Journal of Food Technology 4 (1) : 16-21, 2006

Effects of Local Spices on Physico-chemical Microbiological and Consumer Acceptability of Beef Patties

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Abstract: The effect of substitution of Bovida spices with locally formulated spices on physico-chemical, microbial and consumer characteristics of beef patties was investigated. The control patty (patty I) was prepared using Bovida spices at the dose of 3g spices kg^{-1} ground meat, while in patties II, III, IV and V the Bovida spices were substituted with 3,6, 9 and 12g of locally formulated spices Kg^{-1} ground meat respectively. The type and the levels of added spices did not significantly ($p>0.05$) affect the moisture, the lipid and the ash content of the raw and the cooked beef patties. The protein content of the samples increased with the levels of added spices in the beef patties. Physico-chemical studies showed that the pH and the TBA value of the raw and the cooked samples were significantly ($p<0.05$) influenced by the type of spices used. The same trend was observed with the WHC of the raw samples. The penetration index and the cholesterol content were not significantly ($p>0.05$) affected by the treatment groups. The highest phenolic content was recorded for the patty containing Bovida spices. The lowest values of magnesium and phosphorus were found in the Bovida spices and in the corresponding formulated patties. An inverse trend was observed as far as calcium and iron were concerned. The highest flavour and overall acceptability score were obtained for the patties formulated with Bovida spices and the patties containing 3g and 6g locally formulated spices. Salmonella and shigella, clostridium and staphylococcus were not present in any of the samples analised.

Keys words: Patties, local spices, bovida spices

INTRODUCTION

Consumer demand plays a major role in the formulation of new products that are natural and free of synthetic additives. The attention in recent years have been focused on the used of extract from herbs and spices to improve sensory characteristics and extend the shelf life of food products[1-3]. Beef patties are common meat product processed from lower – value trimmed red meat to produce a high – value meat product.

Modern trends towards production of pre – cooked, refrigerated ready to eat products have made the control of lipid oxidation increasingly important. Processed meat which are minced and cooked are susceptible to accelerate a lipid oxidation. Lipid oxidation is one of the main factors responsible for loss of quality of meat products besides microbiological deterioration[4,5]. On the other hand, cooked meat products such as beef patties have a high potential for bacterial growth due to ideal nutrient composition. Therefore, the application of a mixture of different type of spices may be useful to maintain the quality and extend the shelf life of meat products. Among compound present in spice mixtures, phenolic compounds were found to exert antimicrobial activity[6,7]. In spit of being strongly contaminated with micro-organisms, spice mixture reduced bacterial count of pork and inhibited growth of various meat-spoiling micro-organisms in a model medium[8]. Phenols are also one of the most important groups of natural anti oxidants. They occur only in material of plant origin and they are known to protect constituent of food from oxydation. Madsen and Bertelsen[9] demonstrated that spices inhibit rancidity, often showing synergism. Dominguez and Zumalocarregui[10] have observed that during ripening of dry fermented sausages lipid oxydation did not occur, the reason was attributed to the antioxydative action of the spices, curing agent and smoke. Paprika was even able to inhibit the peroxydation effect of salt in chirizo, a dry fermented sausage[11].

In the present study a mixture of four local spices in powder form were used to substitute the four Bovida™ spices and then determine their effect on physico-chemical, microbial and consumer acceptability of beef patties.

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MATERIALS AND METHODS

Materials: Beef semi-membranous muscle (top round) was purchased from a local slaughter-house. Beef muscle was trimmed of all visible extra-muscular fat and ground through a 3mm plate using a meat grinder (Moulinex 505, France). The ground meat was sealed in 15x25 cm^2 polyethylene bag (1000 g package) and store at - 4°C. Two spices formulations were used in the present study: a commercially available Bovida spices (Bovida, France) and a locally formulated spices. The locally formulated spices consist of a mixture of fruits from *Hua gabonii*, *Xylopia aethiopica*, *Monodora myristica* and the pulp of the wing of the fruits of *Tetrapleura tetraptera*. The exact composition of this spice mixture is deposited at the department of Food Science and Nutrition, University of Ngaoundéré, Cameroon.

