beef and pork burger patties during storage.
- To assess the free radical scavenging activity of *P. oleracea* extract in beef and pork burger patties.
- To compare the oxidative stability and free radical scavenging activity of treated patties with untreated control patties.

1.3 Research Questions

1. Can the incorporation of *Portulaca oleracea* phytochemicals into beef and pork burger patties improve their oxidative stability and free radical scavenging activity?
2. What is the effect of treating beef and pork burger patties with *P. oleracea* phytochemicals on their oxidative stability?
3. Is there significant variation in the potency of *P. oleracea* phytochemicals in preservation of beef or pork burger patties?

1.4 Statement of the Problem

There has been a growing demand for natural food additives that promote good health. This has seen preferential use of alternatives of plant origin compared to synthetic antioxidants in meat products. Synthetic antioxidants are plagued with a plethora of health risks in spite of them being very effective (Ivane et al., 2024). This has resulted in negative consumer perceptions about them. The most critical factor the shelf life and quality of meat products is oxidative stability. Lipid peroxidation causes off-flavor, rancidity and compromises nutritional value (Nieto et al., 2023). Moreover, free radicals produced during the oxidative processes pose significant health hazards to consumers. *P. oleracea* is underutilized despite its wide availability and phytochemical endowment comprising of antioxidants among other bioactive principles.

1.5 Significance of the study

Investigating the stabilizing potential of purslane on meat products critical in finding natural antioxidant solutions that are competent alternatives to synthetic ones in a bid to improve the quality and safety of beef and pork patties by minimizing potentially harmful compounds produced by lipid peroxidation. The plant under study is a natural source of potent antioxidant compounds. Therefore, demonstrating scavenging free radical scavenging and stabilizing potency can further consolidate the adoption of natural preservatives in the food industry. *P. oleracea* is rich in omega-3 fatty acids, vitamins, and minerals and its incorporation in meat products will not only help in preservation but most probably will improve their nutritional profile (Basuny et al., 2024). In the long run, the risk of chronic diseases emanating from oxidative processes can be minimized. This study bridges the gap existing in literature regarding specific application of *P. oleracea* extracts in preserving meat products. Most importantly, the study inarguably contributes to the development of sustainable and healthier meat products fortified with natural antioxidants.

1.6 Delimitations

The focus of the study is on beef and pork burgers excluding other types of meat and meat products the study examines specific concentrations of purslane-derived metabolites. Any other concentrations outside the specified range will not be considered. The study is also limited to a specific temperature of refrigeration within a defined time frame hence long-term changes over long periods of storage are beyond the scope of the study.

1.7 Limitations

The current study has a specific formulation of burger patties made from pork and beef. Therefore, the results obtained here in are not generalizable to all types of meat of formulations. The sole focus of this research is on *P. oleracea* phytochemicals and the time frame of the study is relatively short to capture long term effects and stability of the patties fortified with the said compounds.

1.8 Hypotheses

H_0: Treatment of beef and pork burger patties with *P. oleracea* phytochemicals has no significant effect on their oxidative stability and free radical scavenging activity.

H_1: Treatment of beef and pork burger patties with *P. oleracea* phytochemicals has significant effect on their oxidative stability and free radical scavenging activity.

Chapter 2

2.1 Introduction

Over the past 70 years, the global demand for dietary supplements fortified with bioactive compounds, such as those derived from meat and meat products, has experienced rapid growth. This can be attributed to the health benefits associated with these supplements, as well as increasing consumer awareness (Amoli et al., 2021; Cheng et al., 2020). Research on the global meat consumption market indicates that the market size has increased by 58% over the 20 years leading up to 2018, reaching 360 million tons. This growth can be attributed to two main factors: 54% due to population growth, and the remaining 46% due to increase per capita consumption, which is driven by changes in consumers' dietary preferences and incomes (Whitnall & Pitts, 2019). In industrialized countries, the consumption of meat products typically fulfills a substantial portion of an individual's daily protein requirement. One of the major challenges faced by meat manufacturers is providing products with desirable flavor, color, and freshness attributes that can be maintained throughout the shelf life of the meat and meat products, while minimizing costs (Cmlp & Cyc, 2021). However, lipid oxidation during processing and storage can lead to a decline in the quality attributes of meat products (Domínguez et al., 2019).

Oxidation determines acceptability and quality of meat products because it is responsible for loss of flavor and taste. Meat is prone to oxidative degradation due to high concentrations of metal catalysts, unsaturated lipids, oxidants and heme pigments found in the muscles (Domínguez et al., 2019). Oxidative deterioration in meat and meat products results in development of off-flavor, discoloration, generation of toxic compounds, reduced shelf-life and nutrient loss (Amoli et al., 2021). This causes generation of compounds that are toxic to the cells and genes (Domínguez et al., 2021). According to Falowo et al., (2014), metabolic reactions and cellular respiration are driven by oxygen and the element undergoes some reactions that form free radicals as part of normal reactions in animal physiology with 2-5 % of it being converted to reactive oxygen species (ROS).

To retard oxidative reactions, antioxidants that are hydrogen donors are added to meat products and many of them are synthetic. Owing to current trends advocating minimal use of synthetic food additives, natural compounds have gained popularity. Therefore, advanced research needs to unearth natural products that are novel and possess potency for application in the meat industry

(Lorenzo et al., 2019). In a quest to reduce the threat posed by lipid/protein susceptibility to oxidative degradation, exploration of plant-based natural alternative antioxidants is important. Their major advantages include being economical and beneficial health-wise (Munekata et al., 2020). Therefore, the objective of this study is to explore the application of methanolic extracts of purslane as natural antioxidants to retard oxidative degradation in beef and pork burger patties.

