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Effect of whey protein concentrate on quality and shelf life of buffalo meat emulsion sausage

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Abstract: Incorporation of whey protein concentrate (WPC) at a level of 1, 2, 3 and 4% in buffalo meat was investigated for production, quality characteristic and shelf life of buffalo meat emulsion sausage (ES). Quality of emulsion sausage was evaluated in terms physicochemical characteristics like moisture content, pH, protein content, water holding capacity (WHC), Extract release volume (ERV), microbiological characteristics like total plat count (TPC) and Yeast and mold count (Y&M), Coliform count, and sensory characteristic including instrumental colour measurement. It was found that moisture content, ERV, pH and WHC were significantly ($P<0.05$) decreased of emulsion sausage in period of storage at 0º C as result of incorporation of 1-4% of WPC. But protein content significantly ($P<0.05$) increased in period of refrigerate storage. TPC, Y&M and Coliform count of emulsion sausage were found in range of 7.11-7.39 log cfu/g, 3.34-3.86 log cfu/g and 2.25-2.54 log cfu/g respectively. Emulsion sausage incorporated with whey protein concentrate was acceptable to the panelist. The numerical value of 'L' for the samples in 25th days of storage condition found in the range of 25.02%-32.55% Thus sample had 32.55% of maximum lightness as compared to 66.45% darkness.
Keywords: Whey protein concentrate, Emulsion sausage, Incorporation, Sensory characteristic, Shelf life

INTRODUCTION

Meat and meat product are nutritionally rich, providing a wide range of nutrients, such as protein, fat, minerals and vitamins and constitute an important part of the European diet [1]. Meat has long been considered a highly desirable and nutritious food, and has become a mass consumer product throughout the world with the highest consumption rates being recorded in industrialized Western countries. Meat and meat products make an important nutritional contribution to the diet of the people. A significant percentage of the recommended dietary allowances for proteins, vitamins-B, magnesium, iron and zinc are contributed by red meat and poultry [2]. Indian buffalo meat production is growing significantly. Although no official production statistics are available, industry sources and export data indicate that continued strong export demand is triggering an expansion in buffalo meat supplies in India. As a result, new slaughterhouses are emerging, providing farmers with a new market for non-productive buffalo heifers, bulls and bull calves. Calendar year (CY) 2013 Indian buffalo meat production is thus forecast to rise to a record 4.16 million tons (on a carcass weight equivalent basis), up 14 percent from CY 2012. CY 2012 buffalo meat production is estimated at 3.64 million tons (up 12% from CY 2011), and CY 2011 production has been slightly revised up to 3.24 million tons [3]. Buffalo meat can be very well used for production of sausage, a ready to eat and serve product. Sausage is a food that is prepared from comminuted and seasoned meat and is usually formed into a symmetrical shape. Sausage making had developed over a number of countries, beginning simple process of salting and drying meat. This was done to preserve fresh meat that could not be consumed immediately. The typical flavors, texture, and shape of the many sausages known today, such a frankfurter, braunschweiger, and salami were named due to the geographical location of their origin. The emulsion sausages cook sausages that have been finely comminuted to the consistency of a fine paste. Hot dog, Frankfurter, Mortadella, Bologna, liver sausage are typical examples. Emulsion sausage will be successful if the enough lean meat has been selected and enough myosin has been extracted. The lean meat is the main source of myosin. The more myosin extracted, the thicker and stronger protein coat develops around particles of fat. In cause of myosin depends on how vigorous the cutting process was and how much salt (and phosphates) was added. Whey proteins are by-products of the chesses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Now a day, great efforts are being made to find new uses for whey proteins, e.g. production of edible film [4]. Whey protein improves emulsion stability, provide better

color properties and result in lower chewiness and elasticity [5]. Whey protein consists of a number of individual protein components. The two most abundant proteins are β-Lac (50-55%) and α-Lac (20-25%) (Van) [6]. Emulsion sausage incorporated with whey protein products is one product, which can be prepared from buffalo meat using other ingredient salt, spices, condiment, whey protein (isolate, concentrate, whey powder), fat and animal fat. The product will have a pleasant taste, excellent flavor and increased juiciness. The addition of WPC to meat products an increase of WHC, with the result of reduces of the cooking weight loss. This addition also improves sensory quality and enhances nutritional values of meat products [7]. The addition of WPC had a lesser influence in the total yield when the NaCl concentration was higher than 1.6% (w/w) when the sausage vide cooking pasteurization protocol was 70 °C–2 min at the slowest heating point of muscles[8]. [9] reported chicken sausage with a total lipid content in the range of (0.22%, to 6.09%), with increasing WPC decrease the sausage hardness and cohesive was obtained. Exhaustively washed chicken breast muscle improved by the addition of WPC in the emulsion stability heated cream layers[10]. The objective of our study was to assess the quality and shelf lifeof emulsion sausage on buffalo meat incorporated with whey protein concentrate with a level of 1-4%.

MATERIAL AND METHOD

Preparation of meat and non meat ingredient

Meat samples collected from the local meat shop in the study were from buffaloes slaughtered according to the traditional halal method at the slaughter house of the municipal corporation, Aligarh. Meat samples from a round portion (biceps fermoris muscle) of 2.5, 3 and 3.5 years aged female carcasses of good finish were obtained from the meat shop within 4 hr. of slaughter. The meat chunks and buffalo fat were packed in combination film packaging and brought to the laboratory within 20 min and kept at 2ºC in low temperature cabinet (Yarco, India). The connective tissue portions of the samples were removed. Other non-meat ingredients like spices, salt, condiments, casings and HDPE film were procured from the local market. Whey protein concentrate provided by Mahaan proteins Ltd, New Delhi, India. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at 2º C for about 20 hours [11].