Methods

Patties preparation: Prior to processing, the frozen ground meat was thawed at 4°C for 16 h. For partial sterilisation, beef fats were boiled in water for 15 min. and ground through a 2 mm plate. The ground meat (80) and beef fat (20%), 2% sodium nitrate and spices were thoroughly mixed for 5 min. The treatment tests were: (1) control, 3g Bovid spices Kg^{-1} ground meat; (2), 3g locally formulated spices Kg^{-1} ground meat; (3) 6g locally formulated spices Kg^{-1} ground meat; (4) 9g locally formulated spices Kg^{-1} ground meat and (5) 12g locally formulated spices Kg^{-1} ground meat. The mixtures were formed into 300g patties using cardboard meat boxes with no overwrapping.

Beef patties were cooked at 90°C in an oven (Memmert, UL 40, West Germany) to an internal temperature of 70°C. Cooked product were then cooled at room temperature (22-25°C) for 30min. Cooled patties were cut into 25-30g portions, wrapped with aluminium foil and stored at 4°C until required for analyses.

Proximate analysis: Moisture, protein, fat and ash contents were determined on raw and cooked products as well as on spices, using AOAC methods[12]. Moisture was determined as weight loss of 3g sample after drying for 18h at 102°C. Crude protein was analysed by micro Kjeldahl method (Nx6.25). Fat was determined by weight loss after 16h extraction in a soxhlet apparatus with petroleum ether and ash by incineration of 3g sample at 550°C until a light grey ash result. Carbohydrate content was determinate using 3.5-dinitro salicylic acid[13].

pH determination: For pH determination, 10 g of raw and cooked patties were homogenised with 90 mL of distilled water and the pH was determined using a pH-meter (Eutech Cybernetics, Cyberscan 1000, Singapore)[14].

Water holding capacity: The Tsai and Ockerman (1981) press technique was used with modification to measure the Water Holding Capacity (WHC) of the raw patties. A sample (0.05g) was placed on the filter paper (Whatman n°1, stored over saturated KCL) which was placed between two Plexiglas sheets and pressed for 20min. by $1kg^{-1}$ weight. The area of pressed meat and a spread juice was measured and the water holding capacity was calculated as follows:

$$\% \text{ free water} = \frac{(\text{Total surface area - meat film area})(mm^2)(6.11)}{(\text{Total moisture (mg) in meat sample})} \times 100$$

% WHC = 100- free Water

Cooking loss: Three patties from each treatment group were weighed immediately after they were formed and after cooking and cooling to determined cooking loss.

Texture measurement: Penetration depths were determined on samples of cooked patties from each treatment group using a penetrometer (Model PNR 10-404, Sommer and Runger Co., Germany) equipped with a 162 mm long needle plunger (ASTMD 1321, standard) following the procedure described by Dzudie and Okubanjo[15].

Phenolic compounds analysis: For quantitative determination of the phenilic compounds, extracted component from defatted cake was analysed by high-pressure liquid chromatography (HPLC) on a Varian 5000 analytical liquid chromatograph equipped with a 10μl loop injection valve (Valco) and connected with a variable wavelength UV detector (Violet) at 280nm, a U-Bondapack C18 10μm column (Waters, 300x309mm I.D.) and LiChrosob 100 RP-18 (4x4mm I.D., systeme (Perkin Elmer). Methanol (HPLC grade, Trimital) and 5% HCOOH were used as eluent with a gradient program from CH_3OH/5%. HCOOH in the ratio 1:9 (9mi), increasing to CH_3:5%. HCOOH 4:6 in 11min. the flow rate was 2mL $min.^{-1}$. Before injection, the samples were filtered on Milex-SR 0.5μm filter (Millipore). Phenolic compound were identified by co-elution with the following standards: benzoic, p-hydroxybenzoic, syringic, gallic, protocachuic, cinnamic, cinapic, ferulic, p-coumaric acid and tyrosol, 2-(4-hydroxyphenyl ethanol)[16].

