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Effect of humectants on the shelf life of chicken sausage stored at ambient temperature

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Abstract
Inadequate cold storage facilities are among the most important factors that have impeded the development of the processed meat sector in developing countries, including India. Hence, there is a need to develop safe, nutritious and acceptable shelf-stable meat products using milder techniques that can be stored/marketed without refrigeration. Hence, a study on the effect of humectants on the shelf life of chicken sausages stored at ambient temperature was undertaken. Sorbitol at 1%, Texturized Soy Protein (TSP) at 2% and a combination of 0.5% Sorbitol and 1% TSP were used as humectants to reduce the water activity of the sausage to 0.93, and they served as treatments, T_1, T_2 and T_3, respectively. The pH and aw were significantly ($P \leq 0.01$) lower in the treatments. Tyrosine value was significantly different between the treatments at 30 hrs ($P \leq 0.05$) and 36 hrs ($P \leq 0.05$), TBARs values were significantly different between storage periods and in the overall storage means only. Hardness, gumminess and chewiness were significantly different between the treatments. Microbial counts were significantly lower in treatments. Treatments secured significantly higher sensory scores. Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the treatments (at 0 day and 30 hours), between the storage periods, between overall treatment means and between overall storage means. Further, the control group was microbiologically stable only at 24 hours of storage, whereas all the treatment groups were microbiologically stable up to 30 hours of storage.

Keywords: Chicken sausage, humectants, texturized soy protein (TSP)

Introduction

Meat is a nutritious, protein-rich food that is highly perishable and has a short shelf-life, unless preservation methods are used (Addis, 2015) [1]. Maintaining food safety and quality is critical for public health and food security (Mao *et al*., 2021) [15]. Food preservation represents an important industrial challenge in terms of preserving nutritional and organoleptic qualities and food safety (Braïek and Smaoui 2021) [8]. In addition, consumer desires range from freshness, long-term storage, absence of chemical preservatives, and reasonable prices of foods (Braïek and Smaoui 2021) [8]. Fresh raw meat constitutes a highly perishable ecological niche (Borch *et al*. 1996) [6], due to intrinsic parameters and the direct exposure of the carcass to the environment once the natural anatomical barrier of the skin/hide is removed. The high-water content (aw - 0.99), the pH that corresponds to the optimal range for microbial growth (5.5-6.5), and the availability of energy-yielding nutrients (e.g., glucose, ribose, amino acids, and nucleosides), as well as vitamins and minerals, account for meat being a foodstuff that is very vulnerable to spoilage and as a consequence, enjoys a very a short shelf-life.

The various forms of microbiological spoilage are preventable to a large degree by a wide range of preservation techniques, most of which act by preventing or inhibiting microbial growth (e.g., chilling, freezing, drying, curing, vacuum packing, modified atmosphere packing, acidifying, fermenting and adding preservatives). A smaller number of techniques act by inactivating microorganisms (e.g., pasteurisation, sterilisation, and irradiation). Additional techniques restrict the access of microorganisms to products (e.g., aseptic processing and packaging).

But the spoilage and poisoning of foods and meat in particular, by microorganisms, is a problem that is not yet under adequate control, despite a range of preservation techniques available. Processing measures such as chopping and/or grinding of meat result in an increased surface-to-weight ratio and rapid product quality deterioration due to additional microbial contamination. Furthermore, processing and storage conditions may greatly affect product shelf life (Mead, 1990) [16].

Consumers demand high-quality and convenient meat products, with natural flavour and taste, and very much appreciate the fresh appearance of minimally processed food. Besides, they require safe and natural products without additives such as preservatives (Hugas *et al.* 2002) [12]. Consumers all over the world, especially in industrialised and developed countries, increasingly demand minimally processed foods and foodstuffs, which retain natural flavour, colour, texture, nutritional characteristics, and contain fewer chemical additives (Ohlsson and Bengtsson, 2002) [18]. Conventional food preservation methods, such as pasteurisation and dehydration, often change the overall organoleptic quality of the food products (Mao *et al*., 2021) [15].

Thus, the need to preserve foodstuffs using natural preservatives and environmentally friendly technologies is obvious (Coma, 2008) [9], due to the constantly growing consumer awareness regarding the relation between food and health (Viuda-Martos *et al.,* 2008) [28]. Thus, it has become necessary for the food industry to constantly investigate the replacement of traditional food preservation techniques (intense heat treatments, salting, acidification, drying and chemical preservation) by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products (Devlieghere *et al.*2004) [11]. Thus, the challenge of modern food preservation is to develop techniques that have a high impact on deleterious microorganisms in foods with little effect on food quality attributes. The necessity of food processors to adopt only minimal processing, such as refrigeration, modified atmosphere packaging, etc., is one of the most important factors that have impeded the development of the processed meat sector in developing countries, including India, as these countries lack efficient cold storage facilities and hence do not maintain cold chains efficiently for their effective marketing (Thomas *et al.* 2008a) [25]. Processing techniques have been standardised for several meat products, but most of them have a limited shelf life at ambient temperature. Studies have indicated that fermented sausages (shelf-stable) are less palatable to consumers (Mir Salahuddin and Sharma, 2003) [17], especially Indians.