Preparation of emulsion sausage

The emulsion sausage (ES) prepared from a comminuted mixture of meat, fat, salt, condiments, spices mixtures and whey protein concentrate (WPC). The recipe was; meat 2 kg, fat 400 g, spices mix 32 g, salt 45 g, Condiments 50 g and WPC with a level of 1, 2, 3, 4%. The buffalo meat was ground on a grinder (PRS Technologies, India) at (11°C temperature, through a 0.95cm plate). The ground meat was transferred to bowl chopper (PRS Technologies, India) for further Comminution. It was chopped at slow speed (17 rpm) for two minutes, and then ice cubes (50 g) were added and further comminuted for two minutes. As the mix absorbed the moisture received from molten ice, the other ingredients like fat, salt, spices, condiment and whey protein were added and chopping was further continued for five minutes and the remaining ice addition brought temperature in range of 14-16°C during chopping. Entire mix was filled in the stuffing machine (PRS Technologies, India) and collagen casing (25mm dia) was used for filling sausage. The finished sausage was cooked in sausage cooker (Yarco, India, operated by steam) for 20 min at 110°C temperature. Cooked sausages were exposing to chilled water or chill water was spread over cooked sausage. This operation led to the cracking of casing and finally the sausages were packed in HDPE packageing. The finished sausages were stored at 0°C in an ultra low temperature cabinet for future study [11]. Fig 1 shows the emulsion sausage incorporated with whey protein concentrate.

Fig-1: Buffalo meat emulsion sausage incorporated with whey protein concentrate

***Physico-chemical analytical methods*:**

Moisture analysis

Moisture content of sausage was evaluated as given in the Food Industry Manual [12] by using a hot air oven (Model ASO, Yarco, New Delhi, India) thermo statically controlled at 150±5° C.

pH measurement

pH of sausage samples were determined by digital pH meter (Model XT 22, Metzer, New Delhi, India). The electrode of pH meter was calibrated with the help of 2 buffer solutions of pH 4 and 7. Ten grams of finely ground sample were blended with 50 ml of distilled water in a test tube in a Cyclo Mixer (CM-101, Yarco, New Delhi, India). The extract was filtered through Whatman No.1 filter paper. Electrode of pH meter was dipped in the filtrate and the pH of the sample was recorded.

Protein analysis

Protein of sausage sample was analytically estimated by determining the amount of total nitrogen by AOAC method as described in [13]. 2 g of finely minced emulsion sausage along with 2g of catalyst mixture was transferred in to digestion tubes. 25 ml concentrated sulphuric acid was poured into the mixture and kept for digesting in DK6 heating digester (VELP Scientific, Europe) for 3 hours. At the end part of digestion, the mixture became colourless. After cooling the tubes were transferred to distillation section of Kjeldhal apparatus. The Ammonia liberated from the reaction mixture was absorbed in 20 ml of 2% boric acid solution. Distillation was continued for five minutes. This solution was titrated against 0.01N HCl using mixed indicator. The blank was running in the second test of experiment and the titration was done in a similar way.

Water holding capacity

Water holding capacity (WHC) was determined according [14]. 10 gm of sausage sample placed in glass jars and heated at 90°C for 10 min in a water bath. After heating sample was carefully removed from glass jars and cooled to room temperature, wrapped in cotton cheesecloth, and placed in 10 ml polycarbonate centrifuge tubes, then centrifuged 9000 rpm in 4°C for 10 min. After centrifuging samples were re weighed and WHC was calculated below formula.

$$\%WHC= 1-T/M\times100= 1-B-A/M\times100$$

Where,

T= total fluid loss during heating and centrifugation

M= total water content in the sample

B= weigh of the sample before heating

A= weight of sample after heating and centrifugation

Extract release volume

Extract release volume (ERV) of meat samples was evaluated as given in manual for analysis of meat and meat products [15]. 20 g sausage sample was homogenized with 100 ml of distilled water for 2 min. Homogenate was Poured directly in to the funnel, lined with Whatman filter paper No.1. Allowed the homogenate and collected extract in 100 ml graduated cylinder for 15 min and extract release volume was recorded.

Interpretation

ERV (ml)	Sausage quality
>25 ml	good quality
>20 ml	incipient spoilage
<20 ml	spoilage sausage

Microbiological analysis methods:

All the samples were evaluated for the direct plate count using serial dilution spread plate technique with nutrient agar medium for total plate count and potato dextrose agar for yeast and mold count [16]. The microbiological characteristics of sausage samples were evaluated in fresh conditions after constant intervals. For the determination of the total plate count, yeast and mold count, the samples were taken with sterile knife, committed to fine particles in a tissue Homogenizer (Yarco, India) and then transferred to a test tube containing 9 ml of normal saline solutions. The samples were homogenized in the cyclomixer (mode CM-101, India). Serial dilutions were made by transferring 1 ml of the extract from each dilution and finally the samples were inoculated in the petridishes containing the solid medium. The colonies were counted after 24-48 hr incubation in BOD incubator (York Scientific, India).

Instrumental colour

The sample of ES was made flat by pressing. The tip of the hunter Lab (Mini scan XE plus, USA) instrument was kept over the samples at room temperature (25° C) and L, a, b values measured.

Sensory characteristics

Sensory characteristics of emulsion sausages were evaluated on 9 points scale by Hedonic rating tests [13] for colour, flavour, texture, taste, mouth coating, juiciness, palatability and overall acceptability using 8-10 panelists. The panelists were selected from the staff and students of the Department of Post Harvest Engg. & Technology, Faculty of Agricultural Sciences, Aligarh Muslim University (AMU), Aligarh.

Statistical analysis

Data obtained from experimental observations (n = 6), were subjected to analysis of variance (ANOVA) as described by [17].

