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DEVELOPMENT AND EVALUATION OF BEEF SAUSAGES USING DIFFERENT FAT SOURCES, SHELF STABLE AT AMBIENT TEMPERATURE

Muhammad Toheed Akbar[1*], Kashif Nauman[1], Shahid Mehmood[2], Bilal Asghar[1],
[1]Department of Meat Science & Technology, University of Veterinary and Animal Sciences, Lahore; [2]Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore

[*]Corresponding author:-

The study was undertaken to find out the effects of beef kidney fat, sheep tail fat, palm kernel butter fat, storage temperature and storage duration on the thiobarbituric acid values (TBARs), total plate count (TVC), cooking loss, water activity (A_w) and color values of the sausages made from buff calf (*Bubalus bubalis*). Sausages were stored at chilling 2±2˚C and ambient temperature 35±2˚C for 11 days. Shelf stable beef sausages were developed employing hurdle technology. Hurdles incorporated were reduced water activity, saturated fats incorporation, ascorbic acid, phosphates and nitrite addition, respectively. Spraying the sausages prior to cooking with 5% potassium ascorbate solution was also investigated. Hurdle treatment collectively increased the shelf life stability whereas quality deterioration was significantly reduced during storage. Lowest lightness (L*) values were recorded in animal based fat sausages. The redness (a*) values increased during the storage whilst greatest increase was observed during storage at ambient temperature. Highest TBARs, TVC values were determined for sheep tail fat sausages (STF). STF sausages had lowest cooking loss. During storage at ambient temperature various physico-chemical features namely TBARs, TVC, color and A_w differed significantly. Ambient temperature stored samples markedly decreased in quality characteristics throughout the storage duration. Irrespective of the treatments, TBARs, TVC, color significantly increased whilst A_w values decreased during storage period. On the 7th day, sausages placed at ambient temperature become unacceptable resulting in spoilage whereas threshold limits exceeded on the 11th day of trial in case of sausages stored at chilling temperature.

Keywords: KPH fat, sheep tail fat, palm kernel butter fat, beef sausages, shelf stable

Livestock is backbone of socio-economic development of Pakistan by having 58.9% contribution in the agriculture and 11.1% in the national GDP. Meat sector is amongst one of the most vibrant section of livestock sector still growing with a great pace e.g. 5.25% since the period 2017-18 with estimated production of 4,262 thousand tons of meat (Anonymous, 2018). Meat and meat products are enriched with essential nutrients including proteins, fatty acids, minerals and vitamins, important for the optimal growth of body tissues (López-López et al., 2011). In Pakistan, the food consumption pattern has endured massive changes over the last two decades (Aziz & Shahnawaz, 2005). A rapid development of urban areas of Pakistan has been observed in the recent past which has indicated the ever increasing demand of the value added products particularly meat products (Anonymous, 2018).

Demands for food supply, food preferences, handling of food are altered by the change in life styles of major portion of the human population worldwide and a complete transformation in structure of households (Duchin, 2005). This prompt increase in consumption of several meat and meat products is linked with upturn interest of people in urbanization having more disposable money, no adequate time availability for conventional cooking ultimately diversity in diet desires of human (Fessler and Navarrete, 2003). It has lead towards value addition in fresh, chilled meat and meat products through presenting quality

cuts, yield grading and the introduction of novel products into the local market.

However, the processed meat sector is facing major problem in many segments regarding cold supply chain components particularly chilled transportation and storage i.e. freezers and reefers are required for the efficient distribution of perishable meat products (Thomas, Anjaneyulu, & Kondaiah, 2008a). Meat products are discarded in significant amounts at various levels of supply chain and major portion of this loss is due to microbial spoilage (Dave & Ghaly, 2011). Meat and meat products are highly perishable commodities require refrigeration for extended shelf life during marketing and storage though a significant amount of energy input needed to cater the loss generated by discarding these products (Ahmad and Srivastava, 2007). Meat safety occupies a central position in consumer minds now-a-days with pivotal importance in food alarms of current era throughout the world. Occurrence of pathogenic bacteria causing spoilage of food is a worldwide growing issue resulting in meat quality and safety issues today.