Cholesterol analysis: Cholesterol was determined on cooked Patties using the Mannheim total cholesterol kit

procedure (N°139050, Boehringer Mannheim GmbH D-68298 Mannheim, Germany). Cooked patties (2.5g) were heated in 10 mL of methanol potassium hydroxide solution (1.0 mol L^{-1}) under reflux condenser for 25 min. while stirring. The supernatant was then transferred into 25 mL volumetric flask and the residue was boiled twice with portioned of 6mL isopropanol each under a reflux condenser for 5min. The solution was collected in a volumetric flask, cooled and diluted to the mark with isopropanol. The mixture was filtered and clear solution used for the essay.

Mineral analysis: Patties as well as spices for mineral analysis were prepared according to AOAC[14] procedure and the minerals (except phosphorus) were analyses spectroscopically using an atomic absorption spectrophotometer (Model 1100, Perkin-Elmer, Nor-walk, USA) Phosphorus was determined following the colorimetric molybdenum (Mo)-blue method[14].

Microbiological analysis: Microbiological analysis was determined on cooked beef patties. 25 g of meat sample from each treatment group were homogenized for 2 min in 225 mL of sterile 0.1% peptone water using a lab blender 400 stomacher. Serial dilutions of the previous homogenate were then prepared and microorganisms enumerated in triplicate using a plate technique. Total plate counts were enumerated onto Plate Count Agar (PCA) (Oxoid, Basingstoke,UK) incubated aerobically at 37°C for 2 days. Salmonelles/Shigelleses research requires a culture on S.S middle (gélose for Salmonellas and Shigelleses), incubated aerobically at 37°C for 24-48 hours. Streptococci have been counted on Litsky middle incubated aerobically at 37°C for 2 days and Clostridium TSN middle under the same conditions. Results were converted to $\log_{10}$ UFC/g.

TBA values: The degree of lipid oxidation of the raw and cooked beef patties was determined by the 2-Thiobarbituric Acid (TBA) cold extraction method, described by Wite, Krause and Bailey[18]. The results are expressed as mg malonaldehyde Kg^{-1} meat.

Consumer acceptability: samples for consumer acceptance were carried out on cooked beef patties. Each treatment group were cut into slices and given to a consumer panel. This panel was composed of non-specialist panellist (n=40) randomly recruited within the students and the lecturers of the University of Ngaoundere without care of age or sex. The patties were evaluated on a 9 point hedonic scale ranging from: dislike extremely (flavour, overall acceptability),extremely dry (juiciness), extremely tender (tenderness), extremely white (colour), to like extremely, extremely tender, extremely juicy, extremely pink[19].

Statistical analysis: The experiments were performed in triplicate and the means±standard deviation of three values were reported. Data were subjected to analysis of variance and Duncan's multiple range test[20] was used to determined the significant differences among means.

RESULTS AND DISCUSSION

Physico-chemical composition of Bovida and locally formulated spices are shown in Table 1. With respect to their composition, the two spices mixtures were significantly different ($p<0.05$) from each other. Among organic constituents carbohydrate was identified as the most abundant component in both Bovida and locally formulated spices. The highest protein content was recorded in the locally formulated spices. Phenolic compounds a natural antioxidant was significantly higher in Bovida spices than in locally formulated spices.

Beef patty composition is presented in Table 2. The moisture, the lipid and the ash contents of the raw and cooked patties were not significantly ($p>0.05$) affected by the treatments groups. The type of the spices has no significant effect ($p>0.05$) on the protein contents of the products, meanwhile the protein contents of the raw and cooked patties significantly increased as the spice dose increased from 3 to 6g spices Kg^{-1} meat. The carbohydrate contents of the beef patties treated with the locally formulated spices were significantly higher ($p<0.05$) compared to those treated with the Bovida spices. This was expected and can be attributed to the differences in the carbohydrate contents of the two spices mixtures.

