

# Protein hydrolyzates from seal meat as phosphate alternatives in food processing applications

# F. Shahidi\* & J. Synowiecki

Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X9

(Received 3 May 1996; revised version received 12 August 1996; accepted 12 August 1996)

Protein hydrolyzates were prepared from mechanically separated seal meat (MSSM) and used as phosphate alternatives in meat products in order to improve their water-binding capacity. The drip volume from thermally processed MSSM containing 3% (w/w) seal protein hydrolyzate (SPH) was 5.8% (v/w) as compared with 12.8% (v/w) for meats cooked without any added SPH. The cooking loss of MSSM was minimum at a SPH concentration of 3%, similar to that of polyphosphates at the same level, but much higher than that of polyphosphates at their 0.5% maximum allowable limit of use. When compared to different phosphates, the drip volume resulting from use of SPH (54.7% of the drip from MSSM without additives) was lower than that for samples treated with sodium pyrophosphate (72.6%), sodium tripolyphosphate (66.4%) or sodium hexametaphosphate (60.0%). © 1997 Elsevier Science Ltd

### INTRODUCTION

Phosphates are important functional additives used in a variety of foods, including meat, poultry, seafood, fruit and vegetable products. Phosphates influence water-binding, colour, rancidity development, texture, emulsification and curing of various foods (Hamm, 1970; Steinhauer, 1983; Tompkin, 1984). In meat products, phosphates are generally used to enhance water-holding capacity and to improve cooking yield. This is brought about by an increase in the pH of the meat from its isoelectric point (Hamm, 1960; Shults et al., 1972; Shahidi et al., 1994) and ionic strength (Seman et al., 1980; Trout, 1984). The effect of phosphates in increasing waterholding capacity is also due to their ability to sequester divalent metal ions, to bind to meat proteins and to dissociate actomyosin (Trout, 1984). When added to meat, phosphates can also attach to positively charged groups of protein, while the rest of the molecule can attract water molecules and increase the water-holding capacity by acting as a polyanion (Steinhauer, 1983). However, pH and ionic strength are perhaps the most important factors responsible for improving the waterholding capacity of meat proteins. For maximum water binding and cook yield, a pH of 6-7 and an ionic strength of 0.6 are usually required. The maximum amount of food-grade phosphates permitted for incorporation in meat products is 0.5% (USDA, 1982).

\*To whom correspondence should be addressed.

Presence of excessive amounts of phosphates in the diet influences the calcium, iron and magnesium balance in the human body and increases the risk of bone diseases. The improvement of water-binding capacity and other functional properties of muscle proteins may be achieved by addition of protein hydrolyzates (Schnepf, 1992). Among these, seal protein hydrolyzates (SPH) possess a well-balanced amino acid composition and are bland in taste. Thus, the present study was designed to investigate the suitability of SPH as a water-binding agent and a phosphate replacer in processed meats.

# MATERIALS AND METHODS

## Materials

Adult harp seals (*Phoca groenlandica*), hunted in the coastal areas of Newfoundland during the months of April–June, were skinned, blubber removed and eviscerated. Carcasses weighing up to  $30 \, \text{kg}$ , without head and flippers, were placed inside plastic bags and stored on ice for up to 3 days. Each carcass was then washed with a stream of cold water ( $+10^{\circ}\text{C}$ ) for about 15s to remove most of the surface blood. Mechanical separation of meat from carcasses of 15 seals was carried out using a Poss deboner (Model PDE 500; POSS Ltd, Toronto, Canada). Small portions of mechanically separated seal meat (MSSM) were vacuum-packed in polyethylene pouches and kept frozen at  $-20^{\circ}\text{C}$  for up to 2 months until used.

SPH, with a degree of hydrolysis (DH) of 19%, was prepared using Alcalase 2.4 L (Novo Nordisk, Bagsvaerd, Denmark) according to a procedure described elsewhere (Shahidi *et al.*, 1994).

