Effect of Incorporation of whey Protein Concentrate on Quality Characteristic 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 of 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, and sensory characteristic including instrumental colour measurement. It was found that moisture content, protein content, ERV and WHC were significantly (P<0.05) increased in emulsion sausage as result of incorporation of 1-4% of WPC. Addition of 1-2% WPC decreased the pH. However 3-4% addition of WPC caused no change in pH and it remained comparable the pH of the control sample. TPC of ES was found in range of 6.23-6.37 log cfu/g. Emulsion sausage incorporated with whey protein concentrate was acceptable to the panelist. The numerical value of 'L' for the samples in fresh condition found in the range of 29.50%- 33.25%. Thus sample had 33.25 % of maximum lightness as compared to 66.75% darkness. The sample of fresh emulsion sausages was greyish brown colour in appearance.

Keyword: Sausage, Whey, Buffalo, Meat, Quality characteristic.

INTRODUCTION

Consumers have demand a meat product which is safe, nutritious, convenient and attractive. By this approach meat industry in recent year is aimed to develop healthier meat products. Buffalo meats are cheaper compared to other type of meat. It is better to convert buffalo meat into value added products such as sausage. Sausage is a popular and highly relished meat product recognized world over [1]. The increase in meat production gave rise to various problems in handling, processing, preservation and storage, marketing and distribution till it reaches to the consumer table. These problems can be solved through research and development and the person involved in the meat industry need significant training to improve their traditional skill. Sausage formulations were designed by experts who, based on their experience were able to obtain the desired properties for sausage [2].

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. Whey proteins are by-products of the cheeses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Nowadays, great efforts are being made to find new

uses for whey proteins, e.g. production of edible film [3]. Whey protein improves emulsion stability, provide better colour properties and result in lower chewiness and elasticity [4]. Whey and whey products are used in sausage to improve the flavour, taste, texture and increase the protein of finished products. Sweet whey, whey protein concentrate has 34-80% protein and whey protein isolate has more than 90% protein. These products are among the most common whey products used in processed meats [5].

Quality improvement, nutritional optimization and cost effectiveness are key drives in using whey protein ingredients in processed meats. 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 [6]. 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 sous vide cooking pasteurization protocol was 70 °C–2 min at the slowest heating point of muscles [7].

Andres *et al.* [8] 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. Low-fat chicken sausages incorporated with xanthan and guargums and WPC had good acceptable sensory scores and functional properties [8]. Exhaustively washed chicken breast muscle improved by the addition of WPC in the emulsion stability heated cream layers [9]. The addition of 2% of WPC in Bockwurst sausage, the sausage had higher stability than with 0.00, 1.75, or 3.75% addition

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of WPC [10]. The objective of our study was to assess the effect of whey protein concentrate with a level of 1-4% on quality characteristic of emulsion sausage on buffalo meat.

MATERIALS AND METHODS

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 were packed in combination film packaging and brought to the laboratory within 20 min. Buffalo fat was also packed in combination film packaging and brought to the laboratory. 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 2^oC for about 20 hours.

Preparation of Emulsion Sausage

The emulsion sausage (EM) 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 20min 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 HDFE packageing. The finished sausages were stored at 0°C in an ultra low temperature cabinet for future study. Figure **1** shows the emulsion sausage incorporated with whey protein concentrate.

Figure 1: Buffalo meat emulsion sausage incorporated with whey protein concentrate.

Physico-Chemical Analytical Methods

Moisture content of sausage was evaluated as given in the Food Industry Manual [11] by using a hot air oven (Model ASO, Yarco, New Delhi, India) thermo statically controlled at 150±5° C.

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 of sausage sample was analytically estimated by determining the amount of total nitrogen by AOAC method as described in Ranganna [12]. Two grams 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 run in the second test of experiment and the titration was done in a similar way.

Water holding capacity was determined according to Lianji and Chen [13]. Ten grams 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.

$$\%WHC = 1-T/M \times 100 = 1-B-A/M \times 100$$

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 (ERV) of meat samples was evaluated as given in manual for analysis of meat and meat products [14]. 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 [15].

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 (model CM 101) Yarco, 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 at 37°C (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, and b values were measured.

Sensory Characteristics

Sensory characteristics of emulsion sausages were evaluated on 9 points scale by Hedonic rating tests [12] for colour, flavour, texture, taste, mouth coating, juiciness, palatability and overall acceptability using 8-10 panellists. The panellists were selected from the staff and students of the Department of Post Harvest Engg. & Technology, Faculty of Agricultural Sciences, Aligarh Muslim University (AMU), Aligarh.

