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Effect of Fat and Essential Oil on the Microbial Qualiy of Beef Patties

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Abstract: Combined effect of temperature and incorporation of animal fats, vegetable oils or essential oil on microbial characteristics of beef patties were investigate. Animal fats (beef and pork fats), and vegetable oils (ground nut and maize oils) were added at 20% level and essential oils (ginger and basilica essential oils) at 0.2% level. A pure culture of *E. coli* was inoculated in all patties before heat treatment to a core temperature of 65°C . Samples were stored at 4°C for 30days and evaluated periodically for enterobacteriacea, *E. coli*, Lactic acid bacteria and mesophiles. Formulation containing vegetable oils and essential oils exibited the greatest microbial reduction during cooking and consequently the higher shelf-life periods. Enterobacteriacea and *E. coli* growth was decelerated for at least 12 days. However no bacterial decreases was observed during storage. For all treatment pH values increased with lactic acid bacteria growth .

Key words: Beef patties, fats, essential oil, microbial stability

INTRODUCTION

Microbial growth in meat product is one of the major factor affecting product quality. Because of their nutrient composition and lack of competitive or repressive organisms, cooked meat products have a high potential for bacterial growth[1]. Among characteristics affected by bacterial growth, loss of red color and development of off flavors are those that influence consumers decisions regarding safety and acceptability of meat products[2]. Since consumer demand is currently driven towards natural foods and those free of additives[3] extension of meat products shelf life by natural antimicrobial agents have been a focus for many food scientists. These concerns have led to an increased interest in incorporation of a variety of additive with an antimicrobial potential. Investigations were made on compounds such as organic acids and their salts[4,7], biopreservatives such as lactic acid bacteria[8,9]. Herbs, spices and plant extracts have also been used to improve shelf life of food products[10,11,13].

More over as consumer concerns regarding health and dietary fat intake have increased the demand for reduced fat meat products[14] much attention has been focused on the partial replacement of animal fat with vegetable oils[15,17]. Little information is available on the effect of direct incorporation of vegetable oils in meat products on microbial stability.

Bloukas, Paneras and Fournitzis[18] who studied the replacement of pork fat with olive oil in fermented sausages found no difference among treatments as far as bacteria counts were concerned. Liu *et al.*,[17] also reported that incorporation of soybean oil or hydrogenated soybean oil had no effect on microbial stability of lean ground beef patties. Ockerman and Sun[18] reported that addition of different garlic products did not reduce bacterial level in chinese-style sausages. However, antimicrobial activity of fatty acid and their ester have been shown on *Listeria monocytogenes*[19], *Staphyloccocus aureus*[20,21] and on *Clostridium botulinum*[22]. Application of garlic-derived organosulfur compounds in ground beef significantly enhanced colour, lipid and microbial safety[10]. Because the previous work have shown an antioxidant effect of ginger and basilica essential oil[33], the objective present work was to study the effect of lipids sources and essential oils on microbial quality of beef patties during storage.

MATERIALS AND METHODS

Materials: Beef semi-menbranous muscle (top round) and fats (beef and pork fats) were purchased from a local slaughter house. Beef muscle was trimmed of all visible extramuscular fat; then ground through a 3 mm plate using a meat grinder (Moulinex 505, France). The ground meat was sealed in 15 x 25 cm^2 polyethylene bags (1000

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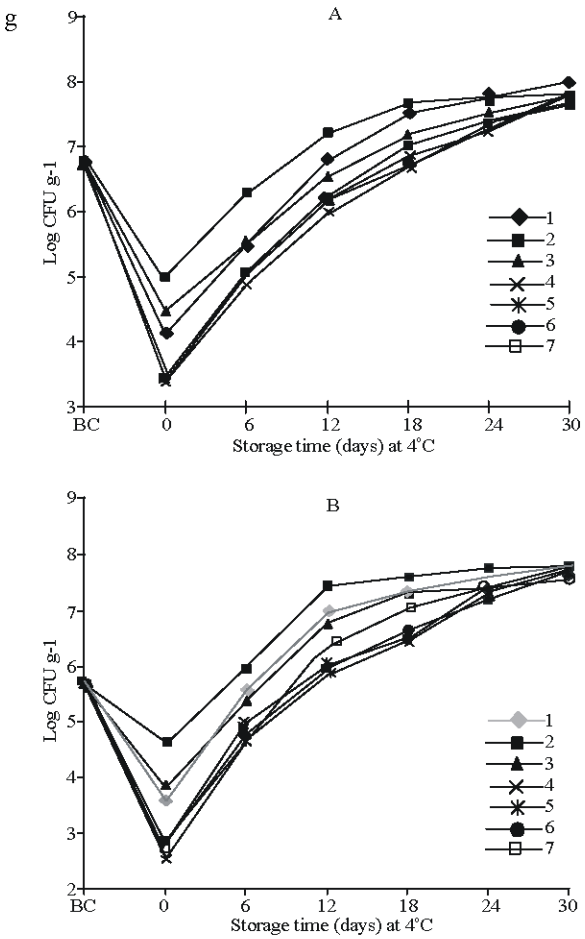