RESULTS AND DISCUSSION

Physiochemical analysis of emulsion sausage

Moisture content

Moisture contentof emulsion sausages (ES) is important property, which relates to the quality and shelf life of the product. In cause of shelf life controlled moisture content and reduced pH are the main point to extend shelf life of emulsion sausages. Moisture content of emulsion sausages incorporated with levels of (1, 2, 3 and 4%) were found to be in between 64.40% and 64.82% (on wet basis) in fresh condition and control sample was 63.40%, which shows the amount of moisture content of control sample less than the incorporated products. The analysis of variance (ANOVA) demonstrated that the use of different level of whey protein concentrate significantly ($p<0.05$) increased the moisture content of sausages sample. It was shown that the addition of whey protein concentrate slightly increased the moisture content of samples as compared to control sample. [18] Found similar results, that the dairy ingredients significantly increased ($P<0.05$) moisture content of sausage sample. The results also were in agreement with [19]. During refrigerated storage, moisture content of these samples

significantly ($p<0.05$) decreased. The moisture contents of samples were found in the range of 60.28% -60.73% on the 25th days of storage (Table 1). These finding are in agreement with [20], addition of whey powder had moisture content in range of 62.0% in cooked beef sausages.

pH values

pH of meat changes during postmortem glycolysis and it decreases to the blast stage (common as ultimate) pH stage. pH plays important role in preservation. Meat is a such of bacterial and mold spoilage sausage contains ingredients like spices, condiments, fat and antioxidant which offer with in microbial growth. pH values of the samples were found in range of 6.23- 6.39 after product preparation (Table 1). pH of emulsion sausage samples are evaluated before and during refrigerated storage at 0°C. Different levels of whey protein concentrate (WPC) (1, 2, 3 and 4%) made a slight change the pH value of sausage samples. [21] demonstrated that the WPC increased the pH value of cooked ground turkey breast compared to the control sample. That the pH increased linearly with WPC until the maximum concentration studied (3.5%) [22]. During refrigerated storage at 0°C, pH values were found to decrease consistently and pH measurement was carried out every after 5th days, till the end of shelf life. Refrigerated storage significantly ($p<0.05$) reduced the pH value of emulsion sausages samples incorporated with whey protein concentrate at the levels (1, 2,3and 4%). At the end of 25 days of storage pH values were found to be in range 5.71-5.78. Similar result found by [23], changing occurred during refrigerated at 4±1°C storage pH of chicken emulsion patties. The pH value of control sample was 5.83 in end of 25 days.

Protein content

Protein content is indicative of nutritional quality. In case of meat products, the quality of protein is very high, because of most of essential amino acids present in it like important constituents. Protein is also degraded by micro organisms in during storage. The protein contents of all samples of emulsion sausages incorporated with different levels whey protein concentrate (1, 2, 3 and 4%) in fresh condition were found 23.55, 23.96, 24.06 and 24.10% respectively. Treated samples had significantly higher percentage of protein content as compared to the control sample (22.58%). Refrigerated storage significantly ($p<0.05$) increase the protein content of emulsion sausages. At the end of 25th day of storage protein content of emulsion sausage incorporate with whey protein concentrate with levels (1, 2, 3 and 4%) were found 26.43, 26.94, 27.06 and 27.13% respectively (Table 1).

Water holding capacity

The water holding capacity (WHC) of emulsion sausages in incorporated with different level of whey protein concentrate was found in range 71.18% - 71.50% (on wet basis) in fresh condition. However WHC of control sample was 69.69%. An increasing trend of WHC of emulsion sausage samples, similar results was also found by [18] who reported addition of dairy ingredient significantly ($P<0.05$) increased WHC and emulsion satiability.Milk proteins enhance physical properties including gelation, fat emulsification, and water holding capacity [24]. In another study [21] reported whey proteins contain many hydrophilic groups that are exposed upon heating and react with water, thus increasing the meats water holding capacity. Similar result reported by [25; 26; 27] whey protein concentrate (WPC) was employed to increase water holding capacity. The analysis of variance (ANOVA) showed that the use of different level of whey protein concentrate significantly ($p<0.05$) increased the water holding capacity of sausages sample. During refrigerated storage, water holding capacity of these samples significantly ($p<0.05$) decreased. The moisture contents of samples were found in the range of 66.20% -66.72% on the 25th days of storage (Table 1).

Extract release volume

The extract release volume (ERV) of emulsion sausage samples in which incorporated with different level of whey protein concentrate (1, 2, 3 & 4%) were found to be in range 34.2 ml and 36.4 ml and control sample was 32.2 ml in fresh condition. The analysis of variance (ANOVA) shows that the use of different levels of whey protein concentrate significantly ($p<0.05$) increased the (ERV) of sausage samples. During refrigerated storage, extract release volume of these samples significantly ($p<0.05$) decreased. The (ERV) of samples were found in the range of 25.2 – 27.3 ml on the 25th days of storage (Table 1). It was noticed from the interpretation of extract release volume, that quality in storage condition the emulsion sausages samples had good quality.

Instrumental measurement of colour of emulsion sausage

Table2 present the results of colour evaluation of emulsion sausage incorporated with whey protein concentrate. The measurements of colour of samples were done in fresh condition and during refrigerated storage while sample was packed in combination film. The numerical value of 'L' in 25 days of storage samples condition found in the range of 18.86 - 27.77 for emulsion sausage incorporated with whey protein concentrate.The sample emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3, 4%) on end of storage (25th days) had 27.77% of maximum lightness as compared to 72.23% darkness. It was brown red colour in appearance. Hue values were found in the range 4.78 – 6.74. This indicated that maximum red colour was 6.74 as compared to yellow colour 'Chroma' value were found in the range of 6.43 – 9.24. This value describes the saturation of red colour, assuming maximum saturation at 60. L and b values were found to decrease dramatically refrigerated

Storage (0^0 C) while 'a' values of first decreases and then increase on storage (0^0C). The emulsion sausage lost its colour during refrigerated storage at end of 25 day. [27] reported the addition of whey protein increased the lightness and decreased both the redness and yellowness values. Similarly [21] explained that lightness of ground turkey increased with the addition of whey protein powder. [28] stated there was no significant different in lightness and redness values on emulsified meat batters prepared at different protein levels. Researchers like [18] stated that sample with addition of dairy ingredients had no effect on a^* values but into lower b^* values.

Microbiological characteristics of emulsion sausage during refrigerated storage 0°C.

