STRATEGIES FOR THE DEVELOPMENT OF NOVEL AND FUNCTIONAL BEEF PATTIES TO REDUCE THE RISK OF HYPERTENSION.

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ABSTRACT: *In the functional examination of minced beef patties, estimation for total sodium contents was assessed to check the level of risk of hypertension along with proximate analysis. The minced beef patties were also treated with a chitosan-mint mixture @ 0.1% to minimize the presence of microorganisms, reduce oxidation, and to increase the shelf life of the product by acting as a natural antioxidant as well as antimicrobial agent results showed the moisture contents decreased in the final product from 65% in the raw minced beef to 60% in the final product, whereas protein contents were about 25%, with 15.5% to 16% of fat content, 0.6% ash contents and almost 3% crude fiber content with slight reduced Na^+ and Potassium contents essential to reduce the risk of hypertension.*

Keywords, Antimicrobial, Hypertension, Salt Replacement, Natural Antioxidant.

1. INTRODUCTION

The subject of meat functionality is as important as the consumers' interest in the meat they eat. It has been an established fact that relates meat especially beef to an increased risk for cardiovascular disease (CVD) and hypertension. Meat is a good medium for the multiplication of many microorganisms as it is high in moisture, nearly neutral in pH, and rich in sodium [6].

Product development and studies concerning the reduction of salt should address the scientific effects that it may have on such technological functions as water-holding capacity, fat binding, texture, sensory, stability, and shelf life. Hypertension is a major risk factor in the development of cardiovascular disease. The results of the DASH sodium study (Dietary Approaches to Stop Hypertension) showed a graded linear relation between salt intake and blood pressure [3].

Meat is relatively low in sodium, containing only 50-90 mg of sodium per 100g [18]. However, the sodium in meat derivatives is much higher because of the salt content, which can be as much as 2% in heat-treated products (e.g. sausages) and as much as 6% in uncooked cured products, in which drying (loss of moisture) increases the proportion even further [23].

There is convincing evidence that reducing the salt contents or its partial substitution with KCl in ground beef patties does not change its flavour [1]. On the other hand, adding chitosan and mint mixture to beef patties improves their shelf life without affecting the taste, color, and appearance of patties [8]. Meat is prone to both microbial and oxidative spoilage; therefore, is desirable to use a preservative with both antioxidant and antimicrobial properties [9].

Chitosan exhibits antimicrobial activity against a range of food-borne microorganisms especially Gram-positive bacteria and consequently has attracted attention as a potential natural food preservative [4]. Beef patties are a popular food in Pakistan in which minced beef and all other ingredients are interleaved by egg coating, followed by shallow frying. Patties are puffs or flaky masses with savoury filling such as minced meat. Ground beef used in patties can easily be modified as to the nature and proportion of ingredients. In this investigation processing conditions such as ingredient types and their quantity, boiling time, optimum frying time, and temperature conditions were determined by conducting a number of trials. The effects of using salt substitute i.e. (KCl) on the quality of patties were also studied. Different treatments of salt with lower quantity i.e. to ensure 2.4% salt or its partial replacement with KCl to decrease Na^+ contents, to decrease the risk of hypertension and cardiovascular disease, and chitosan-mint mixture at 0.1 % level was given to minimize the microbial contamination and to increase the shelf life of the product without any disturbance to consumer perception about the product.

2. MATERIALS AND METHODS

At the time of starting the study, fresh beef, oil/ghee, and gram pulse (daal chana)/starch were procured from the local market for the preparation of beef patties. All other ingredients essential for the preparation of the product such as eggs, soy sauce, onions, red chilies, salt (NaCl and KCl), spices, and chitosan-mint mixture as a natural antioxidant and antimicrobial agent, were procured from PCSIR Labs Karachi with a degree of deacetylation 85-90%. Further, all the preparatory operations were done, including deboning and mincing beef and boiling and mixing ingredients.

2.1. Chemical Analyses of Minced Beef and Beef Patties

Minced beef was tested for moisture, protein, fat, fiber, ash, and NFE as detailed below.

2.1.1. Moisture

Moisture contents of the minced beef and patties were determined by making a paste of the beef by grinding 2 g of the sample was then taken in an aluminum dish and kept in an oven at 105 ± 5 ^{0}C till constant weight was obtained, 950.46 [2].