Fig.1: Beef patties treatment effect on Aerobic mesophilic bacteria (A)and Lactic acid bacteria (B) count over storage time. For treatment formulations see Table1. BC = Before Cooking

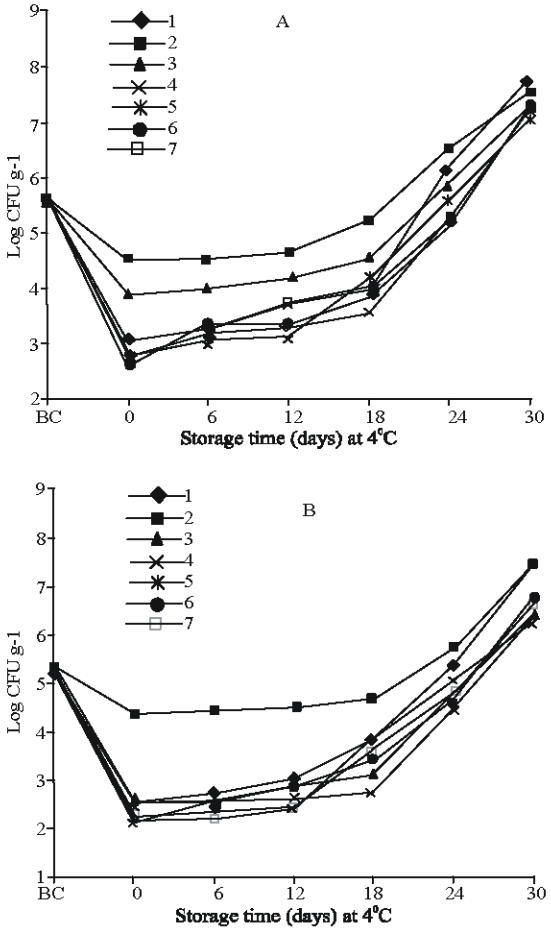

Fig. 2: Beef patties treatment effect on enterobacteria (A) and *E. coli* (B) count over storage time.For treatment formulations see Table1. BC = Before Cooking

package) and store at –20°C. Refined vegetable oils were obtained from a retail store. Essential oils from Ginger (*Zingiber officinale*) and Basilica (*Occimum gratissimum*) were obtained following the procedures described by Ngassoum[24]. *E. coli* was obtained from the culture collection of the Microbiology Laboratory, University of Ngaoundere (Cameroon)

Patties preparation: Prior to processing, the frozen meat was thawed at 4°C for 16 h. Beef and pork fats were boiled in distilled water for 15mn for partial sterilisation and separately ground through a 2 mm plate. The meat, 2% sodium chloride, fats, different oils and 1% (v/w) *E. coli* suspension (10^8 UFC/mL) were thoroughly mixed for 5 min. *E. coli* culture was inoculated within the first minute. The treatments test were : (1) control, no lipids and essential oils, (2): 20%(w/w) beef fat ;(3) 20% (w/w) pork fat, (4) 20% (w/w) ground-nut oil; (5) 20% (w/w) maize oil; (6) 0.2% (w/w) ginger essential oil; (7) 0.2% (w/w) basilica essential oil. The mixtures were then formed into 300g patties using cardboard meat boxes with no overwrapping. Patties were then cooked at 90°C using an oven (Memmert, UL 40, West Germany) to an internal temperature of 65°C. The cooked products were cooled at room temperature (22 – 25°C) for 30 min. The cooled patties were cut into 25-30 g portions, wrapped with aluminium foil and stored at 4°C until required for analysis.

