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The Potential Application of *Syzygium aromaticum* and *Cymbopogon citratus* Essential Oils as Natural Preservatives of Beef Patties

Fabrice Bruno Siewe[1], Pierre Désiré Mbougueng[2], Léopold Ngoune Tatsadjieu[3*], Thierry Ngangmou Noumo[1], Carl M. F. Mbofung[1,4]

[1]Department of Food Science and Nutrition, National School of Agro-Industrial Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[2]Department of Process Engineering, National School of Agro-Industrial Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[3]Department of Food Engineering and Quality Control, University Institute of Technology, University of Ngaoundere, Ngaoundere, Cameroon
[4]College of Technology, University of Bamenda, Bamenda, Cameroon
Email: [*]tatsadjieu@yahoo.fr

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Abstract

The effect of essential oils from *Syzygium aromaticum* and *Cymbopogon citratus* on the lipid oxidation and microbial growth in beef patties was investigated in the present study. Essential oils were incorporated into the beef patties at 0.1% and 0.2% (w/w). The beef patties were then inoculated with cultures of *E. coli* and *S. aureus* and stored at 4˚C. The control patties were processed without essential oil but inoculated with *E. coli* or *S. aureus*. The proximate composition, lipid oxidation and microbial counts were carried out after 7, 14, 21 and 28 days. Results showed that the incorporation of essential oils in beef patties did not significantly ($P < 0.05$) influenced the chemical composition of the beef patties but significantly ($P < 0.05$) reduced the TBA (Thiobarbituric acid) during storage period. The incorporation of the essential oil of *S. aromaticum* at 0.2% reduced the *E. coli* growth by 1.48 log CFU/g and that of *S. aureus* by 6.52 log CFU/g while the incorporation of *C. citratus* at 0.2% reduced the *E. coli* growth by 1.21 log CFU/g and that of *S. aureus* by 1.4 log CFU/g after 28 days of storage. The pH measurement during the storage period showed a slight drop during the first 7 days of storage and an increase during the last 21 days in all samples. The sensory test of the beef patties showed that the consumers accepted patties formulated with the two

[*]Corresponding author.

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essential oils. But the one made with 0.1% essential oil of *S. aromaticum* was the most accepted. The analysis of the color of beef patties between 0 and 28 days of storage revealed that the incorporation of essential oils retarded the degradation of the color of patties. Results obtained in the present study indicate the possibility of exploiting *Syzygium aromaticum* and *Cymbopogon citratus* essential oils to protect beef patties against lipid oxidation and microbial growth.

Keywords

S. aromaticum*, *C. citratus*, Essential Oil, Beef Patties, Lipid Oxidation, Microbial Stability, *Escherichia coli*, *Staphylococcus aureus

1. Introduction

Lipid oxidation and growth of undesirable microorganisms in food products results in the development of spoilage, off flavor, rancidity and deterioration, rendering such products unacceptable for human consumption [1] and yielding many compounds that contribute to the pathogenesis of cancer, atherosclerosis, heart and allergic diseases [2] [3]. Contaminations are generally due to the bacteria strains *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. [4]. During their growth on foodstuffs, they can on one hand deteriorate the organoleptic quality (the color, the flavor and texture) and on the other hand cause foodborne and infectious diseases [5].

Lipid oxidation in meat product is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavors and changes in color of meat products [6]. This oxidation of meat lipids is a complex process and its dynamics depends on numerous factors, including the chemical composition of the meat, light and oxygen access, as well as storage temperature. The rate of the oxidation process is also affected by technical procedures to which meat is subjected during its processing. Because lipid oxidation leads to the formation of numerous other compounds which have an adverse effect on the quality attributes and nutritive value of meat products [7], this process frequently limits the shelf-life of the processed meat.

Refrigeration storage is usually the most common preservative method of meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially those of synthetic origin such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are added to meat products. However, consumers increasingly demand for the use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in the last years [8]. Much attention has been focused on extracts from herbs and spices which have been used traditionally to improve the sensory properties and extend the shelf-life of foods [9]. In this respect, essential oils are regarded as natural alternatives to chemical preservatives and their use in foods meets the demand of consumers for minimally processed products [10]. They have been classified as GRAS (Generally Recognized as Safe). *Syzygium aromaticum* (L.) Merr. & Perry (clove) and *Cymbopogon citratus* Stapf (Lemon grass) are widely used plant in tropical countries, especially in Africa. These two plants have been used for many purposes since ancient times in various food applications. Studies indicated that *Cymbopogon citratus* possesses various pharmacological activities such as anti-amoebic [11], antibacterial [12]; antidiarrheal, antifilarial, antifungal and anti-inflammatory properties [13]. Various other effects like antimalarial, antimutagenicity, antimycobacterial, antioxidants [14], hypoglycemic and neurobehaviorial [15] have also been studied. Previous studies also reported the antibacterial activities of essential oil of *Syzygium aromaticum* in the agar assay [16]. These results are very encouraging and indicate that the essential oils of these two plants should be studied more extensively to reveal their preservative potential in food matrix. Therefore, the aim of this work was to investigate the antibacterial activity of *S. aromaticum* and *C. citratus* essential oils on *Escherichia coli* O157:H7 and *Staphylococcus aureus* inoculated in beef patties.