Total plate count

Buffalo meat emulsion sausages are food with high moisture and good in nutrition and therefore they are center for attraction of microorganism especiallybacteria because of favorable pH for their growth. Microbial contamination may be added or reduced at different stages of processing of buffalo sausage. Total plate count (TPC) of emulsion sausage samples were evaluated and reported as log (cfu)/g.(TPC) of samples of emulsion sausages were enumerated in fresh condition and periodically after every 5 days during refrigerated storage at 0ºC. Table 3 represent the microbial profile of emulsion sausages incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%) and controlled during refrigerated storage at 0°C. Refrigerated Storage significantly ($p<0.05$) increased TPC of emulsion sausages incorporated with whey protein concentrate at 0ºC. The TPC was found to be range of 7.11- 7.39 log cfu/g, on 25^{th} day of storage for samples prepared at the levels of (1, 2, 3, 4 %) of whey protein concentrate. After 25 days of refrigerated storage (0ºC) TPC of all samples were found to be in the safe limit. Similar result of TPC reported by [29], total plate counts (log cfu/g) buffalo sausage significantly ($p<0.05$) increased period of 35 days of storage with different packed (packed in vacuum and nitrogen packed). But In nitrogen packed sausage, increase in TPC was more from 4th day onwards. Although [30] reported TPC of pork sausages increased significantly ($P<0.01$) and progressively as the storage (7±1°C) period increased. This might be due to the permissive temperature and relative availability of moisture and nutrients for the growth of the aerobes. A similar trend of significant increase in the mean TPC under refrigerated storage was observed by [31] in pork sausages, [32] in chicken patties and [33] in smoked chicken sausages. Meat is rich in nutrition and therefore it is centre of all attraction for micro-organisms especially for bacteria due to their desirable pH for growth. Bacteria even at 0^0C are surviving though the rate of growth is slow. Increased microbial population caused the degradation of protein and fat into simpler compounds like fatty acid, amino acid, ammonia, sulphur dioxide and carbon dioxide.

Yeast and Mold count

The results of yeast and mold (Y & M) counts of sausages samples expressed as log cfu/g has been presented in Tables 3. Yeast and mold count were not affected in samples till 5 days of refrigerated storage at 0ºC. A very low count was observed at 10^{th} days of storage. The results are in agreement with [23] Y & M counts were observed on days 9 and 12 during refrigerated storage for chicken emulsion patties. However, countable colonies noted on 25^{th} days of storage in emulsion sausage incorporated with whey protein concentrate. Refrigerated storage significantly ($p<0.05$) increased the yeast and mold count of emulsion sausages. The Y & M count was found to be between 3.34 - 3.86 log cfu/g for emulsion sausages incorporated with whey protein concentrate. The ANOVA tests indicated that the different levels of whey protein concentratesignificantly ($p<0.05$) increased yeast and mold count of emulsion sausages in duration of storage. After 25 days of refrigerated storage (0ºC) yeast and mold count of all samples was found to be less than 4 log cfu/g. This particular value of yeast and mold count defined the spoilage condition. When log cfu/g of Y & M count increase to 4.0, spoilage of food samples starts [34]. [35] observed that the growth of yeast and mold in Italian style sausages were controlled during storage after inoculation with LAB starter cultures. They concluded that it could be due to the antagonistic activities of the latter. [36] reported similar observation of Turkish sausage after inclusion with LAB strains as protective cultures. [37] noted a reduction in the yeast and moulds counts in fresh beef inoculation with LAB starters.

Coliform count

Coliform count of emulsion sausages produced with different levels of whey protein concentrate (1, 2, 3 and 4 %) were enumerated and they were found there were no sign of coliform bacteria on plated containing MacConkey agar till 20^{th}days of storage (0ºC) in four levels of whey protein concentrate. However, coliform count was found to be in the range of 2.06- 2.34log cfu/g in emulsion sausages incorporated with whey protein concentrate after 15days of storage (Table 3). The results are in agreement with [23] the coliforms were totally undetected from any of the emulsions chickenpatties during 12 day of storage. Refrigerated storage significantly ($p<0.05$) increased the coliform count of all samples at the levels 1-4% of whey protein concentrate. In final stage of storage, coliform count was found to be in the range of 2.16-2.54log cfu/g. Reduction in counts of *Enterabacteriaceae* and *Staphylococcus* in meat has been reported by studies of [37; 38; 39]. Usually, the presence of total coliform in food indicates improper heat treatment or post processing contamination. Coliform were not usually

pathogenic. They also indicated inadequate sanitation and disinfection of appliance [34].