Microbiological analysis: Twenty five grams of meat sample were homogenized for 2 min in 225 mL of sterile 0,1% peptone water using a lab blender 400 stomacher. Serial dilution of the previous homogenate were then

Table 1: Proximate composition (%) and pH of raw and cooked beef patties)

		Treatments*						
Analysis		1	2	3	4	5	6	7
Moisture	raw	75.75±0.75^a	67.74±0.68^b	66.68±0.86^b	67.81±0.86^b	69.14±0.78^b	77.1±0.69^a	75.98±0.90^a
	cooked	67.59±0.66^a	62.73±46^b	63.77±0.70^b	60.25±0.75^b	63.8±0.80^b	67.2±0.67^a	68.24±0.85^a
Proteint	raw	23.44±0.58^a	19.55±0.80^b	20.68±0.45^b	14.55±0.55^c	14.56±0.68^c	22.88±0.72^a	22.46±0.75^a
	cooked	26.45±0.56^a	22.28±0.75^b	21.36±0.55^b	19.15±0.75^c	18.54±0.76^c	26.4±0.84^a	26.5±0.68^a
Fat	raw	3.77±0.25^c	15.8±0.80^b	16.21±0.85^b	17.23±0.40^a	18.46±0.90^a	3.58±0.77^c	3.47±0.56^c
	cooked	6.26±0.60^b	14.17±0.95^a	15.19±0.70^a	16.56±0.52^a	15.54±0.55^a	5.45±0.56^b	5.4±0.54^b
Ash	raw	1.14±0.15^a	1.07±0.14^a	1.07±0.13^a	1.01±0.16^a	1.03±0.13^a	1.11±0.12^a	1.14±0.12^a
	cooked	1.28±0.16^a	1.34±0.14^a	1.23±0.13^a	1.19±0.15^a	1.16±0.13^a	1.37±0.16^a	1.19±0.13^a
pH	raw	5.51±0.07^b	5.70±0.09^a	5.68±0.07^a	5.53±0.05^b	5.52±0.04^b	5.51±0.06^b	5.58±0.04^b
	cooked	5.71±0.15^b	5.90±0.10^a	5.84±0.12^a	5.72±0.09^b	5.75±0.09^b	5.70±0.18^b	5.72±0.15^b

abc Means on the same row with different superscripts are significantly ($p<0.05$) different Treatments: (1) control, (2): 20%(w/w) beef fat , (3) 20% (w/w) pork fat, (4) 20% (w/w) ground-nut oil, (5) 20% (w/w) maize oil, (6) 0.2% (w/w) ginger essential oil, (7) 0.2% (w/w) basilica essential oil.

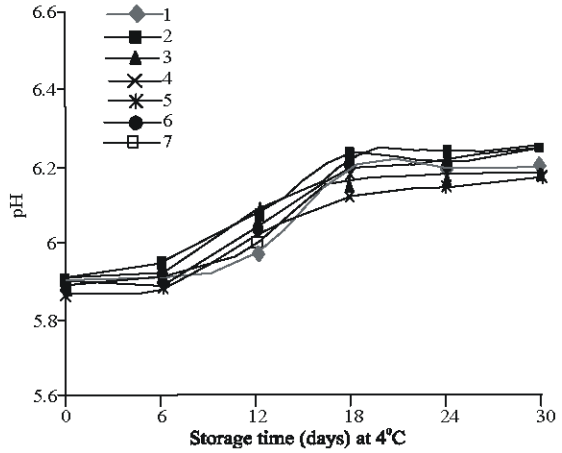

Fig. 3: pH variation of the different beef patties formulations during storage time

prepared and microorganisms enumerated using a pour plate technique in duplicate. The aerobic mesophilic bacteria (AMB) were enumerated onto Plate Count Agar (PCA) (Oxoid, Basingstoke,UK) incubated aerobically at 37°C for 2 days. Enterobacteria were detected on Violet Red Bile Glucose agar (Difco laboratories) incubated at 37°C for 24h. *E. coli* was determined on eosine methylene blue(EMB) Agar (Difco) after an incubation of 24-48h at 44°C. Lactic acid bacteria were enumerated on De Man Rogosa and Sharp agar (MRS) containing actidione. Plates were incubated anaerobically at 30°C for 48h in anaerobic jars using a carbon dioxide generator. Samples were analysed at 6 days intervals up to 30 days. Results were converted to $\log_{10}$ UFC/g sample and averages of two replicates were reported for each treatment.

