Haryana Vet. (December, 2016) **55 (2)**, 125-129 **Research Article**

EFFECT OF DIFFERENT KINDS OF PHOSPHATE, ITS LEVELS AND TUMBLING TIME ON THE PROCESSING CHARACTERISTIC OF BUFFALO CALF MEAT

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Received: 05.05.2016; Accepted: 26.10.2016

ABSTRACT

The objective of the present study was to select the suitable phosphate, its level of incorporation and tumbling time to prepare male buffalo calf meat emulsion with good processing characteristics. Three phosphates viz. sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHM) and tetrasodium pyrophosphate (TSPP) were tried each at 0.3, 0.4 and 0.5% level of incorporation in minced meat (2% salt added) and vacuum tumbled at 0, 1, 2 and 3 h of tumbling time. One level of incorporation and tumbling time from each class of phosphate was selected on the basis of water holding capacity and emulsion stability followed by comparing three different phosphate selected levels on the same ground. The water holding capacity and emulsion stability showed significant increase from 0.3 to 0.4% level and non-significant increase at 0.5% level of incorporation. The parameters also got improved with increase in tumbling time. For further scrutiny 0.4% levels of each phosphate at 2 h of tumbling time were selected. The water binding and stability of emulsion was significantly higher in TSPP followed by STPP and SHM. The results concluded that TSPP at 0.4% level and 2 h of tumbling time is best suitable for the processing of buffalo calf meat emulsion.

Key words: Emulsion stability, male buffalo calf meat, TSPP, water holding capacity

In India, buffalo meat has gained importance in the recent years because of its domestic usage and export potential. Buffalo meat has registered a growth of 27% in export during the financial year 2012-13 (APEDA, 2012). In India, buffalo meat comes from spent dairy buffalo after full utilization of their productive life. Such meat is a by-product and the average carcass yield varies from animal to animal. Spent buffalo meat is dark, coarse and tough in texture and has poor organoleptic and processing characteristics (Kandeepan *et al*., 2009; Naveena *et al*., 2011). This issue can be resolved by using meat of male animals of lower age (below 18 months) with more collagen solubility (Kandeepan *et al*., 2009). But the processing characteristics of buffalo calf raw meat must be evaluated before utilizing in meat products preparation.

However, it has been observed that the meat obtained from young cattle (less than approximately 24 months of age) is comparatively less juicy. One of the reasons may be the low marbling. Marbling is considered "late maturing", meaning that higher levels of marbling are usually seen later in life, as the animal matures (Greenwood, 2012). Marbling contributes juiciness to some extent and numerous studies have been undertaken on the relationship between marbling score and quality beef palatability attributes in the past four decades (Spehar *et al*., 2008; Oler *et al*., 2016).

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Phosphates have been reported to enhance water holding capacity due to rise in pH, inside the meat product. The higher pH of the blend thus, changes the pH of the solution, shifting the isoelectric point, increasing electrostatic repulsion, stimulating protein unfolding and water binding (Schroeder, 2013). Moreover, sequestration of metal ions such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+} etc, by phosphates to form a complex is an important function of phosphates in food applications (Lampila and Godber, 2002). Maximum concentration of phosphate permitted in the finished meat product is 0.5% (de Hall, 1981).

Tumbling ensures meat emulsion formation with more uniform cured colour, improved tenderness, juiciness and palatability. Theno *et al*. (1976) observed disruptions in the muscular tissue of tumbled hams that resulted in higher levels of extracted myofibrillar proteins. In addition, the uniform diffusion of the marination sauce to the tissues is also facilitated by tumbling, which further improves the WHC, juiciness and emulsion stability (ES). To utilize buffalo calf meat for meat products development, the processing characteristics must be evaluated. The study was thus conducted for the selection of suitable phosphate and tumbling time for the preparation of raw meat emulsion.

