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Research article

EFFECT OF INCORPORATION OF WHEY PROTEIN POWDER ON QUALITY CHARACTERISTIC OF BUFFALO MEAT EMULSION SAUSAGE

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ABSTRACT: Investigations were carried out to study the effect of incorporation of different levels of whey protein powder (1, 2, 3 and 4%) on quality characteristics of buffalo meat emulsion sausage (ES) in fresh condition. The quality of ES was based on physicochemical characteristics namely moisture content, ash content, fat content, protein content, TBA number (Thiobarbituric acid), extract release volume (ERV), water holding capacity (WHC) and microbial characteristic viz total plat count (TPC), yeast and mold (Y&M), coliform count, salmonellashingella and sensory characteristic including instrumental colour measurement. The moisture content, protein content, ash content, TBA number, ERV and WHC were significantly ($P<0.05$) increased in emulsion sausage samples as result of incorporation with 1-4% of whey protein powder (WPP) as compared to control sample. However fat content of emulsion sausage significantly ($p<0.05$) decreased. TPC of ES was found in range of 3.70-3.93 log cfu/g. The numerical value of 'L' for the samples in fresh condition was found in the range of 19.33%- 20.57%.
Key words: Emulsion sausage, Whey protein powder, Extract release volume, Water holding capacity, Physicochemical characteristic

INTRODUCTION

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 [1]. Whey protein improves emulsion stability, provide better color properties and result in lower chewiness and elasticity [2]. 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%). β-Lac has a molar mass of 18.3 kDa and diameter of about 2 mm. The isoelectric point is 5.1 and the denaturation temperature is 78°C. β-Lac is largely responsible for solubility, gelation, foaming, emulsification, and flavour binding of whey protein. Because β-Laos is the most abundant protein in whey, it has been suggested to be one of the main determinants of the properties of whey protein gels [3]. Consumer demand healthier meat products that are low in fat, salt, cholesterol, nitrates and calories in general and contain in addition health-promoting bio active components such as carotenoids, unsaturated fatty acids, sterols and fibers on the other hand, furthermore, consumer accept these level meat products with altered formulations to taste, look and smell the same way as they're traditionally formulated and processed counterparts. Emulsion sausage incorporated with whey protein powder 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. This study was designed to determine whether whey powder at the levels (1, 2, 3 and 4%) added in the formulation of emulsion sausage prepared with buffalo meat would help to improve the quality characteristic of sausage.

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 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 powder have purchased in local market of Aligarh city. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at 2^{o} C for about 20 hours [4].

Preparation of emulsion sausage

The emulsion sausage (ES) prepared from a comminuted mixture of meat, fat, salt, condiments, spices mixtures and whey protein powder (WPP). The recipe was; meat 2 kg, fat 400 g, spices mix 32 g, salt 45 g, Condiments 50 g and WPP with a levels 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 packaging. The finished sausages were stored at 0°C in an ultra low temperature cabinet for future study [4]. Fig 1 shows the emulsion sausage samples incorporated with whey protein powder.

Physico-chemical analytical methods:

Moisture content of sausage was evaluated as given in the Food Industry Manual [5] 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 content was determined by the Kjeldahl method [6]. Water holding capacity (WHC) was determined according [7]. Extract release volume (ERV) of meat samples was evaluated as given in manual for analysis of meat and meat products. Extract release volume (ERV) of meat samples was evaluated as given in manual for analysis of meat and meat products. 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 [8].

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 [9]. 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).

Sensory characteristics

Sensory characteristics of emulsion sausages were evaluated on 9 points scale by Hedonic rating tests [10] 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.

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.

Statistical analysis

Data obtained from experimental observations (n = 6), were subjected to analysis of variance (ANOVA) as described by [11].

