Microstructure of smoked buffalo meat sausages containing whey protein concentrate

Rao KH[1*], Deenathayalan P[2], Anjaneyulu ASR[2], Yadav PL[2]

[1]Division of HRD, National Academy of Agricultural Research Management, Hyderabad-500 030, India
[2]Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar-243 122, India
* E-mail:khrao@naarm.ernet.in

The structural characteristics of smoked buffalo meat sausages incorporated with whey protein concentrate (WPC) were evaluated using scanning electron microscopy (SEM). Results indicated that the control samples had a dense matrix. The fat globules observed were irregular in shape, less in numbers and covered by the protein. In sausages containing 1 and 2% WPC, the fat globules were fine, numerous and regularly arranged. The entire surface of emulsified fat globules was abundantly covered with WPC aggregates. At higher WPC (3 and 4%), particles were lodged between the spaces in the protein matrix as a filler. Addition of up to 2% WPC improved structural characteristics of the sausages.

Keywords: Buffalo meat sausages, Whey protein concentrate, Microstructure

Many food processing operations are designed to create the microstructure that gives the food product certain characteristic properties. Meat products such as sausages, milk products such as cheeses and yogurts and cereal products such as pasta are based on colloidal structures such as gels, emulsions and/or their combination. The relationship between the microstructure and physical properties of foods will be of importance in assessing the quality or functionality of the added ingredient in the formulation (Hermansson et al 2000). Buffalo meat in India, mainly originates from aged, unproductive and spent animals. Consequently, its poor marbling, coarse and fiber toughness adversely affect the quality and palatability of meat products. The quality of communited meat products could be improved by addition of different milk products to the formulations (Rao et al 1999). Milk proteins substitute more expensive meat proteins, maximize the yield (Allen Lee et al 1980), improve the emulsifying capacity by dispersing themselves in the salt soluble muscle proteins matrix (Lucca and Tepper 1994) and interact to provide improved textural characteristics to the product like sliciability (Haines 2004). It has been observed that a fibrous network of protein matrix (Theno and Schmidt 1978, Ray et al 1981) and finely comminuted fat particles give stability to the dispersed matrix of meat emulsion in sausages (Schut 1978). When combination gel containing salt soluble protein (SSP) and whey protein concentrate (WPC) were heated to 65°C, the expressible moisture and hardness decreased (Beuschel et al 1992). Highly soluble WPC gels heated at 90°C for 15 min contained grape like globular, clusters (Hung and Zayas 1992). The present study was aimed to understand the effect of incorporation of WPC on the microstructure of smoked buffalo meat sausages.

Materials and methods

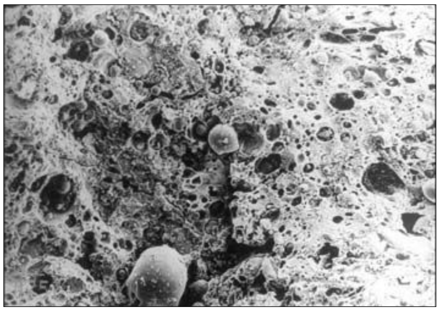
Preparation of buffalo meat sausages: Hot deboned lean meat from the round portion of adult buffalo carcasses with good finish was obtained from local market. Meat was packed in polyethylene bags and chilled for 24 h prior to use. WPC with a protein concentration of 54.2% was obtained from National Dairy Research Institute, Karnal, India. Control formulation consisted of lean buffalo meat (70%), vegetable refined oil (10%), chilled water (10%), salt (1.8%), sodium pyrophosphate (0.4%), spice mix (1.5%), refined wheat flour (2.5%), condiments (3.8%) and sodium nitrite (150 ppm). WPC was incorporated additionally at 1, 2, 3 and 4% levels in the treated formulations while preparing emulsions. Chilled meat was cut into small chunks and minced in meat cutter/chopper (Seydelmann, Model WS – 114, Germany) using 13 mm plates followed by 8 mm plate. Emulsions of 1.5 kg sample for each treatment were prepared by using bowl chopper (Seydelmann, Model – K20, Germany) and stuffed into 18 mm diameter natural goat casing using hydraulic sausage filler (Mainca, Model-EP 25, Spain). Sausages were linked manually.