MATERIALS AND METHODS

Materials: Three healthy male buffalo calves of 10-12 months of age, weighing around 130 kg which were reared under similar conditions were slaughtered as per standard procedure with the consideration of animal welfare, in the experimental slaughterhouse of the Department of Livestock Products Technology, LUVAS, Hisar. The dressed carcasses were chilled for 24 h prior to manual deboning. After the removal of fascia, external fat and other connective tissue, the deboned meat was packaged in LDPE bags and stored at -18±1^{0}C till further use. The frozen chunks were drawn as per requirement and thawed overnight in a refrigerator (4±1^{0}C) and were used for further study. The food grade ingredients of the established brands were procured from local market. The chemicals and readymade media used in the study were procured from reputed firms (CDH Chemicals, Sigma Aldrich and Hi-Media).

Processing of Meat: The frozen buffalo calf deboned meat was minced in an electrical meat mincer (3 mm sieve plate) (Mado Primus Meat Mincer, MEW-613; Dr. Froeb India Pvt. Ltd.). In the minced meat, 2% common salt was added and then three types of phosphates i.e. sodium hexametaphosphate (SHMP), sodium tripolyphosphate (STPP) and tetrasodium pyrophosphate (TSPP) were added at 0.3, 0.4 and 0.5% levels. Each treatment was subjected to tumbling for 0, 1, 2 and 3 h under constant vacuum for emulsion preparation (Fig. 1).

Selection Protocol: Out of 12 treatments of each phosphate one best phosphate level at best tumbling time was selected based upon water holding capacity (WHC) and emulsion stability (ES) and again out of these 3 selected treatments, one treatment was selected based upon WHC and ES.

Methods: WHC was estimated according to Wardlaw *et al*. (1973). In a 100 ml polycarbonate centrifuge bottle, finely minced meat sample (20 g) was taken and then 30 ml of 0.6 M NaCl solution was added to it, mixed with glass rod and stirred for 2 min on a mechanical shaker. After holding for 15 min at 4^{0}C, in order to allow the effect of salt to reach equilibrium, the meat slurry was again stirred for 1 minute on a shaker and immediately centrifuged at 5000 rpm for 10 min at -9^{0}C in a refrigerated centrifuge. The supernatant volume was measured and difference between the added and decanted solution was expressed as percentage of the initial weight of meat.

Stability of the emulsions was determined using the method of Baliga and Madaiah (1970). Meat emulsion (20 g) was taken in LDPE bags of 150 gauge and was placed in a thermostatically controlled water bath (Model: NSW 125) at 80±1^{0}C for 20 min. Bags were removed from water bath, cooled to room temperature, cut open, the cooked fluid (fat, water soluble solids) was drained off and final sample weight was recorded. The emulsion