2. Materials and Methods

2.1. Materials

Thigh muscle, liver and bump from a 3 to 4-year beef were purchased from a slaughter house in Ngaoundere

(Cameroon) and rapidly transported to the laboratory. Beef muscle was trimmed of all visible extra muscular fat then cut through a 5 cm bit surroundings and was left to mature for 96 hours at 4˚C after mixing with salt (19 g/Kg).

Essential oils from *S. aromaticum* and *C. citratus* were obtained following the procedures described by Ngassoum *et al*. [17].

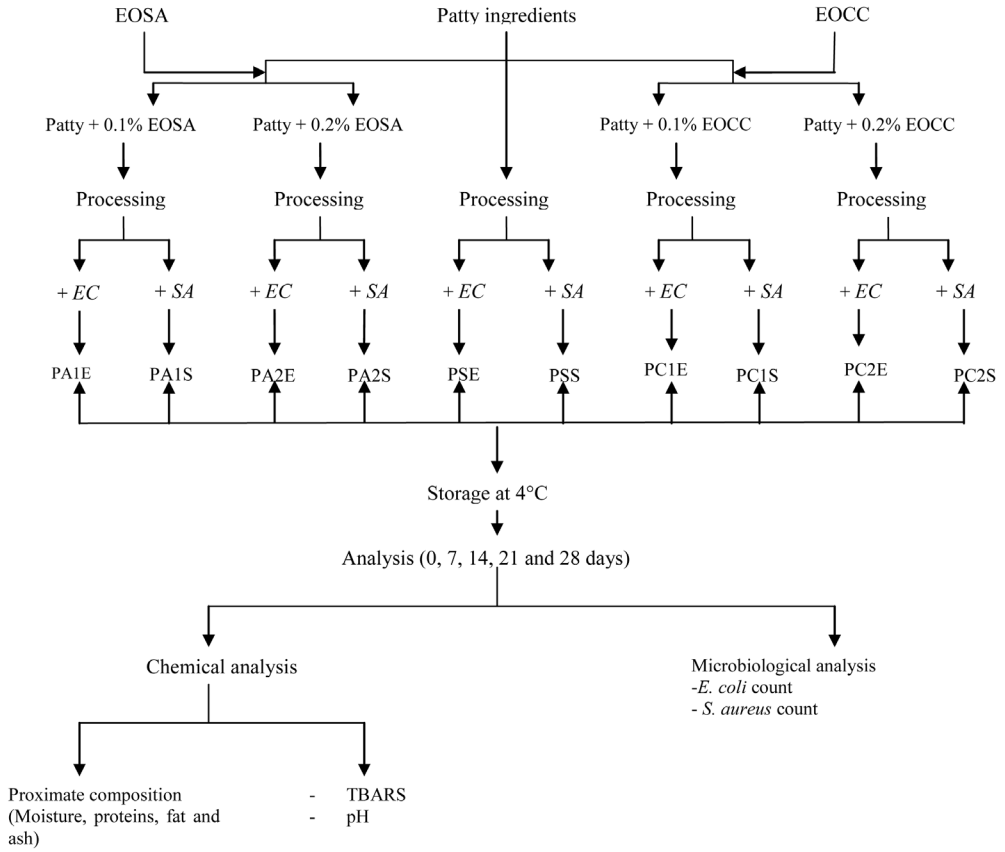
Escherichia coli O157:H7 ATCC 43888 and *Staphylococcus aureus* ATCC 25923 were obtained from the culture collection of the Microbiology Laboratory, University of Ngaoundere, Cameroon.

2.2. Methods

A diagram representing the different steps of samples processing is shown in **Figure 1**.

2.2.1. Patties Manufacture

Beef meat was ground through a 2-mm plate grinder. Fat (hump) and the liver representing each 1/3 of the lean were chopped in a cutter (MTK 666, Mado Grant, Germany) at high speed until obtaining a fine farce. The two and spices were mixed for 5 min in the mixer. The batter which results from the mixture allows formulating various patties. *Essential oils* (EO) of *S. aromaticum* and *C. citratus* were incorporated at 0.1% and 0.2%. The control was formulated without EO. The beef patties were cooked at 90˚C until an internal temperature of 65˚C using an oven (Memmert, UL 40, Germany). The cooked products were cooled at room temperature (22˚C - 25˚C) for 30 min. The cooled samples (treated and control) were cut in sections of 25 ± 1 g



EOSA: *Essential oil* of *Syzygium aromaticum*, EOCC: *Essential oil* of *Cymbopogon citratus*; *EC: Escherichia coli*, SA: *Staphylococcus aureus*; PA1E: Patty + essential oil of *S. aromaticum* (0.1%) + *E. coli*; PA1S: Patty + essential oil of *S. aromaticum* (0.1%) + *S. aromaticum*; PA2E: Patty + essential oil of *S. aromaticum* (0.2%) + *E. coli*; PA2S: Patty + essential oil of *S. aromaticum* (0.1%) + *S. aromaticum*; PSE: Patty + *E. coli*, PSS: Patty + *S. aureus*; PC1E: Patty + essential oil of *Cymbopogon citratus* (0.1%) + *E. coli*; PC1S: Patty + essential oil of *Cymbopogon citratus* (0.1%) + *S. aureus*; PC2E: Patty + essential oil of *Cymbopogon citratus* (0.2%) + *E. coli*; PC2S: Patty + essential oil of *Cymbopogon citratus* (0.2%) + *S. aureus*.

