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Effect of Citrus Seed Extracts on Oxidative Stability of Raw and Cooked Chicken Meat

Research Article

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ABSTRACT

The potential of extracts prepared from seeds of three citrus species (orange, lemon and grapefruit) to stabilize lipid oxidation of cooked and raw broiler meat under refrigeration storage was evaluated and compared with butylated hydroxyl anisole (BHA), a synthetic antioxidant. The citrus seed extracts were separately applied at a rate of 1.5% of the weight of the meat. There was a negative control without any additive and a positive control with 0.01% of BHA. Each sample was divided into 28 parts of 12.5 g each. Fourteen of these were cooked in microwave oven for 1½ minutes while the other 14 samples were left raw. Both cooked and raw samples were stored in a refrigerator for 12 days at a temperature of 4 ˚C. Oxidative stability of the cooked and raw samples was monitored at 2 day intervals using the Thiobarbituric acid (TBA) assay. The result shows that all the additives and BHA were able to reduce lipid oxidation in broiler meat than the negative control. Addition of citrus seed extract was effective in reducing lipid oxidation in both cooked and raw broiler meat under refrigeration. Thus, oxidative stability of chicken meat can be prolonged for 12 days with citrus seed extracts under refrigeration storage.

KEY WORDS antioxidant, BHA, citrus, minced.

INTRODUCTION

Chicken meat is an excellent source of protein,however, its lipids are high in unsaturated fatty acids which make it susceptible to oxidative deterioration. The need to curb reduction in nutritional quality, incidence of off color, off odor and rancid taste or warmed over flavor in meat caused by lipid oxidation necessitate the use of antioxidants in food (Olorunsanya *et al*. 2009). Antioxidants are classified based on their source as synthetic and natural antioxidants. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT) and tertia butyl hydroxy quinone (TBHQ) have been used extensively in meat industry to ensure product preservation and prolonged shelf life (Gray *et al*. 1996).

Syntethic antioxidants are obtained from inorganic chemical compounds are very effective. However, despite the effectiveness of these chemicals, there are some problems associated with their use (Cao *et al*. 1997). Synthetic antioxidants are very scarce, expensive and pose health hazards to consumers (Olorunsanya *et al*. 2009). There is a concern about the safety and toxicity of synthetic antioxidants in relation to their metabolism and accumulation in the body organs and tissues (Cao *et al*. 1997). Synthetic antioxidants are known among other things to cause impairment to blood clothing, lung damage and to act as tumor promoters. Therefore, consumers prefer natural ingredients and there is a growing interest in the potential use of antioxidants from natural sources (Kingstone *et al*. 1998). The need to find alternative sources of antioxidants brought

about the use of spices such as ginger, tomato, garlic etc (Lodikas and Lougovis, 1990). Phenolic extracts from herbs and spices (Abdallah and Roozen, 1999), cereals and legumes (Onyeneho and Hiettiarachchy, 1992) have been reported to retard lipid oxidation in oils and fatty foods. Phenolic compounds exist in considerable amounts in citrus fruits, which triggered scientific interest (Rapsidar *et al.* 1999). Vitamin C in citrus is an effective inhibitor of oxidation, at levels greater than 1000 mg/kg whereas at levels lower than 1000 mg/kg, it has been shown to increase warmed over flavor (Wang *et al.* 2007; Sato *et al.* 1973). Flavonoids are widely distributed group of phenolic compounds presents in citrus fruits with health related benefits, which are based on the antioxidant properties (Roberts and Gordon, 2003). Flavones, flavanols and flavonones are the flavonoids presents in citrus fruits (Roberts and Gordon, 2003). The objective of this study was to evaluate the effectiveness of citrus species that are common in Nigerian markets in improving the shelf life of raw and cooked broiler meat under refrigeration.