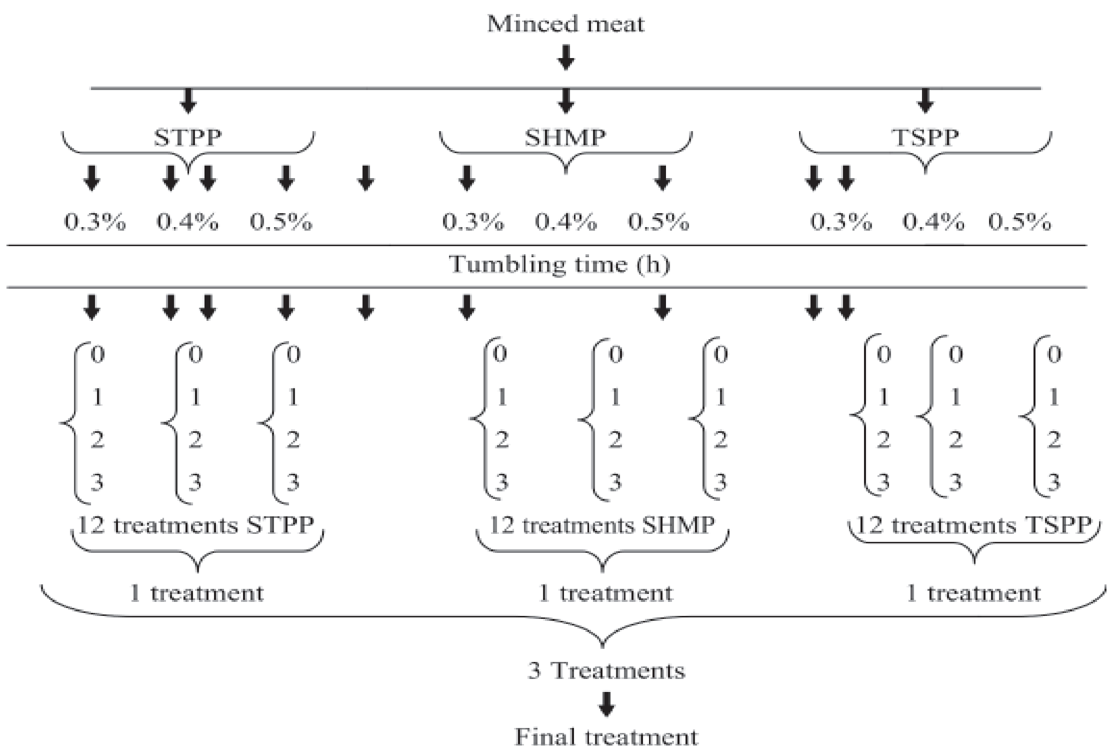

Fig 1. Framework of study carried out on male buffalo calf minced meat (STPP=tetrasodium polyphosphate, SHMP=sodium hexametaphosphate, TSPP=tetrasodium pyrophosphate

Table 1
Effects of different phosphates and their levels at variable tumbling time on WHC and ES (%) of buffalo calf raw meat emulsions (Mean±S.D., n=6)

Parameter	Phosphates	Levels	Effect at different time (h)			
			0	1	2	3
WHC	STPP	0.3%	35.55 ± 2.35^{Aa}	45.53 ± 2.77^{Ba}	55.17 ± 1.77^{Ca}	57.88 ± 2.44^{Ca}
		0.4%	40.88 ± 2.20^{Ab}	50.11 ± 2.65^{Bb}	58.91 ± 2.04^{Cb}	61.43 ± 2.95^{Cab}
		0.5%	43.66 ± 2.82^{Ab}	52.47 ± 3.31^{Bc}	59.86 ± 2.65^{Cb}	62.88 ± 3.27^{Cb}
	SHM	0.3%	33.40 ± 1.19^{Aa}	39.98 ± 2.87^{Ba}	50.98 ± 3.05^{Ca}	53.68 ± 3.25^{Ca}
		0.4%	37.97 ± 2.18^{Ab}	44.40 ± 2.77^{Bb}	57.07 ± 2.71^{Cb}	60.11 ± 3.13^{Cb}
		0.5%	40.09 ± 1.19^{Ac}	47.32 ± 3.00^{Bb}	58.48 ± 2.72^{Cb}	61.23 ± 2.93^{Cb}
	TSPP	0.3%	41.31 ± 1.57^{Aa}	53.22 ± 1.76^{Ba}	62.76 ± 3.56^{Ca}	65.55 ± 2.94^{Ca}
		0.4%	47.10 ± 2.50^{Ab}	59.62 ± 2.79^{Bb}	66.79 ± 2.75^{Cb}	69.97 ± 2.95^{Cb}
		0.5%	49.33 ± 1.84^{Ab}	62.80 ± 1.83^{Bc}	68.67 ± 3.23^{Cb}	71.01 ± 3.40^{Cb}
ES (%)	STPP	0.3%	67.24 ± 3.33^{Aa}	73.50 ± 1.43^{Ba}	75.25 ± 1.97^{BCa}	77.26 ± 1.45^{Ca}
		0.4%	70.91 ± 2.43^{Ab}	75.73 ± 1.57^{Bb}	79.12 ± 1.38^{Cb}	80.97 ± 2.93^{Cb}
		0.5%	72.17 ± 2.94^{Ab}	78.40 ± 1.76^{Bc}	82.01 ± 2.00^{Cc}	85.52 ± 3.06^{Cb}
	SHM	0.3%	64.71 ± 2.54^{Aa}	70.19 ± 1.58^{Ba}	73.55 ± 1.65^{Ca}	74.85 ± 2.07^{Ca}
		0.4%	69.96 ± 2.85^{Ab}	74.55 ± 2.16^{Bb}	77.48 ± 1.97^{BCb}	78.16 ± 3.03^{Cab}
		0.5%	72.22 ± 2.10^{Ab}	76.92 ± 1.50^{Bc}	79.95 ± 1.60^{Cc}	80.95 ± 3.10^{Cb}
	TSPP	0.3%	72.33 ± 1.53^{Aa}	76.19 ± 1.82^{Ba}	80.28 ± 1.71^{Ca}	82.67 ± 1.53^{Da}
		0.4%	76.66 ± 1.55^{Ab}	80.13 ± 1.52^{Bb}	83.46 ± 2.31^{Cb}	85.75 ± 2.51^{Cb}
		0.5%	78.81 ± 1.73^{Ac}	82.51 ± 1.69^{Bc}	85.94 ± 2.40^{Cb}	87.79 ± 2.58^{Cb}