Figure 1. Diagram showing the steps of processing and analysis of beef patties.

then each section was inoculated with 1 mL of bacterial suspension (*E. coli* or *S. aureus*) of concentration 10^6 CFU/mL. The samples were then stored in a refrigerator at 4˚C and analysis were carried out after 7, 14, 21 and 28 days.

2.2.2. Proximate Analysis

Moisture content of samples was determined by oven-drying method at 105˚C [18]. Crude protein, fat, and ash content were determined on cooked products using the standard methods [19].

2.2.3. Microbiological Analysis

Twenty-five grams of sample was ground in a mortar, and then homogenized for 2 min in 225 mL of sterile 0.1% peptone water. Serial dilution of previous homogenate were then prepared and microorganism enumerated using a plate technique in triplicate. *E. coli* was enumerated on to eosine methylene blue agar, incubated aerobically at 37˚C for 48 hours. *S. aureus* was determined on Chapman medium, incubated aerobically at 37˚C for 48 hours. Results were converted to log10 CFU/g samples and averages of three replicates were reported for each treatment.

2.2.4. pH Determination

The pH of the cooked patties was measured using a pH meter (HANNA instrument, Model H1 8424 microcomputer) on a homogenate of 10 g sample in 90 mL of distilled water.

2.2.5. Thiobarbituric Acid (TBA)

The degree of lipid oxidation of the raw and cooked beef patties was determined by 2-Thiobarbituric Acid (TBA) method described by Buedge and Aust [20].

2.2.6. Color Analysis

Color analysis of beef patties samples were carried out using a Chromameter Lovibond RT Color Measurement Kit V2.28 to measure L*, a*, and b* values [21]. The instrument was calibrated (L* = 93.87, a* = 0.18, b* = 2.71) and the L*, a*, and b* values (L* =whiteness, a* = redness, b* = yellowness) of each sample were recorded in triplicate on samples placed in a clear Petri dish. Average of the readings was computed and reported.

2.2.7. Hedonic Evaluation of Beef Patties

A panel of 30 women and men recruited within the students and lecturers of the University of Ngaoundere participated in the evaluation. Four cooked beef patties (patty with 0.1% or 0.2% of each essential oil) were used. Codified beef patties samples (25 g each) were presented in a randomized way to panelists sited under controlled laboratory conditions in partitioned booths illuminated with white incandescent lights. Each panelist was brought to evaluate the acceptability of each patty. The patties were evaluated on a 9 point hedonic scale ranging from: dislike extremely to like extremely (taste, flavor, overall acceptability); extremely dry to extremely juicy (jutoxity); extremely hard to extremely tender (texture); extremely white to extremely pink (color) [22].

2.2.8. Statistical Analysis

All measurements were carried out in triplicate, and all microbial counts were converted to base-10 logarithms of colony forming units per g of beef patties samples (log10 CFU/g). Data were subjected to analysis of variance and the differences among means were obtained using Duncan's multiple range test (differences were considered significant at the $p < 0.05$ level).

3. Results and Discussion

3.1. Chemical Composition of Beef Patties

The proximate analysis of different beef patties are shown in **Table 1**. The raw patties containing essential oils are not significantly different from each other and from the control. The same observation is made for the cooked ones. Essential oils have no significant effect ($p > 0.05$) on the composition of raw and cooked patties.

After cooking, drop in the moisture content of patties is noticed. This drop is link to the drainage and/or evapora-

Table 1. Chemical composition of beef patties.