Value addition in mutton and beef meat by the introduction of innovative and novel products is the utmost demand to fulfill energy needs, protein requirement as well as traditional hunger desires associated with the new pattern of meat consumption. Currently only frozen chicken meat products are frequently available and those require proper cold chains like freezers and cold trucks. Consequently, this scenario must be accounted one way or the other.

Development of value added meat products is an emerging era somehow economical and a profitable sector worldwide particularly in Pakistan. Besides of this robust growth and potential, lack of knowledge regarding value addition of meat and meat products causes major hindrance towards meat sector development at national level. Moreover, due to these circumstances improvement and further potential has been indicated to cater various small issues and threats posed by the saturation of production and export of only chilled or fresh meat (Rehman, Jingdong, Chandio and Hussain, 2017). These challenges are expected to continue at lead of social concerns if not encountered effectively through various methods (Sofos, 2008).

This has emanated the formation of meat products that are safe, healthy and reliable like sausages, can be developed by utilizing KPH fat from buffalo, sheep tail fat and palm kernel butter fat ensuring authenticity for consumption especially among different communities which loathe pork.

This proposed study was designed to determine the shelf life stability of sausages which were prepared from buffalo calf meat by using three different fat sources, stored for 11 days at two different temperatures, evaluated on each periodic interval.

MATERIALS AND METHODS

The study was conducted at meat processing facility located at the department of Meat Science and Technology, University of Veterinary and Animal Sciences (UVAS), Lahore. Animals were slaughtered according to Halal slaughtering method PS 3733:2016. The carcasses were chilled overnight in a walk-in chiller at 0˚C-4˚C. 24 hours post slaughter the carcasses were deboned and meat from brisket region (deep pectoral muscle) was obtained. Components of the recipe mentioned in table 1, were purchased from the local market. Spice mixture was procured from Gevurzmuller Company (Germany). While non-edible collagen based, halal certified 32mm casings were purchased from Shen Guan (China).

Table 1: Composition

Ingredients	Quantity (grams)
Meat	2000
Lean meat	700
Fat	320
Ice	100
Spices	18
Nitrite-NaCl (0.5%)	70
Ascorbic acid	1.5
Phosphate	10

NOTE: NaCl: Sodium Chloride

Buffalo (KPH fat), mutton (sheep tail fat) and plant (palm kernel butter fat) were used for the development of sausages. 2kg meat of buffalo calve from brisket region (deep pectoral muscle) was used. 1 kg meat and

320g fat was minced in meat mincer (Dynasty, Model No. HL-G22S, Taiwan) and placed in chiller at 4˚C for 2 hours (portion 1). Remaining 1 kg meat was firstly minced. Premix was added following mixing in the mixer for one minute (C-EMM 30, Omega Group, Italy). Batter was placed in chiller at 4˚C for about 2 hours to provide curing time (portion 2). Then the first portion of mince was placed in bowl chopper for chopping (DMS - Machinesysteme, DMK 20, Germany). Half of the ice was added during this procedure.

When the first portion was homogenized then portion 2 along with lean meat mince was added. The remaining ice was added to prevent any rise in temperature of the batter. Chopping was done until homogenization to ensure even distribution of ingredients. After batter formation it was packed in bag and placed in chiller for 24 hours for providing curing time. Meat batter was put into the vacuum stuffer (DMS-Machinesysteme, DF 250, Germany) for stuffing into the casing. Before stuffing, artificial collaged based casing was prepared for stuffing by dipping it in water for 15 minutes.

