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Research Paper

Effect of Emulsion pH and Humectants on Sensory and Physico-Chemical Quality of Hurdle Treated Chicken Sausages

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ABSTRACT

The aim of this study was to develop acceptable quality of shelf stable chicken sausages using hurdle technology. Hurdles incorporated were humectants textured soya protein and low emulsion pH. Different parameters evaluated were pH, emulsion stability, cooking yield, moisture, protein, fat and sensory attributes. Incorporation of TSP at 4% level significantly ($p<0.05$) improved texture and overall palatability of chicken sausages, however, above 4% TSP level adversely affected different sensory attributes especially flavour. Addition of TSP was also significantly ($p<0.05$) improved emulsion stability, cooking yield, protein and fat content of chicken sausages. The acidulent used for reduction of emulsion pH was 0.5N lactic acid. Reduction of emulsion pH significantly ($p<0.05$) influenced the processing and quality parameters of chicken sausages. Emulsion pH below 5.70 adjusted with lactic acid affected different sensory attributes as well as physicochemical properties adversely. Findings of the study indicated that, acceptable quality hurdle treated chicken sausages could be prepared by incorporation of TSP at 4% with adjustment of pH at 5.70.

Keywords : *Chicken sausages, Emulsion pH, Hurdle technology, Textured soya protein*

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INTRODUCTION

Consumption of poultry meat in India is increasing in recent years as large number of non-vegetarian population is switching over to poultry meat. Increased consumption of poultry meat is mostly because of rapid growth of poultry industry, relatively its cheaper cost, comparatively higher nutritional value and introduction of newer processed chicken products (Barbut 2001). There has been an increased consumption of convenience foods due to changing life styles. In response to these changes, the food industry has developed a great variety of processed chicken products such as chicken sausages, nuggets, salami, patties and many of the researchers tried to produce value added products such as chicken sausages added with skin, fat, heart and gizzard (Kumar 2009), chicken nuggets added with subcutaneous chicken fat are developed (Ghogare 2009).

Fresh chicken meat and their semi-processed and processed products are quickly perishable due to high moisture and nutrient levels, which promote microbial growth and is the primary factor associated with meat quality reduction, spoilage and economic loss. In order to prevent the microbial spoilage and to maintain the product quality from the time of production to consumption, fresh chicken or semi-processed products have to be preserved, mainly by refrigeration. Refrigeration storage and transport of meat products are common in developed countries, whereas in many developing countries like India, these facilities are limited, especially in rural areas, leading to reduced meat consumption. Moreover, processing of meat and then storage in chilled condition incurs more production. On the other hand, numerous preservation methods including heating, chilling, freezing, freeze-drying, drying, curing, salting, sugar addition, acidification, fermentation, smoking, oxygen removal, carbon dioxide addition and irradiation are used to make meat stable (inhibition of spoilage) and safe (avoidance of food poisoning).However, these preservation methods are based on relatively few parameters (hurdles) i.e. high or low temperature treatment, lowing water activity (a_w), pH and Eh (oxidation-reduction potential), application of preservatives (e.g. nitrite, smoke, carbon dioxide), use of competitive microflora (e.g. lactic acid bacteria), radiation treatment (e.g. gamma rays) etc. Thus more processes are distinguishable than parameters which govern them as pointed out by Leistner (2000). For the microbiological stability of almost all foods a combination of parameters (hurdles) is decisive i.e. hurdle technology. Hence, alternate storage methods, which are cheaper, efficient and acceptable, have to be developed. Heat and serve, processed poultry products were prepared using multi-hurdle technology like chicken curry (dehydrated) which can be stored at ambient temperature for 10 days (Rathod 2006). These techniques include, hygienic processing

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of healthy chicken, pH reduction, preparation of products under aseptic conditions, minimizing the cellular water activity, addition of herbs/spices having anti-microbial, antioxidant and preservative properties.

Shelf stable meat products both traditional and novel are storable without refrigeration facilities. These products are stable and safe due to a combination of factors (hurdles) which inhibit microbial growth; hence hurdle technology can be employed successfully in development of shelf stable chicken products. To maximize the effectiveness of these hurdles in foods, it is necessary to adopt a systems approach (hurdle technology) to meet the challenge posed by the need for food preservation and to avoid the limitations of refrigeration as well as single chemical preservatives.