		Parameters (%)				
		Humidity	**Sugars**	**Proteins**	**Lipids**	**Ash**
Raw patties	**PS**	61.40 ± 0.97[a]	4.46 ± 0.54[a]	22.62 ± 0.27[a]	7.51 ± 0.42[a]	3.19 ± 0.08[a]
	PA1	61.02 ± 1.02[a]	4.67 ± 0.16[a]	22.63 ± 0.31[a]	7.40 ± 0.28[a]	3.15 ± 0.12[a]
	PC2	61.2 ± 0.99[a]	4.64 ± 0.05[a]	22.65 ± 0.21[a]	7.33 ± 0.85[a]	3.17 ± 0.14[a]
	PA2	61.00 ± 1.01[a]	4.55 ± 0.25[a]	22.61 ± 0.32[a]	7.62 ± 0.68[a]	3.14 ± 0.16[a]
	PC1	61.27 ± 0.61[a]	4.62 ± 0.31[a]	22.62 ± 0.22[a]	7.45 ± 0.21[a]	3.17 ± 0.15[a]
Cooked patties	**PS**	59.38 ± 1.01[b]	5.10 ± 0.78[a]	21.57 ± 0.4[b]	8.35 ± 0.49[b]	3.77 ± 0.09[b]
	PA1	59.45 ± 1.69[b]	4.73 ± 0.66[a]	21.52 ± 0.36[b]	8.65 ± 0.49[b]	3.73 ± 0.11[b]
	PA2	59.00 ± 1.00[b]	5.18 ± 0.82[a]	21.64 ± 0.17[b]	8.20 ± 0.7[b]	3.77 ± 0.19[b]
	PC1	59.20 ± 0.04[b]	4.90 ± 0.16[a]	21.53 ± 0.45[b]	8.3 ± 0.14[b]	3.85 ± 0.11[b]
	PC2	59.4 ± 0.98[b]	5.15 ± 0.70[a]	21.56 ± 0.42[b]	8.65 ± 0.07[b]	3.76 ± 0.12[b]

The mark a, b, c, d, e, f in the same column without a common letter indicates significant difference, $p < 0.05$. PS: Control patty (without EO); PA1: Patty + EO of *S. aromaticum* (0.1%); PA2: Patty + EO of *S. aromaticum* (0.2%); PC1: Patty + EO of *C. citratus* (0.1%); PC2: Patty + EO of *C. citratus* (0.2%).

tion of free water and volatiles substances that are present in the patties. This results tie with that of Mbougueng *et al.* [23] who observed a decrease in the moisture content of patty after cooking.

3.2. Bacterial Change during Storage

The effect of *S. aromaticum* and *C. citratus* essential oils on the growth of *E. coli* and *S. aureus* inoculated in beef patties are presented in **Figure 2** and **Figure 3**. During the storage at 4˚C, the microbial growth is characterized by two phases: a fast growth phase observed before the first 7 days during which microbial initial population increased by more than 3 log CFU/g and a deceleration phase after day 7 and day 28, during which an increase of less than 1 log CFU/g of E. coli and *S. aureus* occurred. A study carried out by Essia Ngang *et al.* [24] showed similar effect of essential oil on the microbial quality of refrigerated beef patties. When compared to the control samples, for both microorganisms, the bacterial load decreased with increase of incorporation rate of both essential oils.

In the patties treated with *C. citratus* essential oil, a high effect was perceptible on patties incorporated with 0.2% of essential oil. At this concentration, *S. aureus* load increased from 4.29 log CFU/g to 6.03 log CFU/g at Day 7, to reach 6.54 log CFU/g after 28 days. For *E. coli*, the bacterial load increased from 4.29 log CFU/g to 6.54 log UFC/g at Day 7 to reach 6.8 log CFU/g after 28 days.

In the patties treated with essential oil of *S. aromaticum*, the load of *E. coli* increased from 4.29 log CFU/g to 6.54 and 6.30 log CFU/g at Day 7 to reach 7.04 and 6.58 log CFU/g at Day 28 respectively for the rates of incorporation of 0.1 and 0.2%. On the other hand, the load of *S. aureus* increased from 4.29 log CFU/g at Day 0 to reach 5.96 and 5.85 log CFU/g at Day 7 and 6.15 log CFU/g and 5.43 log CFU/g after 28 days respectively for the rates of incorporation of 0.1% and 0.2%. The results obtained at 0.2% incorporation rate of *S. aromaticum* on *S. aureus* are in agreement with those of Jagadeesh *et al.* [25] which showed that the incorporation of *S. aromaticum* reduced the microbial load in chicken patties during 14 days of storage.

At the same incorporation rates, the essential oil of *S. aromaticum* is significantly ($p < 0.05$) more effective than that of *C. citratus*. The difference in antibacterial activity could be explained by the difference in chemical composition of the essential oils. The antibacterial activity of these essential oils is related to the presence of eugenol for *S. aromaticum*, neral, geranial and myrcene for *C. citrates* [26]-[28]. The antimicrobial mechanism of action of these constituents is generally considered to be the disturbance of the cytoplasmic membrane, disruption of the proton motive force, electron flow, active transport and coagulation of cell contents [29]. In the present study it was observed that the essential oils of *S. aromaticum* and *C. citratus* were more inhibitory to *S. aureus* than to *E. coli* when tested in beef patties. In fact, the speed of growth is higher for *E. coli* compared to