MATERIALS AND METHODS

Meat preparation

Ten broiler chickens at 8 weeks old weighing 2±0.35 kg were obtained from Animal production pavilion, University of Ilorin, Nigeria. The broilers were slaughtered by cutting through the jugular vein with a sharp knife. They were scalded manually by dipping into boiled water for a minute, de-feathered, washed, eviscerated and de-skinned. The carcasses were cut into different parts. The breast, thigh and drumsticks were manually deboned using a sharp knife, pooled and minced together using a food processor (National MK-5080M). The minced meat was mixed thoroughly to form a homogenous mixture.

Citrus Seed processing

Citrus fruits including lemon (*Citrus limonum*), orange (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) were obtained from Ipata market within Ilorin metropolis. Seeds were taken from the fruits and dried. Subsequently, they were finely ground and extracted with 100% methanol through soxhlet extractor. Each type of seed was extracted separately.

Treatments

The minced meat was divided into five sample groups of 350 g. These samples were treated as follows: no additive (Control), addition of 1.5% citrus seed extract from lemon, grape or orange, or addition of 0.01% BHA. Each of the treated meat samples was divided into 28 parts of 12.5g each. Fourteen of these were cooked for 1½ minutes using a microwave oven (National-NN-55WF) while the other parts were left raw. Both cooked and raw meat samples were wrapped in foil paper with labeling corresponding to the applied treatments and stored in a refrigerator (HR-170T) for 12 days at a temperature of 4 ˚C. The oxidative stability was monitored for the first time at day 0 and then at 2 day interval.

Analysis

Lipid oxidation in meat

The oxidative stability was monitored at two day-intervals. Lipid oxidation in the meat samples was evaluated using the 2-thiobarbituric acid (TBA) assay. The thiobarbituric acid reactive substance (TBARS) values were measured on a duplicate 10g samples at each sampling day using the distillation method of Tarladgris *et al.* (1964). Ten grams of the meat sample was homogenized with 47.5 mL of distilled water in a specimen bottle using glass pestle. The homogenized mixture was rinsed with 50 mL of distilled water into a round bottom flask. Thereafter, 2.5 mL of diluted hydrochloric acid (0.02 M) was added and the mixture was distilled through a condensing assembly to collect about 15 mL of the distillate. five mL of the distillate was mixed with 5 mL of TBA (0.02M) and boiled for 35 minutes in boiled water. Then, the mixture was cooled for ten minutes under a running tap water for color development. The duplicate absorbance readings were measured at a wavelength of 538 nm against a blank that contains 5 mL of hydrochloric acid solution and 5ml of TBA reagent using a spectrophotometer (COMSPEC-M105). The absorbance values were multiplied by 7.8 (Tarladgris *et al.* 1964) to obtain the TBARS values in milligram per malonaladehyde per kilogram of sample (mg/MDA/kg). Each treatment was replicated four times.

Statistical Analysis

Data were analyzed as a 5 × 2 × 7 factorial arrangement. Treatments were antioxidant treatments (Put names of 1, 2, 3, 4 and 5), state of meat (raw and cooked), and storage days (0, 2, 4, 8, 10, 12, 14 days) respectively. Data obtained were subjected to analysis of variance using a Genstat 5 program package (Payne *et al.* 1987). Difference between treatment means was determined by Duncan multiple range test and significance was defined at $P<0.05$. The Analysis of variance model was as follow:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha i x \beta j) + (\alpha_i x \gamma_k) + (\beta_j x \gamma_k) + (\alpha i x \beta j x \gamma_k).$$

Y_{ijkl} denotes the i^{th} observation arising from level i of antioxidant treatment, level j of state of meat and level k of storage days.

μ: overall mean.
α_i: effect of level i of antioxidant treatment.
β_j: effect of level j of state of meat.
γ_k: effect of level k of storage days.
(αi×βj): interaction effect of antioxidant and state of meat.
$(\alpha_i \times \gamma_k)$: interaction effect of antioxidant and storage days.
$(\beta_j \times \gamma_k)$: interaction effect of state of meat and storage days.
$(\alpha i \times \beta j \times \gamma_k)$: interaction effect of antioxidant, state of meat and storage days.
l: replicate.

RESULTS AND DISCUSSION

The minced meat treated with citrus seed extracts and BHA have lower (P<0.05) TBARS values than the control treatment (Table 1).