After stuffing, ends of the casings were tied using food grade thread. The sausages were then cooked in a hot air oven operating at 85 ˚C for 90 mins to attain internal core temperature of 75˚C to maximize food safety aspect of a particular product(Seo, Yum, Kim, Jeong, & Yang, 2016). Now, sausages were allowed to cool down. Sausages were then sprayed with 5% potassium sorbate solution using filtered water and then allowed to dry out at room temperature for 15 minutes in order to limit mold and fungal growth (Thomas, Anjaneyulu, & Kondaiah, 2008b) For each fat source, same procedure was followed and three batches were made.

Half of the samples were placed in incubator to mimic high ambient temperature at 35 ˚C while remaining samples were placed in chiller to maintain chilling temperature 2 ˚C. On the 1st, 3^{rd,} 5th, 7th, 9th and 11th all the parameters were measured.

Parameters studied

Color

For measurement of color, the sausages were cut longitudinally and calorimeter was placed on the center of the samples. Observations were recorded from three samples per treatment selected randomly. The calorimeter was calibrated each time before recording the observations with a standard white tile. The color of both chilled and incubator stored sausage samples was measured by D65 angle for calorimeter on 1st, 3^{rd,} 5th, 7th, 9th and 11th day whilst color reading data recording included lightness (L*), redness (a*), yellowness (b*), Chroma (c) and Hue (h) values using colorimeter (Konica Minolta® CR-410, Japan).

Cooking loss

The weight of raw and cooked sausages samples whole batch was recorded for the determination of cooking loss (Candogan & Kolsarici, 2003). Cooking loss was determined using this formula:

$$\text{Cooking loss} = \frac{\text{Weight before cooking} - \text{weight after cooking}}{\text{Weight before cooking}} \times 100$$

Water activity

For the determination of water activity of the samples, 2g grounded sample from each treatment was placed inside the water activity meter and readings were noticed after the Aw meter shows stable sign. Readings were noted down on apparatus Aqua lab 4TE, Decagon, USA. Observations were recorded for both temperature stored samples on the 1st, 3^{rd,} 5th, 7th, 9th and 11th day of storage, mean of three readings were taken.

Thiobarbituric Acid Reactive Substances (TBARS)

Readings were obtained on the 1st, 3^{rd,} 5th, 7th, 9th and 11th day of trial by using a 2-TBA reactive substances (TBARS) method. 2 grams of each sample was weighed through an analytical weighing balance and poured into 50ml falcon tube. 50 µL of butylated hydroxytoluene (7.2%) was added into the tube along with 15ml of double distilled water and then homogenized using a Polytron homogenizer (type PT 10/35, Brinkman Instruments Inc.) for 20-30 seconds at max speed. When the sample was homogenized then 1ml of sausage sample homogenate was transferred to a disposable test tube (13 × 100 mm) along with the 2ml solution mixture of TBA/TCA. The mixture was then mixed thoroughly using vortex. After this tubes were incubated in a boiling water bath

operating at 90°C for 15 min to develop color. Then samples were allowed to cool down in ice water for 10 min. Test tubes were vortex mixed again and then centrifuged for 15 min at 2000 rpm at 4°C (Ohkawa, Ohishi and Yagi, 1979).

The absorbance of the resulting supernatant solution was determined at 531 nm in spectrophotometer against a blank containing 1 mL of deionized distilled water and 2 mL of TBA/TCA solution. The amounts of TBARS were expressed as milligrams of malondialdehyde (MDA) per kilogram of meat. A standard curve was constructed and TBARS value of the samples were calculated using that curve. Calculation was made as mentioned below:

TBARS value (mg MDA/kg of meat) = 4 * absorbance at 531nm

Total Viable Count (TVC)

1 gram sample was cut into thin particles, weighed and poured into the 0.9% normal saline solution gently shaked through vortex mixer to prepare a dilution of 10^{-1}. Using separate tips for each dilution 10 fold dilutions of 10^{-2}, 10^{-3}, 10^{-4} up to 10^{-5} were prepared from sausage sample homogenate by transferring 1 ml of previous dilution into the 9 ml of next diluent and 1 ml was discarded from the last dilution made. All dilutions were manually shaked for 12 seconds. 2ml from three dilutions i.e. no. 3,4 and 5 was poured onto agar medium into appropiately labelled petri dishes with the help of micropipette using different tips for each dilution (APHA, 1992).