#### Methods

Frozen MSSM was thawed at 4°C overnight and mixed well with 3% NaCl (w/w). To 150 g of untreated or salttreated MSSM in Mason jars, tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP) or seal protein hydrolyzate was added at concentrations ranging from 0 to 5% (w/w of meat). The samples were mixed manually with a glass rod for 10 min and then stored for 1 h at 4°C. Sealed jars were thermally processed at 95°C for 1 h and cooled under a stream of cold tap water for 30 min. Seals were then opened and the drip volume measured.

Crude protein concentration in the drip liquid was calculated based on the nitrogen content as determined by the AOAC (1990) method (i.e. %N×6.25). Ten millilitres of cooking drip, previously diluted with distilled water to a volume of 100 ml, were used in these determinations.

The pH values of meat were measured according to AOAC (1990) following the homogenization of one portion of cooked meat with one portion of distilled water (w/v) in a Waring blender (Model 33BL73; Dynamic Corp., New Hartford).

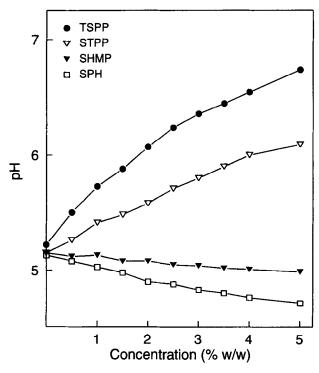


Fig. 1. pH values of MSSM cooked for 60 min at 95°C in the presence of tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP) or seal protein hydrolyzate (SPH).

## Statistical analysis

Analysis of variance and Tukey's studentized range test (Snedecor & Cochran, 1980) were used to determine differences in mean values based on the data presented. Significance was determined at 95% probability.

### RESULTS AND DISCUSSION

In order to compare the influence of phosphates and SPH on the cook yield of MSSM, the most commonly used phosphates in the food industry, namely TSPP, STPP and SHMP, were examined. Although the maximum allowable addition of polyphosphates to foods is limited to 0.5% (USDA, 1982), in this study addition of up to 5% TSPP, STPP and SHMP was considered in order to mimic conditions necessary to attain comparable results to those exerted by using SPH. As shown in Fig. 1, TSPP was most effective in increasing the pH of MSSM. On the other hand, SHMP slightly decreased the pH of MSSM. Existing differences in pH of 4% solutions of TSPP (10.4), STPP (8.9) and SHMP (6.4) may explain these observations.

The dripping curves obtained after thermal processing of MSSM for 1 h at 95°C with 0-5% (w/w) of TSPP, STPP or SHMP are shown in Fig. 2. The dripping curves for all phosphates used exhibited a minimum;

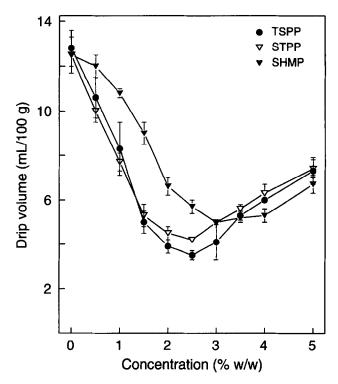


Fig. 2. The drip volumes of mechanically separated seal meat (MSSM) cooked in the presence of different amounts of tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP). Results are mean values of three determinations and error bars depict standard deviations.

both TSPP and STPP showed a minimum drip volume at the 2.5% (w/w) level and their influence on cook yield of MSSM was very similar. However, SHMP-treated samples exhibited a minimum drip at a 3% (w/w) level of addition. The pH of MSSM samples at minimum dripping in the presence of TSPP, STPP or SHMP was 7.24, 6.71 and 6.04, respectively (Table 1). The polyphosphates, TSPP and STPP, decreased the drip volume by approximately 72.7 and 66.4%, respectively, compared with that of MSSM, which was heat-processed in the absence of phosphates. Sodium hexametaphosphate decreased the drip volume by only 60% (Table 1). These findings lend further support to those of Trout &

Schmidt (1984), who reported that TSPP and STPP were most effective in increasing water-binding capacity of beef rolls. The concentration of protein in cook drip from MSSM in the presence of different phosphates was 1.49–1.64 times higher than for MSSM cooked in their absence (Table 1). However, the smaller drip volume had an overall lowering effect on protein loss from MSSM treated with TSPP (0.94% of total protein), STPP (1.08%) and SHMP (1.22%) compared with MSSM cooked without phosphates (2.66%).