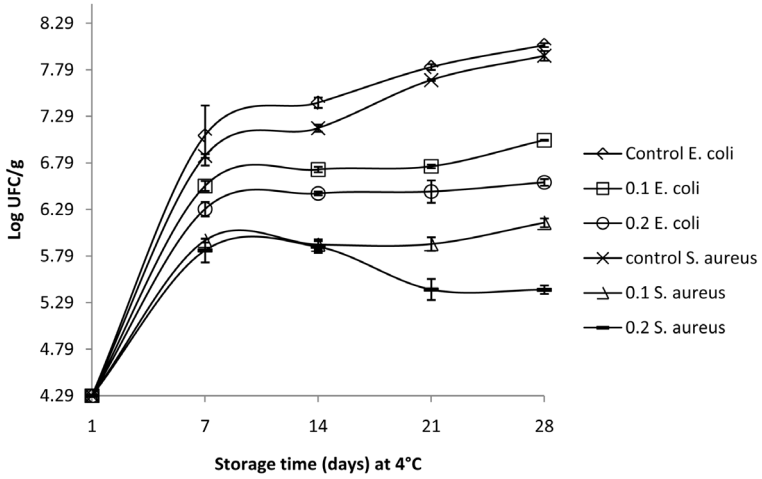


Figure 2. Effect of *S. aromaticum* essential oil on *E. coli* and *S. aureus* growth during storage.

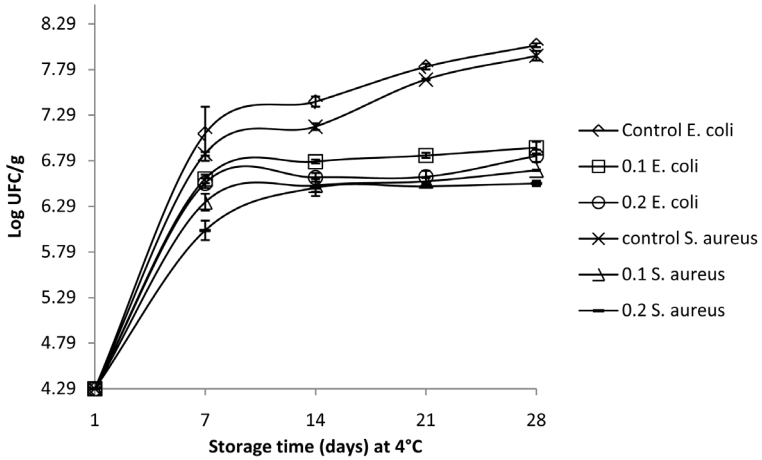


Figure 3. Effect of *C. citrates* essential oil on *E. coli* and *S. aureus* growth during storage.

that of *S. aureus* irrespective of the essential oil concentration. This result is in agreement with previous studies which demonstrated that Gram-positive bacteria are more sensitive to plant extracts than Gram-negative bacteria [30].

3.3. Changes in the pH Values of Beef Patties during Storage

Changes in the pH of beef patties during storage at 4˚C are shown in **Figure 4** and **Figure 5**. The type of microorganism, the type of essential oil, the rate of incorporation and the storage time significantly ($p < 0.05$) influenced the pH values of patties. The differences between the pH values of essential oil-treated beef patties and essential oil free-beef patties were relatively small during the first 7 days of storage. At Days 14, 21 and 28, however, significant differences were observed between the pH values of controls and the others. In fact, the pH rapidly increased in essential oil free-beef patties and reached 6.7 and 6.6 in those inoculated with *E. coli* and S. *aureus* respectively after 28 days whereas in the essential oil treated-beef patties, the values were around 6.1 - 6.2. These results are consistent with Devendra and Tanwar [31], who showed that the pH of chicken patties incorporated with 0.1% of *S. aromaticum* powder increases during the storage. The increase in the pH values of beef patties during the refrigerated storage may be due to the proteins degradation and the formation of basic nitrogen compounds [32].

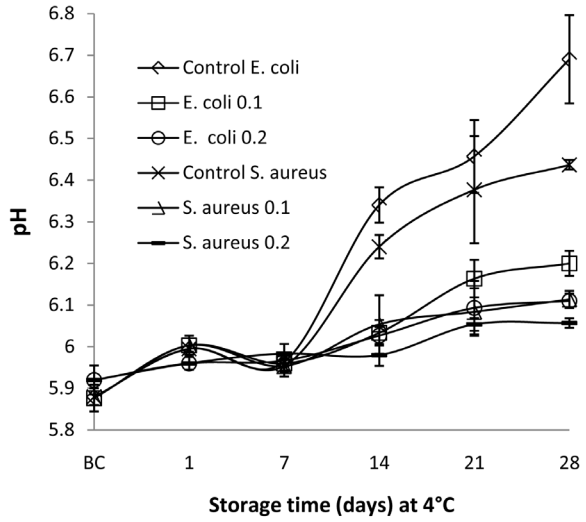


Figure 4. Influence of *S. aromaticum* rate on pH variation of beef patties during storage (BC = Before Cooking).