Immediately spreaded the dilutions on the plate with the help of glass spreader which was dipped in alcohol solution and flamed on a spirit lamp. After that petri plates were inverted and promptly placed in incubator for 48 ± 2 hour at 35˚C. Plates of each dilution having 30-300 colonies were counted.

CFU per gram was calculated by using the following formula:

$$N = \frac{No.\,of\ Colony\ count}{volume\ usedm\ in\ ml} * Dilution\ Factor$$

N = No. of CFU per gram of the sample

Statistical Analysis

The data was analyzed through factorial ANOVA, assuming fat source as main effect and storage temperature and duration as sub-effects and their interaction was also checked. PROC GLM was employed in SAS software (Version, 9.1). Significant treatment means were separated through Duncan's Multiple Range test at $P \leq 0.05$ probability level. Following mathematical model was used:

$Y_{ijkl} = \mu + F_i + T_j + D_k + (F\times T)_{ij} + (F\times D)_{ik} + (T\times D)_{jk} + (F\times T\times D)_{ijk} + \varepsilon_{ijkl}$

Where,

Y_{ijkl} = Observation of dependent variable recorded on i^{th} fat source, j^{th} storage temperature and k^{th} storage duration

μ = Overall population mean

$(F\times T\times D)_{ijk}$ = Interaction among fat source, storage temperature and storage duration

RESULTS AND DISCUSSION

Color

Sausages made from palm kernel butter fat (PKBF) were lighter than sausage made from remaining fat sources. These findings are consistent with the findings of Dzudie, (2005) who reported more lightness (L*) values in sausages made from hydrogenated sunflower oil than sausages made from other fat sources like pork fat, beef fat and mutton fat. This might be associated to the degree of fat dispersion in the sausages. Fat present in the PKBF sausages was more finely broken up than the sausages prepared from animal fats thus showing a greater impression of lightness. Likewise, higher L* values were observed in case of chicken frankfurters formulated with palm oil (Tan, S., Aminah, Mohd, Atil and Babji, 2001).

Irrespective of the treatments, the results have shown that lightness (L*), yellowness (b*) and hue (h) instrumentally decreased whereas redness (a*) and Chroma (c) values slightly increased throughout the experiment. Samples stored at 35°C showed more pronounced instrumental red color than the samples stored at 2°C, although that was visually imperceptible. Furthermore, samples placed at 35°C showed higher a* but lower L* and b* values than the samples stored at 2°C. The results narrated that with the advancement in the storage duration i.e. moving towards 11^{th} day, the sausages instrumental color changed to a darker color. These findings are in accordance with the results of (Cavalheiro, Piovesan, Terra,

Table 2: Influence of different fat sources, storage temperature and storage duration on CIE instrumental CIE color attributes of cooked beef sausages.