2.1.2. Crude protein contents

The nitrogen contents of each beef sample and patties were determined by using Kjeldhal's method as described in, 928.08 [2]. The samples were digested in Kjeldhal's flasks with concentrated sulfuric acid in the presence of digestion mixture tablets for 3-4 hours. The ammonia trapped in sulfuric acid was liberated by adding 40 % NaOH through

distillation in a conical flask containing 4 % boric acid solution. To determine the nitrogen content in the samples, these were titrated against standard H_2SO_4 solution.

2.1.3. Crude fat contents

The crude fat contents were determined by taking 2 g dried sample of minced beef using petroleum ether as a solvent in a Soxhlet apparatus for about two hours according to the instructions of the manufacturer and the method given in, 960.39 [2].

Table 2.1. Formulations for Patties

ingredients	Patty A (T_1)	Patty B (T_2)	Patty C (T_3)	Patty D (T_4)	Patty E (T_5)
Salt	NaCl 2.4g & KCl 0.0g	NaCl 1.8g & KCl 0.6g	NaCl 1.2g & KCl 1.2g	NaCl 0.6g & KCl 1.8g	NaCl 0.0g & KCl 2.4g
Water	500ml	500ml	500ml	500ml	500ml
Egg (whole)	1	1	1	1	1
Minced beef	100g	100g	100g	100g	100g
Red chillies	3g	3g	3g	3g	3g
Spices	1.5g	1.5g	1.5g	1.5g	1.5g
Onions (prepared)	100g	100g	100g	100g	100g
Soy sause	5ml	5ml	5ml	5ml	5ml
Starch/Dal chana	100g	100g	100g	100g	100g
Chitosan-mint mixture	0.1ml	0.1ml	0.1ml	0.1ml	01ml
Oil or ghee	250ml	250ml	250ml	250ml	250ml

T1 = Standard beef patties (Control)
T2 =Beef patties with 75 % NaCl and 25 % KCl
T3 = Beef patties with 50 % NaCl and 50 % KCl
T4 = Beef patties with 25 % NaCl and 75 % KCl
T5 = Beef patties with 0 % NaCl and 100 % KCl

2.1.4.Crude fiber contents

The crude fiber contents were determined by taking a 2 g dried sample of minced beef, which was firstly defated, then digested in 1.25 % H_2SO_4, after filtration frequent washings with distilled water were given, then again the residues were digested with 1.25 % NaOH. After frequent washings, the material was ignited in a muffle furnace at 600ºC till grey or white ash. As according to the method given in [2].

2.1.5.Crude ash contents

Crude ash contents were determined by placing 2 g minced beef sample in the already weighed crucible and then it was ignited in a muffle furnace at 550-650ºC till white grey ash was obtained and ash contents were calculated as in, 900.02A or B [2].

2.1.6. Nitrogen-free extract (NFE)

NFE comprises of sugar, starch, and hemicellulose was determined by calculating the difference of moisture, crude protein, crude fat, crude fiber, and ash from 100. all the above-mentioned parameters were calculated, summed up, and then subtracted from 100 as described in [2] method.

2.2. Mineral Determination of Minced Beef and Beef Patties

2.2.1. Determination of Na contents

Na contents were determined by using 935.47 [2] method. A standard solution containing 10 mg Na per 100ml distilled water was sprayed on a flame after setting the galvanometer reading to zero using distilled water, and the galvanometer reading was noted. Then again the reading of the galvanometer was set to zero by using distilled water and then sample solution was sprayed and the reading was recorded.

2.2.2. Determination of K contents

1g sample was taken and was transferred into a 100 ml Kjeldhal's flask, then 20 ml conc. HNO_3 was added and the material was heated for 15-30 minutes. Then 5 ml $HClO_4$ added and the same was heated for 15-30 minutes till 1-2 ml remained. Then it was diluted to 100 ml. 5 ml of diluted sample was taken in a china dish and 20 ml distilled water and then 0.5 ml buffer solution (NH_4Cl 67.5 g in 300 ml distilled water +570 ml NH_4OH, and volume was made 1000 ml) was added. Then 4-5 drops of erichrome black-T was added and purple colour was recorded

Then the contents were titrated against N/100 = EDTA and the volume of EDTA was recorded and the amount of K in the beef sample was calculated

2.3. Determination of Peroxide Value of Beef and Beef Patties

Peroxide value was determined by the method of [2], and as modified by Watts *et al.,* (1991) by the 2-Thiobarbituric acid (TBA) method and the result was expressed in mg malonaldehyde Kg^{-1} of meat.

2.4. Sensory Evaluation

Patties were evaluated for appearance, colour, taste, tenderness, juiciness, hardness; beef flavour intensity and puffing characteristics. A trained panel of judges assessed these parameters on the basis of the 9-point hedonic scale [12].

