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Title : THE REGULATORY ROLE OF FOOD SPICES AGAINST LIPID PEROXIDATION IN MEAT PRODUCTS

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Abstract

Spices are plant-based materials used for enhancing the flavor, taste and aroma of meat and have been employed in cooking and meat processing for many decade. Interestingly, some of these spices have found relevance in meat preservation as they not only enhance the flavor and taste of meat product but can also reduce meat spoilage. One of the major causes of meat spoilage outside those induced by microbes is Lipid peroxidation [LPO]. This is a multifaceted reaction which begins with the destabilization of tissue stable oxygen molecules [O_2] to unstable oxygen molecules [O*]. This and many other processes lead to the oxidation of tissue lipids hence creating a chain reaction that can disrupt the cell membrane, and also combine with microorganism leading to food spoilage, creating rancidity and odor. Methodology employed here are those developed by Tang *et al.,* 2002 and Melink *et al.,* 2006. From the results obtained, some of the spices were able to reduce the oxidation of lipids in the meat products as compared to the natural antioxidants and synthetic (alpha tocpherol [α-TC] and Butylated Hydroxyl Toluene [BHT]) respectively. It can be concluded that the incorporation of specific spices to food and meat products can prolong their shear life by reducing or delaying the oxidation of the lipids hence preventing rancidity in the meat products although some may not be able to preserve food for long time.

Key words: Lipid peroxidation, food, meat, cell membrane, spices, antioxidant.

1.0.INTRODUCTION

Spices are flavorful and aromatic substances that are commonly used as condiments and preservatives (Amorati *et al.,* 2013). They contain chemical constituents that have significant biological properties, such as antimicrobial, antioxidant, anti-inflammatory and anti-cancerous properties. These properties have been well documented in various scientific reports (Jiang,

2019). Because of their antibacterial and antioxidant qualities, spices can therefore be utilized as preservatives in a variety of food processes[1].

On the other hand, Lipid peroxidation (LPO) is the primary cause of cellular damage which leads to destabilization in the antioxidant attribute of the cells. This leads to oxidative damage and result in metabolic diseases. It affect all organs in the body and the overall process leads to oxidative stress and eventually metabolic diseases that affect all organs in the bodyAccording to Yin et al. (2011), LPO is a three-step process that includes initiation, propagation, and termination. Conversely, lipid peroxidation involves the following steps; initiation, propagation and termination. During the initiation process, pro-oxidants like hydroxyl radicals abstract the allylic hydrogen forming the carbon-centered lipid radical (L·). Also, in the propagation phase, lipid radical (L·) reacts spontaneously with oxygen to form a lipid peroxy radical (LOO·) which abstracts hydrogen from another lipid molecule generating a new L· (that continues the chain reaction) and lipid hydroperoxide (LOOH). Finally, during the termination reaction, antioxidants like vitamin E donate a hydrogen atom to the LOO· species and form a corresponding vitamin E radical that reacts with another LOO· forming non-radical by-products (Figure 1).[2,3]

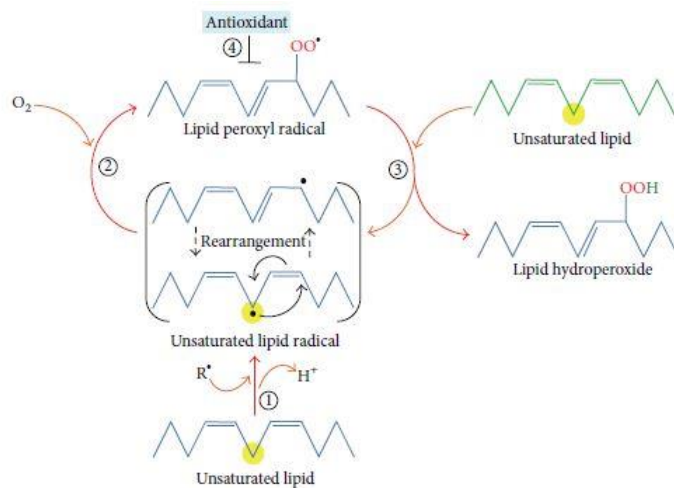

Figure 1: Lipid peroxidation process.