pH determination: 10 g of meat samples were homogenised with 90 mL of distilled water and pH was determined using a pH-meter (Eutech Cybernetics, Cyberscan 1000, Singapore)

Statistical analysis: The effect of each treatment was analysed from seven different preparations (n = 7). Data were subjected to analysis of variance and Duncan's multiple range test[25] was used to determined the significant differences among means.

RESULTS

Microbial quality of beef patties was assessed before and after cooking to screen the effectiveness of additives during cooking.

When compared to the control group, the microflora of the beef patties were significantly reduced by the addition of vegetable oils and essential oils during cooking (Fig. 1 and 2) . In contrast to product formulated with vegetable oils and essential oils tha showed similar microbial reduction, patties containing beef fat showed higher microbial count than those containing pork fat after cooking.

Which ever the patties, the Aerobic Mesophile Bacteria (AMB) in cooked samples increased with storage time. Growth curves of those microorganisms were characterized by two phases (Fig. 1A): A rapid growth phase for at least 12days during which AMB initial population increased by more than 2 log CFU g^{-1} and a decelaration phase in the remainder storage time, during which an increase of less than 1 log CFU g^{-1} in AMB occurred.

Durind the first phase the cell number reached a concentration of 7log CFU g^{-1}, which is described as the index of spoilage[5]. This spoilage index was achieved withing 18 days when vegetable oils or essentials oils were used compared to 12 days for the control group and product with animal fats.

Although the index of spoilage was reached faster in patties containing animals fats, growth rate of AMB were more important in products containing vegetables oils or essential oil than in patties containing animals.

Effect of lipid sources and essentials oils on lactic acid bacteria (LAB) count presented in figure 2B show similar growth curves to those of AMB during storage. No significant difference ($p>0.05$) was observed between growth rate of LAB and AMB in the same group of

patties. The results were much perceptible on patties formulated with beef fat that contained the higher initial count of both microorganisims compared to other patties. In this case indeed, LAB and AMB increased respectively from 4.6 log CFU g^{-1} and 4.9 log CFU g^{-1} to reach 7.4 log CFU g^{-1} and 7.2 log CFU g^{-1} after 12 days and 7.8 log CFU g^{-1} after 30 days.

The behavior of enterobacteria and *E. coli* during storage is presented in figure 3. Regardless of the initial concentration of those microorganisims, enterobacteria and *E. coli* growth showed a lag phase for at least 12 days , time after which growth resumed until the end of storage period. Once growth began, the growth rate of those bacteria on patties treated with vegetable or essential oils were not different from the control group.

As microbial growth are affected by the product composition[6], physico- chemical analysis of these patties previously done[23] were resumed in Table 1. As previously describe, the raw and cooked patties containing animals and vegetable oils had a lower water content ($p<0.05$) than the control and those formulated with vegetable oils.

In contrast to water content, fat content was higher in raw and cooked patties containing animal fat and vegetable oils. Formulations had no significant effect on ash content of the products and pH values of all the patties increased upon heating.

Because organic acids are the principal microbial metabolites produced during bacterial growth, the pH values that are generally correlated with the acidity of the media was evaluated during storage. Figure 3 that present this pH evolution with storage time revealed that pH was unaffected during the first 6 days of storage. Samples showed then a light increase from day 6 to day 18 followed by a stabilization period from day 18 to the end of the storage.

DISCUSSION

The addition of non meat ingredients to extend shelf-life of meat product from a microbiological standard point without altering palatability or visual characteristics is one of the preoccupation of meat industry. Since vegetable fats are perceive as less health risk than animal fats, and because of their higher content of unsaturated fatty acid known to affect microbial growth, they were tested for microbial stability of beef patties.

