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Effect of whey protein products on microbiological characteristics of buffalo meat emulsion sausage

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Abstract

Incorporation of whey protein products (WPPs) namely whey protein concentrate, isolate and whey protein powder with the different levels (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 term of microbiological characteristics like total plat count (TPC) and Yeast and mould count, coliform count and *Salmonella shigella* count. Refrigerated Storage (0ºC) significantly ($p<0.05$) increased total plate count, yeast and mould count, coliform count of emulsion sausages incorporated with different levels of whey protein concentrate, isolate and powder. The total plate count was found to be between 7.11- 7.39 log cfu/g, 6.74- 7.23 log cfu/g and 6.76-7.21 log cfu/g, yeast and mould count was found to be 3.34-3.86 log cfu/g, 3.45-3.57 log cfu/g and 3.58-3.95 log cfu/g on 25th day of storage for samples prepared with different levels (1, 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder respectively. After 25 days of refrigerated storage (0ºC) total plate count and yeast and mould count of all samples were found to be in the safe limit (3.32 - 3.93 log cfu/g). Coliform count were detected in emulsion sausages samples and it was found to be in the range of 2.06-2.34 log cfu/g, 2.15-2.28 log cfu/g and 2.08-2.22 log cfu/g in emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively after 15 days of storage. In final stage of storage, coliform count was found to be in the range of 2.16-2.54 log cfu/g, 2.30-2.41 and 2.30-2.43. *Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C for 25 days.

Keyword: Whey Protein Products, Buffalo Meat, Emulsion Sausage, Microbiological Characteristics

Introduction

Meat and meat products 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 (Cosgrove *et al.,* 2005). 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 is a very versatile culinary product and has become a vital element of both cuisine and culture. 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 (Pearson and Brooks, 1978). India has a major show in world buffalo population; Pakistan and China are second and third in the world of the buffalo population. India has 105.1 million and they comprise approximately 58 percent of the total world buffalo population, which Pakistan and China have respectively 29.0 million and 23.27 million and India has share 50.34 % of buffalo meat production. (Badpa and Ahmad, 2014a). India's per capita buffalo meat consumption is estimated at approximately two kg per year. Meat consumption increases will likely occur primarily in the poultry segment. CY (current year) 2013 buffalo meat consumption in India is forecast at 2 million tonnes (on a carcass weight equivalent basis), CY 2012 buffalo meat consumption is estimated at 1.98 million tonnes, and CY 2011 buffalo meat consumption is kept unchanged at 1.95 million tonnes. Note that despite an increase in overall consumption, per capita

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consumption may not arise due to India's growing population. 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. The word sausage comes from the Middle English sausige, which came from sal, Latin for salt. In France they are sausissons and in Germany, Wurst. In practice for over a millennia sausage-making was originally a method used to preserve meats, especially lesser cuts (Huda *et al.,* 2012; Marianski and Marianski, 2010). 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 (Anker *et al.,* 1998). Whey protein improves emulsion stability, provide better color properties and result in lower chewiness and elasticity. (Yetmin *et al.,* 2001). Whey protein is a high quality, complete protein with all the essential amino acids. Whey protein is richest source of naturally occurring branched chain amino acids (leucine, isoleucine and valine). These are important for active individuals, exercise and professional athletes. Adding whey protein to the diet is great ways to jump start a weight loss program. Whey protein is a key ingredient in numerous weight loss and meal replacement products. Emulsion sausage incorporated with whey protein products, can be prepared from buffalo meat using other ingredient salt, spices, condiment, whey protein (isolate, concentrate, whey powder), fat and animal fat. Emulsion sausage incorporated with whey protein products, will have a pleasant taste, excellent flavor and improved juiciness along with bioactive ingredients like immuglobulins and lacto-ferrin, which boost the immune system. Emulsion sausage incorporated with whey protein products will have rich abundance of branched chain amino acids and its quick absorption rate. These are important to help repair and rebuild muscles. Emulsion sausage treated with whey protein products have fresh, natural taste and do not contain isoflavones and any other component with potential hormonal effects. There are chances of contamination of meat and meat products during slaughtering, handling and processing. The person, equipment and machineries are potential source of microbiological penetration in the developed products. The most important part of the production of meat and meat products is to make the system hazard free. The industry environment must be hygienic and to ensure the safety and security, hazard Analysis Critical Control Point (HACCP) system should be followed. The study was conducted with the following objectives of the investigation: 1. To develop emulsion sausage of buffalo meat, incorporating whey protein products namely whey protein concentrate, whey protein isolate and whey protein powder. 2. To study the microbiological properties of the developed product.