Yin and colleagues (2011) : - Pro-oxidants absorb the allylic hydrogen during the lipid peroxidation's initial phase, creating a carbon-centered lipid radical. This radical is then stabilized by a chemical rearrangement to create a conjugated diene (step 1). Moreover, the lipid radical quickly reacts with oxygen to create a lipid peroxy radical (step 2) when lipid

peroxidation reaches the propagation phase. This radical then extracts hydrogen from another lipid molecule to create a new lipid radical and lipid hydroperoxide (step 3). Antioxidants give a hydrogen atom to the lipid peroxide radical species when the reaction reaches the termination phase, which leads to the creation of nonradical products (step 4). Following the initiation of lipid peroxidation, a series of chain reactions will continue until termination products are generated (Yin et al., 2011)[4].

2.0.2.0.Conversely, antioxidant qualities are associated with a substance's ability to shield food ingredients that contain lipids and oils from oxidative deterioration, hence averting food spoiling. They slow the production of harmful oxidation products and control the rancidity process (Yin et al., 2011). Antioxidants function in two ways. First, oxidative damage triggers the antioxidant defense mechanisms, which prevent ROS from forming and trap and block the radicals that are produced (Thomas, 2015). These systems can be either enzymatic or non-enzymatic and are found in the membrane and aqueous compartments of cells. The first line of defense against the production of free radicals is the enzymatic antioxidant system, which includes glutathione peroxidase, catalase, and superoxide dismutase. Furthermore, the second line of defense against the production of free radicals is the use of thoils and mon-enzymatic antioxidant systems. This group includes metabolic or hydro- and lipo-soluble chemicals (Cadet and Davies, 2017). Second, the elimination of malfunctioning and damaged biomolecules prior to their aggregation alters cell metabolism during the antioxidant repair process. The repair systems' intervention includes the use of certain enzymes to repair oxidatively damaged nucleic acids, proteolytic systems to remove oxidized proteins, and phospholipases, peroxidases, or acryl transferases to repair oxidized lipids (Thomas, 2015). Oxidizing radicals are immediately scavenged by antioxidants, which also help organisms repair oxidized biomolecules.Due to the problem created by lipid peroxidation, and the protective effect of spices against oxidative damage, this research was aimed at the role of food spices as a regulator of lipid peroxidation in various meat products.

3.0. Objective of the research

The following are the research objectives:

i. To prepare meat products.
ii. Incorporate spices in meat products.
iii. Compare the antioxidant power of the spices against lipid peroxidation with natural and synthetic antioxidants using TABARS assay for 14 days.

4.0.MATERIALS AND METHODS

4.1.Collection of samples

The various plants sample used for this research was gotten from uchi market in Auchi Edo state Nigeria, and authenticated by a botanist. The various meat used for the research was gotten freshly from a slaughter house in a place called Zongo in Auchi.

4.2.Sample preparation

Sample preparation was done according to the modified method of Tang *et al.,* 2002. Fresh meat was chopped into tiny pieces and homogenized in a blender till a homogenous paste was formed. After this, the homogenized meat samples were divided into four equal halves each weighing 100kg. Each part was mixed thoroughly with 0.75g of the various spices and with Butylated Hydroxyl Toluene (BHT) and alpha-tocopherol (α-TC) separately. Latex glove was worn to prevent contamination and ensure optimum productivityFor comparison, BHT and α-TC were employed. In addition to that, a control without the spices, BHT, or α-TC was also included. Following that, each sample was put in a cellophane bag and securely fastened. After which they were cooked for 30 mins in a water bath at 75± 1˚C after the cooking was terminated, a thermometer was dipped into each sample to monitor the cooling rate and to ensure that the first stage of the experiment was taken at the exert time the sample was cooled. This served as the day one reading after that, the samples were placed in the refrigerator at 4˚C for further analysis in subsequent days[5,6].