2.2 Plant metabolites as natural antioxidants

More than a billion tons of food including meat and meat products is spoilt annually in the supply chain (from production up until final household utilization). Unfortunately, this loss which is a public concern is also directly linked to outbreaks of food related illnesses (Salami et al., 2016). Domínguez et al., (2019) state that up to 50 % of the gross meat spoilage and wastage happens at household level as a result of poor methods and facilities for meat preservation. Chemical and microbial spoilage are the leading factors in meat wastage. The consequent effects of these factors are economic losses, food insecurity as well as food-borne diseases.

The food industry currently prefers natural products to synthetic ones because the later are thronged with prohibitive regulations. Besides, consumer awareness with regards to their toxic effects and health threats is on the rise. According to Tomasevic et al., (2021), foods that contain antioxidants remain fresh and maintain their desirable sensory characteristic for longer. Plant-derived food additives contain phenolic compounds that are potent antioxidants (Nik-maram et al., 2018). Seeds, herbs, grains, cereals, vegetables, fruits and spices contain bioactive compounds in abundance and can restrain oxidation. Potent plant-derived antioxidants must have no adverse effect on odor, taste and color. They must be efficacious at low concentrations (1-100 mg per 100g) besides compatibility with food. Other factors that count include ease of application, being economical and stability in processing and shelf-life. More importantly, they should be harmless at concentrations higher than meal-type ones. This also applies to their metabolites. (Pateiro et al., 2018).

2.3 Mechanisms of oxidation in Meat: Protein and Lipid Degradation

Cellular homeostasis is controlled by a battery of reactions in living tissues some of which are related to redox reactions that control certain compounds such as free radicals; ROS and reactive nitrogen species (RNS). Without regulatory homeostatic mechanisms in the cells, free radicals tend to build-up and react with fatty acids and proteins producing by-products reduce the shelf-life

of meat products (Manessis et al., 2020). A deep understanding and mitigation of the mechanisms of oxidation is vital for meat preservation and ensuring the safety of consumers.

2.4.1 Lipid Oxidation in Meat: Mechanisms and Stages

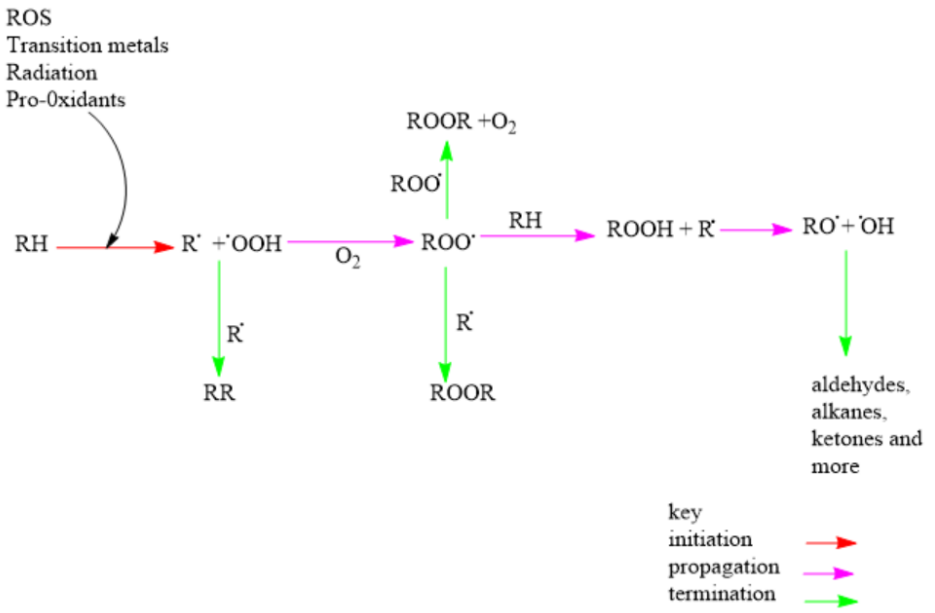

Figure 1: Mechanism for lipid peroxidation

The process of lipid peroxidation occurs in three main steps namely initiation, propagation and termination. During initiation, a hydrogen atom is stripped off an unsaturated fatty acid resulting in an alkyl radical. The alkyl radicals generated during initiation step react with molecular oxygen forming peroxide radicals under propagation. More alkyl radicals (primary products of lipid peroxidation) are produced through interaction of peroxide radicals with unsaturated fatty acid molecules. The peroxide radicals further react with other peroxide or alkyl radicals producing organic peroxides. Alkoxy and hydroxy radicals are formed from decomposition of the hydroperoxides. In the termination stage, secondary products of lipid peroxidation (alkanes, aldehydes, ketones) are formed from the decomposition of alkoxy radicals (Falowo et al., 2014; Manessis et al., 2020). Basically, this is a free radical chain reaction where reaction of free radicals

forms non-radical compounds stopping propagation. As a matter of fact, antioxidants react and eliminate free radicals thereby delaying lipid peroxidation.