Table 1 Main effects of antioxidant treatments and state of meat on oxidative stability of broiler meat

Factor	TBARS				
Antioxidant treatment	Control (0%)	BHA	LSE	GSE	OSE
	4.70^{b}	3.00^{a}	2.70^{a}	2.60^{a}	3.40^{a}
SEM	0.183				
State of meat	Cooked		Raw		
	3.60^{b}		2.40^{a}		
SEM	0.22				

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

There was no difference (P>0.05) in the TBARS value between lemon seed extract, grape seed extract and BHA. This thus shows that lemon seed extract and grapefruit seed extract are equally potent as conventional BHA. This result is in line with the report given by Roberts and Gordon, (2003) that citrus by-products represents a rich source of naturally occurring flavonoids which possesses significant antioxidant properties.

The TBARS values in raw meat samples were less than that of the cooked samples. This result agrees with the general observations of oxidation in cooked meat (Asghar *et al.* 1988; Rhee, 1999; Cotelle *et al.* 1996).

Cooking was reported to disrupt lipid membrane system causing an interaction of chemicals such as oxygen and molecular weight metal with unsaturated fatty acids resulting in the generation of free radicals and propagation of oxidative reactions (Tims and Watts, 1958; Asghar *et al.* 1988).

In raw meat samples, there was no difference (P>0.05) in the antioxidant potency of grapefruit seed extract, orange seed extract, lemon seed extract and BHA (Table 2). The antioxidant properties of the citrus species may be due to the presence of phenolic compounds such as flavonoids, phenol and tannin.

In cooked meat samples, the control samples had the highest TBARS value, which was significantly different from the additives and BHA. At storage days 2, 4 and 6, there was no significant difference in the TBARS value of all additives and BHA (Table 3).

Table 2 Interaction effect of antioxidant treatment and state of meat on oxidative stability of broiler meat

State	TBARS (mg/MDA/kg)				
	Control (0%)	BHA	LSE	GSE	OSE
Raw	3.80_{x}^{b}	2.78_{x}^{a}	2.20_{x}^{a}	2.40_{x}^{a}	2.85_{x}^{a}
Cooked	5.60_{y}^{c}	3.20_{x}^{a}	3.20_{y}^{a}	2.80_{x}^{a}	3.90_{y}^{b}
SEM	0.13				

a, b, c: the means within the same row with at least one common letter, do not have significant difference (P>0.05).
x, y: the means within the same column with at least one common letter, do not have significant difference (P>0.05).

The TBARS value of the control samples was higher than the other treatments. This agrees with the report of Branen, (1975) which asserted that BHA could reduce lipid oxidation in fatty foods. Banon *et al.* (2007) also state that grapefruit seed extracts have better potential as a shelf life extending antioxidant in cooked meat systems. At storage day 8, there was no significant difference in the TBARS value of samples treated with grapefruit seed extract and lemon seed extracts. After 10 days of storing samples in refrigerator a sharp reduction in TBARS value for all the treatment levels were occurred.

This was unexpected because lipid oxidation has been reported to increase as storage days increases (Dawson and Schierhotz, 1976). At storage day 12, there was no significant difference in the TBARS value of all the additives and BHA. In fresh samples, the TBARS values for all treatments including cooked and raw meat samples were not significantly different (Table 4). At storage day 2, there was no significant difference in the raw samples. In the cooked samples, the control treatment had the highest TBARS value that was significantly different (P>0.05) from other treatments. BHA and the additives are not significantly different (P>0.05).

At storage day 4, there was no significant difference among the treatments in the raw state. In the cooked samples, grapefruit seed extract and lemon seed extract had a TBARS value of 3.70 and 3.00 respectively, which was significantly different from the TBARS value of other treatments. The TBARS value of BHA was high and not significantly different from the control samples.

The high TBARS value of BHA was unexpected and cannot be explained. This is because BHA has been reported not to loose it antioxidant properties during cooking (Kirk-othner, 1979). At storage day 6, in the raw samples, there was no significant difference (P>0.05) among the treatments.