The lowest bacterial count found after cooking in patties formulated with vegetable oils in comparison to the product with the animals fats could be linked to their lower percentage of saturated fatty acid that are known to affect melting point of fat. With respectively 18.6% and 14.3 % of saturated fatty acid for groundnut and maize oil[26] compared to 53.7% and 41.5% for beef and pork fat[16] heat transfer could have been increased in patties containing vegetables oils than in patties containing animal fats. Consequently the microflora in products with vegetable oils would have been more affected. The higher percentage of saturated fatty acid in beef fat in comparison to pork fat could also explain the difference of microbial count between animal fat patties.

As unsaturated fatty acid are known to be more active against microorganisms than the corresponding saturated fatty acid[27], the microbial reduction in patties with vegetable oils could have been increased by the higher content of unsaturated fatty acid in vegetable oils.
Essential oils that are commonly appreciated in food for specific taste showed similar microbial reduction to vegetable oils. Because the level of essential oil and vegetable oils used (0.2% and 20% respectively) were too different, and as fat content was very different in both group of patties, effect of fatty acid composition on heat transfert could not be accounted for patties containing essential oils.

Reduction of microbial count in product containing essential oils would be linked to the inhibitory effects of those additives. Antimicrobial activities of essential oils have been described by many authors[28,30]. These biological activities were attributed to the presence of volatiles compounds such as Cinnamaldehyde and eugenol that were identify as the main active compound of cinnamomum and clove oil[27,28]. Isothiocyanate have also been found as the antimicrobial compounds of essential oil of mustard[7].

Antimicrobial activities of both essential oils previously tested in vitro have been attributed to the presence of thymol in those essential oil[31]. In contrast to result obtained in culture media, which showed that essential oil of *Occimum gratissimum* was more active than that of *Zingiber officinale* on *E. coli*, There was no significant difference in microbial count between patties containing each essential oil.

The addition of lipids or essential oil did not appear to alter growth of AMB or LAB during storage. This findings is consistent with those of Liu *et al.*,[17] who reported no effect of soybean oil and hydrogenated soybean oil on microbial stability.

Despite demonstration of the antimicrobial activities of essential oil in culture media[31,32] and contrary to results of Lemay *et al.*,[1] that showed increase of AMB lag phase and reduction of *E.coli* count by essential oil of mustard, no AMB lag phase or *E.coli* reduction was observed in our test.

The coincidence of the lag phase of enterobacteria and *E.coli* with the growth phase of LAB and the recovery of *E. coli* growth with the deceleration of LAB

growth could suggest the inhibition of the gram negative bacteria by the LAB. Lactic acid bacteria are indeed known to suppress food spoilage bacteria and food borne pathogens through decrease of pH due to the production of lactic and acetic acid as well as other antibiotic substances[8]. Our results of pH variation did not agree with results of many authors who reported decrease of pH during storage[33,35]. Decrease in pH was reported with increasing LAB population and organic acids such as lactate, acetate, succinate, or formate produced by those bacteria were accounted for the decline in pH observed[6,34].

Despite important number of work that correlated decrease in pH with increasing LAB population, increase of pH during some period of storage have been found by some authors. Maca *et al.*,[4] who evaluated the effects of storage time on pH during 84 days reported that pH of cooked beef top round increase with storage for the first 28 days then decrease to the initial level with further storage. This period of pH increase correlated with our results.

Results of LAB growth during the first week and the minimal variation of pH values observed during the same period are also in agreement with the work of Papadopoulos *et al.*,[35] who observed with high concentation of sodium lactate that the minimal fluctuation in pH were not due to the suppression of bacterial growth.

The lack of a decrease in pH in our study despite LAB growth would be linked to initial composition of our products. As no glucose was added to our products as usually done in meat process, lack of sugar could have justify limitation of acidification. Then increase of pH observed could be accounted to the use of amino acid and organic acid of meat by microorganisms as it is known that pH tends to rise as compound are deaminated.

CONCLUSION

Different lipids sources and essential oils were tested for their potential to extend beef patties shelf-life. Results obtained showed that addition of vegetable oils particularly maize oil and essential oil reduce microbial cells during cooking.

Consequently microbiological shelf-life (i.e time to reach AMB of 7logCFU/g) of beef patties formulated by maize oil and essential oil was prolonged by 6 days compared to animal fats- treated patties. Because of their volatil character essential oils was not effective in bacterial growth during storage. So addition of these compound in combination with vegetable oils after heat treatment could enhance microbial stability of beef patties.

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