RESULTS AND DISCUSSION

Physicochemical analysis

The results of physicochemical analysis of buffalo meat emulsion sausage samples incorporated with different levels of whey protein powder (1, 2, 3 and 4%) have been presents in Table 1. Moisture content of fresh emulsion sausage sample is an important characteristic, which relates to the quality and shelf life. It also influences storage stability and texture of foods. Furthermore high moisture foods are more prone to microbial spoilage, but they have a softer texture. In fresh condition the moisture content of control sample was 63.40% and that of emulsion sausage samples incorporated with of whey protein powder with levels of 1, 2, 3 and 4% were found 65.70, 64.71, 66.12 and 64.96 % respectively. It showed that the addition of whey protein powder increased the moisture content of samples as compared to control sample. Similar results were found by [12]. He advocated that whey powder and skim milk powder (SMP) significantly increased ($p<0.01$) moisture retention values of meat patties. He also explained that patties formulated with WP had higher moisture retention than those formulated with SMP and control samples. Similarly [13] found that result by addition of 0.2% WP increased moisture content of meatball.

Fat content is the important ingredient for making emulsion in sausage, it has high calorific value it provides energy to the body and gives palatability to the product. The fat content of treated samples with whey protein powder at the levels of (1, 2, 3 and 4%) were found 12.23, 12.34, 12.20 and 11.95% respectively and control sample of ES was 13.47%. That shows a significant difference between control sample and emulsion sausages incorporated with whey protein powder. It represented that a significantly ($p<0.05$) decreased fat content of emulsion sausage incorporated whey protein powder with levels 1, 2, 3 &4% as compared to control sample. [14] Explained that whey protein did not affect fat contents of the raw and cooked meat balls. Similar results found by 15], these study revealed that incorporation of whey powder did not affect the fat content of cooked beef sausage. But the present study concluded that addition of whey protein powder in emulsion sausages caused a slight decrease of fat content of sausage samples.

Protein content of emulsion sausage is indicator of nutritional value as meat protein is a good source of essential amino acids. Further, addition of whey protein increased its nutritional value. The protein content of control sample was 22.58% and the samples of emulsion sausage treated with whey protein powder at different levels had protein contents 24.90, 24.96, 25.12 and 25.22% respectively. It showed that the whey protein powder significantly ($P<0.05$) increased the protein of emulsion sausage. [16] stated emulsion type Turkey roll incorporated with whey protein had lower protein content than control. This lower may due to lower quantity of protein (13%) in WP.

Evaluation of the extract release volume (ERV) phenomenon is a rapid test for determining spoilage in meat. Extract release volume appears to have considerable possibilities for assessing the spoilage of beef [17]. It also has a highly significant correlation with Water holding capacity. The ERV decreases as spoilage progresses and no filtrate is obtained from putrid meat [18]. In fresh condition, ERV of control sample was 33.2 ml and emulsion sausage with the incorporated of whey protein powder at levels of 1, 2, 3 and 4% were found 33.4 ml, 37.2 ml, 36.2 ml and 36.6 ml respectively. The emulsion sausages were found to be in good quality.

The ash content of emulsion sausage showed the quantity of mineral matter in the samples. In fresh condition the ash contents of emulsion sausages samples of control sample was 2.36% and samples incorporated with whey protein powder at levels of 1, 2, 3 and 4% had 2.36, 2.18, 2.54 and 2.34% respectively. Incorporation of whey protein powder brought a slight change in ash content of emulsion sausage. It was may due affect of mineral of whey protein powder to treated emulsion sausage. According to [14] addition of whey protein in raw meatballs increased slightly the ash content, and four percent WP almost doubled the ash content when compared with that of the control.
The sausages contain good quantity of fat and therefore the products is prone to lipid oxidation, which leads to onset of rancidity and finally warm over flavour. Thiobarbituric acid number is estimated to know the extent of oxidation of fat. TBA number of emulsion sausages incorporated with whey protein powder at the levels of 1, 2, 3 and 4 % were found to have 0.195, 0.208, 0.202 and 0.196 respectively, while control sample had TBA number as 0.179. Addition of whey protein powder slightly increased the TBA number of emulsion sausage as compared to control sample. The treatment of whey protein powder significantly ($p<0.05$) increased the TBA number of the samples. [19] reported the pork sausage incorporated with low, medium and high calcium milk product recorded significantly ($P<0.01$) higher mean TBA values compared to control.