Physico-chemical properties of beef patties are shown in table 3. The lowest pH and WHC values were recorded for the raw patty treated with 3g locally formulated spices Kg^{-1} meat and the highest for the beef patty contening 12g spices Kg^{-1} meat. The same trend as far as pH was concerned was observed after cooking. The pH values increased upon heating. Similar results were

Table 1: Proximate composition of Bovida and locally formulated spices

	Locally formulated spices	Bovidaspices
Moisture (g $100g^{-1}$)	11.69 ± 1.13^{b}	12.05 ± 1.64^{b}
Protein (g $100g^{-1}$)	1.32 ± 0.06^{a}	0.76 ± 0.02^{b}
Lipid (g $100g^{-1}$)	5.92 ± 1.27^{b}	4.93 ± 0.76^{b}
Ash (g $100g^{-1}$)	3.27 ± 0.05^{b}	4.12 ± 0.10^{a}
Charbohydrate (g $100g^{-1}$)	32.81 ± 1.48^{a}	21.92 ± 1.27^{b}
Phénolics compound (g $100g^{-1}$)	0.37 ± 0.03^{b}	0.49 ± 0.02^{a}

[ab] Means on the same line with different superscripts are significantly difference ($p<0.05$).

Table 2 : Proximate composition of raw and cooked beef patties (g/100)

		Treatments*				
		I	II	III	IV	V
Moisture	Raw	63.13±2.99^{a}	62.86±1.51^{a}	63.02±1.05^{a}	62.91±3.08^{a}	65.90±2.46^{a}
	Cook	61.50±2.47^{a}	59.92±2.97^{a}	60.63±2.41^{a}	61.01±3.21^{a}	62.66±2.19^{a}
Protein	Raw	24.27±0.72^{b}	24.14±0.07^{b}	26.64±0.59^{a}	26.98±1.09^{a}	27.59±0.79^{a}
	Cook	24.74±0.23^{b}	25.70±0.08^{b}	26.74±0.61^{a}	27.64±0.08^{a}	27.84±0.73^{a}
Lipid	Raw	7.08±1.13^{a}	6.94±0.44^{a}	7.38±0.53^{a}	6.96±0.90^{a}	6.99±0.64^{a}
	Cook	7.64±1.45^{a}	7.66±1.38^{a}	7.85±1.15^{a}	7.41±1.19^{a}	7.16±0.77^{a}
Ash	Raw	2.71±0.08^{a}	2.68±0.08^{a}	2.80±0.07^{a}	2.81±0.06^{a}	2.82±0.08^{a}
	Cook	2.93±0.01^{a}	2.84±0.03^{a}	2.94±0.04^{a}	2.95±0.03^{a}	2.96±0.03^{a}
Carbohydrate	Raw	2.89±0.23^{d}	4.15±0.36^{c}	4.18±0.06^{cb}	4.31±0.19^{ab}	4.57±0.15^{a}
	Cook	3.12±0.15^{e}	4.15±0.34^{d}	4.97±0.29^{c}	5.78±0.27^{b}	6.59±0.27^{a}

^{abcd} Means on the same line with different superscripts are significantly difference ($p<0.05$).
*Pattie I: Control Patty containing 3g Bovida spices Kg^{-1} ground meat;
Pattie II: Patty containing 3g locally formulated spices Kg^{-1} ground meat;
Pattie III: Patty containing 6g locally formulated spices Kg^{-1} ground meat;
Pattie IV: Patty containing 9g locally formulated spices Kg^{-1} ground meat;
Pattie V: Patty containing 12g locally formulated spices Kg^{-1} ground meat.

Table 3 : Physico-chemical properties of beef patties

		Treatments				
		I	II	III	IV	V
pH	Raw	5.38±0.05^{b}	5.04±0.07^{c}	5.34±0.09^{b}	5.34±0.10^{b}	5.54±0.09^{a}
	Cook	5.83±0.05^{b}	5.61±0.06^{c}	5.80±0.07^{b}	5.82±0.06^{b}	5.91±0.03^{a}
WHC (%)	Raw	87.48±2.56^{b}	83.86±2.59^{c}	87.43±2.92^{b}	87.46±3.18^{b}	89.01±3.25^{a}
Cooking loss (%)		10.57±1.34^{b}	13.10±0.80^{C}	11.91±1.01^{b}	11.24±1.76^{b}	9.84±0.94^{a}
Penetration depth (mm)	Cook	11.62±1.6^{a}	11.4±1.84^{a}	11.35±1.09^{a}	11.45±2.05^{a}	11.82±1.59^{a}
Phenolics Compound (%)	Cook	0.16±0.02^{a}	0.13±0.011^{a}	0.12±0.01^{b}	0.11±0.01^{b}	0.10±0.01^{b}
Cholestérol (%)	Cook	0.15±0.01^{a}	0.16±0.02^{a}	0.16±0.04^{a}	0.16±0.02^{a}	0.18±0.03^{a}

^{ab} Means on the same line with different superscripts are significantly difference ($p<0.05$).