Material and methods

Preparation of buffalo meat and non meat ingredient

Meat samples were collected from the local meat shop in the study was 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

LDPE film were procured from the local market. Whey protein concentrate provided by Mahaan proteins Ltd, New Delhi, India, and whey protein isolate and whey protein powder provided from market. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at 2^{o} C for 20 hours (Badpa and Ahmad, 2014b).

Meat sample preparation

Twenty six Kg meats were taken for preparation of emulsion sausage. Four different type samples of emulsion sausages namely control, emulsion sausage incorporated with whey protein concentrate (WPC), whey protein isolate (WPI) and whey protein powder (WPP) were prepared. The sample size was kept 2.0 Kg lean meat each (excluding other ingredient like fat, whey protein, salt, spices and condiment). Different levels (1, 2, 3, and 4%) of whey protein products namely whey protein concentrate, isolate and whey protein powder was taken for the study of emulsion sausage.

Preparation of emulsion sausage

The emulsion sausage (EM) prepared from a comminuted mixture of meat, fat, salt, condiments, spices mixtures and WPC, WPI, WPP. The recipe was; meat 2 kg, fat 400 g, spices mix 32 g, salt 45 g, Condiments 50 g and WPPs 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 70°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 packaging. The finished sausages were stored at 0°C in an ultra low temperature cabinet for future study (badpa and Ahmad 2014b).

Microbiological analysis methods:

All 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 mould count (APHA, 1992). The microbiological characteristics of sausage samples were evaluated in fresh conditions and during refrigerated storage (0^{o} C) after constant intervals. For the determination of the total plate count, yeast and mould count, Coliform count and *Salmonella shigella*, 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).

$$\text{TPC (cfu/g)} = \frac{\text{Number of colonies}}{\text{Amount used for inoculation} \times \text{dilution factor}} \quad (1)$$

Sensory Characteristics

Sensory characteristics of emulsion sausages were evaluated on 9 points scale by Hedonic rating tests (Ranganna, 1994) 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 observation (n=5), were subjected to analysis of variance (Two ways ANOVA) variance (Cochron and Cox, 1992). Linear regression was also determined to study the storage behavior of microbial properties of emulsion sausage incorporated with different levels 1, 2, 3 and 4 % of whey protein products.

Analysis of Variance

To test the significance of the effect of treatment and storage period on quality parameters, analysis of variance (ANOVA) of the collected data for different properties was carried out (Dospekhov, 1984). The least significant differences (LSD) or critical differences (CD) of mean values were calculated using formula

$$\text{LSD or CD} = \left[\sqrt{\frac{2 \times S^2}{n}}\right] \times t \qquad (2)$$

Where,

S= error of total sum of deviation squares

n= No. Of replications

t= value at 0.05 obtained at the degree of freedom

Results and discussion

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 especially bacteria 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 of emulsion sausages were evaluated and reported as log (cfu)/g. Total plate count of samples of emulsion sausages were enumerated in fresh condition and periodically after every 5 days during refrigerated storage at 0ºC. Table 1, 2 and 3 presents the microbial profile of emulsion sausages (controlled and treated) during refrigerated storage at 0°C. The ANOVA results indicated refrigerated storage significantly ($p<0.05$) increased total plate count of emulsion sausages incorporated with whey protein products at 0ºC .The total plate count was found to be between 7.11- 7.39 log cfu/g for WPC, 6.74-7.23 log cfu/g for WPI and 6.76-7.21 log cfu/g for WPP on 25th day of storage prepared with different levels (1, 2, 3 and 4%). The end of 25th day of refrigerated storage (0ºC) total plate count of all sausage samples were found to be in the safe limit (3.32- 3.93 log cfu/g). Similar result of TPC were reported by Sachindra *et al.,* (2005), who advocated that total plate counts (log cfu/g) buffalo sausage significantly ($p<0.05$) increased till 35 days of storage with different packaging system (packed under vacuum and nitrogen flush system). But the sausage sample packed under nitrogen atmosphere had more increased value of TPC on compared to vacuum packaging from fourth day onward. Although Bhaskar *et al.,* (2009) also reported total plate count 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 total plate count under refrigerated storage was also observed by Murthy (1986) in pork sausages, Nath *et al*., (1995) in chicken patties and Rao *et al.,* (1996) in smoked chicken sausages. Ranken and Kill (1993) described that the spoilage condition which are detected when total plate count increase to 10^7 per g in meat and meat products. The results are also in an agreement with Hytainen *et al*., (1966) and Essory *et al.,* (1985). Kala *et al.,* (2007) described the total plate count showed an increasing trend for chicken emulsion patties in during the storage. This finding is in agreement with Foster *et al*., (1977) in ground beef and beef soy patties. Bacteria even at 0°C 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.