RESULTS AND DISCUSSION

Physicochemical Quality

Moisture content of fresh emulsion sausage is an important characteristic, which relates the quality and shelf life. It also influences storage stability and texture of foods. High moisture foods are more prone to microbial spoilage, but they have a softer texture. Table **1** presents the results of moisture contents of buffalo meat emulsion sausage incorporated with whey protein concentrate with level (1, 2, 3 and 4%). The moisture content of control sample was 63.40 % and the sample of emulsion sausage incorporated with whey protein

Table 1: **Evaluation of Physicochemical of Fresh Buffalo Meat Emulsion Sausage Incorporated with whey Protein Concentrate**

Sample code	Moisture content%	pH value	Protein content%	ERV	WHC%	TPC logTPC/g
C.S	63.40±0.011	6.39±0.071	22.58±0.52	33.2±0.442	69.69±0.089	3.31±0.017
WPC1	64.54±0.012	6.23±0.037	23.55±0.016	34.2±0.836	71.22±0.036	3.52±0.016
WPC2	64.82±0.010	6.24±0.028	23.96±0.018	35.8±0.836	71.50±0.246	3.60±0.015
WPC3	64.70±0.013	6.35±0.081	24.06±0.023	36.4±0.894	71.48±0.225	3.64±0.140
WPC4	64.40±0.010	6.37±0.009	24.10±0.130	36.2±0.836	71.18±0.032	3.72±0.015

C.S = control sample, WPC1, 2, 3, 4= whey protein concentrate with levels 1, 2, 3 and 4%.

concentrate in different levels (1-4%) had moisture contents 64.54, 64.82, 64.70 and 64.40 % respectively. It was shown that the addition of whey protein concentrate slightly increased the moisture content of samples as compared to control sample. Meltem and Eylem [17] found similar results, that the dairy ingredients significantly increased (P<0.05) moisture content of sausage sample. The results also were in agreement with Hung and Zayas [18].

The pH value is one of the important characteristics of meat products. The pH value has a significant impact on colour, shelf life, taste, microbiological stability, yield and texture of meat and meat products. The pH value referred to acidity of meat and it indicated the extent of post mortem glycolysis. It was found that, the pH value of the sausage was more than the value of fresh meat. The pH value of control sample was 6.39.Incorporation of whey protein concentrate brought a slight change in pH of emulsion sausage. The result of incorporation of whey protein concentrate (1-4%) has been present in Table **1**.

Protein content of emulsion sausage is indicator of nutritional value as meat protein is a good source of essential mino acids. Further additionof whey protein concentrate important increased its nutritional value. The protein contents of fresh emulsion sausages incorporated with whey protein concentrate at the levels of 1 to 4%, were found to have 23.58, 23.55, 24.06 and 24.10% respectively (Table **1**). The protein content of control sample with zero percent of whey protein concentrate was 22.58%. It showed that the whey protein concentrate significantly (P<0.05) increased the protein contents of emulsion sausage.

Water holding capacity (WHC) is broadly defined as the ability of meat to retain moisture. This includes the moisture inherent to the muscle tissue as well as any fluids that may be added to the meat during further processing. Grinding of meat increased WHC by

enhancing the number of polar groups available for binding with the water molecule and the water is bound better when added in to the ground meat [19]. Water holding capacity of buffalo meat emulsion sausages incorporated with whey protein concentrate with level (1, 2, 3 and 4%) has been presented in Table **1**. The WHC of control sample was 69.69% and that of emulsion sausages samples incorporated with 1, 2, 3 and 4% of whey protein concentrate was found respectively 71.22%, 71.50%, 71.48% and 71.18. An increasing trend of WHC of sausages sample. Similar results were noticed by Meltem and Eylem [17] who reported addition of dairy ingredient significantly (P<0.05) increased WHC and emulsion satiability. A high WHC in the lean meat is a decisive factor for producing a high quality sausage [19]. The low ultimate pH generally increased tenderness, which is positively correlated to WHC [20].

Extract release volume (ERV) of buffalo meat emulsion sausage incorporated of whey protein concentrate with different level (1, 2, 3 and 4%) has been presented in Table **1**. The ERV of control sample was 33.2 and developed fresh sample of emulsion sausage with the incorporation of whey protein concentrate with levels of 1, 2, 3 and 4% found 34.2 ml, 35.8 ml, 36.4 ml and 36.2 ml respectively. It was noticed from the interpretation of extract release volume, that quality in fresh condition the emulsion sausages samples had good quality.

Microbiological Quality

Total plate count of the fresh sausage represents the bacterial load which in turn is indicative of quality of fresh sausage. Ranken and Kill [11] described the relation of bacterial populations and meat product quality in the following manner: Bacterial count 10^2 per g-excellent quality, 10^4 per g-very good quality, 10^6 per g-medium quality but rejection limit in many commercial contract, 10^7 per g-spoilage begins, 10^8 per g-meat

Table 2:   Effect of Colour on Fresh Buffalo Meat Emulsion Sausages Measured by Hunter Lab

Samples code		C.S	WPC1	WPC2	WPC3	WPC4
Colour measurement	L	33.25	31.23	30.28	29.50	30.37
	a	3.9	4.92	4.91	4.36	4.53
	b	9.22	8.76	8.85	8.4	8.8

C.S = control sample, WPC1, 2, 3, 4= whey protein concentrate with levels 1, 2, 3 and 4%.

smells, 10^9 per g-meat becomes slimy. The emulsion sausages prepared in hygienic condition in the present study were found to have loge TPC/g values 3.52, 3.60, 3.64 and 3.72 for samples with 1, 2, 3 and 4% of whey protein concentrate respectively (Table **1**). The TPC/g value of control sample was found 3.31. Different levels of whey protein concentrate did not significantly (P<0.05) affect the total plate counts of emulsion sausages. The results are also in an agreement with Hytiainen *et al*. [21]. Yeast and mold count, Coliform count and *Salmonella shigella* were not detected in the emulsion sausages samples in the fresh condition.