Means with different capital letter superscript in a row and small letter superscript in a column within a group differ significantly ($P\leq0.05$).

stability was calculated and expressed as percentage of cooked meat.

Statistical Analysis: Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, Il USA) software package as per standard methods (Snedecor and Cochran, 1994). The experiment was run three times and duplicate samples were drawn for each parameter in each trial to have total six observations for all parameters. The data was statistically analyzed by two way analysis of variance (ANOVA) at 5% level ($p\leq0.05$) and evaluated with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

WHC: Results revealed that different phosphates variably affected the WHC of buffalo calf meat emulsion incorporated with 2% salt (Table 1). In addition, the different phosphate levels and tumbling time affected the WHC. The meat showed significant ($P\leq0.05$) increase in WHC with increase in phosphate level from 0.3-0.4%, irrespective of type of phosphate level at 0 h of tumbling time which might be due to an increase of ionic strength and subsequently increased water binding. Increase in water-binding capacity with increased ionic strength via salt or phosphate addition has been reported by Trout and Schmidt (1983). Addition of phosphates could also have resulted in increased phosphate–protein interactions, thereby increasing phosphate–protein interactions i.e. unfolding of the three-dimensional protein network by the high ionic strength that causes the muscle to swell and the protein to solubilize thereby increasing the net negative charge of the protein and subsequently greater protein repulsion and water binding ability (Trout and Schmidt, 1983; Torley *et al*., 2000). Baublits *et al*. (2005) also documented that the increased water-binding ability with increased phosphate concentrations could be related to changes in ionic strength or increased phosphate binding to proteins. However, the rise was not significant from 0.4% to 0.5% level of incorporation which suggested that phosphate had a limitation for increasing effect in water-holding of emulsion or the saturation of water binding sites in protein. Similarly, Lu and Zhang (2000) observed that the water-binding curve was not linear with added phosphates.

Further, vacuum tumbling resulted in significantly higher WHC (Table 1). The WHC of minced meat increased with increase in the time of vacuum tumbling. A significant rise in WHC was found from 0 to 2 h of tumbling, but the increase from 2 to 3 h was non-significantly higher in raw meat emulsion. According to the results, tumbling effectively increased WHC of raw meat emulsion. The application of tumbling in the presence of phosphate aided in the extraction of more salt soluble proteins such as actin and myosin (Siegel *et al*., 1978) which made the emulsion much more uniform and with higher WHC. It is, therefore, evident that tumbling process probably disrupts the cell membrane and causes more uniform distribution of these phosphates (Cheng and Ockerman, 2003). With increase in tumbling time, the

WHC increased which could be attributable to higher extraction of proteins with time. Solomon and Schmidt (1980) showed that there was a linear relationship between the tumbling time and the amount of crude myofibrillar protein extracted from a sample. They also observed that there was an increased production of crude myosin with increase in tumbling time. Increasing tumbling time has been reported to improve protein binding and subsequent processing and quality traits (Schroeder, 2013).

A similar trend was found in SHM and TSPP as with rise in level of incorporation and tumbling time, the WHC increased except at 1 h of tumbling time where, the WHC did not vary significantly from 0.4 to 0.5% level of incorporation. Therefore, 0.4% level at 2 h of tumbling time was adjudged best amongst different levels of STPP, SHM and TSPP on the basis of WHC for further scrutiny.