1.1. Determination of the extent of Lipid peroxidation in cooked meat using TBARS (Thiobabituric Acid Reactive Species).

The extent of Lipid peroxidation in the various meat samples was done according to Mielnik *et al.,* 2006 with some modifications. Lipid peroxidation was monitored in the various meat samples for 14 days at 4-day intervals. From the 100g of cooked meat initially weighed[7], 10g of the sample was weighed and mixed with 34 ml of 7.5% TCA (Tri Chloro Acetic Acid) and homogenized in a blender. After which, it was filtered with a Whatman filter paper No. 4. Into a 50ml measuring cylinder, thereafter, the residue was discarded and the filtrate made up to 50 ml with 7.5% aqueous TCA. Moreso, 5.0ml aliquot of the filtrate was transferred into a screw cap test tube, and 5.0ml of aqueous TBA (Thiobarbituric Acid) solution was added to the initial TCA. This mixture was incubated at 80˚C for 20 minutes in a water bath and cooled at room temperature (25˚C) for 20 minutes this was done to initiate and terminate TCA, and TBA reaction and facilitate MDA formation which is an indicator of Lipid peroxidation this was shown with corresponding color change in the test tube after incubator. The Absorbance Value was taken with a U/V spectrophotometer (Jenway 7205) at 532nm. The amount of lipid peroxidation was measured using Thio Barbituric Acid Reactive Substance (TBARS), which is expressed as milligrams of malondialdehyde (MDA) per kilogram of meat[88,9].

. Using the formula below (Pikul *et al.,* 1989)

TBARS Value (mg MDA/Kg of meat) = Absorbance x 5.5 mg MDA/kg meat

1.2.Statistical analysis

Statistical analysis was done with IBM SPSS Statistics version 20. One-way Analysis of Variance (ANOVA) and the least significant was carried out to compare the means of the TBARS value in the cooked meat samples.

2.0.RESULTS

Table 3.1: The effect of nutmeg, BHT and α-TC on the extent of lipid peroxidation on cook beef.

PARAMETERS	TBARS VALUE (MG MDA/KG OF COOKED BEEF MEAT)				
	STORAGE DAYS				
	1	4	8	12	14
Control	0.451±0.13	0.534±0.13	0.611±0.18	1.72±0.26	1.337±0.84
Nutmeg	0.182±0.05[a,c]	0.259±0.05[a,b,c]	0.259±0.08[a,b]	0.732±0.17[a,c]	1.089±0.26[a,b,c]
BHT	0.143±0.04[a,c]	0.193±0.04[a,c]	0.193±0.06[a,c]	0.435±0.10[a,b,c]	0.919±0.22[a,c]
α-TC	0.413±0.12[b]	0.484±0.12[b]	0.484±0.14[a,b]	0.798±0.18[a,b]	0.396±0.10[a,b]

Data are reported as mean± SD. [a]significant different from control sample (p<0.05), [b]significant different from BHT sample (p<0.05). [c]significant different from α-tocopherol group (<0.05). The experiment was done in triplicates.

Table 3.2: The effect of thyme, BHT and α-TC on the extent of lipid peroxidation on cook cow kidney.

PARAMETERS	TBARS VALUE (MG MDA/KG OF COOKED KIDNEY MEAT)				
	STORAGE DAYS				
	1	**4**	8	**12**	14
Control	0.226±0.07	0.391±0.12	1.353±0.40	2.305±0.68	2.882±0.86

Thyme	0.215±0.06[b,c]	0.222±0.07[c]	1.711±0.51[a,b,c]	1.716±0.51[a,b,c]	1.843±0.55[a,b,c]
BHT	0.116±0.03[a]	0.209±0.06[c]	1.259±0.37[a,c]	1.469±0.44[a,c]	1.705±0.51[a,c]
α-TC	0.066±0.02[a]	0.116±0.03[a,b]	0.682±0.20[a,b]	1.029±0.31[a,b]	1.155±0.34[a,b]

Data are reported as mean± SD. [a]significant different from control sample ($p<0.05$), [b]significant different from BHT sample ($p<0.05$). [c]significant different from α-tocopherol group (<0.05). The experiment was done in triplicates.

Table 3.3: The effect of Tumeric, BHT and α-TC on the extent of lipid peroxidation on cook cow liver.