MATERIALS AND METHODS

Chicken meat: Broilers of approximately 8 weeks old were procured from the local market of Parbhani and were dressed following traditional Halal method. All tendons were removed and separable connective tissues and fat were trimmed off. Dressed meat packed in LDPE pouches and stored in deep freezer at -18 ± 1^0C for overnight.

Textured soy protein: Textured soy protein powder was prepared from commercially available soy granules (Nutrela, Ruchi Soya Industries Ltd, Manglia, M.P.).

Condiments: The external covering of onion and garlic were peeled off, cut into small pieces and were blended in 3:1 ratio using electric grinder to obtain fine paste.

Spice mix: All the spice ingredients were cleaned to remove extraneous matter, dried in hot air oven at 60^oC for $2^1/_2$ hr and then ground in a grinder using proportionate quantity to obtain dry spices mix for preparation of chicken sausage. Spice mixture so obtained was stored in airtight plastic container and used subsequently.

Formulation of chicken sausages: A typical formulation as suggested by Biswas *et al*. (2007) with slight modification was used for preparation of chicken sausage. The formulation includes meat (74.50%), chicken fat (14%), salt (1.80%), sodium tripolyphosphate (0.05%), monosodium glutamate (0.50%), spice mix (1.50%), condiment paste (3.63%), sugar (1.0%), refined wheat flour (3.0%) and sodium nitrite (0.02%). Quantity of meat was proportionally replaced by textured soy protein as shown in experiment I.

Preparation of sausage: Broiler meat after deboning was kept at freezing temperature (-18 ± 1^0C) overnight. After adequate thawing, it was cut into small chunks and minced in meat mincer. Minced meat was chopped in bowl chopper for desired duration. Chicken sausage was subjected to sensory evaluation and physico-chemical analysis.

Emulsion stuffing: Sausage batter was stuffed into 20 mm diameter artificial cellulose casing using mechanical hand stuffer and linked manually.

Cooking: The process of cooking of sausage was carried out as per the method outlined by Thomas (2007). Stuffed raw sausages in cellulose casing were suspended inside steam oven without pressure till the internal temperature reaches 75±1^0C. The sausages were cooled to room temperature followed by peeling off casings.

Analytical procedures

pH: The pH of chicken sausage was determined by the method of Trout *et al*. (1992).

Emulsion stability: Emulsion stability of sausage was determined as per the procedure of Baliga and Madaiah (1971). Twenty gram of sample was taken in polyethylene bag and sealed airtight. The bags were heated in a thermostatically controlled water bath at 80^0 C for 30 min. The bags were removed from the water bath, cut at one end, cooked fluids were drained out and the cooked samples were weighed. Weight after cooking was calculated and expressed as percent emulsion stability.

$$\text{Emulsion stability (\%)} = \frac{\text{Weight of cooked mass}}{\text{Initial Weight}} \times 100$$

Cooking yield: The weight of sausage was recorded before and after cooking. The yield was calculated and expressed as percentage.

$$\text{Cooking yield (\%)} = \frac{\text{Weight of cooked sausage}}{\text{Weight of raw sausage}} \times 100$$

Proximate composition: The moisture, fat and protein content of chicken sausages were determined by following the method of AOAC (1995).

Sensory evaluation: The sensory panelists consisting of academic staff were involved to assess the quality of fresh and stored chicken sausages on the basis of sensory attributes viz., appearance, flavour, juiciness, texture and overall acceptability using 8 point descriptive scale (Keeton 1983) where '8' denoted extremely desirable and '1' denoted extremely poor. The stored product was observed for any objectionable flavour and colour before evaluation. Chicken sausages were warmed before serving to the members of sensory panel for evaluation.

Statistical analysis: The data generated during the study were analysed by analysis of variance technique following standard procedure of Snedecor and Cochran (1989).

RESULTS AND DISCUSSIONS

Effect of different levels of textured soya protein as a humectant on quality of chicken sausages: Experimental trials were conducted to prepare acceptable quality chicken sausages incorporated with different levels of textured soya protein (TSP). Textured soya protein was incorporated at different levels (2, 4 and 6%) replacing proportionate amount of meat and compared with the control. The acceptable level of textured soya protein was selected on the basis of sensory quality as well as physico-chemical characteristics.