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 increase WHC by enhancing the number of polar groups available for binding with the water molecule and the water is bound better when meat in brought to gronned form [20]. The water holding capacity (WHC) of control sample was 69.69% and that of emulsion sausages samples incorporated with 1, 2, 3 and 4% of whey protein powder were found 75.33, 74.90, 75.22 and 74.60 % respectively in fresh condition. It represented that a significantly ($p<0.05$) increase in WHC of ES incorporated whey protein powder with levels varying in through 1-4 % as compared to control sample.

Microbial analysis

The emulsion sausages were prepared in hygienic condition. The results of microbial of emulsion sausages samples incorporated with 1, 2, 3 and 4 % of WPP have been presented table 2. Total plate count (TPC) of the fresh sausage represents the bacterial load which in turn is indicative of quality of emulsion sausage. In present study, the samples of emulsion sausages treated with 1, 2, 3 and 4 % of WPP had log TPC/g values 3.70, 3.93, 3.92 and 3.92 respectively. The TPC/g value of control sample was found 3.31. Different levels of whey protein powder significantly ($P<0.05$) increased the total plate counts of emulsion sausages. The results are also in an agreement with [21]. Yeast and mold count, Coliform count and *Salmonella shigella* were not detected in the fresh sausages samples in the fresh condition.

Sensory characteristics of buffalo meat emulsion sausages incorporated with different levels of whey protein powder

Sensory characteristics of fresh emulsion sausage samples expressed in terms of sensory attributes, namely colour, aroma, texture, taste, mouth coating, juiciness. These sensory scores for treatment attributes were evaluated by a group of panel members on a nine point Hedonic scale. The results of sensory evaluation of emulsion sausage samples incorporated with different levels of whey protein powder (1, 2, 3 & 4%) have been presented in Table 3. The colour score of controlled emulsion sausage sample was found 7.7. It showed 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 has found in the range of 7.9- 8.3. It explained like very much to like extremely conditions. The score values of aroma were found to be in the range 8.2-8.4, which indicated very good condition of the treated sausages samples. The control sample had a score of 8.4 for aroma. Texture, taste, mouth coating and juiciness of fresh emulsion sausage incorporated with whey protein powder were found above eight score, while the score of control sample was found less than eight. [13] quoted that addition of 0.2% whey protein increased hardness of cooked meat ball compared to control samples and treated samples had a significant effect on the chewiness of meatballs. Likewise, another study showed that hardness and chewiness increased when WP was added to Frankfurters [22]. [12] reported that addition of 1 and 2% whey protein and skim milk powder were make no significant differences in appearance, interior colour, juiciness and flavour scores of patties. [23] Claimed that additions of whey powder to comminuted beef patties increased flavour scores.

Table 1: Evaluation of physicochemical of buffalo meat emulsion sausage incorporated with whey protein powder

Sample code	Moisture content%	Fat content%	Protein content%	Ash content%	ERV	TBA No.	WHC%
Cs	63.40± 0.011	13.47± 0.015	22.58± 0.052	2.36± 0.027	33.2± 0.449	0.179± 0.004	69.69± 0.089
$Swpp_1$	65.70± 0.058	12.23± 0.051	24.90± 0.032	2.36± 0.056	33.4± 0.894	0.195± 0.004	75.37± 0.098
$Swpp_2$	64.71± 0.016	12.34± 0.074	24.96± 0.035	2.18± 0.032	37.2± 0.839	0.208± 0.006	74.90± 0.063
$Swpp_3$	66.12± 0.021	12.20± 0.063	25.12± 0.048	2.54± 0.074	36.2± 0.836	0.202± 0.002	75.22± 0.077
$Swpp_4$	64.96± 0.019	11.95± 0.032	25.22± 0.041	2.34± 0.045	36.6± 0.547	0.196± 0.008	74.60± 054