Sensory characteristics of emulsion sausage during refrigerated storage 0°C

Sensory characteristics of fresh emulsion sausages incorporated with different levels of whey protein concentrate 1, 2, 3 and 4% expressed in terms of sensory attributes, namely colour, aroma, texture, taste and juiciness were evaluated by a group of panel members on a nine point Hedonic scale. The results of sensory evaluation have been presented in Table 4. The emulsion sausages had bright red colour after cooking. All the fresh emulsion sausages had score values of colour between 8-9. It represented condition between liked very much and liked extremely. Different levels of whey protein products significantly ($p<0.05$) affected the colour score values of fresh emulsion sausages as compared to control sample. The colour score values during storage were found to be decreasing. Refrigerated storage significantly ($p<0.05$) decreased the colour score values of emulsion sausage. The findings of the current study are consistent with [23], who found the colour score values significantly decrease for chicken emulsion patties as the storage days. After 25 days of storage, the highest score for colour was given for sample of emulsion sausage incorporated with whey protein at the levels of 4%. The decline in colour scores during storage was due to lipid oxidation and subsequent oxidized compounds reacting with amino acids during non enzymatic browning of the product. [30] reported irrespective of type of formulations and type of storage conditions, the mean colour, flavor, juiciness, tenderness and overall acceptability scores of pork sausages decreased significantly ($P<0.01$) with increasing storage period. The characteristic aroma of emulsion sausages mainly originates from the breakdown of carbohydrates, lipids and proteins through the action of microbial and endogenous meat enzymes [40]. The development of aroma is also influenced by several variables such as product formulation (especially spices), processing condition and starter culture [41; 42; 43]. Different levels of whey protein concentrate did not significantly ($p<0.05$) affected the aroma of emulsion sausages. But the interaction between whey protein concentrate and storage period significantly ($p<0.05$) affected the aroma score values. During refrigerated storage the score values of aroma significantly ($p<0.05$) decreased and 25 days of storage it was between of 6.3 -7.0 for emulsion sausages incorporated with whey protein concentrate. [44] reported that the aroma scores of buffalo meat decreased significantly ($p<0.05$) with increase in storage period. Similar result found by [23], the aroma scores values chicken emulsion patties progressive decreased in during storage at 4±1°C. The samples incorporated with whey protein concentrate at the level of 4% highest score values. The lowest score values were for the samples incorporated with whey protein concentrate at the level of 2%. Different levels of whey protein concentrate significantly ($p<0.05$) affected the texture score values of emulsion sausages. During the refrigerated storage (0°C) the score values of texture of emulsion sausages incorporated with whey protein concentrate at levels of 1 to 4% significantly ($p<0.05$) decreased. The mean score values of texture on 25th day of storage for all samples were between of 5.5-6.6. [45] reported that ambient storage significantly ($p<0.05$) decreased the score values of texture of meat keema. It should be noted that the changes of other chemical contents such as protein, moisture, fat collagen and pH value during storage might result in the change of texture of cooked sausages [46]. All the emulsion sausages in fresh condition had the score values of taste in range of 8.1 to 8.7. It represented condition between liked very much and liked extremely. The score value of control sample without whey protein was 7.9. Different levels of whey protein concentrate significantly ($p<0.05$) affected the taste score values in fresh condition of emulsion sausages compared to the control sample with 0 percent of whey protein concentrate. Also the interaction between whey protein products and storage period significantly ($p<0.05$) affected the taste score values. At the end of storage period, there was a significant effect of whey concentrate on the taste score values. This might be due to the different pH and TBA number values. During refrigerated storage at 0°C the score values of taste significantly ($p<0.05$) decreased. This finding is in agreement with [47], sensory evaluation of the product revealed that the taste decreased steadily during chilled storage. The means score values of taste on 25th days of storage were between of 6.9-7.5for all samples. Sample incorporated with whey protein concentrate at the level of 3% significantly ($p<0.05$) scored the highest value for the taste among all samples. Whey protein concentrate significantly ($p<0.05$) affected thejuiciness of emulsion sausages. Interaction between whey protein products and storage period significantly ($p<0.05$) affected the juiciness score values. Moreover at the end storage period, there was a significant effect of different levels of whey protein concentrate on the juiciness score values. During refrigerated storage (0°C) the score values of juiciness significantly ($p<0.05$) decreased due to the loss of moisture content during refrigerated storage. However the score values of juiciness on 25th day of storage were between of 6.6 – 7.1 for all samples.

Table 1: Evaluation of physicochemical of buffalo meat emulsion sausage incorporated with different levels of whey protein concentrate during storage at 0°C

Parameter	Days of storage	Cs	$Swpc_1$	$Swpc_2$	$Swpc_3$	$Swpc_4$
Moisture content	0	63.40±0.011	64.54±0.012	64.82±0.010	64.70±0.013	64.40±0.010
	5	63.16±0.066	63.94±0.033	63.79±0.053	64.12±0.035	64.11±0.042
	10	62.19±0.035	62.54±0.080	63.13±0.044	63.25±0.081	63.59±0.053
	15	61.81±0.069	62.08±0.040	62.19±0.045	62.47±0.073	62.52±0.055
	20	60.83±0.045	61.79±0.068	61.82±0.060	61.73±0.070	61.23±0.036
	25	59.47±0.036	60.67±0.071	60.82±0.059	60.28±0.068	60.73±0.051
pH	0	6.390±0.071	6.236±0.037	6.242±0.028	6.358±0.081	6.378±0.009
	5	6.286±0.009	6.180±0.023	6.124±0.023	6.260±0.048	6.261±0.033
	10	6.167±0.016	6.054±0.028	6.046±0.018	6.136±0.020	6.154±0.024
	15	6.011±0.007	5.989±0.008	5.915±0.003	6.010±0.011	5.990±0.002
	20	5.976±0.004	5.815±0.003	5.818±0.005	5.881±0.011	5.857±0.006
	25	5.834±0.009	5.750±0.021	5.711±0.004	5.783±0.048	5.769±0.038
Protein	0	22.58±0.052	23.55±0.016	23.96±0.018	24.06±0.023	24.10±0.013
	5	23.35±0.020	24.02±0.040	24.46±0.040	24.88±0.032	24.93±0.047
	10	23.95±0.023	24.83±0.054	25.22±0.036	25.49±0.027	25.65±0.053
	15	24.56±0.051	25.36±0.038	25.85±0.036	25.91±0.054	26.06±0.043
	20	25.12±0.039	25.91±0.066	26.34±0.035	26.41±0.025	26.51±0.047
	25	25.82±0.034	26.43±0.038	26.94±0.043	27.06±0.048	27.13±0.024
Water holding capacity	0	69.69±0.089	71.22±0.036	71.50±0.246	71.48±0.225	71.18±0.037
	5	68.56±0.044	70.22±0.016	70.24±0.070	70.18±0.038	70.31±0.079
	10	67.72±0.077	69.59±0.042	69.46±0.066	69.77±0.050	69.28±0.043
	15	66.79±0.020	68.91±0.053	69.17±0.043	68.91±0.051	68.70±0.052
	20	65.19±0.044	67.83±0.065	67.76±0.075	67.37±0.094	67.48±0.063
	25	64.20±0.056	66.52±0.058	66.72±0.090	66.27±0.051	66.20±0.063
Extract release volume	0	32.2±0.447	34.2±0.836	35.2±0.836	36.4±0.894	36.2±0.836
	5	31.6±0.894	32. 2±0.836	33.8±0.570	33.7±0.758	33.0±0.790
	10	30.2±0.570	32.0±0.707	30.2±0.836	30.2±0.758	29.8±0.836
	15	28.5±0.114	29.3±0.282	30.0±0.610	28.1±0.502	27.8±0.570
	20	26.4±0.296	27.7±0.559	27.3±0.311	28.3±0.336	27.8±0.739
	25	24.4±0.250	26.5±0.360	25.2±0.230	26.3±0.356	27.3±0.342