2.4.2 Protein Oxidation in Meat: Mechanisms and Implications

Protein oxidation is a common phenomenon in cells, body fluids and extracellular tissues and it contributes to oxidative stress directly impacting on cell signaling, structure as well as metabolic processes. Removal of a hydrogen atom from a protein forms a protein radical that reacts with oxygen forming an alkyl proxyl radical. The alkyl proxyl radical either reacts with water (to produce alkoxyl radicals, more water and oxygen) or reacts with another protein molecule to form alkoxyl radicals (Manessis et al., 2020). The latter the reacts with transition metals in their reduced states forming more alkoxyl radicals. These are converted to hydroxyl derivatives that lead to carbonylation of proteins (Guyon et al., 2016). According to Dominguez et al., (2021), the meat industry uses physical and heat treatment on meat which tend to release iron, copper or manganese from metalloenzymes found in meat. These metals initiate protein oxidation. It is imperative to understand the mechanism of protein oxidation in order to develop methods that can effectively alleviate the impact of protein oxidation and preserve the quality of meat and meat products.

2.5 Controlling Protein-Lipid Oxidation in Meat and Meat Products

The quality, safety and shelf-life of meat and meat products is largely affected by lipid and protein oxidation although the later causes deterioration to a lesser extent. The impact of the two phenomena can be retarded by preventing free radicals and ROS from initiating chain reactions. This can be achieved by neutralizing them through the use of antioxidants Das et al., 2020; Velazquez et al., 2021). Antioxidants interfere with reactive free radicals by reacting to form compounds that are stable (Dominguez et al., 2019). This occurs through donation of a hydrogen atom to a given free radical, scavenging metal cations or directly reacting with oxygen.

Living tissues have natural antioxidants that retard oxidation but right after slaughter, their level decline and oxidation processes become dominant. The meat industry according to Echegaray et al., (2020) either feed the animals with a diet rich in antioxidants prior to slaughter or they add antioxidants directly to the meat after slaughter. This study focuses on antioxidants added after slaughter.

2.6 Mechanism of Action of Plant Antioxidants

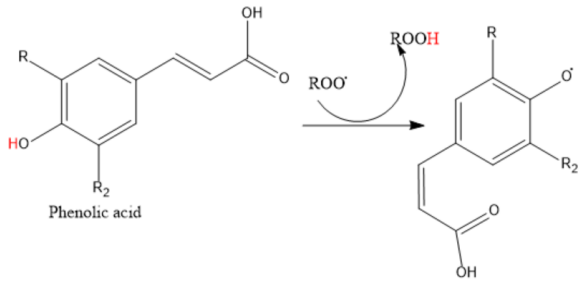

Figure 2: Antioxidant action of phenolic acids

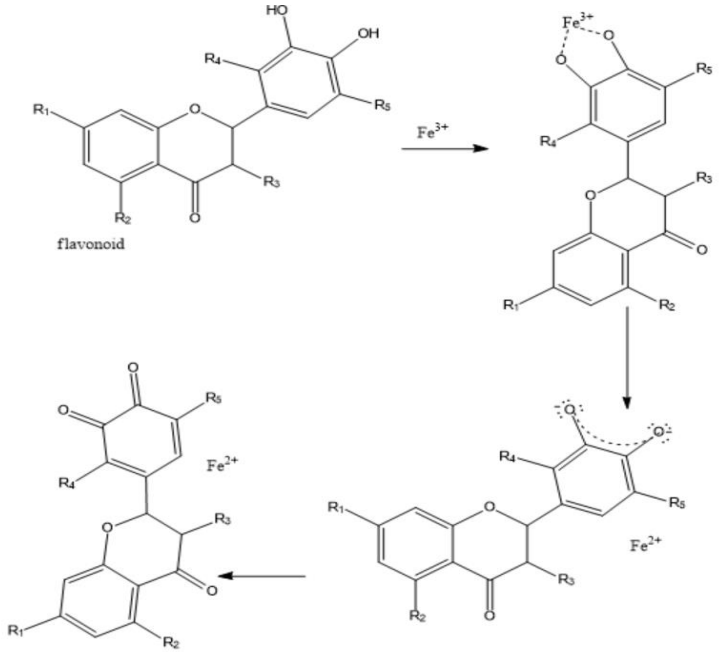

Figure 3: Mechanism of action of flavonoids

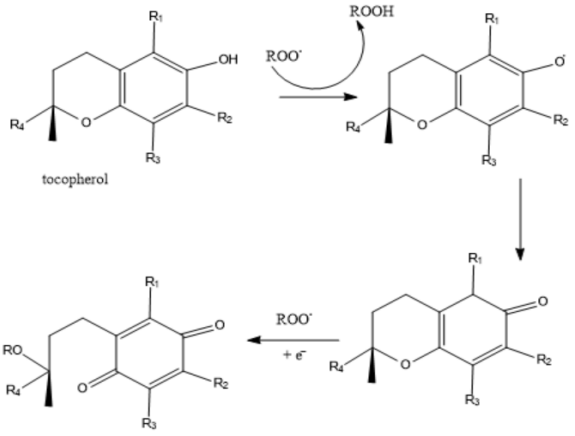

Figure 4: Mechanism of action of tocopherol

Flavonoids, terpenoids and phenolic compounds exhibit ability to control the creation of free radicals and chelate metal cations. Phenolic compounds for example exert their activity through their skeletal structure, number and position of their functional groups (Kumar et al 2015) as shown in figure 3. Structures that contain more hydroxy groups are more potent antioxidants but glycosylation of functional groups tends to reduce antioxidant activity.