The cooking loss of MSSM was minimum at a phosphate concentration of 2.5-3%, which is much higher than the 0.5% level permitted by the USDA (1982).

Table 1. Influence of tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP) and seal protein hydrolyzate (SPH; DH 19%) on water and protein loss of mechanically separated seal meat (MSSM) cooked for 60 min at 85°C

Specification	MSSM without phosphates	MSSM with addition of:			
		TSPP	STPP	SHMP	SPH
Concentration of additives for attaining a minimum drip (%)		2.50	2.50	3.00	3.00
pH of MSSM without or with additives	$6.22 \pm 0.02$	$7.24 \pm 0.02$	$6.71 \pm 0.02$	$6.04 \pm 0.02$	$6.13 \pm 0.02$
Drip volume (ml per 100 g of MSSM)	$12.8 \pm 0.3$	$3.5 \pm 0.2$	$4.2 \pm 0.1$	$5.0 \pm 0.1$	$5.8 \pm 0.2$
Drip volume as percent of drip volume of MSSM cooked without additives	100	27.3	33.6	40.0	45.3
Protein concentration in drip liquid (mg ml <sup>-1</sup> )	$37.9 \pm 0.8$	$62.2 \pm 0.5$	$59.8 \pm 0.5$	$56.7 \pm 0.2$	
Protein loss after cooking (% of total protein in MSSM)	2.06	0.94	1.08	1.22	_

Results are mean values of three determinations  $\pm$  standard deviation.

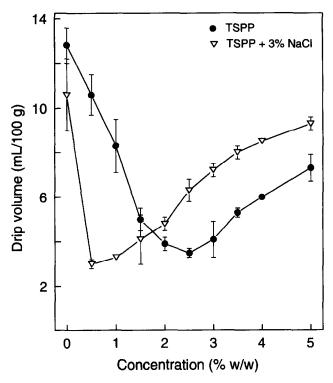


Fig. 3. The drip volumes of mechanically separated seal meat (MSSM) cooked with different amounts of tetrasodium pyrophosphate (TSPP) in the absence or presence of 3% (w/w) of NaCl. Results are mean values of three determinations and error bars depict standard deviations.

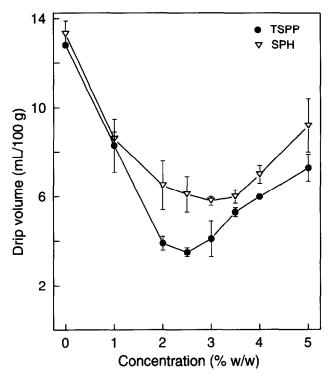


Fig. 4. The drip volumes of mechanically separated seal meat (MSSM) cooked with different amounts of tetrasodium pyrophosphate (TSPP) or seal protein hydrolyzate (SPH). Results are mean values of three determinations and error bars depict standard deviations.

However, it is possible to decrease the amount of phosphates needed for this purpose by employing a combination of NaCl and phosphates. Figure 3 shows the comparison of drip volumes at different dosages of TSPP added to MSSM as such or MSSM previously tumbled with 3% (w/w) of salt. Both dripping curves exhibited a minimum at different dosages of TSPP. In the case of NaCl-treated MSSM, the minimum volume of drip (3 ml per 100 g of meat) was obtained at a 0.5% (w/w) phosphate concentration and was 76.57% lower than the volume drip from MSSM processed without additives (12.8 ml per 100 g of meat).