Microprocessor controlled smoke oven (Enviro-Pak, Model – UV-350T, USA) was used for smoking and cooking of raw sausages. The sausages were thermally processed using 4 stage schedule to an internal temperature of 72°C as follows: drying 30 min at 40°C, smoking 1 h at 45°C, 45% RH, cooking 85°C with a delta temperature of 10°C to the core temperature of 72°C and showering 10 min.

Microstructure of sausages: Microstructure was examined under a scanning electron microscope (SEM) following the procedure described by Cavestany et al (1994). Smoked buffalo meat sausages incorporated with 1, 2, 3 and 4% WPC were cut into 5 mm thickness using a razor blade and fixed in 2.5% gluteraldehyde in phosphate buffer solution (pH 7.2) for 60 h at 4°C. The fixed samples were freeze fractured in super cooled isopentane using liquid nitrogen. They were kept in fresh gluteraldehyde (2.5%). Post-fixation was done by osmium tetroxide (OsO_4) for 24 h, followed by 2 washings with PBS. Washed samples were dehydrated with ascending grade of alcohol. Finally, the samples were washed with hexamethyl disilazane (HMDS) 3times, air-dried and coated using sputter coater (JFC-1100 model) with 1 mm gold plates. The samples were mounted in brass stubs and scanning was done using SEM (JEO1: JSM 840A, Japan).

Results and discussion

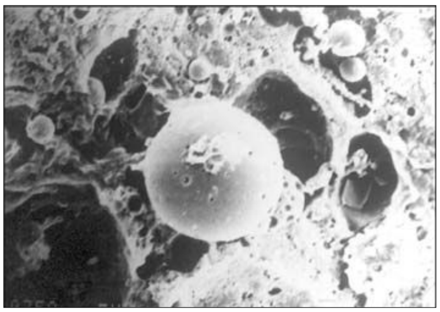
The ultrastructure micrographs obtained from the SEM examination indicated that the control formulation had a dense matrix (Fig.1a). This could be the protein matrix formed from the gelation of salt soluble protein at the cooking



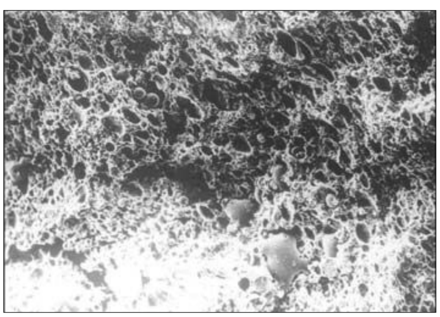
a. Control formulation showing dense matrix with less number of fat globules and irregular craters (x 500)

temperature of 72^{0}C, which existed as a continuous area (Ray et al 1981). With this protein matrix, irregular craters, without distinct void spaces were present which might be the place of fat globules, washed away by the dehydration process with graded alcohol. This supports the findings of Ray et al (1981) and Yost and Kinsella (1996). The fat globules observed were of irregular shape and less in numbers and were covered by the protein matrix. The fat globules were covered with the meat protein matrix showing a latticed structure (Fig. 1b) similar to one reported by Theno and Schmidt (1978). However, the protein matrix appeared to be a coagulum.

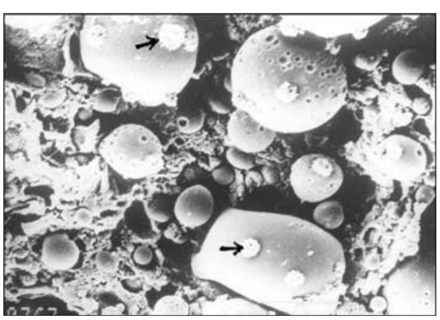
Micrographs of 1% WPC incorporated samples revealed that even though they were like the control formulation (Fig.1c), under higher magnification they were having more irregular fat, globules surrounded by WPC particles (Fig.1d). The WPC added might have emulsified the free fat available and saved the salt soluble protein for more water binding.