2.7 Techniques used in evaluating antioxidant capacity of substances

Thiobarbituric acid reactive substances (TBARS) assay.

This assay is based on the interaction of malondialdehyde (a lipid oxidation product) and thiobarbituric acid producing quantifiable compounds that are pink-colored absorbing at 532 nm. TBARS monitors the magnitude of lipid oxidation by tracking production of MDA. However, it is important to note that this assay lacks specificity since malondialdehyde is not solely produced by lipid oxidation. It is a widely accepted assay though (Manessis et al., 2020).

DPPH assay

DPPH is a stable, purple-colored nitrogen-based radical that can be reduced by antioxidant compounds to a yellow diphenyl-picrylhydrazine. The reduction of DPPH causes a decrease in absorbance which can be measured at 517 nm and the magnitude of the decrease in absorbance is to assess the antioxidant potential of a compound (Gülçin, 2012).

Ferric Reducing Antioxidant Power (FRAP) Assay

This technique measures the ability of antioxidant compounds to reduce a $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ complex to $[Fe^{2+}\text{-}(TPTZ)_2]^{2+}$ at low pH, where iron remains soluble. A characteristic blue-colored ferrous-tripyridyltriazine compound is formed and the FRAP value is determined by measuring the absorbance at 593 nm. The intensity of the blue color signifies the amount of Fe^{2+} produced. This method however, is limited to antioxidants that can reduce iron (iii) fast enough for accurate determination. Moreover, any compound that has a lower reduction potential than $Fe^{3+/}Fe^{2+}$ system is likely to contribute to the measured values (Guyon et al., 2016).

2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

It measures the antioxidant capacity to neutralize the stable $ABTS^{\bullet+}$ radical cation. The maximum absorption of the blue-green chromophore is at 734 nm and its intensity decreases in the presence of antioxidants. The degree of discoloration, quantified as a drop in absorbance at 734 nm is dependent on reaction time, the intrinsic antioxidant activity as well as the sample concentration (Guyon et al., 2016).

Oxygen Radical Absorption Capacity (ORAC) Assay

This assay measures the ability of antioxidants to inhibit the oxidation of peroxyl radicals (the predominant free radicals involved in lipid oxidation in biological systems and food). It monitors the inhibition of this radical chain reaction by antioxidants and the resulting ORAC values are considered by some researchers to be a biologically relevant measure of antioxidant efficiency (Munteanu & Apetrei, 2021).

Other methods for determining protein oxidation

The methods typically involve the detection of protein oxidation products or modifications in amino acids such as loss of thiol groups. When proteins undergo oxidation, the cysteine and methionine residues can lose their thiol groups due to degradation by oxidizing compounds. This thiol group loss can be measured using 5,5′-dithiobis(2-nitrobenzoate), (DTNB) which binds to free thiol groups, releasing a colored thiolate ion that has an absorption maximum at 412 nm (Nollet & Toldrá, 2009).

Determination of carbonyl compounds which are produced in large quantities as a result of protein degradation is another method typically used. This involves reacting the carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) to produce 2,4-dinitrophenylhydrazone. The hydrazone can

be detected by spectrophotometry at 370 nm. The total protein level is measured at 280 nm for comparison, without the addition of DNPH (Nollet & Toldrá, 2009).

Chapter 3

3.1Sample collection, preparation and extraction

Whole fresh *P. oleracea* plants were collected, washed with tap water, rinsed three times in distilled water and cut into small pieces. The plant material was left to dry under a shade until they were completely dry. The dry matter was ground into fine powder using a high-speed blender and the powder was stored in dry air-tight polyethene bags until needed.

3.2 Extraction procedure

Dry powder was extracted using absolute methanol in a solid to solvent ratio of 1:10 on a shaker for 48 hours after which the extract was filtered using a Whatman No. 1 filter paper. The filtrate was evaporated at 40 °C until the volume was 25% of the original volume. The concentrated extract was centrifuged at 3000 rpm for 15 minutes and the supernatant was collected and stored at 4°C until time of use.

3.3 Qualitative Phytochemical Analysis

The method described by Al-Moghazy et al., (2017) was adopted with modifications. The procedure and positive test results are tabulated in table 3.1.

Table 3.1:Qualitative phytochemical analysis of purslane polar extracts

Phytochemical	**Test procedure**	**Positive result**
Alkaloids	2 ml extract + 2 drops Mayer's reagent along the sides of the test tube	Creamy or yellow precipitate
Flavonoids	2 ml extract + 10% Ferric chloride solution	Green precipitate
Phenolic compounds	1 ml extract + 4 drops dilute iodine solution	Transient red color
Tannins	0.5 g plant extract + 10 ml bromine water	Bromine is decolorized
Phytosterols	3 ml extract + a pinch of Sulphur	Sulphur sinks to the bottom
Terpenoids	5 ml plant extract + 2 ml chloroform + 3 drops sulphuric acid boiled on water bath	Grey colored solution
Saponins	2 ml extract + 5 ml distilled water, shake vigorously	Stable foam

3.4 Quantitative Phytochemical Analysis

Estimation of Alkaloids

1 g of extract was placed in a 250 ml beaker. 200 ml of 10 % ethanolic acetic acid were added. The beaker was covered and allowed to stand for 4 hours after which the mixture was filtered and concentrated on a water bath to 50 ml. Concentrated NH_4OH was added drop wise to the extract until precipitation was complete. The solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Estimation of Flavonoids

To 1 ml of extract, 4 ml of distilled water were added together with 0.3 ml of 5 % sodium nitrite solution. 0.3 ml of 10 % aluminum chloride solution was added after 5 minutes followed by 2 ml of 1 mol dm^{-3} sodium hydroxide after exactly 6 minutes. The mixture was immediately diluted with 3 ml distilled water. Absorbance was measured at 510 nm against a quercetin standard. Results were expressed as milligrams quercetin equivalents per gram of sample (mg QE/g) according to the equation:

$$C = \frac{cV}{m}$$

where, C = total flavonoid content mg QE/g dry extract, c = concentration of quercetin obtained from calibration curve in mg/mL, V = volume of extract in mL, m = mass of extract in gram.