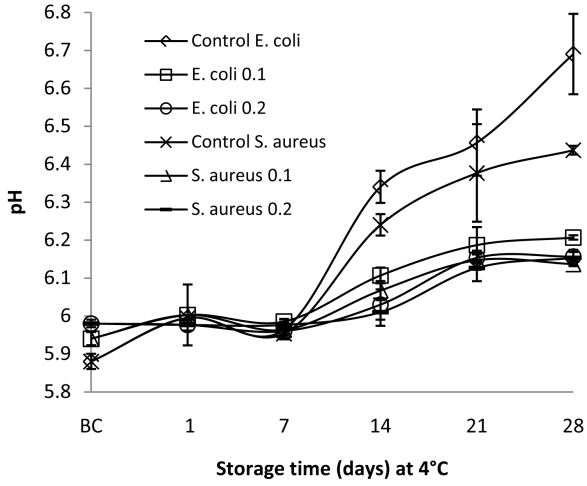


Figure 5. Influence of *C. citrates* rate on pH variation of beef patties during storage (BC = Before Cooking).

3.4. Changes in TBA Values of Beef Patties during Storage

The influence of essential oils of *S. aromaticum* and *C. citrates* on the TBA values of patties after cooking and during storage at 4˚C is presented in **Figure 6** and **Figure 7**. TBA values after cooking were significantly affected ($p < 0.05$) by the rate of incorporation and the type of essential oil. After cooking, a significant increase ($p < 0.05$) was noted in TBA value of beef patties compared to their raw counterparts. This increase would be due to the oxidation of the lipids during cooking. These results are consistent with Ngah [33], who observed an increase in TBA value of 6.7 times during cooking at 190˚C/13min of turkey meat products. Compared with controls, the essential oils treated-beef patties were less sensitive to lipid oxidation; this testifies the antioxydant properties of the two essential oils during cooking.

In general, the TBA values of all beef patties increased significantly after 14 days and decreased after 21 days and the TBA values of essential oil free-beef patties were significantly ($p < 0.05$) high compared with those of essential oil treated-beef patties.

For patties treated with essential oil of *S. aromaticum*, between Day 0 and Day 14, the concentration of essential oil did not significantly ($p > 0.05$) influenced the values of TBA. However, between Day 14 and Day 21,

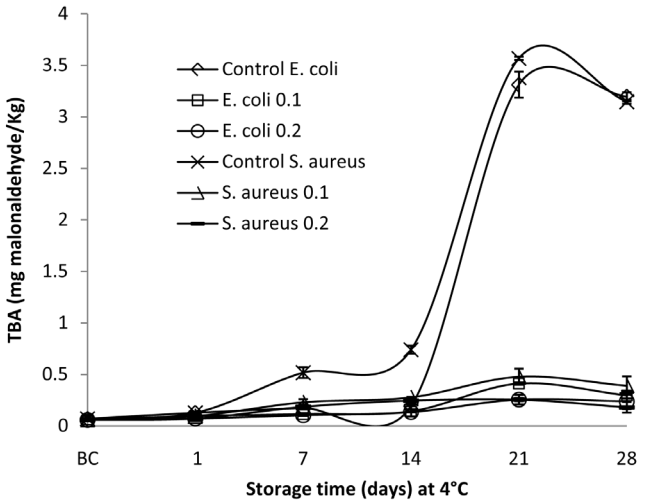


Figure 6. Effect of *S. aromaticum* essential oil on TBA value of beef patties inoculated with *E. coli* and *S. aureus*.

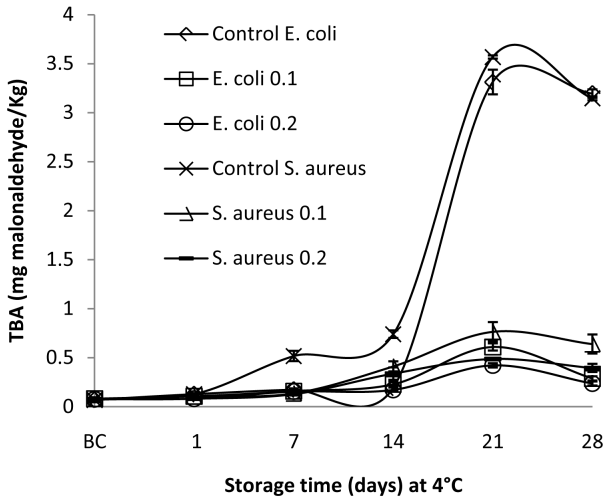


Figure 7. Effect of *C. citratus* essential oil on TBA value of beef patties inoculated with *E. coli* and *S. aureus*.

a significant ($p < 0.05$) difference was observed between the two rates of incorporation. After 21 days of storage of beef patties inoculated with *S. aureus*, the TBA values were 0.477 meq and 0.255 meq respectively for the concentration of 0.1% and 0.2%. These values fell respectively to 0.391 meq and 0.185 meq after 28 days. For patties inoculated with *E. coli*, the TBA values reached 0.411 meq and 0.250 meq at Day 21 respectively for the concentration of 0.1% and 0.2%, then fell to 0.297 meq and 0.238 meq after 28 days.