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($P<0.05$), Cs = Control sample, $Swpc_{1, 2, 3, 4}$= Sausage whey protein concentrate at the levels 1, 2, 3 and 4%

Table 2: Evaluation of instrumental measurement of colour of emulsion sausages incorporated with different levels of whey protein concentrate during storage at 0°C

Sample Code		Instrumental measurement of colour					
		Storage Period (Days)					
		0	5	10	15	20	25
Cs	L	33.22	32.43	23.75	18.27	27.83	24.89
	a	3.9	4.15	3.4	3.16	4.55	5.49
	b	9.22	9.27	7.73	5.26	8.06	6.28
$Swpc_1$	L	31.23	22.98	24.07	23.88	28.23	27.77
	a	4.92	3.56	5.8	6.12	7.67	6.74
	b	8.78	6.27	7.03	7.56	7.79	9.24
$Swpc_2$	L	30.28	20.97	19.92	22.99	24.67	25.06
	a	4.91	3.55	5.25	5.76	6.47	5.80
	b	8.85	6.36	7.91	7.11	7.77	6.70
$Swpc_3$	L	29.50	26.69	28.32	20.75	27.42	18.86
	a	4.36	3.92	7.22	5.54	7.24	4.78
	b	8.4	7.86	9.57	6.78	8.14	6.43
$Swpc_4$	L	30.37	32.55	25.27	25.25	26.92	25.02
	a	4.53	5.09	6.06	6.51	6.6	4.15
	b	8.80	9.60	8.57	8.13	7.98	7.70

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($P<0.05$), Cs = Control sample, $Swpc_{1, 2, 3, 4}$= Sausage whey protein concentrate at the levels 1, 2, 3 and 4%

Table 3: Evaluation of mmicrobiological characteristicsof emulsion sausages incorporated with different levels of whey protein concentrate during storage at 0°C

Parameter	Days of storage	Cs	$Swpc_1$	$Swpc_2$	$Swpc_3$	$Swpc_4$
Total Plate Count	0	3.32±0.017	3.52±0.016	3.60±0.015	3.64±0.014	3.72±0.015
	5	3.95±0.032	4.65±0.007	4.51±0.018	4.66±0.014	4.50±0.015
	10	4.46±0.013	5.12±0.011	5.22±0.015	5.45±0.013	5.32±0.008
	15	5.12±0.016	5.97±0.016	6.09±0.024	6.33±0.021	5.94±0.026
	20	5.91±0.038	6.54±0.027	6.83±0.027	6.94±0.032	6.64±0.028
	25	6.65±0.027	7.12±0.019	7.33±0.035	7.39±0.020	7.11±0.039
Yeast and Mould Count	0	ND	ND	ND	ND	ND
	5	TFTC	TFTC	TFTC	TFTC	TFTC
	10	1.74±0.041	1.85±0.038	1.84±0.028	2.15±0.041	2.19±0.051
	15	2.08±0.028	2.26±0.058	2.22±0.043	2.43±0.037	2.40±0.070
	20	2.61±0.079	2.94±0.044	2.96±0.029	2.93±0.024	2.90±0.042
	25	3.23±0.050	3.34±0.034	3.45±0.031	3.86±0.031	3.82±0.052
Coliform count	0	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND
	10	ND	ND	ND	ND	ND
	15	ND	ND	ND	ND	ND
	20	2.06±0.022	2.30±0.028	2.21±0.036	2.34±0.025	2.11±0.022
	25	2.16±0.022	2.54±0.031	2.35±0.022	2.54±0.025	2.25±0.036

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($P<0.05$)
Cs= Control sample, $Swpc_{1, 2, 3, 4}$= Sausage whey protein concentrate at the levels 1, 2, 3 and 4%, ND = Not detected, TFTC = To few to count

Table 4: Evaluation of sensory of emulsion sausages incorporated with different levels of whey protein concentrate during storage at 0°C

Parameter	Days of storage	Cs	$Swpc_1$	$Swpc_2$	$Swpc_3$	$Swpc_4$
colour	0	7.7±0.01	8.7±0.02	8.6±0.01	8.1±0.09	8.4±0.02
	5	7.7±0.03	8.1±0.07	8.4±0.04	8.1±0.08	8.3±0.02
	10	7.4±0.05	7.8±0.03	8.1±0.07	8.0±0.01	8.1±0.01
	15	7.1±0.01	7.5±0.05	7.6±0.05	7.9±0.02	8.1±0.06
	20	7.1±0.02	7.5±0.04	7.3±0.03	7.6±0.03	7.8±0.04
	25	6.8±0.04	7.1±0.03	7.1±0.03	7.2±0.06	7.6±0.04
Aroma	0	8.4±0.13	8.4±0.25	8.4±0.15	8.5±0.20	8.3±0.26
	5	8.0±0.11	8.1±0.11	8.0±0.18	8.2±0.08	7.9±0.08
	10	7.5±0.11	7.5±0.14	7.7±0.18	7.8±0.18	7.6±0.14
	15	6.8±0.16	7.1±0.22	7.3±0.11	7.5±0.16	7.2±0.64
	20	6.4±0.28	6.7±0.18	7.0±0.18	7.2±0.24	6.9±0.19
	25	6.1±0.19	6.3±0.33	6.4±0.66	7.0±0.35	6.4±0.35
Texture	0	7.5±0.07	8.1±0.01	8.5±0.07	8.4±0.07	8.7±0.10
	5	7.2±0.15	7.4±0.26	8.2±0.14	8.3±0.19	8.5±0.32
	10	6.9±0.23	7.2±0.21	7.7±0.19	8.0±0.19	8.1±0.25
	15	6.4±0.14	6.8±0.21	7.2±0.17	7.4±0.35	7.8±0.18
	20	5.9±0.13	6.4±0.37	6.9±0.59	6.6±0.20	7.2±0.24
	25	5.5±0.37	5.9±0.35	6.2±0.22	5.7±0.17	6.6±0.15
Taste	0	7.9±0.08	8.1±0.08	8.6±0.07	8.7±0.05	8.3±0.13
	5	7.7±0.19	8.0±0.11	8.4±0.11	8.5±0.21	8.3±0.18
	10	7.5±0.21	7.7±0.17	8.1±0.16	8.3±0.15	8.1±0.12
	15	7.3±0.18	7.5±0.28	7.8±0.14	8.0±0.17	7.9±0.21
	20	7.0±0.11	7.2±0.13	7.6±0.17	7.8±0.21	7.7±0.15
	25	6.9±0.08	7.1±0.13	7.3±0.22	7.5±0.19	7.4±0.22
Juiciness	0	7.7±0.14	8.3±0.10	8.7±0.89	8.3±0.13	8.2±0.44
	5	7.5±0.35	8.0±0.13	8.1±0.21	7.9±0.13	7.8±0.26
	10	7.3±0.15	8.0±0.35	7.7±0.20	7.6±0.39	7.6±0.37
	15	6.8±0.11	7.8±0.18	7.5±0.23	7.2±0.21	7.2±0.26
	20	6.6±0.16	7.5±0.37	7.0±0.25	6.9±0.21	7.0±0.19
	25	6.6±0.14	7.1±0.18	6.7±0.46	6.5±0.13	6.8±0.18