Fat Source	Storage Temp	Storage Duration	L*	a*	b*	c	h
KPH fat	2°C	1	52.86^{c}±0.05	17.83^{op}±0.02	10.81^{de}±0.04	20.85^{l}±0.00	31.23^{b}±0.23
		3	51.78^{de}±0.01	17.91^{o}±0.02	10.69^{fg}±0.00	20.86^{l}±0.01	30.82^{c}±0.04
		5	51.57^{def}±0.05	18.72^{kl}±0.00	10.62^{ghi}±0.03	21.52^{g}±0.01	29.55^{ef}±0.06
		7	50.99^{fgh}±0.02	18.84^{hijk}±0.01	10.43^{jk}±0.04	21.53^{g}±0.02	28.96^{hijk}±0.11
		9	50.48^{hij}±0.09	18.95^{efgh}±0.04	10.28^{l}±0.04	21.55^{fg}±0.05	28.47^{lm}±0.04
		11	50.09^{ij}±0.04	19.17^{c}±0.03	10.22^{lm}±0.05	21.72^{de}±0.00	28.07^{no}±0.15
	35°C	1	52.74^{c}±0.36	18.60^{mn}±0.01	11.85^{a}±0.04	22.05^{b}±0.02	32.51^{a}±0.09
		3	51.41^{def}±0.20	18.79^{ijkl}±0.03	11.01^{c}±0.02	21.78^{cd}±0.01	30.36^{d}±0.10
		5	50.41^{hij}±0.33	19.18^{c}±0.01	10.50^{ij}±0.05	21.87^{c}±0.03	28.70^{klm}±0.10
		7	49.02^{l}±0.01	19.69^{b}±0.04	10.13^{mn}±0.02	22.14^{a}±0.03	27.22^{p}±0.09
		9	-	-	-	-	-
		11	-	-	-	-	-
Palm Kernel Butter Fat	2°C	1	54.76^{a}±0.02	17.28^{q}±0.01	11.15^{b}±0.05	20.56^{m}±0.02	32.84^{a}±0.12
		3	54.54^{a}±0.02	18.48^{n}±0.01	10.74^{efg}±0.04	21.37^{ijk}±0.03	30.16^{d}±0.10
		5	53.63^{b}±0.48	18.59^{mn}±0.01	10.44^{jk}±0.02	21.32^{jk}±0.01	29.32^{efgh}±0.05
		7	53.68^{b}±0.29	18.74^{jkl}±0.03	10.34^{kl}±0.00	21.40^{hij}±0.03	28.88^{ijkl}±0.03
		9	52.94^{c}±0.05	18.98^{efg}±0.01	10.24^{lm}±0.01	21.56^{fg}±0.01	28.35^{mn}±0.04
		11	52.75^{c}±0.04	19.12^{cd}±0.03	10.13^{mn}±0.00	21.63^{ef}±0.02	27.92^{o}±0.03
	35°C	1	51.84^{de}±0.19	18.53^{n}±0.06	10.88^{d}±0.02	21.49^{gh}±0.06	30.41^{d}±0.05
		3	51.55^{def}±0.05	18.86^{ghij}±0.02	10.62^{ghi}±0.05	21.64^{ef}±0.04	29.37^{efg}±0.10
		5	51.25^{efg}±0.23	19.12^{cd}±0.01	10.44^{jk}±0.03	21.78^{cd}±0.01	28.64^{lmk}±0.08
		7	49.39^{kl}±0.07	19.81^{ab}±0.04	10.06^{no}±0.04	22.21^{a}±0.01	26.93^{pq}±0.13
		9	-	-	-	-	-
		11	-	-	-	-	-
Sheep tail fat	2°C	1	52.80^{c}±0.08	17.78^{p}±0.10	10.78^{def}±0.05	20.79^{l}±0.06	31.22^{b}±0.28
		3	51.90^{d}±0.09	18.67^{lm}±0.01	10.64^{gh}±0.04	21.49^{gh}±0.03	29.67^{e}±0.09
		5	51.52^{def}±0.02	18.50^{n}±0.02	10.51^{hij}±0.01	21.28^{k}±0.02	29.60^{ef}±0.00
		7	50.94^{fgh}±0.12	18.73^{kl}±0.04	10.48^{j}±0.00	21.46^{ghi}±0.03	29.22^{fghi}±0.06
		9	50.57^{hi}±0.08	18.91^{fghi}±0.05	10.26^{l}±0.09	21.51^{g}±0.00	28.48^{lm}±0.27
		11	50.26^{ij}±0.00	19.04^{def}±0.11	10.09^{n}±0.02	21.54^{fg}±0.08	27.93^{o}±0.18
	35°C	1	53.36^{bc}±0.58	18.57^{mn}±0.04	10.84^{de}±0.07	21.50^{gh}±0.01	30.28^{d}±0.21
		3	51.65^{de}±0.04	18.94^{efgh}±0.03	10.56^{hij}±0.02	21.68^{de}±0.02	29.13^{ghij}±0.07
		5	50.71^{ghi}±0.23	19.06^{cde}±0.03	10.48^{j}±0.01	21.75^{d}±0.02	28.80^{jkl}±0.06
		7	49.89^{kj}±0.07	19.86^{a}±0.09	9.95^{o}±0.06	22.21^{a}±0.05	26.62^{q}±0.24
		9	-	-	-	-	-
		11	-	-	-	-	-