For patties treated with the essential oil of *C. citratus*, after 21 days of storage, the TBA values of beef patties inoculated with *S. aureus* were 0.764 meq and 0.488 meq respectively for the concentration of 0.1% and 0.2% then fell to 0.639 meq and 0.395 meq at Day 28. For beef patties inoculated with *E. coli*, the TBA values reached 0.609 meq and 0.413 meq at Day 21 then fell to 0.288 and 0.237 at Day 28 of storage respectively for the concentration of 0.1% and 0.2%.

The results obtained showed that the antioxidant activity of *S. aromaticum* essential oil is higher than that of *C. citratus* over the storage time. The high antioxidant property of *S. aromaticum* essential oil is due to its high content of eugenol which has the capacity to react with the free radicals and hydroxyls, transforming them into more stable forms. These results are consistent with Kumar & Tanwar [34] who showed that the incorporation of the *S. aromaticum* powder at 0.1% enabled the slowdown of oxidation in chicken patties compared to the control.

Olorunsanya *et al*. [35] also studied the influence of the rates of incorporation of the *C. citrates* powder on the oxidation of raw and cooked patties during 9 days and noted that the incorporation of 1.5% of the leaves powders not only protect raw and cooked patties during cooking but also during the storage time. They observed that the oxidation rate was higher in control.

3.5. Changes in Beef Patties Color, before and after Storage

Meat and meat products purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness. The differences in the colors are illustrated in **Table 2** as trichromatic coordinates. The lightness reflected as the L* value was significantly high in essential oil-treated beef patties ($p < 0.05$) than essential oil free-beef patties. During the storage at 4˚C for 28 days, the value of L* decreased by 20% and 188% in cooked beef patties inoculated respectively with *E. coli* and *S. aureus*. In beef patties treated with *C. citratus* essential oil, the decrease of L* value ranged between 8.5% - 9.06% and there was no significant difference between the incorporation rate of 0.1% and 0.2%. In beef patties treated with *S. aromaticum*, the decrease of L* value ranged between 8% - 8.37% for the incorporation rate of 0.1% and between 2.75% - 3.05% for incorporation rate of 0.2%.

Studies on instrumentally measured meat color often focus on the a* value (redness), because the redness of meat is an important component of visual appeal to consumers [36]. Generally, the redness of all the beef patties was not significantly different ($p > 0.05$) at Day 0 and ranged between 6.78 - 6.81. In essential oil free-beef patties, the values of a* highly decreased after 28 days of storage. These decreases were 82% and 75.22% in patties inoculated respectively *E. coli* and *S. aureus*. These results are consistent with Sajid and Soottawat [37], who observed a decrease of a* index in patties incorporated with tannic acid and preserved in modified packages at 4˚C. The decrease of a* values was significantly lower in essential oil-treated beef patties (46% - 68% in patties treated with essential oil of *C. citratus* and 35% - 51% in patties treated with essential oil of *S. aromaticum*) than essential oil free-beef patties. The work carried out by Shan *et al.* [38] also reported the protective effects of spices and herb extracts against the decrease in a* value in raw pork during storage. The yellowness, b* values, of the control increased significantly ($p < 0.05$) with storage time, but those of the treated samples increased only slightly.

Table 2. Effect of essential oils on cooked beef patties color after storage time.

	Parameters					
	L*		a*		b*	
	Days		Days		Days	
Patties	0	28	0	28	0	28
PSE	44.00 ± 5.03^{b}	35.96 ± 0.52^{a}	6.66 ± 0.48^{a}	1.19 ± 0.07^{a}	22.45 ± 0.39^{a}	28.72 ± 0.26^{a}
PC1E	55.47 ± 0.49^{a}	50.67 ± 0.49^{c}	6.78 ± 0.49^{a}	2.15 ± 0.07^{bc}	22.78 ± 0.31^{a}	26.02 ± 0.4^{c}
PA1E	56.09 ± 1.39^{a}	51.67 ± 2.46^{b}	6.78 ± 0.19^{a}	3.27 ± 0.72^{de}	22.95 ± 0.02^{a}	25.01 ± 0.18^{b}
PC2E	56.68 ± 0.13^{a}	51.86 ± 0.08^{c}	6.79 ± 0.5^{a}	2.85 ± 0.24^{cd}	22.78 ± 0.05^{a}	24.82 ± 0.39^{b}
PA2E	56.34 + 0.55^{a}	54.79 ± 0.36^{d}	6.81 ± 0.05^{a}	3.60 ± 0.07^{def}	22.95 ± 0.11^{a}	23.61 ± 0.16^{a}
PSS	44.00 + 5.03^{b}	36.13 ± 1.22^{a}	6.66 ± 0.48^{a}	1.65 ± 0.15^{ab}	22.45 ± 0.39^{a}	27.07 ± 0.39^{a}
PC2S	56.68 ± 0.13^{a}	51.54 ± 0.11^{c}	6.79 ± 0.3^{a}	3.63 ± 0.52^{def}	22.49 ± 0.05^{a}	24.43 ± 0.78^{b}
PA2S	56.34 ± 0.55^{a}	54.62 ± 1.48^{d}	6.81 ± 0.05^{a}	4.43 ± 0.44^{f}	22.95 ± 0.11^{a}	23.11 ± 0.04^{a}
PC1S	55.47 ± 0.49^{a}	50.45 ± 0.16^{c}	6.78 ± 0.49^{a}	3.52 ± 0.46^{de}	22.78 ± 0.31^{a}	25.73 ± 0.15^{c}
PA1S	56.09 ± 1.39^{a}	51.39 ± 0.02^{c}	6.78 ± 0.19^{a}	3.90 ± 0.03^{ef}	22.95 ± 0.02^{a}	24.95 ± 0.37^{b}