Table 1: Effect of incorporation of textured soya protein (TSP) on sensory quality of chicken sausage

TSP level (%)	Sensory attributes				
	Appearance	Flavour	Juiciness	Texture	Overall palatability
Control	$7.07^{a} \pm 0.03$	$7.10^{a} \pm 0.07$	$7.11^{a} \pm 0.07$	$7.00^{b} \pm 0.08$	$6.84^{b} \pm 0.06$
2	$7.00^{a} \pm 0.03$	$6.93^{a} \pm 0.08$	$6.90^{b} \pm 0.04$	$7.33^{a} \pm 0.09$	$7.07^{a} \pm 0.05$
4	$6.93^{a} \pm 0.04$	$6.90^{a} \pm 0.10$	$6.84^{b} \pm 0.05$	$7.44^{a} \pm 0.11$	$7.11^{a} \pm 0.02$
6	$6.83^{b} \pm 0.05$	$6.43^{b} \pm 0.06$	$6.50^{c} \pm 0.01$	$6.43^{c} \pm 0.07$	$6.50^{c} \pm 0.10$

Means with common superscripts did not differ significantly ($p > 0.05$)

Sensory quality: The data in respect to sensory quality of chicken sausages as influenced by incorporation of textured soya protein are given in Table 1. It is observed that incorporation of textured soya protein up to 4% level had similar appearance as compared to control and 2% textured soya protein incorporated chicken sausages while incorporation of 6% TSP level resulted in significant ($p<0.05$) reduction in appearance which suggest that members of sensory panelist had less liking for increased yellowness of product with higher level of TSP. Similar trend was reported by Thomas *et al.* (2008) for pork sausages. Flavour scores declined gradually up to 4% TSP level, with further increase in TSP to 6% the scores declined significantly ($p<0.05$) might be due to high level of TSP, which produces beany flavour in products. Present findings are in agreement with that of Deliza *et al.* (2002) for ground beef patties. The sensory scores with regard to juiciness of chicken sausages incorporated with textured soya protein decreased significantly ($p<0.05$) as compared to control. It might be due to increase dryness in products as a result of incorporation of dehydrated powdered TSP. Within the treatment the scores for juiciness declined steadily up to 4% TSP level. With further increase in TSP to 6%, the juiciness scores decreased significantly ($p<0.05$). The findings are in close agreement with the observations of Thomas *et al.* (2008) for shelf stable pork sausages. The sensory scores for texture and overall palatability were significantly ($p<0.05$) higher in chicken sausages incorporated with 4% TSP as compared to control and 6% TSP level. The results indicated that the overall palatability of sausages was mostly influenced by their juiciness, flavour and textural properties. Though sensory panelist detected mild beany flavour in chicken sausages incorporated with 4% TSP, the better textural characteristics resulted in their higher overall palatability. The similar findings were recorded by Matulis *et al.* (1995) for frankfurters and Porcella *et al.* (2001) for vacuum packaged chorizos.

Physicochemical properties: The data with regard to effect of different levels (0, 2, 4 and 6%) of textured soya protein on physicochemical parameters of chicken sausages is presented in Table 2. The pH of chicken sausages increased significantly ($p<0.05$) with incorporation of textures soya protein (TSP). Addition of 2% TSP in the formulation significantly ($p<0.05$) increased pH of the product by 0.04 while addition of 4% TSP increase it by 0.07 units as compared to control. With subsequent addition of TSP at 6% level pH increased non-significantly, this might be due to higher pH of TSP granules (6.8-7.0). Similar observations were reported by Thomas *et al.* (2008) for shelf stable pork sausages. Also an increase of pH with incorporation of isolated soya protein was observed by Decker *et al.* (1986) in frankfurters and chin *et al.* (1999) in low fat bologna.