2.5. Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance to the method described by [2].

3. RESULTS AND DISCUSSION

Process conditions, chemical analysis of minced beef and beef patties, mineral and antioxidant determination of fresh minced beef and beef patties, and results of sensory evaluation of patties are presented accordingly.

3.1. Proximate Analysis of Minced and Cooked beef

Minced beef used as the main ingredient for beef patties was tested for moisture, protein, fat, ash, crude fiber, and NFe contents. Results about these constituents appear in (Table 3.1). The analysis of fresh beef showed that average moisture, protein, fat, ash, crude fiber and Nfe contents are (65.0 %, 18.59 %, 15.50 %, 0.78 %, 2.50 % and 2.50 %) respectively and in cooked beef, these were 60.0 %, 18.59 %, 16.50 %, 2.50 %, 0.82 %, 2.70 % respectively. The composition of beef as 17.5 % protein 22.0 % fat, 0.60 % ash and 60.0 % water were reported by Potter *et al.*, (1995). Proximate composition of cooked beef as moisture 48.2 %, ash 0.9 %, protein 25.2 % and fat 24.2 % were described by Siddique (2000). Minced raw beef contains moisture 63.13 ± 2.99, protein 24.2 ± 0.72, lipids 7.08 ± 1.13, ash 2.71 ± 0.08, and carbohydrates 2.89 ± 0.23 were observed by [13].

Table 3.1. Proximate composition of minced beef

Constituents	Value %)
Uncooked minced beef	
Moisture	65.0
Protein	18.59
Fat	15.50
Fiber	2.50
Ash	0.78
NFE	2.50
Cooked minced beef	
Moisture	60.0
Protein	18.59
Fat	16.50
Fiber	2.50
Ash	0.82
NFE	2.70

3.2. Chemical Analyses of Minced Beef and Beef Patties

3.2.1. Moisture contents

Minced beef was analyzed for moisture content because it affects the quality of the final product. The average moisture contents found in minced beef and fried patties were (65% and 60%) respectively table (3.2). The loss of moisture was due to frying and storage at refrigeration temperature for taperiod of 15 days. Beef composition of moisture was reported as moisture 65% [5].

Table 3.2. Impact of treatments on moisture contents of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	61.20	59.63	59.43	59.33	59.30	59.23	59.16	59.61
	T_2	60.90	59.50	59.23	59.13	59.10	59.03	41.06	56.85
	T_3	59.63	59.00	58.50	58.36	58.26	58.30	58.63	58.67
	T_4	59.66	59.50	59.43	59.33	59.33	59.33	59.30	59.41
	T_5	60.83	59.93	59.66	59.56	59.53	59.56	59.53	59.80
Means		60.44	59.51	59.25	59.14	59.10	59.09	55.54	

Table 3.3. Impact of treatments on Crude Protein of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	18.56	18.58	18.58	18.58	18.58	18.58	18.58	18.58 b
	T_2	18.21	19.01	19.01	19.01	19.01	19.01	19.01	18.90 b
	T_3	18.69	18.23	18.23	18.23	18.23	18.23	18.23	18.30 b
	T_4	18.75	19.86	19.86	19.86	19.86	19.86	19.86	19.71 a
	T_5	18.22	18.76	18.76	18.76	18.76	18.76	18.76	18.68 b
Means		18.48	18.89	18.89	18.89	18.89	18.89	18.89	

LSD (T) = 0.5560

3.2.2. Crude protein contents

It was reported that the protein contents of the minced beef patties were as 17.5% [15], whereas, the protein contents of beef patties were 25.2% [21]. The results of this investigation are similar to those reported in the literature. The data obtained about the protein contents is given in Table 3.3.

3.2.3. Fat contents

The analysis of fresh minced beef and patties showed that the fat contents were 15.5% and 16.5% respectively [11] observed that frying slightly reduces the availability of certain amino acids; therefore severe heat processing should be avoided. Some loss of fat also occurred as it went into the gravy. Fat contents were as pointed at 9.78 % [5]. The effects of cooking on the fat content of beef were determined by [7]. The fat level of raw meat was linearly related to that of cooked meat. an equation was given for calculating fat contents (Y) after cooking from the fat content of raw meat (X) i.e. Y = 0.197 0.854(X), where fat content is expressed as g/100g raw meat. The data obtained statistically was highly significant for treatments and storage time but was

nonsignificant for interaction between treatments and storage time Table 3.4.