Table 4: Minerals content and microbiological count of raw and cooked beef patty and spices

	Spices			Treatments				
	Bovida™	Local		I	II	III	IV	V
Phosphorus (mg $100g^{-1}$)	125.53±8.05^{b}	154.59±6.35^{a}	Raw	17.13±0.67^{e}	25.81±0.52^{d}	31.64±3,67^{c}	35.60±3.96^{b}	47.29±3,47^{a}
			Cook	19.20±0.15^{e}	27.72±4.07^{d}	46.26±1,08^{c}	50.83±2.12^{b}	55.84±1,33^{a}
Magnesium (mg $100g^{-1}$)	507.26±36.27^{b}	691.37±37.35^{a}	Raw	150.23±7.33^{b}	161.38±4.20^{b}	161.37±15.45^{b}	169.98±9.87^{b}	196.54±1.82^{a}
			Cook	170.59±16.52^{a}	183.84±3.72^{a}	190.74±0.2^{a}	222.11±29.07^{a}	216.55±15.01^{a}
Calcium (mg $100g^{-1}$)	294.77±10.69^{a}	89.96±7.48^{b}	Raw	36.48±0.49^{a}	20.20±0.82^{d}	23.11±1.72^{c}	25.68±3.30^{b}	26.62±1.82^{b}
			Cook	48.33±3.72^{a}	22.23±0.97^{d}	25.85±0.01^{c}	32.57±0.94^{b}	33.16±0.88^{b}
Iron (mg $100g^{-1}$)	32.04±3.70^{a}	14.48±0.62^{b}	Raw	2.98±0.06^{a}	1.94±0.08^{c}	1.99±0.08^{c}	2.03±0.08^{b}	2.07±0.08^{b}
			Cook	3.22±0.05^{a}	2.10±0.05^{b}	2.14±0.05^{b}	2.18±0.05^{b}	2.23±0.04^{b}
Total plate count	222x10^{4b}	144x10^{5a}	Cook	1,28 x10^{2b}	1,65x10^{3a}	1,32x10^{3a}	1,98x10^{3a}	2,36x10^{3a}
Streptococcus	Abs	Abs	Cook	*Abs	Abs	Abs	Abs	Abs
Salmonella and shigella	Abs	Abs	Cook	Abs	Abs	Abs	Abs	Abs
Clostridium	Abs	Abs	Cook	Abs	Abs	Abs	Abs	Abs

Mean on the same line withing treatment and withing spices with the same letter superscript do not present a significant difference at 5% level. * not present

Table 5: Acceptability characteristics of beef patties

	Treatments				
Parameters	I	II	III	IV	V
Juiciness	6.39±0.65^{a}	6.42±0.15^{a}	6.17±0.12^{a}	6.15±0.02^{a}	6.11±0.1^{a}
Colour	6.37±0.20^{a}	6.11±0.05^{a}	6.21±0.29^{a}	6.29±0.26^{a}	6.33±0.47^{a}
Tenderness	6.61±0.49^{a}	6.73±0.57^{a}	6.23±0.48^{a}	6.18±0.1^{a}	6.18±0.13^{a}
Flavour	6.67±0.04^{a}	6.76±0.09^{a}	6.71±0.2^{a}	6.07±0.2^{b}	6.04±0.47^{b}
Overall Acceptability	6.26±0.15^{a}	6.25±0.2^{a}	6.31±0.32^{a}	5.61±0.04^{b}	5.32±0.1^{b}

^{ab} Means on the same line with different superscripts are significantly difference ($p<0.05$).

observed on beef patties formulated with various animal fats and essential oils[3]. Cooking loss was signicantly higher for the beef patty contening 3g locally formulated spices Kg^{-1} meat and significantly lower for the patty containing 12g spices Kg^{-1} meat. These results were consistent with the values recorded for pH and WHC of the respective products. The highest phenolic compound was recorded for the patties containing Bovida spices.