Therefore sausage developed off-flavour after complete spoilage (Ahmad, 2005). As reported by Brooks *et al.,* (2008) some authors stated that microbial population on raw beef must reach approximately 10^8cfu/g to show tackiness when touched, whereas others have claimed that proteolytic changes do not occur until bacterial populations are greater than 3.2×10^9cfu/cm^2 are reached. The ANOVA result indicated that the different levels of whey protein products significantly ($p<0.05$) affected total plate count of emulsion sausages.

Table 1. Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein concentrate

Sample Code	TPC (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	3.32± 0.017a	3.95± 0.032	4.46± 0.013	5.12± 0.016	5.91± 0.038	6.65± 0.027e
$Swpc_1$	3.52± 0.016b	4.65± 0.007	5.12± 0.011	5.97± 0.016	6.54± 0.027	7.12± 0.019f
$Swpc_2$	3.60± 0.015c	4.51± 0.018	5.22± 0.015	6.09± 0.024	6.83± 0.027	7.33± 0.035g
$Swpc_3$	3.64± 0.014c	4.66± 0.014	5.45± 0.013	6.33± 0.021	6.94± 0.032	7.39± 0.020g
$Swpc_4$	3.72± 0.015d	4.50± 0.015	5.32± 0.008	5.94± 0.026	6.64± 0.028	7.11± 0.039f

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

Table 2. Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein isolate

Sample Code	**TPC (Log cfu/g)**					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	3.32± 0.017a	3.95± 0.032	4.46± 0.013	5.12± 0.016	5.91± 0.038	6.65± 0.027e
$Swpi_1$	3.69± 0.013b	4.13± 0.020	4.85± 0.027	5.64± 0.031	6.15± 0.033	6.90± 0.038f
$Swpi_2$	3.98± 0.012c	4.94± 0.038	5.42± 0.020	5.86± 0.032	6.45± 0.032	7.12± 0.043g
$Swpi_3$	3.85± 0.037d	4.42± 0.018	5.33± 0.020	5.95± 0.035	6.61± 0.032	7.23± 0.014h
$Swpi_4$	3.80± 0.055d	4.33± 0.031	4.95± 0.025	5.44± 0.029	5.95± 0.035	6.74± 0.026i

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

Figures 1, 2 and 3 shows the regression analysis of TPC of emulsion sausages during refrigerated storage at 0ºC produced by whey protein concentrate, isolate and powder respectively. The equation of regression lines and correlation coefficients were shown on the regression graphs. The positive sign in the coefficients of x explain that there was constant increase in TPC during refrigerated storage. The correlation coefficient values exhibited a linear relation between TPC values and storage days. The increasing nature of TPC with storage time was perfect at $R^2=1$, the values of R^2 for all samples were found near 1 which shows that correlation are almost perfect and the graphs may be approximated to a straight line.

Yeast and Mould count

The result of yeast and mould counts of sausages samples expressed as log cfu/g has been presented in Tables 4, 5 and 6. 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} day of storage. The

results were agreement with Kala *et al.,* (2007) who advocated that yeast and mould counts were observed on 9th and 12th days during refrigerated storage for chicken emulsion patties. However, countable colonies were noted in sausage samples on 25th day of storage in three treatments of whey protein products. Refrigerated storage significantly ($p<0.05$) increased the yeast and mould count of emulsion sausages. The yeast and mould count was found to be between 3.34 - 3.86 log cfu/g, 3.45 -3.57 log cfu/g and 3.58 -3.95 log cfu/g for emulsion sausages incorporated with whey protein concentrate, isolate and powder in varied levels (1, 2, 3 and 4 %) respectively. The ANOVA tests indicated that the different levels of whey protein concentrate isolate and powder significantly ($p<0.05$) increased yeast and mould count of emulsion sausages.