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($P<0.05$), Cs = Control sample, Swpc $_{1, 2, 3, 4}$= Sausage whey protein concentrate at the levels 1, 2, 3 and 4%

CONCLUSIONS

Whey protein concentrate incorporated at the levels of 1, 2, 3 and 4% with buffalo meat brought considerable improvment in quality characteristics of emulsion sausages. It had shown that addition of whey protein concentrate increase the protein content, moisture content, water holding capacity and extract release volume in fresh emulsion sausages samples. pH values buffalo meat emulsion sausages decreased with incorporation of 1-2% of whey protein concentrate. In duration of 25th days of refrigerate storage moisture content, pH, water holding capacity and extract release volume decreased. Protein content of emulsion sausage increased in duration of refrigerate storage. The treated sample at the end of storage (25th days) had 27.77% of maximum lightness as compared to 72.23% darkness. Whey protein concentrate improved the sensory characteristics of emulsion sausage.

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REFERENCES

1. Cosgrove M, Flynn A, Kiely M; Consumption of red meat, whit meat and processed meat in Irish adult in relation to dietary. British J. of Nutrition, 2005; 93: 933-942.
2. Pearson A M, Brooks R.F; The contribution of meat to the diet of man. Proc. Relip Meat Conf. 1978; 31:64.
3. Agricultural and Processed Food Products Export Development Authority. 2013. World Buffalo meat market: India in the driving seat. Processed Food Industry. 40-41.
4. Anker M, Stading M, Hermanson A M; Mechanical properties, water vapor permeability and moisture contents of beta- lactoglobulin and whey protein films using multivariate analysis. J. of Agricultural and Food Chemistry, 1998; 46:1820-1829.
5. Yetim H, Muller W D, Eber M. 2001. Using fluied whey in comminuted meat products: effects on technological, chemical and sensory properties of frankfurter- type sausages. Food Research International. 34:97-101.
6. Van Vliet T, Lakemond C M.M, Visschers R W; Rheology and structure of milk protein gels. Current Opinion Colloid Interface Science, 2004;9(5): 298-304
7. Thomsen B; Milk proteins in meat products. Meat International, 1996; 6(1), 32–33.
8. Szerman N, Gonzalez C B, Sancho AM, Grigioni G, Carduza F, Vaudagna S R; Effect of whey protein concentrate and sodium chloride addition plus tumbling procedures on technological parameters, physical properties and visual appearance of sous vide cooked beef. Meat Sci., 2007; 76: 463–473.
9. Andres S, Zaritzky N, Califano A;The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. International J of Food Science and Technology, 2006; 41: 954–961.
10. 10. Imm J Y, Regenstein J M; Emulsification of commercial dairy proteins with exhaustively washed muscle. J. of Food Sci., 1998;63:1012–1017.
11. Badpa A, Ahmad S; Effect of incorporation of whey protein concentrate on quality characteristic of buffalo meat emulsion sausage. Journal of Buffalo Science. 2014; 3:48-54.
12. Ranken M D, Kill R C; Food industry manual. 23rd edn. Chapman and Hall, 2-6 BoundryRaw, London SEI8 HN, UK.p33, 9297.298.
13. Ranganna S. 1994. Handbook of analysis and quality control for fruit and vegetable products. Tata Mc Graw Hill Pub. Co. Ltd. New Delhi.1993; p 3-5, 9-10, 623-625.
14. Lianji M, Chen N; Research in improving the water holding capacity of meat in sausage products. Proceedings of the 35th International Congress of Meat Science and Technology, Copenha-gen, Denmark, 1991; pp. 781±786.
15. Food safety and standard authority, India; Manual for analysis of meat and meat products, fish and fish products. Meat and Fish Products. 2012; 29-31.
16. APHA; Compendium of methods for microbiological examination of foods. American Public Health Association, Washington DC. 1992.
17. Cochron W G, Cox G M; ANOVA. In Factorial experiments. Experimental design (pp. 177–180). John Wiley and Sons. 1992.
18. Meltem S, Eylem E D; Chemical composition and quality characteristics of emulsion type turkey rools formulated with dairy ingredients. J. of Food Technol. 2004; 2(2): 109-113.
19. Hung S C, Zayas J F; Fuctionality of milk proteins and corn germ protein flour in comminuted meat products. J. of Food Quality,1992; 15:139-152.
20. Serdaroglu M, Ozsumer M S; Effect of soy protein, whey powder and wheat gluten on quality characteristics of cooked beef sausage formulated with 5, 10 and 20 % fat. Series Food Science and Technology. 2003; 6(2):1-9.
21. Sammel L M, Claus J R; Whey protein concentrate effects on pink colour developed in a cooked ground turkey breast model system. Meat Sci., 2003;65:1293-1299.
22. Szerman N, Gonzalez C B, Sancho A M, Grigioni G, Carduza F, Vaudagna S R; Optimization of whey protein concentrate and sodium chloride concentrations and cooking temperature of sous vide cooked whole-muscle beef from Argentina. M Sci, 2008; (79)557–567.