Estimation of Total Phenolic content

The Folin method was used against a gallic acid standard 1 ml of sample solution was mixed with a 10-fold dilution of a mixture of 7.5 % sodium carbonate and Folin-Ciocalteu reagent. The samples were incubated in the dark for 30 minutes. The absorbances were measured using a UV-vis spectrophotometer at 760 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

3.5 Preparation of sample patties

Beef and pork burger patties were prepared (with modifications) using varying concentration of and without *P. oleracea* extract as described by Basuny et al., (2024). Table 2 summarizes the formulations used in making the burger patties.

Table 3.2: Formulation of burger patties

Main ingredients	% composition					
	control	A	B	C	D	E
Meat	65	65	65	65	65	65
Bread crumbs	10	10	10	10	10	10
Iced water	9.78	9.28	8.78	8.28	7.78	7.28
Eggs	6	6	6	6	6	6
Grated onion	6	6	6	6	6	6
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Black pepper	1.5	1.5	1.5	1.5	1.5	1.5
Additives						
Triphosphoric acid	0.2	0.2	0.2	0.2	0.2	0.2
Sodium ascorbate	0.02	0.02	0.02	0.02	0.02	0.02
Portulaca extract	0	0.5	1	1.5	2	2.5

3.6 Lipid Oxidation Analysis and Free Radical Scavenging Activity assays

Analyses were done 1 day after preparation and thereafter every fortnight for 1 month.

3.6.1 Extraction of oil from the burger samples

The Folch method was used to extract oil from the burger patties. Briefly, the sample was homogenized with a mixture of saline (0.9 % w/v sodium chloride solution), methanol and chloroform. The extracts were left to separate into two phases. Excess solvent was removed by concentration on a rotary evaporator at 40°C (Saher et al., 2023).

3.6.2 Total antioxidant content of burger patties

1ml of reagent solution prepared from mixing 0.6 mol dm^{-3} sulphuric acid, 2.8 x 10^{-4} mol dm^{-3} sodium phosphate and 4.0 x 10^{-3} mol dm^{-3} ammonium molybdate was mixed with 0.1 ml of sample and heated to 995 °C for one and a half hours after which the absorbance was measures at 695 nm against ascorbic acid as the standard.

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3.6.3 Thiobarbituric acid reactive substances assay

5 grams of each of the prepared patty samples were weighed and thoroughly homogenized using a blender. A 0.375% solution of thiobarbituric acid (TBA) in 15% trichloroacetic acid (TCA) was prepared. The homogenized burger sample was mixed with the TBA-TCA reagent in a test-tube. The mixture was heated in a hot water bath for 30 minutes and quickly cooled in an ice bath. After cooling, the samples were centrifuged to separate the colored malondialdehyde-TBA complex from any insoluble residue. The absorbance of the supernatant was measured at 532 nm against a malondialdehyde (MDA) standard. All measurements were done in triplicate. Results were expressed as milligrams of MDA per kilogram of sample using the equation

$$\frac{mgMDA}{kg}\ sample = \frac{Absorbance\ of\ sample\ \times\ Mr_{(MDA)} \times volume\ of\ extract\ \times dilution\ factor\ \times 1000}{mass\ of\ sample\ \times slope\ of\ standard\ curve\ \times 1000}$$

3.6.4 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of burger patty samples

7.8 mg of DPPH were dissolved in 100 ml of ethanol to make 0.2 mM of DPPH solution. 1 ml of the solution was added to 1 ml of sample and control solutions in a 1:1 v/v ratio. The mixtures were dark-incubated for half an hour at room temperature. The absorbances were measured at 517 nm against ascorbic acid as the standard with concentration ranges of 5 to 500 μg/ml. The DPPH radical scavenging activity of the patties was expressed as mg ascorbic acid equivalent (AAE) per gram of dried sample. All measurements were in triplicate. Percent DPPH radical scavenging activity was calculated as follows:

$$\%\ inhibition = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \times 100$$

3.7 Measurement of pH of burger patty samples

Values of pH of the prepared samples of burger patties were determined by homogenizing 5 g samples with 45 ml of distilled water for 60 seconds and measuring the pH using a digital pH meter.

Chapter 4: Results

4.1 Qualitative and quantitative phytochemical analysis

4.1.1 Qualitative phytochemical testing results

Table 4.1 outlines the phytochemical testing results of methanolic extracts of *P. olareceae*. Of the compounds tested, only saponins were absent.