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

Table 2: Evaluation of microbiological of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein powder

Sample code	Total plate count logTPC/g	Yeast and mold count, log Y&M C/g	Coliform count	Salmonella shigella
Cs	3.31±0.017	ND	ND	ND
$Swpp_1$	3.70±0.082	ND	ND	ND
$Swpp_2$	3.93±0.032	ND	ND	ND
$Swpp_3$	3.92±0.030	ND	ND	ND
$Swpp_4$	3.92±0.048	ND	ND	ND

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

Table 3: Evaluation of sensory characteristics of buffalo meat emulsion sausage incorporated with whey protein powder

Samples code		Cs	$Swpc_1$	$Swpc_2$	$Swpc_3$	$Swpc_4$
Score of Sensory	**Colour**	7.7±0.01	8.3±0.023	8.3±0.014	8.1±0.018	7.9±0.016
	Aroma	8.4±0.13	8.4±0.012	8.4±0.019	8.3±0.016	8.2±0.016
	Texture	7.5±0.07	8.3±0.021	8.4±0.020	8.4±0.016	8.4±0.020
	Taste	7.9±0.08	8.5±0.070	8.0±0.083	8.3±0.089	8.4±0.083
	Mouth coating	8.1±0.14	7.9±0.070	7.6±0.089	7.9±0.083	8.1±0.083
	Juiciness	7.7±0.14	8.1±0.089	7.9±0.054	8.0±0.054	8.0±0.089

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

Table 4: Effect of colour on buffalo meat emulsion sausages incorporated with whey protein powder measured by Hunter lab

Samples code		C.S	SWPP1	SWPP2	SWPP3	SWPP4
Colour measurement	**L**	33.25	20.49	20.57	19.33	20.76
	a	3.9	4.61	3.35	3.99	3.85
	b	9.22	6.09	4.96	5.2	5.41

Cs = Control sample, $Swpp_{1, 2, 3, 4}$= Sausage whey protein powder at the levels 1, 2, 3 and 4%

Fig 1: Buffalo meat emulsion sausage incorporated with whey protein powder

Instrumental measurement of colour

The instrument describes the colour in three dimensional system indicating value, hue and chroma (L, a and b). Value refers to lightness/darkness and it distinguishes light colour from dark or white from black. Maximum brightness is reported as 100. Hue is the colour attribute by which an object/food is judged to red, yellow, green, blue and so forth. It actually describes the shade of red/ yellow/ green/ blue. The four basic colours are given max numerical value 60. Chroma refers to vividness, depth, purity and saturation of a particular colour. It describes how much a particular object/ foundation is yellow/red/ green/ blue.Table 4 present the results of colour evaluation of emulsion sausages by Hunter Lab. The measurement of colour of samples was done in fresh condition. The numerical value of 'L' sample in fresh condition incorporated with whey protein powder found in the range of 20.49%- 20.76%. Thus sample had 20.76 % of maximum lightness as compared to 79.33% darkness. The sample of fresh emulsion sausages was grayish brown colour in appearance. Hue values were found in the range 3.35- 4.61. This showed that maximum red colour was 4.61 as compared to yellow colour 'Chroma' value were found in the range of 4.96-6.09. [14] reported the fat level (20%) and Whey protein significantly affected L* values of cooked meat balls ($p < 0.05$). Increasing the fat level in formulation resulted lower a^* values but whey protein had no effect on a* values of meatballs. Neither the fat level nor the whey protein level affected b* values of samples ($p > 0.05$).

CONCLUSIONS

It had shown that addition of whey protein powder significantly ($p<0.05$) increased the moisture content, protein content, ash content, TBA number and water holding capacity and extract release volume in fresh emulsion sausages samples. Fat content of buffalo meat emulsion sausages decreased with incorporation with addition of whey protein powder. The emulsion sausage had 20.57% of maximum lightness as compared to 79.43% darkness. The emulsion sausage samples were found to safe in microbial characteristic. Whey protein powder improved the sensory characteristics of emulsion sausage.

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