23. Rajani K, Kala Napa K, Anne S R, Anjaneyulu Rajendran T; Evaluation of quality of chicken emulsions stored refrigerated (4 ± 1 _C) for chicken patties. International Journal of Food Science and Technology, 2007; 42: 842–851.
24. Sammel L M, Claus J R, Greaser M L, Lucey J A; Identifying constituents of whey protein concentrates that reduce the pink color defect in cooked ground turkey. Meat Science, 2007; 77:529-539.
25. Corrales D D, Gonzalez C B, Szerman N, Sancho A M, Carduza F, Sanchez G, and others;. Addition of whey concentrate and sodium chloride to beef muscles. Effects of tumbling procedures and sous vide cooking treatment on technological parameters, physical properties and sensory quality. In Proceedings of the international congress of meat science and technology, CD Copy, 9–13 August 2004,Helsinki, Finland.
26. Hayes J E, Desmond E M, Troy D J, Buckley D J, Mehra R; The effect of enhancement with salt, phosphate and milk proteins on the physical and sensory properties of pork loin. MeatScience, 2006; 72, 380–386.
27. Hughes E, Cofrades S, Troy D J. 1998. Effects of fat level, oat fiber and carragenan on frankfurters formulated with fat 5%, 12% and 30%. Meat Sci., 1998; 45**:** 273–281.
28. Youssef M K, Barbut S; Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Sci. 2011; 87**:** 54-60.
29. Sachindra N M, Sakhare P Z, Yashoda K P, Narasimha Rao; Microbial profile of buffalo sausage during processing and storage. Food Control, 2005; 16(1):31-35.
30. Bhaskar Reddy GV, Moorthy P R S, Reddy K P; Effect of milk co-precipitates on quality characteristics of pork sausages. Tamilnadu J. Veterinary & Animal Sci., 2009; 5(6): 257-263.
31. Murthy N G; A study on yield and quality of pork sausages as affected by genetic group, level of added fat and phosphate. MVSc Thesis, APAU, R.Nagar, Hyderabad. 1986.
32. Nath RL, Mahapatra C M, Kondaiah N, Anand S K, Singh J M. 1995. Effect of levels of chicken fat on the quality and storage life of chicken patties. Indian Journal of Poultry Science, 1995; 30**:** 52–57.
33. Rao K H, Singh R R B, Anjaneyulu A S R, Rao K V S S, Yadav P L; Evaluationof caseinates and refined wheat flour as emulsion stabilizer in chicken nuggets. XX World's Poultry Congress, 1996; 4: 435.
34. CQIASA; Guidelines standards for analytical results in food microbiology. Govt. du. Quebec, Deptol legal, Canada.2003.
35. Casaburi A, Aristoy M C, Vavella S, Di Monaco R, Ercolini D, Toldra F; Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. Meat Sci., 2007; 76: 295-307.
36. Erkmen O; Modeling the effects of sucuk production technique on Listeria monocytogenes, aerobic bacteria and lactic acid bacteria during ripening and storage. Food and Bioproducts Processing, 2008; 86: 220-226.
37. Olaove O A, Onilude A A; Investigation on the potential use of biological agents in the extension of fresh beef in Nigeria. World Journal of Microbiological and Biotechnology, dio, 2010; 10.107/ s 11274-010-031905, in press.
38. Gomolka- Pawlika M, Uradzinski J, Wiszniewska A. 2004. Effect of chosen lactic acid bacteria strains on Staphylococus aureus in vitro as well as in meat and raw sausages. Polish J. of Veterinary Sci. 2004; **7**: 251-259.
39. 39. Kaban G, Kaya M. 2009. Staphylococcus xylosus on the quality characteristics of dry fermented sausage (sucuk). J. of Food Sci., 2009; 74: 58-63.
40. Kaban G, Kaya M; Effect of starter culture on growth of Staphylococcus aureus in sucuk. Food Control, 2006; 17: 797-801.
41. Stahnk LH; Aroma components from dried sausages fermented with Staphylococcus xylosus. Meat Sci., 1994; 38: 39-53.
42. Lucke F K; Fermented sausages. In: Microbiology of Fermented Foods. Wood, B.J.B. editor. London, Blackie Academic and Professional.1998; pp.441-483.
43. Toldra F, Sanz Y, Flores M; Meat fermentation technology. In : Meat Science and applications, Hui, Y.H., Shorthose, R., Young, O., Koohmaraie, M.and Rogers, R. editor. Marcel Dekker Inc. New York, USA. 2001; 537-561.
44. Kandeepan G, Biswas S; Effect of low temperature preservation on quality and shelf life of buffalo meat. Amirican J. of Food Technolo. 2007; 2(3):126-135.
45. Kandeepan G, Anjaneyulu A S R, Kondaiah N, Mendiratt S K; Quality of buffalo meat keema at different storage temperature. African J. of Food Sci., 2010; 4(6): 410-417.
46. Dong QL, Tu K, Guo L Y, Yang J L, Wang, Hand Chen Y.Y; The effect of sodium nitrite on the textural properties of cooked sausage during cold storage. J. of Texture Studies. 2007; 38: 537-554.
47. Balve D K, Stavkov A S. Ivanov G Y, Dragoev S G, Filizov E H; Colour stability improvement of chilled beef by natural antioxidant treatment and modified atmosphere packaging. American J. of Food Technol.,2011;6(2):117-128.