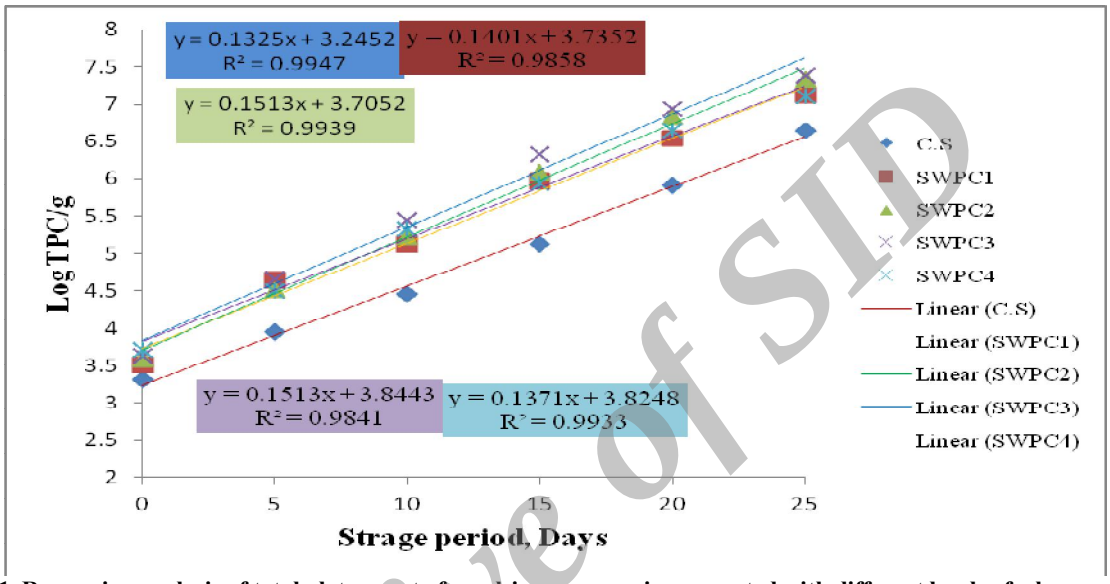

Fig. 1. Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

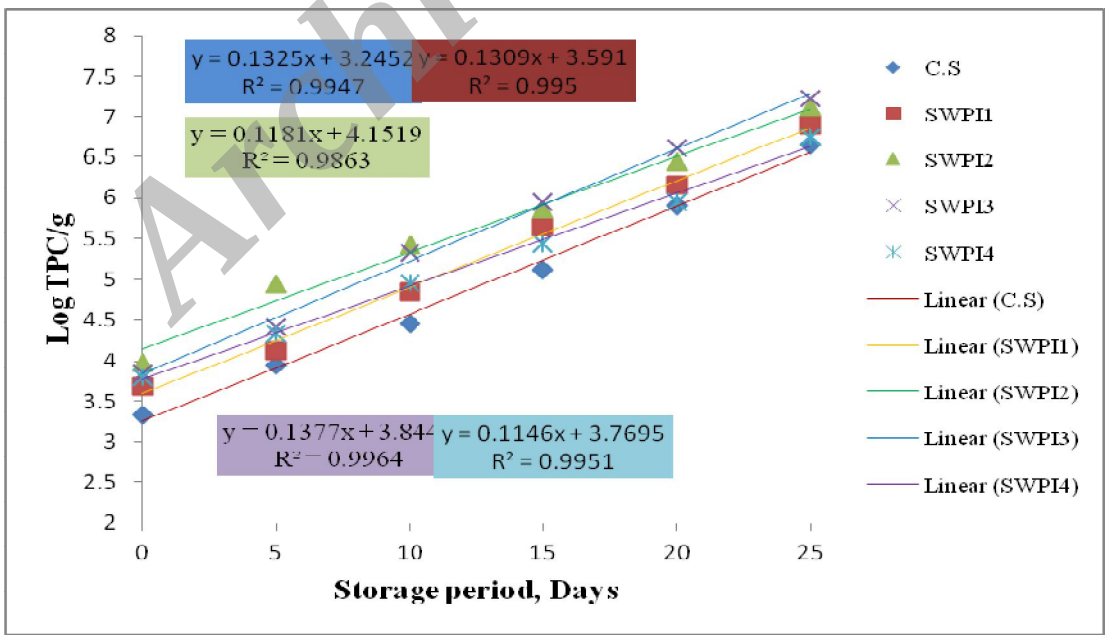

Fig. 2. Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

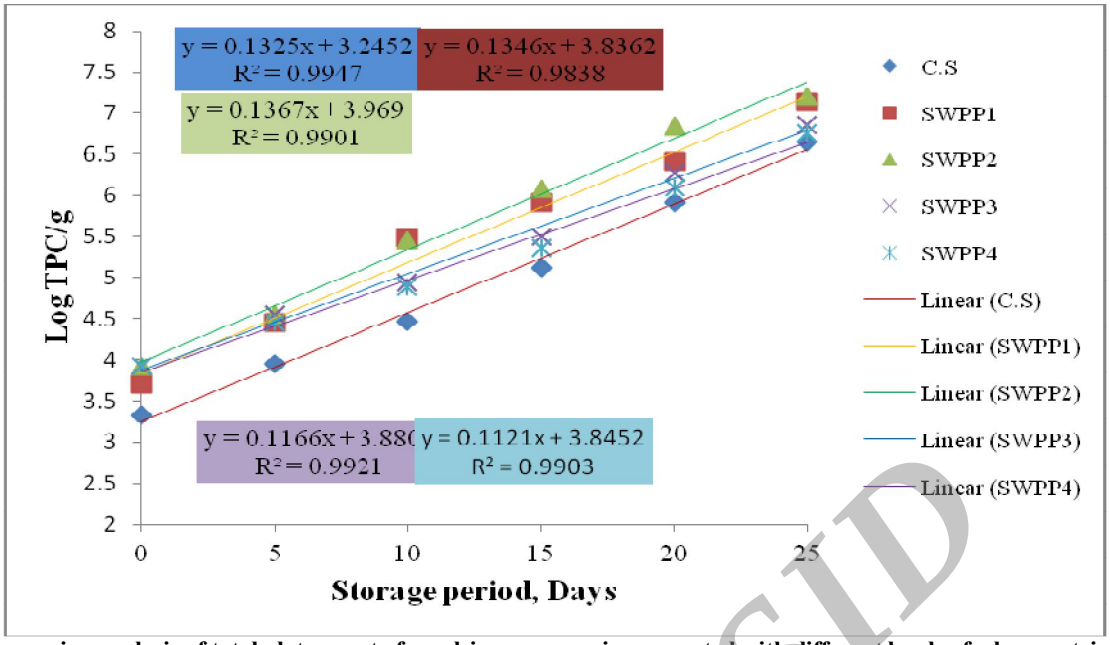

Fig. 3. Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)

After 25 days of refrigerated storage (0ºC) yeast and mould count of all samples was found to be less than 4 log cfu/g. This particular value of yeast and mould count defined the spoilage condition. When log cfu/g of yeast and mold count increased to 4.0, spoilage of food samples starts (CQIASA, 2003). Casaburi *et al.,* (2007) observed that the growth of yeast and mould 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. Erkmen (2008) reported similar observation of Turkish sausage after inclusion with LAB strains as protective cultures. Likewise, Olaoye and Onilude (2010) noted a reduction in the yeast and moulds counts in fresh beef inoculated with LAB starters.

Coliform count

Coliform count of emulsion sausages produced with different whey protein product namely whey protein concentrate, isolate and powder were enumerated and it was found that there were no sign of coliform bacteria on plates containing MacConkey agar till 20th day of storage (0ºC) the incorporation of whey protein concentrate, isolate and powder in four levels (1, 2, 3 and 4%) were selected for study. However, coliform count of sausages samples were found to be in the range of 2.06-2.34 log