Emulsion Stability: The stability of buffalo calf raw meat emulsion varied both with level of incorporation and tumbling time (Table 1). Perusal of results revealed that the emulsion was more stabilized as phosphate incorporation level increased from 0.3% to 0.5%. Higher stability of emulsion with increase in phosphate incorporation level is mainly due to the increase in pH and ionic strength (Zorba *et al*., 1993). Phosphates act as polyelectrolytes and are able to change the ionic charges distributions. Thus, the addition of phosphate increases the ionic strength of the meat and consequently, an increased ionic strength leads to a more severe degree of swelling of the muscle fibers and activation of protein. Enhanced levels of activated and swollen protein support the immobilization of the water added to meat products and the emulsification of fat (Feiner, 2006; Shu Qin *et al*., 2009). In addition, the use of phosphate in meat emulsions enhances the structural homogeneity of the product, provides better emulsification of oil and increases the stability (Yetim, 2000). Similar results were obtained by Yapar *et al*. (2006). With tumbling time, the ES increased which might be due to better stabilization of emulsion with time. Further, the same trend was recorded in WHC of each phosphate, therefore each phosphate at 0.4% level and 2 h tumbling were selected for final selection.

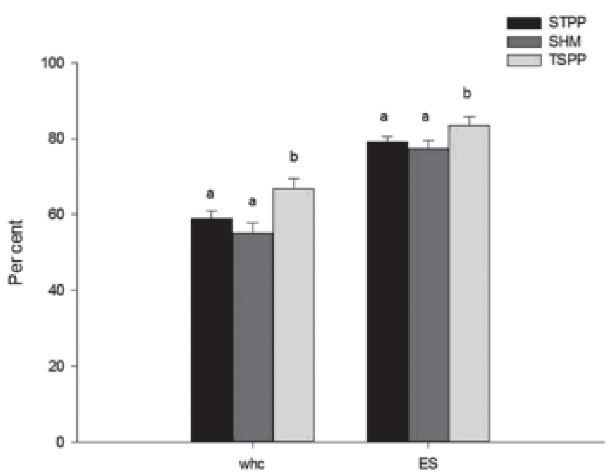

Fig 2. Comparison of STPP, SHM and TSPP (0.4% level, 2 h tumbling time) selected levels of three phosphates on the basis of WHC (%) and ES (%).

Selection of Best Phosphate, its Level and Tumbling Time: The selected level of incorporation and tumbling time of three different phosphates were compared to select the best suitable phosphate level and tumbling time for the further study (Fig. 2). The critical analysis revealed that WHC and ES followed the trend as TSPP>STPP>SHM. This effect might be due to the molecular structure of phosphates and pH of the solution; i.e., the effectiveness of phosphates decreased when the chain length increased and pH decreased. Pyrophosphate is analogous to ATP in regards to cleaving the actomyosin bonds. Tripolyphosphate must first be hydrolyzed to pyrophosphate in order to be as effective at improving WHC and extracted myosin. Hexametaphosphate contains a ring in its structure and undergoes lower dissociation (Trout and Schmidt, 1984). Therefore, the effectiveness of phosphates from high to low was TSPP>STPP>SHM. Sun and Holley (2011) also indicated that tetrasodium pyrophosphate has the greatest binding ability, followed by sodium tetrapolyphosphate, and sodium hexametaphosphate. Different phosphate types have been shown to dissociate actomyosin to differing degrees as well. Yasui *et al*. (1964) discussed that STPP and TSPP caused increased actomyosin dissociation compared to SHM. Thus, in the present study, STPP and TSPP could have caused greater actomyosin dissociation and subsequent solubilization and water binding than SHM.

Therefore, it is concluded that tetrasodium pyrophosphate enhances processing parameters (WHC and ES: indicative of cooking yield and juiciness) of buffalo male calf meat best at 0.4% level of incorporation at 2 h of tumbling time. Thus, processing characteristics of buffalo calf meat can be improved by employing tumbling and phosphates.

ACKNOWLEDGEMENT

The authors sincerely express gratitude to the Department of Science and Technology, New Delhi, India for the award of Inspire fellowship to pursue Ph.D. programme by first author.

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