Table 4.1: Qualitative phytochemical profiling of methanolic extracts of purslane

Phytochemical	Test procedure	Result obtained
Alkaloids	2 ml extract + 2 drops Mayer's reagent along the sides of the test tube	Creamy or yellow precipitate
Flavonoids	2 ml extract + 10% Ferric chloride solution	Green precipitate
Phenolic compounds	1 ml extract + 4 drops dilute iodine solution	Transient red color
Tannins	0.5 g plant extract + 10 ml bromine water	Bromine was decolorized
Phytosterols	3 ml extract + a pinch of Sulphur	Sulphur sank to the bottom
Terpenoids	5 ml plant extract + 2 ml chloroform + 3 drops sulphuric acid boiled on water bath	Grey colored solution
Saponins	2 ml extract + 5 ml distilled water, shake vigorously	no stable foam

4.1.2 Quantitative phytochemistry of purslane

In this study, three groups of phytochemicals were quantified. The values of total flavonoid, phenolic and alkaloid content are compared in figure 4.1. Methanol extracts of purslane were found to contain 14.89±2.73 mg QE/g; 63.12±1.21 mg GAE/g and 65.00±1.33 mg/g total flavonoids, phenolics and alkaloids in that respective order

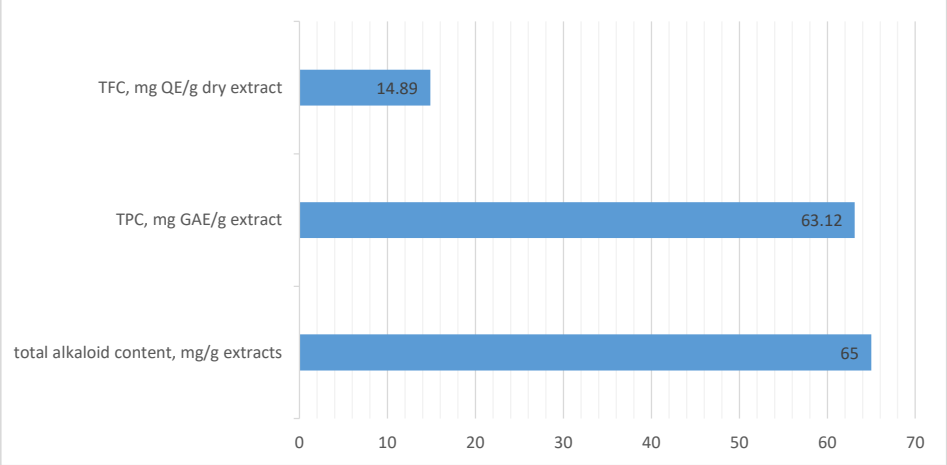

Figure4.1: Estimated alkaloid, flavonoid and phenolic content of methanolic extracts of purslane

4.2 Total antioxidant content of burger patties

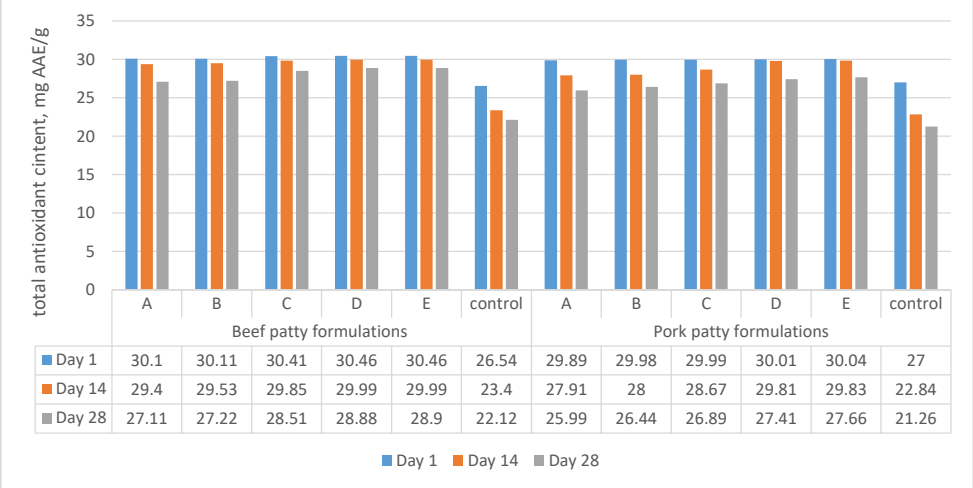

Figure4.2: Total antioxidant content of patty samples

Generally, higher antioxidant content was observed in patty formulations with higher content of purslane extract (figure 2). Total antioxidant content decreased over the 28 days of evaluation. Both control formulation had the highest decrease in total antioxidant content. A 21.25 % decrease was observed in the control pork formulation while that of the beef control was 16.54 %. An

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increase in concentration of purslane resulted in an increase in total antioxidant content with a significant difference at 95 % confidence interval.

4.3 Thiobarbituric acid reactive substances assay

The second stage of autoxidation produces TBARS such as malondialdehyde by oxidation of peroxides to ketones and aldehydes. Figure 3 illustrates the effect of concentration of purslane on oxidation of lipids in beef and pork burger patties on day 1, 14 and 28 under refrigeration. On day 1 of evaluation, there was no significant difference in the TBARS values of the burger patty formulations across all concentrations at $p<0.05$. The beef control patty reached 0.90±0.05 mg MDA/kg while the pork one was at 0.97±0.15 mg MDA/kg on day 28. The value of TBARS in burger patty formulations varied with concentration. Minimum values were observed in higher dose formulations. Beef patties with 0.5 % extract exhibited an increase in the TBARS value from 0.28±0.02 to 0.5±0.05 mg MDA/kg on day 28 while in pork it was 0.29±0.01 to 0.61±0.03 mg MDA/kg. 2.5 % plant extract formulations reached 0.39±0.04 mg MDA/kg in both pork and beef patties. There was significant difference in the TBARS values of the control and experimental patties at $p<0.05$ as the controls breached the permissible limit of 0.9 mg MDA/kg as per standardization and quality for frozen burger patties.