SPH was found to improve the water-binding capacity of seal meat similar to that of phosphates. Augmenting the number of ionizable amino and carboxyl groups brought about by the hydrolysis process, increased the hydrophilicity of meats containing hydrolyzates (Phillips & Beuchat, 1981). Possible interaction of divalent ions such as calcium and magnesium with hydrolyzed peptides and protection against formation of myosin-actin complex also prevents the loss of bound water from meat proteins (Schnepf, 1992). As with phosphates, the dripping curve of MSSM cooked in the presence of SPH exhibited a minimum (Fig. 4). The minimum volume of cooking drip was obtained at 3% (w/w) addition of hydrolyzate in MSSM devoid of salt, which is slightly higher than that of TSPP (2.5%, w/w, of meat). However, the minimum volume of cooking drip caused by SPH and TSPP decreased by 54.7 and 72.6%, respectively, compared with the drip from MSSM processed without additives. The effect of SPH (DH 19%) on water-binding capacity of meat was similar to that of the hydrolyzate (DH 17%) prepared from bovine red blood cells via an Alcalase-assisted process. The drip volume of ground beef containing 3% (w/w) of bovine red blood cell hydrolyzate was 5% (v/w) compared to 5.8% (v/w) for MSSM containing SPH. However, bovine red blood cell hydrolyzate had a bitter taste, contrary to SPH which was bland (Synowiecki et al., 1996).

In conclusion, seal protein hydrolyzates possessing a well-balanced essential amino acid composition and a bland, non-bitter taste might serve as phosphate alternatives for enhancing the water-binding capacity and improving the functional properties of thermally processed meat products. However, optimization of

process conditions requires consideration of additional parameters such as degree of hydrolysis and peptide chain lengths of the hydrolyzed proteins.

## REFERENCES

- AOAC (1990). Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Washington, DC.
- Hamm, R. (1960). Biochemistry of meat hydration. Adv. Food Res., 10, 355-463.
- Hamm, R. (1970). Interactions between phosphates and meat proteins. In Symposium on Phosphate in Food Processing, eds J. M. Deman & P. Melnychyn. AVI Publishing Co., Westport, CT, p. 65.
- Phillips, R. D. & Beuchat, L. R. (1981). Enzyme modification of proteins. In *Protein Functionality in Foods*, ed. J. P. Cherry. American Chemical Society Symposium Series 147. ACS, Washington, DC, pp. 275–298.
- Schnepf, M. I. (1992). Protein-water interactions. In *Biochemistry of Food Proteins*, ed. B. J. F. Hudson, Elsevier Science, London, pp. 1-33.
- Seman, D. L., Olson, D. G. & Mandigo, R. W. (1980). Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J. Food Sci., 45, 1116– 1121.
- Shahidi, F., Synowiecki, J. & Balejko, J. (1994). Proteolytic hydrolysis of muscle proteins of harp seal (*Phoca groelan-dica*). J. Agric. Food Chem., 42, 2634–2638.
- Shults, G. W., Russell, D. R. & Wierbicki, E. (1972). Effect of condensed phosphates of pH, swelling and water-holding capacity of beef. J. Food Sci., 37, 860-864.
- Snedecor, G. W. & Cochran, W. G. (1980). Statistical Methods, 7th edn. The Iowa State University Press, Ames, IA.
- Steinhauer, J. E. (1983). Food phosphates for use in the meat, poultry and seafood industry. *Dairy Food Sanit.*, 3, 244–247.
- Synowiecki, J., Jagielka, R. & Shahidi, F. (1996). Preparation of hydrolyzates from bovine red blood cells and their debittering following plastein reaction. *Food Chem.*, 57, 435–439.
- Tompkin, R. B. (1984). Indirect antimicrobial effects in foods: phosphates. J. Food Safety, 6, 13-27.
- Trout, G. R. (1984). Effect of ionic strength, phosphate type, pH and cooking temperature on meat protein functionality. PhD Dissertation, Colorado State University, Fort Collins, CO.
- Trout, G. R. & Schmidt, G. R. (1984). Effect of phosphate type and concentration, salt level and method of preparation on binding in restructured beef rolls. *J. Food Sci.*, 49, 687-694.
- USDA (1982). Meat and poultry products: phosphates and sodium hydroxide. Fed. Regist., 47(49), 10779.