cfu/g, 2.15-2.28 log cfu/g and 2.08-2.22 log cfu/g in emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively after 15days of storage (Tables 7, 8 and 9). The results are in agreement with Kala *et al.,* (2007) who found that the coliforms were totally undetected from any of the emulsions chicken patties during 12 day of storage. The refrigerated storage significantly ($p<0.05$) increased the coliform count. In final stage of storage, coliform count was found to be in the range of 2.16-2.54, 2.30-2.41and 2.30-2.43 log cfu/g. Reduction in counts of *Enterabacteriaceae* and *Staphylococcus* in meat has been reported by (Gomolka-Pawlicka *et al.,* 2004; Kaban and Kaya 2006; Olaoye and Onilude, 2010). Usually, the presence of total coliform in food indicates improper heat treatment or post processing contamination. Coliform were not usually pathogenic. They also indicate inadequate sanitation and disinfection of appliance (CQIASA, 2003).

Table 3. Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein powder

Sample Code	TPC (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	3.32± 0.017a	3.95± 0.032	4.46± 0.013	5.12± 0.016	5.91± 0.038	6.65± 0.027d
$Swpp_1$	3.70± 0.082b	4.45± 0.060	5.48± 0.067	5.92± 0.048	6.42± 0.035	7.14± 0.031e
$Swpp_2$	3.93± 0.032c	4.55± 0.030	5.45± 0.031	6.08± 0.041	6.85± 0.031	7.21± 0.040f
$Swpp_3$	3.92± 0.030c	4.55± 0.033	4.94± 0.035	5.49± 0.049	6.27± 0.054	6.86± 0.037g
$Swpp_4$	3.92± 0.048c	4.45± 0.020	4.89± 0.050	5.36± 0.033	6.10± 0.064	6.76± 0.035h