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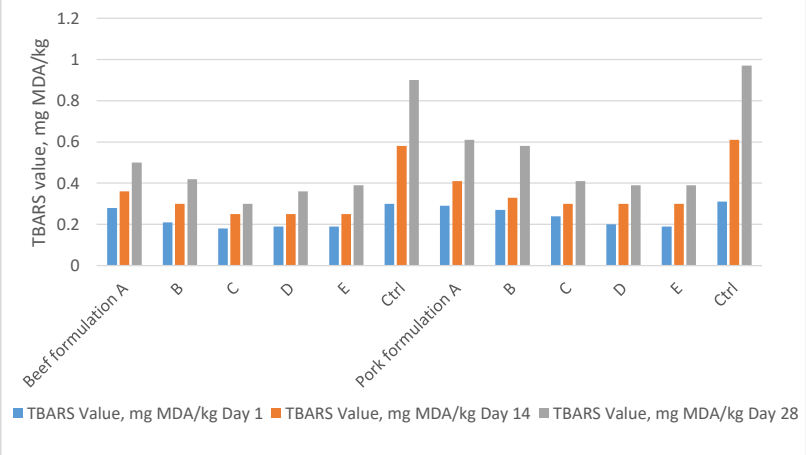

Figure4.3: Comparison of TBARS values of sample patties

4.4 DPPH free radical scavenging activity of burger patty samples

The extent of DPPH discoloration is an indication of the scavenging potency of an antioxidant in relation to the ability to donate a hydrogen. There was a significant increase in the DPPH scavenging activity of burger patties with plant extract at 95 % confidence level compared to the control samples. Moreover, increase in concentration of plant extract proportionally increased the scavenging activity of the patty formulations. In beef patties the scavenging activity was generally constant at each concentration reaching a maximum of 39 % at day 28 at 2.5 % extract formulation. A similar pattern was observed in pork formulations. The maximum scavenging activity was 33 % on day 28 down from 35 % on day 1 in pork patties with 2.5 % plant extract (figure 4.4).

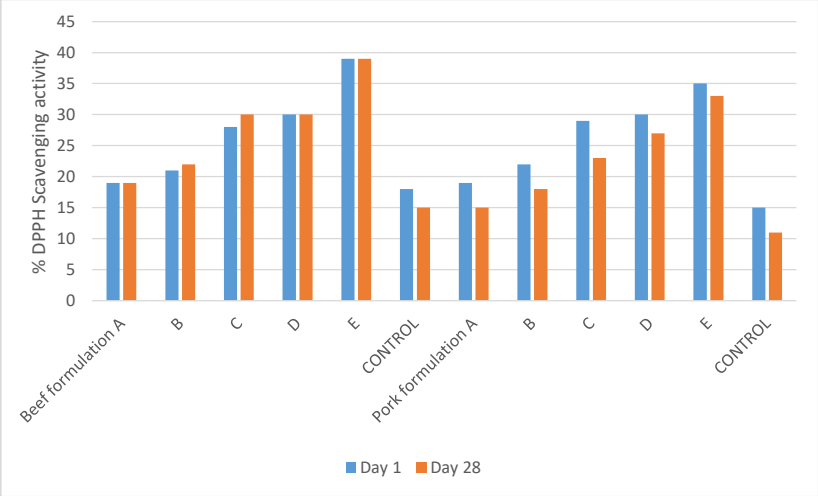

Figure4.4: Percent scavenging activity of burger patty samples

4.5 pH values of burger patties

Table4.2: pH values of burger patties

	pH value	
	Day 1	Day 28
Beef formulation A	6. 01	5.19
B	6.00	5.20
C	6. 00	5.36
D	6.01	5.80
E	6.01	5.91
CONTROL	6.02	5.10

Pork formulation A	6.01	5.21
B	6.00	5.30
C	6.03	5.46
D	6.01	5.60
E	6.01	5.71
CONTROL	6.02	5.00

pH is an important quality parameter of meat and meat products that affects shelf life. Table 3 shows the effect of plant extract concentration in burger patties on pH values against control formulations over a 28-day evaluation period. For beef and pork patties with concentrations 0.5 to 5 % purslane extracts, the pH values were 6.00±0.03 on day 1 of preparation without any significant difference ($p<0.05$). However, significant differences in pH change after 28 days of incubation were noted across patties with 0.5 to 1.5 % purslane extract concentration (5.19- 5.36 for beef and 5.21-5.46 for pork) against 5.10 and 5.00 for their respective control formulations. At 2 and 2.5 % plant extract concentration, the decrease in pH values over the 28 days was negligible. The values were 5.80 and 5.91 for beef while for pork they were 5.60 and 5.71 in that respective order.