Note: Superscripts on different means within column differ significantly ($P \leq 0.05$);

Lovato, Terra and Fries, 2013) according to which a* values were increased whereas b* values were decreased at the end of experiment. This trend i.e. reduced b* values could be possibly due to the oxygen consumption by the microorganism during their exponential growth phase thus decreasing the oxymyoglobin content. In another study, conducted by (kaminek, 2002) it was noted that the sausages stored at a temperature of 15°C had a higher proportion of red color and a lower proportion of yellow

Table 3: Influence of different fat sources, storage temperature and storage duration on the TBARs, TVC and Water Activity of cooked beef sausages

Fat Source	Storage Temp	Storage Duration	TBARs	TVC	Water Activity
KPH fat	2°C	1	$0.25^{p}\pm0.01$	$4.04^{pq}\pm0.00$	$0.97^{ab}\pm0.01$
		3	$0.62^{mn}\pm0.02$	$4.24^{no}\pm0.24$	$0.96^{abc}\pm0.00$
		5	$0.83^{l}\pm0.01$	$4.48^{lm}\pm0.01$	$0.96^{bcd}\pm0.01$
		7	$1.15^{ij}\pm0.03$	$4.90^{hi}\pm0.01$	$0.95^{cde}\pm0.01$
		9	$1.41^{fg}\pm0.02$	$6.41^{c}\pm0.00$	$0.95^{cde}\pm0.01$
		11	$1.73^{cd}\pm0.06$	$7.20^{b}\pm0.02$	$0.94^{de}\pm0.00$
	35°C	1	$0.40^{op}\pm0.00$	$4.22^{no}\pm0.18$	$0.94^{de}\pm0.00$
		3	$1.23^{hi}\pm0.01$	$4.98^{gh}\pm0.01$	$0.93^{gf}\pm0.01$
		5	$1.62^{de}\pm0.11$	$5.28^{f}\pm0.01$	$0.91^{hi}\pm0.01$
		7	$2.08^{b}\pm0.02$	$6.37^{dc}\pm0.01$	$0.90^{i}\pm0.01$
		9	-	-	-
		11	-	-	-
Palm Kernel Butter Fat	2°C	1	$0.22^{p}\pm0.01$	$3.98^{q}\pm0.02$	$0.97^{a}\pm0.00$
		3	$0.47^{no}\pm0.00$	$4.17^{op}\pm0.06$	$0.96^{abc}\pm0.00$
		5	$0.85^{kl}\pm0.05$	$4.81^{ij}\pm0.07$	$0.96^{bcd}\pm0.01$
		7	$1.15^{ij}\pm0.02$	$4.93^{hi}\pm0.01$	$0.95^{cde}\pm0.01$
		9	$1.58^{def}\pm0.05$	$5.11^{g}\pm0.02$	$0.96^{bcd}\pm0.01$
		11	$1.92^{b}\pm0.07$	$6.25^{de}\pm0.01$	$0.95^{cde}\pm0.00$
	35°C	1	$0.38^{op}\pm0.02$	$4.57^{kl}\pm0.01$	$0.95^{cde}\pm0.00$
		3	$0.61^{mn}\pm0.09$	$4.96^{h}\pm0.08$	$0.95^{cde}\pm0.01$
		5	$1.07^{ij}\pm0.10$	$5.37^{f}\pm0.01$	$0.95^{cde}\pm0.01$
		7	$1.57^{def}\pm0.04$	$6.29^{cde}\pm0.02$	$0.93^{gf}\pm0.01$
		9	-	-	-
		11	-	-	-
Sheep tail fat	2°C	1	$0.62^{mn}\pm0.03$	$4.00^{q}\pm0.00$	$0.96^{abc}\pm0.00$
		3	$0.86^{kl}\pm0.13$	$4.47^{lm}\pm0.05$	$0.96^{bcd}\pm0.01$
		5	$1.38^{gh}\pm0.06$	$4.68^{jk}\pm0.03$	$0.96^{bcd}\pm0.01$
		7	$1.51^{efg}\pm0.07$	$5.10^{g}\pm0.01$	$0.95^{cde}\pm0.01$
		9	$1.90^{bc}\pm0.00$	$6.28^{cde}\pm0.01$	$0.95^{cde}\pm0.01$
		11	$2.32^{a}\pm0.06$	$7.45^{a}\pm0.00$	$0.94^{de}\pm0.00$
	35°C	1	$0.70^{lm}\pm0.00$	$4.36^{mn}\pm0.08$	$0.95^{cde}\pm0.00$
		3	$1.02^{jk}\pm0.10$	$5.13^{g}\pm0.05$	$0.95^{cde}\pm0.01$
		5	$1.73^{cd}\pm0.12$	$6.22^{e}\pm0.03$	$0.94^{ef}\pm0.00$
		7	$2.44^{a}\pm0.03$	$6.35^{cde}\pm0.00$	$0.92^{gh}\pm0.01$
		9	-	-	-
		11	-	-	-