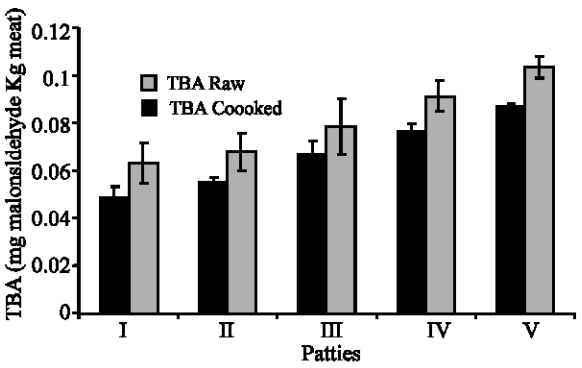

Fig. 1: Thiobartituric acid values of cooked and raw beef patties

The result was expected since Bovida spices contained more phenolic compound than locally formulated spices. Treatments groups had no significant effect ($p>0.05$) on penetration depth and cholesterol content of the beef patties. TBA values have been considered as an index of lipid oxidation. Figure 1 indicated that the degree of lipid oxidation was higher for the beef patty containing locally formulated spices compared to the one containing Bovida spices. These results could be attributed to the difference in the phenolic compounds of the two spices. Phenols are one of the most important groups of natural anti-oxidant. They occur only in material of plant origin and they are known to protect easily oxydizable constituents of food from oxidation[21]. Karpin *et al.*,[22] in the study of the use of natural anti-oxidants in ready to serve food reported that addition of mixture of spices retarded the process of oxidation. As the dose of spices in the products increased an increase in the TBA values recorded for the beef patties increased. The dose of spices incorporated in the products did not affect the phenolic compounds of the beef patties. The TBA values of the products increased upon heating. The higher TBA values of the cooked beef patties over the raw ones may be attributed to the effect of cooking which result in an increased heme compound, thus increasing free and lower molecular weight iron compounds that are hypothesized to be responsible for lipid oxidation[23]. Phosphorus, magnesium values and the total plate count of the locally formulated spices were significantly ($p<0.05$) higher than those of Bovida spices (Table 4). Also regardless of the dose of spices added to the meat, phosphorus and Magnesium contents and total plate count of the beef patties containing locally formulated spices were significantly ($p<0.05$) higher than those of the beef patties containing Bovida spices. an inverse trend was observed as far as calcium and iron contents were concerned. The results on mineral content of the beef patties were consistent with those recorded on the spice mixture. Streptococcus, salmonella and sulphites reducer bacteria were not present in any of the samples analysed. Differences observed in the total plate counts of the beef patties due to the type of spices used could be attributed to the differences in the phenolic contents of the spices. phenolic compounds were found to exert antimicrobial activity[7]. The results obtained from microbioloical analysis were within the limit for acceptable standard[24]. This indicates that the products are of good sanitary quality.

Consumer acceptability of the beef patties are presented in table 5. Neither the type, nor the dose of the spices used in the formulation of the beef patties significantly affects ($p>0.05$) juiciness, colour and tenderness of the products. At the same dose, the type of spices used had no significant effect ($p>0.05$) on flavour and overall acceptability. The lowest flavour scores were recorded for the beef patties containing 9 and 12g locally formulated spices Kg^{-1} ground meat. In the present study, the panellists could have been influenced by the poor flavour characteristics of the beef patties containing 9 and 12g locally formulated spices resulting in the low general acceptability rating of the corresponding products.

CONCLUSIONS

Results from the present study showed that the type and the doses of spices used had no significant effect ($p<0.05$) on the moisture, the lipid and the ash contents of the beef patties. The lipid oxidation as measure by the TBA values was significantly ($p<0.05$) affected by the type of spices. Organoleptically acceptable beef patties of a satisfactory microbiological standard can be produced using locally formulated spices.

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