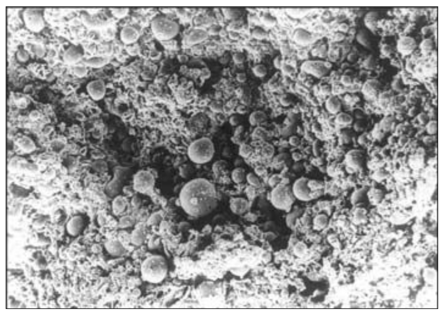
b. Control formulation showing the latticed structure (x 4000)



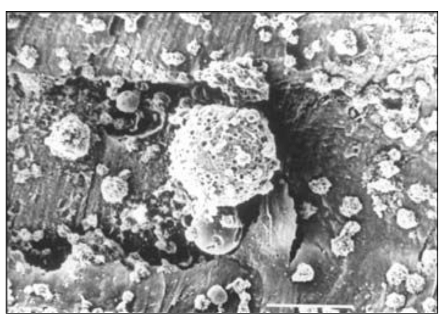
c. Sausages containing 1% WPC, showing clear matrix structure with less number of fat globules (x 500)



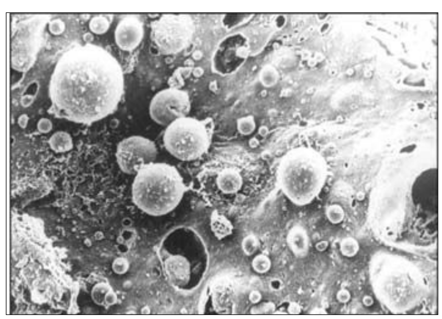
d. 1% WPC incorporation showing, lacked clear interfacial protein film formation with WPC particles, adsorbed on the surface (arrow mark) in different sizes (x 4000)



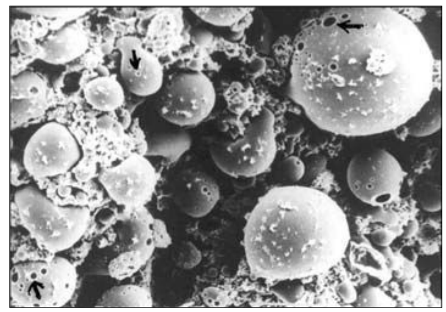
e. Sausages containing 2% WPC showing fine, numerous, regularly arranged fat levels indicating better emulsification (x 500)



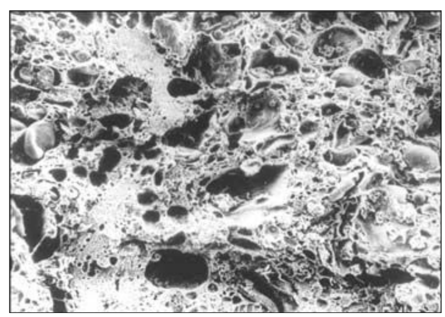
f. Fat globules adsorbed by abundant WPC particles and aggregates in 2% WPC added sausage (x 2000)



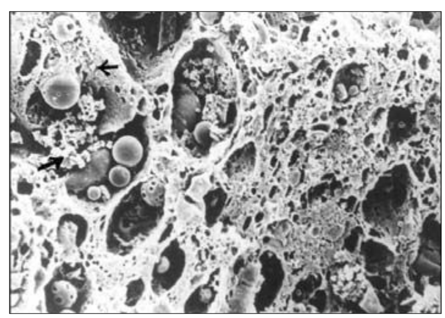
g. Fat globules with clear, numerous interfacial protein film formation (arrow mark), attaching with protein matrix (x 1000) in 2% WPC added sample



h. Sausages containing 2% WPC showing fat globules (x 2000) adsorbed with WPC, immobilized with protein matrix with small exudation points (arrow mark)



i. Sausages containing 3% showing collapsed protein matrix (x 500)



j. Sausages containing 4% WPC showing the WPC aggregates in the spaces of matrix (arrow mark) like filler

Fig. 1. SEM micrographs of buffalo meat sausages containing different levels of whey protein concentrate (WPC)

This supports the observations of Van den Hoven (1987) who reported that the fat globules in larger number surrounded by milk proteins reduced the expulsion of water during cooking by lodging into the protein matrix. But the WPC aggregates were found to be very less over fat globules lacking in interfacial protein strand formation (Fig. 1d).