Note: Superscripts on different means within column differ significantly ($P \leq 0.05$);

color in comparison to the sausages stored at 5°C.

Kaminek, (2002) further concluded that sausages darken in course of the maturing process; a fall in L* value and b* occurred in the course of storage but an increases in a*value. Similarly, (Guo, Liu and Chen, 2003) investigated the color stability of Chinese sausages by studying the effect of starter cultures on them. Likewise, it was reported that the L* and b* values of all sausages decreased but the a* values increased with the progress in storage time. Furthermore, it was determined that main factor effecting color development and stability was processing temperature as well as storage temperature. This color change was probably due to the oxidation of red

oxymyoglobin which was converted into brown metmyoglobin, these chemical changes generally proceeds parallel to rancidity (Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard and Enser, 2004). According to Soyer and Ertas, (2007) only redness values were affected by storage time and the fat content.

Thiobarbituric acid reactive substances (TBARs) assay

The present study revealed highly significant ($P \leq 0.05$) effects on TBARs amongst the various types of fat sources. Sheep tail fat sausages exhibited greater TBARs values whereas lowest values were observed in case of sausages that were made from Palm kernel butter fat whilst sausages prepared from KPH fat showed intermediate values. This shows the probability of Sheep tail fat as more prone to lipid oxidation. This could be associated to the amount of unsaturated fatty acids (USFA) present in a particular fat (Dzudie, Scher, Tchiegang and Hardy, 2005). Moreover, order of unsaturation was sheep tail fat > beef KPH > palm kernel butter fat. Similarly Aksu, (2005) determined more unsaturated fatty acids in sheep tail fat than in beef kidney fat. Hence more oxidation changes could occur in sheep tail fat > KPH fat > palm kernel butter fat merely due to the amount of USFA present respectively. Unsaturated fatty acids with double or multiple bonds tend to oxidize at much higher pace thus greater USFA more accelerated would be the lipid oxidation (Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard and Enser, 2004). In this experiment, results have revealed that storage duration showed significant effects ($P \leq 0.05$) on the thiobarbituric acid reactive substances in beef sausages. These findings are in accordance with the results of Naveena and Kiran (2014) who concluded that the TBA values of duck meat sausages increased significantly as storage progressed to 14 days so unsaturated fatty acids may undergo oxidative changes with the advancement of storage period. This increment in the TBA values might be attributed due to the oxidation of fatty acids during storage duration. Similar trend was determined by evaluating the effect of storage duration on the TBA values of kilishi samples. It was found that overall TBA values increased periodically in all the samples over time (Iheagwara and Okonkwo, 2016). Rubio, Martinez, Garcia-Cachan, Rovira and Jaime, (2008) found that in control group, TBARS were increased exponentially until the10th day of storage.