3.2.4. Ash contents

It was described comprehensively by [15] that the ash contents in beef patties were 0.60% and as described by [21] 0.9%. It was reported that ash contents in beef patties were 2.7% [13]. Further, it was reported that ash contents of beef were estimated at 0.85% [5]. The results were similar to described in the literature. The data obtained in Table 3.5 statistically was found highly significant for storage time and nonsignificant for both treatments and their interaction with storage time. The results were found similar as described in the literature

Table 3.4. Impact of treatments on fat contents of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	15.36	16.43	16.43	16.43	16.43	16.43	16.43	15.29 b
	T_2	15.26	16.53	16.53	16.53	16.53	16.53	16.53	16.40 a
	T_3	15.26	16.33	16.33	16.33	16.33	16.33	16.33	16.40 a
	T_4	15.36	16.20	16.20	16.20	16.20	16.20	16.20	16.40 a
	T_5	15.16	16.50	16.50	16.50	16.50	16.50	16.50	16.40 a
Means		15.29 b	16.40 a	16.40 a	16.40 a	16.40 a	16.40 a	16.40 a	

LSD (T) = 0.1510, LSD (I) = 0.1510

Table 3.5. Impact of treatments on Ash contents of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	0.80	0.83	0.83	0.83	0.83	0.83	0.83	0.83
	T_2	0.77	0.83	0.83	0.83	0.83	0.83	0.83	0.82
	T_3	0.81	0.86	0.86	0.86	0.86	0.86	0.86	0.85
	T_4	0.77	0.86	0.86	0.86	0.86	0.86	0.86	0.84
	T_5	0.81	0.85	0.85	0.85	0.85	0.85	0.85	0.84
Means		0.79 b	0.85 a	0.85 a	0.85 a	0.85 a	0.85 a	0.85 a	

LSD (I) = 0.03257

3.2.5. Crude fiber contents

It was described that the fiber contents of beef patties were found 3% [15]. Later, it was observed fiber contents were 2.8% [14]. The results found were similar to described in the literature. Data obtained in Table 3.6 statistically was found highly significant for treatments and nonsignificant for both storage time and their interaction with treatments. The results were found similar as described in the literature.

3.2.6. NFE contents

It was described comprehensively that the NFE contents in beef patties were 3.0% [15] and were in accordance as described by [21] being 3.8%. Later, it was suggested by [13] that NFE contents in beef patties were 2%. Data obtained in Table 3.7 statistically was found highly significant for treatments and storage time but nonsignificant for the interaction of treatments and storage time. The results were found similar as described in the literature.

3.3. Minerals Determination of Minced Beef and Beef Patties

3.3.1. Sodium contents

Due to the role of sodium in the development of hypertension in sodium-sensitive individuals, public health and regulatory authorities have recommended a reduced dietary intake of sodium chloride. However, intake still exceeds the nutritional recommendations in many countries [20]. Meat itself contains sodium but the amount is less than 100mg Na^+ per 100g. The main source of sodium in meat products is sodium chloride which is added during processing. Sodium chloride contains 39.3% sodium as described by [9]. The data obtained in Table 3.8 statistically was found highly significant for storage time, treatments and interaction between treatments and storage time. The results were found similar as described in the literature.

3.3.2. Potassium contents

Research indicates that 25-40% replacement appears to be the range at which the flavour impact is not as noticeable. As the flavour intensity of some flavour increases such as salty, acidic or spice, a higher proportion of KCl may be acceptable [16]. A process has been developed to produce a low-sodium cured meat product by injecting the meat with a brine containing KCl in combination with calcium citrate, calcium lactate, lactose, dextrose, potassium phosphate, ascorbic acid, and sodium nitrite [17]. The use of sodium or potassium lactate with a corresponding reduction in NaCl tends to maintain certain saltiness while reducing the sodium content in products to some degree [16]. The data obtained in Table 3.9 statistically was found highly significant for storage time, treatments, and interaction between treatments and storage time. The results were found similar as described in the literature.

Table 3.6. Impact of treatments on crude fiber contents of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	2.33	2.36	2.36	2.36	2.36	2.36	2.36	2.36 b
	T_2	1.83	3.06	3.06	3.06	3.06	3.06	3.06	2.89 a
	T_3	1.96	2.50	2.50	2.50	2.50	2.50	2.50	2.42 b
	T_4	2.00	2.23	2.23	2.23	2.23	2.23	2.23	2.20 bc
	T_5	2.50	1.86	1.86	1.86	1.86	1.86	1.86	1.96 c
Means		2.13	2.41	2.41	2.41	2.41	2.41	2.41	

LSD (T) = 0.3779

3.4. Per Oxide Value

Peroxide value was increased with the passage of time during storage study ranging from 0.12 to 0.27mg malonaldehyde per 100g of meat [22] and storage time but nonsignificant for the interaction of treatments and storage time. The results were found similar as described in the literature.