In 2% WPC added samples, the fat globules were fine and numerous in numbers and regularly arranged (Fig.1e). The micrographs with emulsified fat globules revealed abundant WPC aggregates covering the entire surface. Excessive whey protein aggregates were also found around these emulsified fat globules (Fig.1f). Yost and Kinsella (1996) also reported about these types of clumps and protein aggregates covering the emulsified fat. The emulsified fat globules showed numerous thin interfacial strands originating from the fat globules and connecting to the protein matrix (Fig.1g). This interfacial film formation might be due to the unfolding of WPC during gelation. During gelation the unfolding of protein molecules occurs at the temperature range of 60-80°C (Myers 1990) and exposes a larger amount of reactive sites for intermolecular reaction, which aggregates and form thiol-disulphide reaction (Clark and Ross Morphy 1987) and dilsulphide crosslinking of unfolded protein molecules (Hillier et al 1980). Addition of WPC at 1-4% levels in low-fat beef patties formed a network of aggregated clusters in which beef myofibrillar protein were embedded and WPC occupied the interstitial space between myofibrillar protein (El-Magoli et al 1996). Monahan et al (1996) reported that these unfolded protein facilitates the protein-protein reaction forming a thick interfacial layer resulting in the formation of emulsion droplets in the matrix. The fat globules covered with adsorbed protein molecules are immobilized (Fig. 1h) by direct reticulation between membrane forming protein (WPC) molecule and bulk phase protein (salt soluble protein) matrix (Jost et al 1986). In addition to the meat proteins in emulsification, the WPC also found to be adsorbed on the fat globules in a better way, which might have enhanced the gel structure of the product. Because of these smaller fat droplets, the surface area available for protein adsorption will be more and hence integration of fat droplets into the gel microstructure upon heating. Similarly, Jost et al (1989) reported about the surface area of fat droplets. In 1 and 2% added samples, fat globules are exhibiting several small, a uniform pockets of exudation points (Fig. 1b, h), which might be the reason for better emulsion stability than 3 and 4% WPC added samples. Gordon and Burbut (1990) also reported that stable batters would have more small packets of exudation points. However, the effectiveness of the functionality of WPC greatly depends on the composition, processing condition, solubility, ionic concentration and pH (Beuschel et al 1992, Aguilera 1995, Mangino 1992).

In 3 and 4% WPC added samples the overall volume of the void space decreased and the interfacial strand thickness increased. Yost and Kinsella (1996) also reported that the increased concentration of whey protein isolate decreased the void space and increased the strand thickness. This increased protein concentration might have facilitated the interaction between thermally denatured protein molecules and protein aggregation (Harwalkar and Kalab 1985) and associated moisture loss phenomena such as synerysis (Tamime et al 1984). Similarly, Mangino (1992) reported if the protein-protein interaction is too strong, the network will collapse (Fig.1i) and water will be expelled from the structure. These results are correlating with the lower moisture content of 3 and 4% WPC added sausages. Furthermore, the increased WPC level might have had more fat content which is not suitable to be an effective emulsifier. Because the fat particles are more surface active than proteins (Damodaran 1989). These lipid molecules might have destabilized the interfacial protein films through competitive displacement of WPC resulted in decreased emulsion stability and reduced functional properties. Similarly, At higher percentage of WPC addition, the micrographs showed that the WPC particles were lodging between the spaces in the protein matrix and showing the behaviour of filler (Fig. 1j).

Addition of WPC in the sausage formulation significantly improved the textural attributes of product. Based on the microstructural studies, WPC incorporation showed a better product structure up to 2% than 3 and 4%. Higher level of addition behaved like a filler without improving structural characteristics. Therefore, addition of 2% WPC is recommended for improving structural characteristics and in turn the textural quality of the product.