Results indicated lower TBARs for samples stored at chilling temperature (2°C) as compared to samples placed at ambient temperature (35°C). Moreover, similar trend was observed with statistically significant differences ($P \leq 0.001$) between types of storage (5°C and 15°C) at end of trial (Kaminek, 2012). Lipid oxidation was markedly effected by the storage temperature because maximum no. of free radicals were detected from meat products placed at higher temperatures (Ladikos and Lougovois, 1990). However, excluding the samples stored at 35°C for more than seven days of the storage, the TBARs values of remaining samples were within the acceptable range of the 1–2 mg/kg of malonaldehyde that was minimum detectable level for oxidized flavor in beef and beef products (Greene and Cumuze, 1981). This could be attributed due to the addition of anti-oxidant ascorbic acid which could have retarded the lipid oxidation processes through sequestering iron thus extending the shelf life of the sausages.

Water Activity (Aw):

Statistically significant ($P \leq 0.05$) differences were observed while considering the fat sources. Animal fat incorporation resulted in slightly lower Aw values than that of plant fat based sausages. No work has been reported so far regarding specie fat incorporation effect on the water activity of sausages. Statistically significant ($P \leq 0.05$) results were found accounting in terms of storage duration and storage temperature. For ambient temperature (35°C) stored samples, Aw values were slightly higher than that of the samples that were stored at chilling temperature (2°C).

Drip loss, chilling loss and moisture loss due to evaporation could be main reason behind the reduction in Aw values of samples stored at (2°C). Evaporative losses or dehydration lead towards decline in Aw values of samples placed at (35°C). These results correlate with the findings of Arief, Reddy and Reddy, (1989) determined that moisture content of

buffalo meat decreased with advancement in chilled and freeze storage, resulting in moisture losses probably due to evaporation of moisture from meat. With the progress in storage days, slight increase in Aw values of sausage samples was recorded. These judgments were in line with the demonstrations of Andres, Zaritzky and Califano, (2006) who explored that the Aw of chicken meat sausages decreased during the 28 days storage and remain constant afterwards.

Cooking Loss

Statistically significant ($P \leq 0.05$) results were observed for cooking losses of sausages prepared through incorporation of different fat sources. Sausages made from sheep tail fat expressed lowest cooking percent loss (%) whilst palm kernel butter fat showed lowest cooking percent losses. However, KPH incorporated sausages showed intermediate losses.

These results matched with the descriptions of Dzudie, Scher, Tchiegang and Hardy, (2005) who concluded that cooking yields were higher for the sausages prepared from mutton fat, intermediate for beef fat sausages whereas plant oil based sausages showed highest cooking loss. Low cooking losses were found in ground beef patties containing beef fat than the sausages containing 50% plant oil (Liu, Huffman and Egbert, 1991).

The reason behind this could be explained through the declarations of an experiment conducted to explore the effects of reduced fat and increased unsaturated fat content on the quality of frankfurters (John, Buyck, Keeton, Leu and Smith, 1986). It was reported that with the increase in the amount of unsaturated fat a marked increase in the processing yield is obtained.

CONCLUSION

Addition of ascorbic acid, phosphate and sodium nitrite was effective for controlling color, lipid oxidation and odor along with inhibition of pathogenic bacteria maximizing shelf life of meat products. Storage temperature and duration had significant effects on TVC and TBARs, gradual and parallel intensification with increase in storage duration and temperature. Sausages placed at 35°C, irrespective of fat sources were spoiled after 7th day of trial whereas sausages that were placed at 2°C spoiled after 11th day of storage. PKBF sausages showed marginally lower values of TVC and TBARs than the KPH and STF sausages. Whereas, cooking loss of PKBF sausages was slightly higher as compared to sausages made from other fat sources.

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