The mark a, b, c, d, e, f in the same column without a common letter indicates significant difference, $p < 0.05$. PSE: Patty + *E. coli*, PC1E: Patty + essential oil of *C. citratus* (0.1%) + *E. coli*, PA1E: Patty + essential oil of *S. aromaticum* (0.1%) + *E. coli*. PC2E: Patty + essential oil of *C. citratus* (0.2%) + *E. coli*, PA2E: Patty + essential oil of *S. aromaticum* (0.2%) + *E. coli*. PSS: Patty + *S. aureus*, PC2S: Patty + essential oil of *C. citratus* (0.2%) + *S. aureus*, PA2S: Patty + essential oil of *S. aromaticum* (0.1%) + *S. aromaticum*, PC1S: Patty + essential oil of *C. citratus* (0.1%) + *S. aureus*, PA1S: Patty + essential oil of *S. aromaticum* (0.1%) + *S. aromaticum*.

Table 3. Score of consumers according to the valued parameters.

Patties	Parameters					
	Taste	Color	Texture	Jutosity	Flavor	General aceptability
PA1	5.00 ± 1.33^{b}	4.30 ± 1.42^{a}	6.30 ± 1.95^{a}	4.28 ± 1.63^{a}	5.00 ± 1.63^{ab}	5.20 ± 1.32^{a}
PA2	4.10 ± 1.37^{ab}	4.30 ± 1.57^{a}	5.00 ± 1.4^{a}	3.55 ± 1.20^{a}	4.70 ± 1.49^{a}	4.70 ± 1.00^{a}
PC1	4.30 ± 1.89^{ab}	4.60 ± 1.74^{a}	5.60 ± 1.20^{a}	3.96 ± 1.19^{a}	3.90 ± 2.13^{a}	4.40 ± 1.85^{a}
PC2	3.40 ± 1.78^{a}	4.10 ± 1.37^{a}	4.60 ± 2.46^{a}	3.90 ± 1.29^{a}	4.50 ± 1.96^{a}	4.40 ± 1.74^{a}
PS	7.00 ± 0.82^{c}	5.10 ± 1.37^{a}	6.60 ± 1.07^{a}	5.65 ± 2.24^{a}	6.60 ± 1.26^{b}	6.10 ± 1.10^{a}

The mark a, b, c, d, e, f in the same column without a common letter indicates significant difference, $p < 0.05$. PA1: Beef patty + essential oil of *S. aromaticum* (0.1%). PA2: Beef patty + essential oil of *S. aromaticum* (0.2%). PC1: Beef patty + essential oil of *Cymbopogon citratus* (0.1%). PC2: Beef patty + essential oil of *Cymbopogon citratus* (0.2%). PS: Beef patty without essential oil.

3.6. Consumer Acceptability

Since the use of naturally occurring preservatives can alter the taste of food due to the associated flavors, the sensory characteristics beef patties supplemented with essential oils of *C. citratus* and *O. gratissimum* were analyzed. The results presented in **Table 3** revealed that the scores of the various parameters lie between 3.9 and 6.7. Apart from the flavor, parameters of beef patties were significantly identical ($p < 0.05$). From all patties treated with essential oil, patties PA1 (patties treated with 0.1% of *S. aromaticum*) obtained the greatest score for all the sensory parameters. The control presented the best score for all parameters. The score of the flavor and general acceptability decreased with an increase in the rate of incorporation of *S. aromaticum* essential oil while the score of these two parameters increased with an increase in the rate of incorporation of *C. citratus* essential oil. At the same rate of incorporation, the patties treated with *S. aromaticum* obtained the best score.

These data showed that the consumers accepted patties formulated with two essential oils. But the scores showed that the beef patties treated with 0.1% *S. aromaticum* essential oil was the most accepted by the panelists.

4. Conclusion

The results presented in the present work show that the incorporation of essential oils of *S. aromaticum* and *C. citratus*in cooked beef patties at the rate of 0.1% - 0.2% was effective method in inhibiting the growth of *E. coli* and *S. aureus* and the lipid oxidation in cooked beef patties during storage at 4˚C for 28 days. Antibacterial and antioxidant properties of *S. aromaticum* essential oil were higher in beef patties than that of *C. citratus*. Therefore, these two essential oils could be used to reduce the microbial growth and extend the shelf life of cooked beef patties during refrigerated storage.

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