Hence, there is a need to develop safe, nutritious, and acceptable shelf-stable meat products using milder techniques that can be stored/marketed without refrigeration. Hurdle technology is a crucial concept in mild preservation methods, as hurdles in a stable product precisely control microbial spoilage and food poisoning in a targeted manner, leaving desired qualities unaffected. The multiple hurdles may be set at lower intensities because of their synergistic effects than would be required if only a single hurdle were used as the preservation technique.

Hence, a study was undertaken to develop a shelf-stable chicken sausage applying hurdle technology. The study aimed to determine the optimum water activity, pH, natural antimicrobial, antioxidant and packaging method for extending the shelf life of chicken sausages stored at ambient temperature. This paper presents the first phase of the study, i.e., the determination of the optimum water activity in the process to develop a shelf-stable chicken sausage, alone.

Materials and Methods

The optimum water activity was determined to be set at 0.93 from the literature. A pilot study was conducted to determine the ideal level of inclusion of sorbitol and Texturized Soy Protein (TSP), to attain a water activity (aw) of 0.93, which revealed that the addition of sorbitol at 1% and TSP at 2% were the ideal level of inclusion to attain a water activity of 0.93. Another pilot study was also undertaken to determine the ideal combination of Sorbitol and TSP to attain a water activity of 0.93, which revealed that a combination of 0.5% Sorbitol and 1% TSP was the ideal level of inclusion to attain a water activity of 0.93. The results of the pilot study are not presented.

Thus, sorbitol at 1%, TSP at 2% and a combination of 0.5% Sorbitol and 1% TSP were used as humectants to reduce the water activity of the sausage to 0.93, and they served as treatments, T_1, T_2 and T_3, respectively, and the control was sausages prepared using the standard methods adopted at the department.

The sausages prepared were subjected to analysis of the following physico-chemical characteristics (Colour, pH, Water activity, Emulsion stability, cooking yield, texture profile analysis, thio-barbituric acid reactive substances number and tyrosine value), microbial qualities (Total viable count, Coliform count and Yeast and mould count), and sensory evaluation. The studies were conducted daily till spoilage occurred. The studies were repeated every six hours after 24 hours of the study. Six trials were conducted, and the optimum level of humectant (s) was determined and selected.

Water activity of sausages was measured using a water activity meter (Aqualab Lite, Decagon Devices, Pullman, WA, USA). Emulsion stability was estimated as per the method outlined by Baliga and Madaiah (1971) [4], as modified by Kondaiah *et al.* (1985) [14]. Cooking yield was calculated using the formula.

$$\text{Cooking yield (per cent)} = \frac{\text{Weight of the sausage after cooking}}{\text{Weight of the sausage before cooking}} \times 100$$

pH of the emulsion and chicken sausage was measured by using a digital pH meter (Cyberscan pH 510, Merck). Tyrosine value was determined by the modified method of Strange *et al.* (1977) [23]. Thiobarbituric Acid Reactive Substances (TBARS) of cooked sausages were determined following the method of Tarladgis *et al.* (1960) [24]. Texture profile analysis was conducted using a Stable Microsystems Texturometer (Model TA_HD plus texture analyser, Stable System Ltd., England, UK) aligned with the software, Texture Expert. The texture profile was analysed as per the procedure outlined by Bourne, (1978) [7]. Colour of the sausages was measured using Hunter Lab Mini Scan XE plus Spectro-colorimeter (Model No. 45/O-L, Reston, Virginia, USA) with geometry of diffuse/80 (sphere - 8mm view) and an illuminant of D65/10 deg (Bindu *et al*., 2007) [5]. Total viable count, anaerobic, coliform, yeast and mould count of processed samples were determined by the method described by the American Public Health Association (APHA), 2015 [3], using ready - made media. Sensory evaluation was conducted by a sensory panel consisting of the staff of the Department of Livestock Products

Technology (Meat Science), who evaluated the chicken sausages for their sensory characteristics. Sausages were evaluated for appearance, flavour, texture, juiciness and overall acceptability using a nine-point hedonic scale.

Results and Discussion

Effect of Humectants on the Physico-chemical Characteristics of Chicken Sausage Emulsion

The mean ± S.E of the physico-chemical characteristics of humectant-incorporated chicken sausage emulsion are presented in Table 1. The pH of the TSP incorporated sausages was very significantly ($P \leq 0.01$) higher than that of the control group, while that of the Sorbitol + TSP incorporated sausages had a higher pH than the control, but the difference was not statistically significant. However, the sorbitol incorporated sausages had a significantly lower pH than the other treatment groups, as well as the control. The findings with respect to TSP are in congruence with the findings reported by Thomas *et al.*(2008a) [25]. He also added that the higher pH observed in the TSP-incorporated sausage emulsion may be attributed to the higher pH of the TSP granules (6.8- 7.0).