Table 2: Effect of incorporation of textured soya protein (TSP) on physico-chemical characteristics of chicken sausage

TSP level (%)	Quality Parameters					
	pH	Emulsion stability(%)	Cooking yield (%)	Moisture (%)	Protein (%)	Fat (%)
Control	$6.01^c \pm 0.02$	$93.49^d \pm 0.10$	$90.84^d \pm 0.06$	$61.95^d \pm 0.05$	$19.27^d \pm 0.12$	$15.99^c \pm 0.07$
02	$6.05^b \pm 0.03$	$95.38^c \pm 0.09$	$92.78^c \pm 0.08$	$62.56^c \pm 0.06$	$20.62^c \pm 0.13$	$16.54^b \pm 0.02$
04	$6.08^a \pm 0.04$	$95.96^b \pm 0.11$	$94.67^b \pm 0.10$	$62.98^b \pm 0.11$	$21.89^b \pm 0.11$	$16.89^b \pm 0.06$
06	$6.10^a \pm 0.03$	$96.33^a \pm 0.14$	$96.95^a \pm 0.12$	$63.30^a \pm 0.10$	$23.13^a \pm 0.14$	$17.30^a \pm 0.04$

Means with common superscripts did not differ significantly ($p > 0.05$)

Incorporation of TSP resulted in significant ($p<0.05$) improvement in emulsion stability and cooking yield as compared to control. Significant ($p<0.05$) increase in emulsion stability and cooking yield might be due to soya protein which acts as an emulsifier and fat encapsulation agent by supplementing myosin as well as actomyosin and thereby prevent fat and water separation while cooking (Reichet 1991). Present findings are in agreement with the results of Pena-Ramos and Xiong (2003) who have stated that stabilizing effect of soya protein in the emulsion is related to high electric charge and more hydrophilic-lipophilic group within the protein structure that increase the protein-lipid and protein-water interactions. They form charged layer around fat droplets causing natural repulsion, reducing interfacial tension and thus prevent coalescence (Alamanou *et al.* 1996). Similar findings were reported by Porcella *et al.* (2001) for chorizos.

It is further seen that the moisture and fat content of sausages differ significantly ($p<0.05$) with increased level of TSP. Significant ($p<0.05$) increase in moisture and fat content was observed in products which may be due to increase in hydrophilic and lipophilic groups that increase the protein-lipid and protein-water interactions. Nevertheless, fat content did not differ significantly upon incorporation of TSP with 2 and 4% level. Similar trend in moisture and fat content was reported by Thomas *et al.* (2008) for shelf stable pork sausages.

Addition of textured soya protein significantly ($p<0.05$) increased protein content of chicken sausages compared to control. The increase in protein content may be due to higher protein concentration of TSP. Similar observations were reported by Chin *et al.* (1999) for low fat bologna and Procella *et al.* (2001) for vacuum packaged chorizos. Based on results it is concluded that incorporation of TSP above 4% level in the formulation had adverse effect on different physicochemical and sensory attributes of chicken sausages, therefore 4% TSP level was selected for subsequent study.

Effect of different levels of emulsion pH adjusted with lactic acid on the quality of chicken sausages

Sensory quality: Sensory attributes of chicken sausages as influenced by different levels of emulsion pH adjusted with lactic acid are presented in Table 3.The emulsion pH was found to have significant ($p<0.05$) influence on juiciness, texture and overall palatability of the chicken sausages. Appearance and flavour of the products prepared from emulsion with pH 5.90 and 5.70 were comparable to control, while those of sausages made from emulsion with pH 5.50 were significantly ($p<0.05$) lower than that of control. Texture and overall palatability of chicken sausages prepared by adjusting pH with 5.90 and 5.70 did not differ significantly. The better juiciness and texture observed for sausages made from emulsion with higher pH 5.90 and 5.70 might be due to the better fat and water binding capacity in them as a result of lower denaturation of meat proteins. Sensory evaluation also indicated that the overall palatability scores for chicken sausages were mostly influenced by their juiciness and texture. Present findings are in agreement with that of Karthikeyan *et al.* (2000) who observed a linear reduction in flavour, juiciness and texture of goat meat keema with decrease in pH from 5.80 to 5.50.