PARAMETERS	TBARS VALUE (MG MDA/KG OF COOKED LIVER MEAT)				
	STORAGE DAYS				
	1	**4**	8	**12**	14
Control	1.089±0.32	1.282±0.38	1.309±0.39	1.320±0.39	2.261±0.67
Turmeric	0.638±0.19[a,b,c]	0.721±0.21[b,c]	0.776±0.23[a,b]	2.800±0.83[a,b,c]	3.641±1.08[a,b,c]
BHT	0.583±0.17[a,c]	0.919±0.27[a]	1.095±0.34[a,c]	1.111±0.33[a,c]	2.723±0.81[a,c]
α-TC	0.693±0.21[a,b]	0.891±0.26[a]	0.765±0.23[b]	0.836±0.25[a,b]	2.156±0.64[a,b]

Data are reported as mean± SD. [a]significant different from control sample ($p<0.05$), [b]significant different from BHT sample ($p<0.05$). [c]significant different from α-tocopherol group (<0.05). The experiment was done in triplicates.

Table 3.4: The effect of Garlic, BHT and α-TC on the extent of lipid peroxidation on cook cow brain meat.

PARAMETERS	TBARS VALUE (MG MDA/KG OF COOKED BRAIN MEAT)				
	STORAGE DAYS				
	1	4	8	12	14
Control	0.066±0.04	0.33±0.04	0.385±0.22	0.413±0.26	1.524±0.28
Garlic	0.462±0.31[a,b]	0.473±0.31[a,b,c]	0.495±0.32[a,b]	0.523±0.33[a,b]	1.562±0.35[a,b,c]

BHT	1.089±0.73[a,c]	1.149±0.73[a,b,c]	1.166±0.70[c]	0.671±0.78[c]	1.755±0.45[a,c]
α-TC	0.457 ±0.31[b]	0.462±0.30[a,b,c]	0.495±0.31[b]	1.205±0.33[b]	2.552±0.81[a,b]

Data are reported as mean± SD. [a]significant different from control sample ($p<0.05$), [b]significant different from BHT sample ($p<0.05$). [c]significant different from α-tocopherol group (<0.05). The experiment was done in triplicates.

3.0.DISCUSSION

Lipid peroxidation which is a major cause of quality deterioration in meat product was measured using the TBARS assay. The principle of the TBARS assay involve the reaction of TBA with TCA to form a corresponding color which indication the formation of manlo-aldehyde the intensity of the color indicate the level of lipid oxidations in the cells. The research spanned for 14 days and assay conducted at four days interval this is done to monitor the extent of lipid peroxidation and the ability of each spices to reduce the effect of lipid peroxidation in the various animal tissues. Result obtained in table 3.1, 3.2, 3.3 and 3.4, shows the result gotten from the role of food spices as a regulator of lipid peroxidation in various meat products. Since one of the major causes of food spoilage is oxidation of lipids by radicals, the TBARS assay have shown that the rate of lipid oxidation on meat when treated with various spices, BHT and αTC (known antioxidant). From the various result obtained, it reveal that on the first day of TBARS assay, the concentration of TBARS decreases in BHT, αTC and nutmeg. The same trend was observed in 3.2 and 3.3 respectively. Furthermore, the concentration of TBARS increases in Day 8, 12 and 14. The same pattern was seen in 3.2 and 3.3 respectively, this was significant at $p <0.05$ as compared to the control.[10] Also, the same trends was observed when comparing BHT, to αTC and BTH to nutmeg, turmeric and Thyme and αTC with nutmeg, turmeric and thyme and αTC with nutmeg, turmeric and thyme. This was significant at $p<0.05$.

CONCLUSION

Spices have many beneficial applications. Numerous scientific studies have thoroughly established their chemical components and important biological characteristics, such as their antibacterial, antioxidant, anti-inflammatory, and anti-carcinogenic qualities.. Although, they have a remarkable impact on the taste and aroma of food, the result from

this study shows that some of them (nutmeg, thyme, turmeric and garlic) can be applied as antioxidant in the preservation of meat.

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