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

Table 4. Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein concentrate

Sample Code	Y&M (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	TFTC	1.74± 0.041	2.08± 0.028	2.61± 0.079	3.23± 0.050b
$Swpc_1$	NDa	TFTC	1.85± 0.038	2.26± 0.058	2.94± 0.044	3.34± 0.034c
$Swpc_2$	NDa	TFTC	1.84± 0.028	2.22± 0.043	2.96± 0.029	3.45± 0.031d
$Swpc_3$	NDa	TFTC	2.15± 0.041	2.43± 0.037	2.93± 0.024	3.86± 0.031e
$Swpc_4$	NDa	TFTC	2.19± 0.051	2.40± 0.070	2.90± 0.042	3.82± 0.052e

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

Table 5. Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein isolate

Sample Code	Y&M (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	TFTC	1.74± 0.041	2.08± 0.028	2.61± 0.079	3.23± 0.050b
$Swpi_1$	NDa	TFTC	2.25± 0.049	2.84± 0.029	3.20± 0.045	3.62± 0.042c
$Swpi_2$	NDa	TFTC	2.18± 0.043	2.35± 0.035	3.23± 0.058	3.55± 0.055d
$Swpi_3$	NDa	TFTC	2.33± 0.020	2.41± 0.021	3.12± 0.035	3.45± 0.051e
$Swpi_4$	NDa	TFTC	2.24± 0.038	2.53± 0.054	3.32± 0.036	3.57± 0.053d

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $SWPI_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%), ND = Not detected, TFTC = To few to count

Table 6. Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein powder

Sample Code	Y&M (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	TFTC	1.74± 0.041	2.08± 0.028	2.61± 0.079	3.23± 0.050b
$Swpp_1$	NDa	TFTC	1.93± 0.041	2.14± 0.037	2.86± 0.074	3.58± 0.049c
$Swpp_2$	NDa	TFTC	2.24± 0.025	2.76± 0.085	3.23± 0.041	3.89± 0.049d
$Swpp_3$	NDa	TFTC	2.11± 0.051	2.65± 0.057	3.15± 0.055	3.79± 0.049e
$Swpp_4$	NDa	TFTC	2.34± 0.028	2.73± 0.079	3.30± 0.061	3.95± 0.088f

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $Swpp_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%), ND = Not detected, TFTC = To few to count

Salmonella shigella count

Salmonella shigella was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C for 25 days. These findings further support the idea of Kala*et al.,* (2007) Salmonella could not be detected from the emulsion patties samples during various periods of storage.

Sensory characteristics of buffalo meat emulsion sausages incorporated with whey protein products

Sensory characteristics of fresh emulsion sausages expressed in terms of sensory attributes, namely colour, aroma, texture, taste, mouth coating, juiciness, palatability and overall acceptability. These sensory scores for different attributes of emulsion sausage samples were evaluated by a group of panel members on a nine point Hedonic scale.

Emulsion sausage incorporated with whey protein products

The results of sensory evaluation have been presented in Tables 10, 11 and 12. The colour scores of controlled emulsion sausage were found to be 7.7. It indicated like very much condition, while the colour scores 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 8.0 - 8.7. These results indicated the like very much to like extremely conditions. The score values of aroma were found to be in the range 8.0-8.5, 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, juiciness, palatability and overall acceptability of fresh emulsion sausage incorporated with whey protein concentrate were found to have more than eight score, while the score value of control sample for these attributes was found to be less than eight. El-Magoli *et al*., (1996) reported that level of 4% WPC was preferred than the lower WPC levels in terms of juiciness and overall acceptability. It shows 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. However, whey protein concentrate incorporation did not significantly ($p<0.5$) improve the aroma of sausages. Ulu (2004) claimed that addition of 0.2% WP increased hardness of cooked meat ball compared to control samples and WP had a significant effect on the chewiness of meat balls. The study indicated that hardness and chewiness increased when whey protein was added to Frankfurters (Hughes *et al*., 1998). Lyons *et al*., (1999) observed that increasing concentrations of whey protein decreased flavour scores.