Table 3: Effect of emulsion pH adjusted with lactic acid on sensory quality of chicken sausage

Levels of emulsion pH	Sensory attributes				
	Appearance	Flavour	Juiciness	Texture	Overall palatability
Control (6.10)	$7.07^a \pm 0.13$	$7.00^a \pm 0.06$	$7.05^a \pm 0.05$	$7.11^a \pm 0.12$	$7.22^a \pm 0.05$
5.9	$7.07^a \pm 0.11$	$7.00^a \pm 0.08$	$6.84^b \pm 0.01$	$6.93^b \pm 0.01$	$6.94^b \pm 0.07$
5.7	$7.07^a \pm 0.07$	$7.00^a \pm 0.10$	$6.78^b \pm 0.10$	$6.87^b \pm 0.04$	$6.83^b \pm 0.11$
5.5	$6.87^b \pm 0.10$	$6.67^b \pm 0.02$	$6.28^c \pm 0.07$	$6.50^c \pm 0.06$	$6.43^c \pm 0.13$

Means with common superscripts did not differ significantly ($p > 0.05$)

Physicochemical properties: The pH of the chicken sausages differed significantly ($p<0.05$) with change in emulsion pH (Table 4). Among the treatments, high pH was recorded for the product made with emulsion having pH 5.90 which did not differ significantly with the products having emulsion pH 5.70. Subsequent decrease of emulsion pH showed significant decrease of product pH. Present findings are in agreement with that of Thomas *et al.* (2008). Emulsion stability and cooking yield decreased significantly ($p<0.05$) with decrease in pH level of emulsion. Among the treatments emulsion stability and cooking yield did not differ significantly for emulsion pH 5.90 and 5.70. Papadima and Bloukas (1999) stated that increase in denaturation occurred in muscle proteins at lower pH might be the reason for lower emulsion stability and cooking yield encountered in chicken sausages with lower pH values of emulsion.

Table 4: Effect of emulsion pH adjusted with lactic acid on physico-chemical characteristics of chicken sausage

Levels of emulsion pH	Product pH	Emulsion stability(%)	Cooking yield(%)	Moisture (%)	Protein (%)	Fat (%)
Control (6.10)	$6.27^a \pm 0.002$	$97.85^a \pm 0.10$	$96.60^a \pm 0.06$	$59.92^a \pm 0.08$	$19.12^a \pm 0.13$	$15.85^a \pm 0.07$
5.9	$6.02^b \pm 0.003$	$96.51^b \pm 0.12$	$95.32^b \pm 0.10$	$58.10^b \pm 0.06$	$20.53^b \pm 0.16$	$15.35^b \pm 0.06$
5.7	$5.95^b \pm 0.004$	$95.28^b \pm 0.14$	$94.27^b \pm 0.12$	$56.17^c \pm 0.03$	$21.57^c \pm 0.12$	$14.84^c \pm 0.10$
5.5	$5.60^c \pm 0.003$	$90.20^c \pm 0.13$	$91.16^c \pm 0.15$	$53.22^d \pm 0.05$	$22.63^d \pm 0.15$	$14.07^d \pm 0.12$

Means with common superscripts did not differ significantly ($p > 0.05$)

The present findings are in agreement with Barbut (2006) who reported that addition of liquid lactic acid caused pH drop, separation of moisture and fat which in turn resulted in lower cooking yield of salami type products. Emulsion pH significantly ($p<0.05$) affected the moisture, protein and fat content of chicken sausages. The moisture content of chicken sausages decreased significantly ($p<0.05$) with decrease in emulsion pH level. A significant increase in protein content was observed with decrease in emulsion pH which might be attributed to the higher moisture and fat loss with decrease in emulsion pH. Hedric *et al.* (1994) and Person and Gillet (1996) reported that decline in pH of the product approaching the isoelectirc point of meat proteins reduce its water and fat binding capacities. On the contrary Pietrasik and Duda (2000) observed an inverse relationship between moisture and fat content which was attributed to the fat substitution by moisture in low fat products.

CONCLUSION

Acceptable quality chicken sausage could be prepared with incorporation of 4% textured soya protein (TSP). Addition of TSP above 4% had no beneficial effect on physicochemical and sensory attributes of chicken sausages. Emulsion pH of about 5.70 adjusted with lactic acid resulted in better quality characteristics in chicken sausages which were comparable to control. Reduction in emulsion pH below 5.70 affected different sensory attributes adversely.