Hunter Colour Measurement

Colour measurement of buffalo meat emulsion sausage prepared by different levels of whey protein concentrate was done by Hunter Lab. The instrument describes the colour in three dimensional system indicating value, hue and chroma (L, a & b). Value refers to lightness/darkness and it distinguishes light colour from dark or white from black. Maximum brightness is reported as 100. The measurement of colour of samples was done in fresh condition. The numerical value of 'L' sample in fresh condition found in

the range of 29.50%- 33.25% (Table **2**). Thus sample had 33.25 % of maximum lightness as compared to 66.75% darkness. The sample of fresh emulsion sausages was grayish brown colour in appearance. Hue values were found in the range 3.9- 4.92. This showed that maximum red colour was 4.92 as compared to yellow colour 'Chroma' value were found in the range of 8.4-9.22.

Sensory Characteristics

Sensory characteristics of fresh emulsion sausages expressed in terms of sensory attributes, namely colour, aroma, texture, taste, mouth coating, juiciness, palatability and overall acceptability were evaluated by a group of panel members on a nine point Hedonic scale. The results of sensory evaluation have been presented in Table **3**. The colour scores of controlled fresh emulsion sausage were found 7.7 showing the like very much condition, while the colour score of treated sausages samples were higher in score values as compared to control sample. The score values of colour of treated sample were found in the range of 8.4- 8.7, showing the like very much to like extremely conditions. The score values of aroma were found to be in the range 8.3-8.5, which indicated very good

Table 3:   Evaluation of Sensory Characteristics of Fresh Buffalo Meat Emulsion Sausage Incorporated with whey Protein Concentrate

	Samples code	C.S	WPC1	WPC2	WPC3	WPC4
Score of Sensory attribute	Colour	7.7±0.01	8.7±0.02	8.6±0.01	8.1±0.09	8.4±0.02
	Aroma	8.4±0.13	8.4±0.25	8.4±0.15	8.5±0.20	8.3±0.26
	Texture	7.5±0.07	8.1±0.14	8.5±0.07	8.4±0.07	8.7±0.01
	Taste	7.9±0.08	8.1±0.08	8.6±0.07	8.7±0.05	8.3±0.13
	Mouth coating	8.1±0.14	7.9±0.22	8.5±0.17	8.2±0.13	8.2±0.17
	Juiciness	7.7±0.14	8.3±0.10	8.7±0.08	8.3±0.13	8.2±0.44
	Palatability	7.2±0.17	7.8±0.21	8.3±.26	8.2±0.20	8.2±0.17
	Overall acceptability	7.5±0.25	8.1±0.22	8.5±0.24	8.4±0.26	8.0±0.50

C.S = control sample, WPC1, 2, 3, 4= whey protein concentrate with levels 1, 2, 3 and 4%.

condition of the treated sausages samples. The control sample had a score of 8.4. Texture, taste, mouth coating, juiciness, palatability and overall acceptability of fresh emulsion sausage incorporated with whey protein concentrate were found above score of eight, while the score level on control sample was found to be less than eight. El-Magoli *et al*. [22] reported that 4% WPC level was preferred than the lower WPC levels in terms of juiciness and overall acceptability. It showed that the addition of whey protein concentrate in buffalo meat emulsion sausage significantly (P<0.05) affected the colour, texture, taste, mouth coating, juiciness, palatability and overall acceptability of emulsion sausages. But in case of aroma whey protein concentrate incorporation did not significantly (P<0.5) improved the aroma of sausages. Ulu [23] reported that addition of 0.2% WP increased hardness of cooked meatball compared to control samples and WP had a significant effect on the chewiness of meatballs. A study showed that hardness and chewiness increased when WP was added to Frankfurters [24]. El-Magoli *et al*. [22] reported that 4% level was found optimum as compared other levels which was lower than 4% levels.

CONCLUSION

Whey protein concentrate incorporated with buffalo meat brought considerable improvement in quality characteristics of emulsion sausages. It was established, that addition of whey protein concentrate Increased the protein content water holding capacity and extract release volume in fresh emulsion sausages samples. pH values of buffalo meat emulsion sausages decreased with incorporation of 1-2% of whey protein concentrate. The emulsion sausage had 33.25 % of maximum lightness as compared to 66.75% darkness. The sample of fresh emulsion sausages was greyish brown colour in Appearance. Whey protein concentrate improved the sensory characteristics of emulsion sausage.

ACKNOWLEDGEMENT

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