Table 7. Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein concentrate

Sample Code	Coliform count (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	ND	ND	ND	2.06±0.022	2.16±0.022b
$Swpc_1$	NDa	ND	ND	ND	2.30±0.028	2.54±0.031c
$Swpc_2$	NDa	ND	ND	ND	2.21±0.036	2.35±0.022d
$Swpc_3$	NDa	ND	ND	ND	2.34±0.025	2.54±0.025c
$Swpc_4$	NDa	ND	ND	ND	2.11±0.022	2.25±0.036e

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%), ND = Not detected

Table 8. Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein isolate

Sample Code	Coliform count (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	ND	ND	ND	2.06±0.022	2.16±0.022b
$Swpi_1$	NDa	ND	ND	ND	2.28±0.068	2.35±0.033c
$Swpi_2$	NDa	ND	ND	ND	2.22±0.087	2.30±0.040c
$Swpi_3$	NDa	ND	ND	ND	2.15±0.032	2.38±0.049c
$Swpi_4$	NDa	ND	ND	ND	2.19±0.061	2.41±0.056d

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%), ND = Not detected

Table 9. Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein powder

Sample Code	Coliform count (Log cfu/g)					
	Storage Period (Days)					
	0	5	10	15	20	25
Cs	NDa	ND	ND	ND	2.06±0.022	2.16±0.022b
$Swpp_1$	NDa	ND	ND	ND	2.15±0.040	2.30±0.034c
$Swpp_2$	NDa	ND	ND	ND	2.11±0.043	2.34±0.044c
$Swpp_3$	NDa	ND	ND	ND	2.08±0.048	2.40±0.060d
$Swpp_4$	NDa	ND	ND	ND	2.22±0.026	2.43±0.065d

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

Table 10. Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein concentrate

	Samples code	Cs	$Swpc_1$	$Swpc_2$	$Swpc_3$	$Swpc_4$	LSD
Score of Sensory attribute	Colour	7.7± 0.01a	8.7± 0.02b	8.6± 0.01b	8.1± 0.09c	8.4± 0.02c	0.022091
	Aroma	8.4± 0.13d	8.4± 0.25d	8.4± 0.15d	8.5± 0.20e	8.3± 0.26f	0.098678
	Texture	7.5± 0.07g	8.1± 0.14g	8.5± 0.07gh	8.4± 0.07gh	8.7± 0.01h	0.045726
	Taste	7.9± 0.08i	8.1± 0.08j	8.6± 0.07jk	8.7± 0.05jk	8.3± 0.13j	0.042184
	Mouth coating	8.1± 0.14k	7.9± 0.22m	8.5± 0.17n	8.2± 0.13mn	8.2± 0.17mn	0.082771
	Juiciness	7.7± 0.14p	8.3± 0.10q	8.7± 0.08q	8.3± 0.13qr	8.2± 0.44qr	0.106926
	Palatability	7.2± 0.17s	7.8± 0.21st	8.3±0.2 6t	8.2± 0.20tv	8.2± 0.17tv	0.099378
	Overall acceptability	7.5± 0.25w	8.1± 0.22x	8.5± 0.24y	8.4± 0.26y	8.0± 0.50x	0.15268

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

Table 11. Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein isolate

	Samples code	Cs	$Swpi_1$	$Swpi_2$	$Swpi_3$	$Swpi_4$	LSD
Score of Sensory attribute	Colour	7.7± 0.01a	8.0± 0.070b	8.6± 0.054c	8.7± 0.89c	8.4± 0.089d	0.032764
	Aroma	8.4± 0.13e	8.0± 0.089ef	8.0± 0.087ef	8.1± 0.089ef	8.1± 0.083ef	0.046032
	Texture	7.5± 0.07g	8.6± 0.054hi	8.3± 0.089i	8.4± 0.083hi	8.2± 0.054hi	0.03401
	Taste	7.9± 0.08j	8.3± 0.089jk	8.2± 0.054k	8.2± 0.044k	8.1± 0.070k	0.03401
	Mouth coating	8.1± 0.14m	8.1± 0.89m	8.0± 0.054m	7.9± 0.083m	8.2± 0.070m	0.044743
	Juiciness	7.7± 0.14n	7.6± 0.070n	7.6± 0.089n	8.0± 0.089o	7.8± 0.054no	0.044243
	Palatability	7.2± 0.17p	8.5± 0.070pq	8.2± 0.070q	8.2± 0.083q	8.3± 0.083q	0.049013
	Overall acceptability	7.5± 0.25r	8.0± 0.044s	8.0± 0.054s	8.1± 0.054s	7.9± 0.083s	0.066028

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

Table 4.10. Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of protein powder