REFERENCES

Alamanou S, Bloukas JG, Paneras ED, Doxastakis G (1996) Influence of protein isolate from lupin seeds on processing and quality characteristics of frankfurters. Meat Sci 42:79-93

AOAC (1995) Official Methods of Analysis, 16th Edn. Association of Official Agricultural Chemists, Washington, D.C

Baliga BR, Madaiah N (1971) Preparation of mutton sausages. J Food Sci 36:607-610

Barbut S (2001) Poultry product processing: an industry guide. 1st edn. CRC Press, Boca Raton, FL, USA

Barbut S (2006) Fermentation and chemical acidification of salami type products. Effect on yield, texture and micro structure. J Muscle Foods17:34-42

Biswas S, Charkraborty A, Sarkar S, Barpuzari RN, Barpuzari T (2007) Effect of incorporation of chicken fat and skin on the quality of chicken sausages. J Poult Sci 44(1): 111-115

Chin KB, Keeton JT, Longneeker MT, Lamkey JW (1999) Utilization of soy protein isolate and konjac blends in a low-fat bologna model system. Meat Sci 53:45-57

Decker CD, Conley CC, Richert SH (1986) Use of isolated soy protein in the development of frankfurters with reduced level of fat, calories and cholesterol. Proc. Eur. Meeting Meat Res. Workers 17:121-132

Deliza R, Saldivar S, Germani R, Benassi VT, Cabral LC (2002) The effects of colored textured soybean protein (TSP) on sensory and physical attributes of ground beef patties. J Sens Stud 17:121

Ghogare RB (2009) Evaluation of quality of nugget from spent hen meat M.V.Sc. Thesis. Submitted to MAFSU, Nagpur

Hedrick HB, Aberle ED, Forrest JC, Judge MD, Merkel RA (1994) Principles of meat Science, Kendall/Hunt Publishing Co., Iowa

Karthikeyan J, Sushil Kumar, Anjaneyulu ASR, Rao KH (2000) Application of hurdle technology for the development of Caprine *keema* and its stability at ambient temperature. J Meat Sci 54:9-15

Keeton JT (1983) Effect of fat and NaCl / phosphate levels on the chemical and sensory properties of pork patties. J Food Sci 48: 787-885

Leistner L (2000) Basic aspects of food preservation by hurdle technology. Int J Food Micobiol 55:181-186

Matulis RJ, Keith FK, Sutherland JW, Brewer MS (1995) Sensory characteristics of frankfurters as affected by salt, fat, soy protein and carrageenan. J Food Sci 60:48-54

Papadima SN, Bloukas JG (1999) Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. Meat Sci 51:103-113

Pearson AM, Gillet TA (1996) Processed Meats. 3rd edn. CBS Publishers and Distributors, New Delhi

Pena-Ramos EA, Xiong YI (2003) Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci 64:259-263

Pietrasik, A, Duda Z (2000) Effect of fat content and soy protein/ carageenan mix on the quality characteristics of comminuted, scalded sausages. Meat Sci 56:181-188

Porcella MI, Sanchez G, Vaudagna SR, Lasta JA (2001) Soy protein isolate added to vacuum packaged chorizos: Effect on drip loss, quality characteristics and stability during refrigerated storage. Meat Sci 57:437-443

Rathod KS (2006) Application of hurdle technology for preservation of ready to eat chicken curry. M.V.Sc. Thesis submitted to MAFSU, Nagpur

Reichet JE (1991) Nutritional importance and technological effect: Use of soy protein in meat products. Fleishwirtshaft 42:381-382

Snedecor GW, Cochran WJ (1989) Statistical Methods, 8th edn. Iowa State University Press, Iowa, USA

Kumar S (2009) Studies on development of value added chicken sausage M.V.Sc. Thesis. MAFSU, Nagpur

Thomas R (2007) Development of shelf stable pork sausages using hurdle technology. Ph. D. Thesis submitted to Deemed University IVRI, Izatnagar, India

Trout ES, Hunt MC, Johnon DE, Claus JR, Kastner CL, Kropf DH, Stroda S (1992) Chemical, physical and sensory characterization of ground beef containing 5 to 30 per cent fat. J Food Sci 57: 25-29