Chapter 5: Discussion, Conclusion and Recommendations

5.1 Discussion

Plant extracts have been shown to be a source of antioxidants that have been considered to be safe and compostable. A shift from synthetic to natural antioxidant has been on the rise in the last few decades. Research has focused on exploitation of natural products in various industry including the meat industry Plant extracts have been found to exhibit several pharmacological and biological properties, (Salehi et al., 2019; Gadekar et al., 2014). This study evaluated the phytochemical content and effect of concentration of methanolic purslane extracts on the quality of beef and pork burger patties over a 28-day period. Quality parameters evaluated are TBARS, DPPH scavenging activity, pH and total antioxidant content.

Alkaloids, flavonoids, phenolic compounds, tannins, phytosterols and terpenoids were found to be present in methanolic extracts of purslane. Saponins were not present (table 2). These results are in tandem with the findings of Okafor and Ezejindu (2014). The total alkaloid, phenolic and flavonoid content of the extracts were found to be 65.00±1.33 mg/g, 63.12±1.21 mg GAE/g and 14.89±2.73 mg QE/g respectively (figure 1). These results are in agreement with Zhou et al., (2015). The presence of these phytochemicals maybe linked to the antioxidant activity that was observed in the burger patty formulations that were under investigation.

In vitro antioxidant activity as per DPPH scavenging assay proved significant increase in the DPPH scavenging activity of burger patties with increase in concentration of plant extract in the patties at 95 % confidence level compared to the control samples. In beef patties the scavenging activity maximum was 39 % at day 28 at 2.5 % extract formulation while the minimum was 19 %. Scavenging activity in beef patties showed very little variation from the initial values. This suggests that the antioxidants of the plant extracts did not deteriorate over the 28 days. A similar pattern was observed in pork formulations. The maximum scavenging activity was 33 % on day 28 down from 35 % on day 1 in pork patties with 2.5 % plant extract (figure 4). According to Conde-Hernandez and Guerrero-Beltran (2014), the presence of phenolic compounds, flavonoids among other phytochemicals contribute to antioxidant properties of plant extracts. It is an

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established fact that lipid peroxidation that is induced by hydrogen peroxide is significantly inhibited by phenolic compounds since they have a scavenging effect on hydrogen peroxide. Additionally, phenolic compounds can neutralize the superoxide anion, peroxy radicals, hydroxy radicals and also quench singlet oxygen.

Assessment of meat quality without assessment of pH would be amiss because it is linked to all the other evaluation parameters. In this study, beef and pork patties with concentrations 0.5 to 5 % purslane extracts had pH values of 6.00±0.03 on day 1 of preparation (Table 3) without any significant difference ($p<0.05$). However, significant differences in pH change were noted across patties with 0.5 to 1.5 % purslane extract concentration after 28 days of incubation. The values were 5.19- 5.36 for beef and 5.21-5.46 for pork against 5.10 and 5.00 for their respective control formulations. At 2 and 2.5 % plant extract concentration, the decrease in pH values over the 28 days was negligible. The values were 5.80 and 5.91 for beef while for pork they were 5.60 and 5.71 in that respective order. Soltanizadeh and Ghiasi-Esfahani (2015) observed similar results in beef burger patties with purslane extracts.

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One of the most widely used methods in assessing secondary oxidation products particularly malondialdehyde is TBARS assay. The products of secondary oxidation cause oxidative rancidity ad off-flavor in fats (Turgut et al., 2016). Figure 3 illustrates the effect of concentration of purslane on oxidation of lipids in beef and pork burger patties on day 1, 14 and 28 under refrigeration. On day 1 of evaluation, there was no significant difference in the TBARS values of the burger patty formulations across all concentrations at $p<0.05$. The beef control patty reached 0.90±0.05 mg MDA/kg while the pork one was at 0.97±0.15 mg MDA/kg on day 28. The value of TBARS in burger patty formulations varied with concentration. Minimum values were observed in higher dose formulations. Beef patties with 0.5 % extract exhibited an increase in the TBARS value from 0.28±0.02 to 0.5±0.05 mg MDA/kg on day 28 while in pork it was 0.29±0.01 to 0.61±0.03 mg MDA/kg. 2.5 % plant extract formulations reached 0.39±0.04 mg MDA/kg in both pork and beef patties. There was significant difference in the TBARS values of the control and experimental patties at $p<0.05$ as the controls breached the permissible limit of 0.9 mg MDA/kg as per standardization and quality for frozen burger patties.

5.2 Conclusion

The study evaluated the phytochemical composition of *P. oleracea* as well as the effect of increasing concentration of its methanolic extracts on the quality of beef and pork burger patties over a 28-day period. Chemical analysis indicated that methanol extracts of the plant studied possess potent antioxidant activity and can effectively retard lipid oxidation in beef and pork burger patties which is paramount to human health. Therefore, the alternate hypothesis 'treatment of beef and pork burger patties with *P. oleracea* phytochemicals significant effects on their oxidative stability and free radical scavenging activity' is accepted and the null hypothesis rejected.

5.3 Recommendations

Since methanol extracts of purslane have been shown to retard peroxidation of beef and pork burger patties, future studies may focus on retardation of protein oxidation factoring in long term stability and shelf-life studies. Additionally, total antioxidant capacity of the meat matrix is dependent on a plethora factors. Therefore, combining a battery of methods of analysis would provide a complete antioxidant profile of the burger patties including hydrophilic and lipophilic antioxidants with their various mechanisms of action.

Only two methods of assessing lipid peroxidation were used in the current study. Generalizability of results is limited. Therefore, a comprehensive study using many methods of evaluating lipid and protein oxidation is recommended.

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