	Samples code	Cs	$Swpp_1$	$Swpp_2$	$Swpp_3$	$Swpp_4$	LSD
Score of Sensory attribute	Colour	7.7± 0.011a	8.3± 0.023b	8.3± 0.014b	8.1± 0.018c	7.9± 0.016d	0.00825
	Aroma	8.4± 0.013e	8.4± 0.012e	8.4± 0.019e	8.3± 0.016ef	8.2± 0.016ef	0.028361
	Texture	7.5± 0.070g	8.3± 0.021h	8.4± 0.020hi	8.4± 0.016hi	8.4± 0.020hi	0.04677
	Taste	7.9± 0.089j	8.5± 0.070k	8.0± 0.083l	8.3± 0.089ml	8.4± 0.083m	0.039459
	Mouth coating	8.1± 0.148n	7.9± 0.070n	7.6± 0.089no	7.9± 0.083n	8.1± 0.083n	0.046689
	Juiciness	7.7± 0.141p	8.1± 0.089p	7.9± 0.054pq	8.0± 0.054pq	8.0± 0.089pq	0.043226
	Palatability	7.2± 0.173r	8.5± 0.007s	8.6± 0.018s	8.4± 0.021s	8.3± 0.005s	0.038954
	Overall acceptability	7.5± 0.258w	8.2± 0.021wz	8.1± 0.011wz	8.2± 0.018wz	8.0± 0.058wz	0.062366

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), LSD = Least significance difference, Cs = Control sample, $Swpp_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

Conclusion

Refrigerated storage significantly ($p<0.05$) increased total plate count, yeast and mould count and coliform count of emulsion sausages treated with whey protein products at 0ºC. On 25th day of refrigerated storage (0ºC) total plate count of all samples were found to be in the safe limit (3.32-3.93 log cfu/g), yeast and mold count was found to be less than 4 log cfu/g and , coliform count was found to be in the range of 2.16-2.54 log cfu/g. *Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C till 25 days. Whey protein products improved the sensory characteristics of emulsion sausage.

Aknowlegment

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اثر محصولات پروتئین آب پنیر بر روی ویژگی‌های میکروبیولوژیکی سوسیس امولسیون گوشت بوفالو

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چکیده

ترکیبی از محصولات پروتئین آب پنیر (WPPs) از جمله کنسانتره، ایزوله و پودر پروتئین آب پنیر در سطوح مختلف (۱، ۲، ۳ و ۴ درصد) بر خصوصیات کیفی سوسیس امولسیون (ES) در گوشت بوفالو، مورد بررسی قرار گرفت. کیفیت سوسیس امولسیون بلحاظ ویژگی‌های میکروبیولوژیکی مانند تعداد کل (TPC)، میزان مخمر و کپک، تعداد کلی‌فرم و سالمونلا شیگلا مورد بررسی قرار گرفت. ذخیره‌سازی یخچالی اثر معنی‌داری ($p<0.05$) بر افزایش تعداد کل، میزان مخمر و کپک و میزان کلی‌فرم سوسیس امولسیونی ترکیب شده با سطوح مختلف کنسانتره، ایزوله و پودر آب پنیر داشت. در روز بیست و پنجم انبارمانی برای نمونه‌های آماده شده با سطوح مختلف (۱، ۲، ۳ و ۴ درصد) از کنسانتره، ایزوله و پودر پروتئین آب پنیر به ترتیب میزان کل بین ۷/۱۱- log cfu/g ،۷/۳۹-۶/۷۴ log cfu/g و ۷/۲۳-۶/۶۷ log cfu/g، ۷/۲۱ میزان مخمر و کپک به ترتیب ۳/۳۴- log cfu/g ،۳/۸۶ log cfu/g-۳/۴۵ ،۳/۵۷ log cfu/g-۳/۴۵ ،۳/۵۷ log cfu/g-۳/۵۸ و ۳/۹۵ log cfu/g مشاهده شد. بعد از ۲۵ روز ذخیره‌سازی در یخچال (0C) میزان کل، مخمر و کپک تمامی نمونه‌ها در محدوده ایمن (۳/۳۲-۳/۹۳ log cfu/g) گزارش شد. بعد از گذشت ۱۵ روز وجود کلی‌فرم در نمونه‌ها شناسایی شد که در مخلوط سوسیس امولسیونی با کنسانتره، ایزوله و پودر پروتئین آب پنیر به ترتیب در دامنه ۲/۰۶-۲/۳۴ log cfu/g، ۲/۲۸-۲/۱۵ log cfu/g، ۲/۲۲-۲/۰۸ log cfu/g بود. در پایان ذخیره‌سازی میزان کلی‌فرم در دامنه ۲/۱۶-۲/۵۴ log cfu/g، ۲/۳۰-۲/۴۱ log cfu/g و ۲/۳۰- log ۲/۴۳cfu/g گزارش شد. سالمونلا شیگلا در هیچ نمونه‌ای در طی ذخیره‌سازی یخچال(0C) در ۲۵ روز مشاهده نشد.

واژه‌های کلیدی: پروتئین آب پنیر، گوشت بوفالو، سوسیس امولسیون، ویژگی‌های میکروبی

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