Table 3.7. Impact of treatments on NFE of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	2.04	1.93	1.93	1.93	1.93	1.93	1.93	1.95 bc
	T_2	2.62	1.44	1.44	1.44	1.44	1.44	1.44	1.61 c
	T_3	5.22	2.45	2.45	2.45	2.45	2.45	2.45	2.85 a
	T_4	4.12	1.66	1.66	1.66	1.66	1.66	1.66	2.02 bc
	T_5	2.52	2.22	2.22	2.22	2.22	2.22	2.22	2.27 ab
Means		3.31 a	1.94 b	1.94 b	1.94 b	1.94 b	1.94 b	1.94 b	

LSD (T) = 0.5999, LSD (I) = 0.7098

Table 3.8. Comparison of mean values of Na mg/100 g of beef

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	67.33 e	960.3 a	960.3 a	960.3 a	960.3 a	960.3 a	960.3 a	832.8 a
	T_2	65.33 e	725.7 b	725.7 b	725.7 b	725.7 b	725.7 b	725.7 b	631.3 b
	T_3	67.67 e	483.3 c	483.3 c	483.3 c	483.3 c	483.3 c	483.3 c	424.0 c
	T_4	67.33 e	240.7 d	240.7 d	240.7 d	240.7 d	240.7 d	240.7 d	215.9 d
	T_5	68.67 e	68.00 e	68.00 e	68.00 e	68.00 e	68.00 e	68.00 e	68.1 e
Means		67.27 b	495.6 a	495.6 a	495.6 a	495.6 a	495.6 a	495.6 a	

LSD (T) = 2.487and LSD (I) = 2.943, LSD (T x I) = 6.581

Table 3.9. Impact of treatments on K mg/100 g of beef of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	30.00 e	29.67 e	29.67 e	29.67 e	29.67 e	122.0cde	122.0cde	56.10 e
	T_2	29.33 e	101.7de	101.7de	101.7de	101.7de	101.7 de	101.7de	91.33 d
	T_3	30.67 e	203.3cd	203.3cd	203.3cd	203.3cd	203.3 cd	203.3cd	178.7 c
	T_4	30.33 e	306.0 b	306.0 b	306.0 b	306.0 b	213.7 bc	213.7bc	240.2 b
	T_5	29.67 e	408.7 a	408.7 a	408.7 a	408.7 a	408.7 a	408.7 a	354.5 a
Means		30.00 b	209.9 a	209.9 a	209.9 a	209.9 a	209.9 a	209.9 a	

LSD (T) = 33.22, LSD (I) = 39.31, LSD (T x I) = 87.90

Table 3.10. Impact of treatments on per oxide value of beef as affected by storage time

		Time interval (I)							Means
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	
Treatments	T_1	0.12	0.17	0.19	0.21	0.23	0.25	0.26	0.21 a
	T_2	0.14	0.17	0.19	0.21	0.23	0.24	0.27	0.21 a
	T_3	0.13	0.17	0.18	0.21	0.22	0.24	0.25	0.20 b
	T_4	0.12	0.14	0.17	0.20	0.21	0.24	0.26	0.20 d
	T_5	0.13	0.15	0.16	0.19	0.21	0.24	0.28	0.20 c
Means		0.13 g	0.16 f	0.18 e	0.20 d	0.22 c	0.24 b	0.27 a	

LSD (T) = 0.00195
LSD (I) = 0.00230

4, CONCLUSION

Meat and meat products enjoy a unique status in the human diet but meat also serves as an excellent substrate for the growth of most microorganisms and is associated with hypertension and cardiovascular disease due to the presence of high sodium and fat contents. It is therefore concluded that the partial replacement of NaCl with KCl showed satisfactory results and further improved trials in industry or animal study is required. Shallow frying followed by a chitosan-mint mixture during the study also showed satisfactory results. Results for total Na and K contents indicated that these varied from sample to sample because of the addition of NaCl and KCl to the formulation in 25% increments ranging from 0% to 100% in five treatments. The noted range was from 63 mg to 890 mg for Na and 29mg to 409 mg for K contents respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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