Table 3 Interaction effect of antioxidant treatment and storage days on oxidative stability of broiler meat

Storage days		Antioxidant treatment				
		Control (0%)	BHA	LSE	GSE	OSE
0	TBARS (mg/MDA/kg)	2.30_x^a	2.20_x^a	2.19_x^a	2.35_x^a	2.30_x^a
2		4.90_y^b	2.30_x^a	2.40_x^a	3.15_x^a	2.45_x^a
4		5.10_y^b	1.80_x^a	2.60_x^a	3.40_x^a	3.70_y^a
6		4.20_y^a	2.40_x^a	3.00_x^a	2.70_x^a	3.80_y^a
8		6.30_z^b	5.80_y^b	3.50_x^a	2.50_x^a	5.70_z^b
10		3.60_x^a	2.80_x^a	2.30_x^a	2.45_x^a	2.80_x^a
12		6.50_z^b	3.20_x^a	2.20_x^a	2.50_x^a	2.80_x^a
SEM	0.74					

a, b, c: the means within the same row with at least one common letter, do not have significant difference (P>0.05).
x, y, z: the means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 Interaction effect of antioxidant, storage days and state of meat on the oxidative stability of broiler meat

State of meat	Antioxidant treatment	TBARS (mg/MDA/kg)						
		Storage days						
		0	2	4	6	8	10	12
Raw	Control (0%)	2.40_x^a	2.30_x^a	2.80_x^a	2.90_x^a	4.10_y^b	2.20_x^a	6.70_y^c
	BHA	2.40_x^a	2.30_x^a	2.20_x^a	2.80_x^a	3.10_x^a	3.50_x^a	2.50_x^a
	LSE	1.50_x^a	3.70_x^a	1.80_x^a	2.10_x^a	2.95_x^a	2.00_x^a	1.99_x^a
	GSE	2.80_x^a	3.20_x^a	3.80_x^a	2.00_x^a	2.65_x^a	1.92_x^a	2.10_x^a
	OSE	3.20_x^a	2.85_x^a	2.55_x^a	2.45_x^a	4.20_y^b	2.00_x^a	2.70_x^a
Cooked	Control (0%)	2.50_x^a	5.40_y^b	7.40_z^c	5.25_z^b	7.10_z^c	2.90_x^a	6.30_y^b
	BHA	2.10_x^a	2.70_x^a	7.40_z^c	2.20_x^a	3.70_x^a	1.98_x^a	3.90_x^a
	LSE	3.15_x^a	2.80_x^a	3.70_x^a	4.00_y^a	3.90_x^a	2.20_x^a	2.30_x^a
	GSE	2.25_x^a	2.00_x^a	3.00_x^a	3.20_x^a	2.40_x^a	2.80_x^a	3.15_x^a
	OSE	1.80_x^a	2.50_x^a	4.80_y^b	5.20_z^b	6.90_z^c	3.63_x^a	2.80_x^a
SEM	0.56							

a, b, c: the means within the same row with at least one common letter, do not have significant difference (P>0.05).
x, y, z: the means within the same column with at least one common letter, do not have significant difference (P>0.05).

In the cooked samples, the TBARS value of orange seed extract was not significantly different (P>0.05) from the control samples. The TBARS value of BHA, lemon seed extract and grapefruit seed extract were not significantly different (P>0.05). At storage day 8, there was no significant difference in the antioxidant potency of BHA, grapefruit seed extract and lemon seed extract in both cooked and raw samples. At storage day 10, there was no significant difference among the treatments in the cooked and raw samples. At storage day 12, in both cooked and raw samples, there was no significance difference in the TBARS value of BHA and all the additives. The control samples had the highest TBARS value, which was significantly different (P<0.05) from other treatments.

CONCLUSION

Citrus seed extracts were effective in lowering lipid oxidation in both cooked and raw meat samples. They were as effective as BHA. Although, their antioxidant effect was more pronounced in raw meat samples than cooked meat samples. The cheap and commonly available citrus seed extracts could therefore be a good source of natural antioxidants for extending the shelf life of chicken meat.

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