References

Aguilera JM 1995. Gelation of whey proteins. Food Technol 49(10):83-89

Allen Lee RY, Cannon C, Huffiman DL 1980. Whey protein concentrates in a processed meat loaf. J Food Sci 45:1278-1304

Beuschel BC, Partridge JA, Smith DM 1992. Insolubilized whey protein concentrate and/or chicken salt soluble protein gel properties. J Food Sci 57:852-857

Cavestany H, Jimenez Colmenero F, Solas MT, Carballo J 1994. Incorporation of sardine surimi to bologna sausages containing different fat levels. Meat Sci 38:27-37

Clark AH, Ross-Murphy SB 1987. Structured and the chemical properties of biopolymer gels. Adv Polym Sci 83:60-192

Damodaran D 1989. Inter-relationship of molecular and functional properties of food protein. In: Food proteins: structure and functional relationships. Kinsella JE, Soucie W (eds), Am Oil Chem Soc, IL, p 21-51

El-Magoli SB, Laroia S, Hansen PHT 1996. Flavour and texture of low-fat ground beef patties formulated with whey protein concentrates. Meat Sci 42:179-193

Gordon A, Barbut S 1990. The role of the interfacial protein film in meat batter stabilization. Food Struct 55:1196-1200

Haines B 2004. New directions in dairy, Prepared Foods. htpp://www.preparedfoods.com

Harwalkar VR, Kalab M 1985. Thermal denaturation and aggregation of β-lactoglobulin in solution. Electron microscopic studies. Milchwissenschaft 40:65-68

Hermansson AM, Langton M, Loren N 2000. New approaches to characterizing food microstructures. MRS Bull, Dec, p 30-36

Hillier RM, Lyster RLJ, Cheeseman GC 1980. Gelation of reconstituted whey powder by heat. J Sci Food Agric 31:1152-1157

Hung SC, Zayas JF 1992. Functionality of milk proteins and corn germ protein flour in communited meat products. J Food Qual 15:139-152

Jost R, Baechler R, Masson G 1986. Heat gelation of oil in water emulsion stabilized by whey protein. J Food Sci 51:440-444

Jost R, Dannenberg F, Rosset J 1989. Heat set gels based on oil/water emulsion: An application of whey protein functionality. Food Microstruct 8:23-29

Lucca PA, Tepper J 1994. Fat replacers and the functionality of fat in foods. Tr Food Sci Technol 5:12-19

Mangino ME 1992. Gelation of whey protein concentrates. Food Technol 46:114-117

Monahan FJ, McClements DJ, German JB 1996. Disulphide-mediated polymerization reactions and physical properties of heated WPI-stabilized emulsions. J Food Sci 61:504-509

Myers CD 1990. Study of thermodynamics and kinetics of protein stability by thermal analysis. In: Thermal analysis of foods. Harwalkar VR, Ma CY (eds), Elsevier Appl Sci, London, p 16-50

Rao KH, Rao KVSS, Singh RRB, Anjaneyulu ASR, Yadav PL 1999. Milk proteins as emulsion stabilizers in comminuted meat products. Indian Food Ind 18:287-298

Ray FK, Miller BG, Van Sickle DC, Aberle ED, Forres JC, Judge MD 1981. Microstructure of liver sausage. J Food Sci 46:694-696

Schut J 1978. Basic meat emulsion technology. Proc. Meat Industry Research Conf, American Meat Institute Foundation, Arlington, VA, p 1-3

Tamime AY, Kalab M, Davis G 1984. Microstructure of set style yoghurt manufactured from cow's milk fortified by various methods. Food Microstruct 3:83-90

Theno DM, Schmidt GR 1978. Microstructural comparison of three commercial frankfurters. J Food Sci 43:845-850

Van den Hoven M 1987. Functionality of dairy ingredients in meat products. Food Technol 41(10):72-77, 103

Yost RA, Kinsella J 1996. Microstructure of whey protein isolates gels containing emulsified butterfat droplets. J Food Sci 57:892-897

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Contact :

E-mail : -vijayfch@iiitr.ernet.in

Phone : 01332-285718