Analysis of variance did not reveal any significant difference in the emulsion stability between the treatments. The emulsion stability of the treatments was higher in the treatments. This is inconsistent with the findings reported by Thomas *et al.* (2008a) [25], who reported a significant increase in emulsion stability in TSP-incorporated pork sausages. An increase in emulsion stability is again anticipated, as TSP is an emulsifier. Soy protein has been reported to act as an emulsifier and fat encapsulating agent by supplementing myosin and actomyosin, and thereby prevents fat and water separation while cooking. (Reichet, 1991) [21]. The stabilising effects of soy proteins in the emulsion are related to high electric charge and more hydrophilic -lipophilic groups within the protein structure, which increase the protein lipid and protein water interactions. Further, Alamanou *et al.* 1996 [2] stated that texturised soy proteins form a charged layer around fat droplets, causing natural repulsion, reducing interfacial tension and thus preventing coalescence. The lack of a significant increase in emulsion stability may be owing to the very high emulsion stability in the control samples themselves, due to high levels of binder in the sausage formulation, 10% refined wheat flour (maida), in contrast to the 2% refined wheat flour (maida) used by Thomas *et al.* 2008a [25].

Cooking yield also followed the same trend as that of emulsion stability, and this finding is along expected lines, as emulsion stability is closely linked to cooking yield. Analysis of variance did not reveal any significant difference between the treatments. The cooking yield of the treatments was higher, in the treatments, though not statistically higher.

Effect of Humectants on the Physico-chemical Characteristics of Chicken Sausage

The mean ± S.E of the physico-chemical characteristics of humectant-incorporated chicken sausages are presented in Table 2.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in pH between overall treatment means and between overall storage means. There was a linear increase in pH on storage, and the overall mean pH of the control group is significantly higher than that of the treatment groups. The lower pH of the control group than TSP incorporated sausages is not in congruence with the findings reported by Thomas *et al.* (2008a) [25], who reported a lower pH in the control group, but that finding was at 0 day alone; however, this includes three readings, and the final reading was after 30 hours of ambient storage. The increase in pH on storage in all the groups, and higher pH in the control group, may be attributed to degradation of lactate and accumulation of metabolites derived from bacterial action and deamination of meat products (Jay, 1996) [13]. When meat is cooked to 70-80°C, the pH value increases by about 0.4 units. In a sausage batter, the difference between raw and cooked pH values is 0.0-0.4, depending on the starting value and the use of phosphates. (Puolanne,2001) [20]. This explains the uniform rise in pH in sausages in all the groups when compared to the emulsion.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the water activity between the overall treatment means and the overall storage means. The water activity of the treatments was significantly lower than that of the control group, and it increased linearly and significantly with storage. The difference between the control and treatment is a direct consequence of the added humectants, which have depressed the water activity in the sausages. Thomas *et al.* 2008b [26] and Das *et al.* 2008 [10] utilised TSP ad full fat soy paste, respectively, to reduce the water activity. Water activity, along with pH and temperature, is the most important hurdles that determine the microbial stability in foods.

The tyrosine values were significantly ($P \leq 0.05$) higher in the control than the treatments, and a progressive and significant ($P \leq 0.01$) increase on storage was also noticed. Ruban *et al.* (2009) [22] recorded similar results and reported a linear and highly significant ($P \leq 0.01$) increase in tyrosine values with the increase in storage days. Although tyrosine value cannot be considered specific for proteolysis, it has been regarded as a good general index of meat protein breakdown (Pearson, 1968) [19] and could prove useful for the assessment of spoilage in meat and meat products (Thomas *et al*., 2008 c) [27]. The increase in storage and the higher values in control in comparison to the treatment groups are corroborative evidence of tyrosine value being a good indicator of spoilage.

The Thio barbituric acid reactive substances of chicken sausages showed a constant linear increase on storage. Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the storage periods, and overall storage means only. Das *et al.* (2008) [10] reported a similar increase in TBARS in goat meat nuggets prepared using full-fat soy paste and soy granules. Though statistically not significant, the hurdle-treated sausages had lower TBARS values than the untreated control.

Effect of Humectants on the Texture Profile of Chicken Sausage

The mean ± S.E of the textural characteristics of humectant-incorporated chicken sausages are presented in Table 3.

The treatment groups had a lesser hardness than the control, a finding that is inconsistent with the one reported by Thomas *et al.* (2008a) [25], but consistent with the findings of Das *et al.* (2008) [10]. Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the treatments, and between overall treatment means only. The hardness of meat products decreased with an increase in moisture content, and this is to be anticipated in the treatments, as higher emulsion stability will result in higher moisture, since the loss of water is less.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the adhesiveness of the treatments, between the storage periods (in treatments 1 and 3), between overall treatment means and between overall storage means. Adhesiveness was lower in the treatments than in the control, except in the case of the sorbitol group, which was statistically similar to the control, and both groups were more adhesive than the other two treatments.

Analysis of variance did not reveal any significant difference in springiness between the treatments, between the storage periods, between the overall treatment and between the overall storage means. Springiness increased on storage, reflecting a higher elasticity. The treatments had a higher springiness than the control, and similar findings were reported by Thomas *et al.* 2008a [25], but lower springiness was reported by Das *et al.*2008 [10] in the full-fat soy paste incorporated sausages.

Analysis of variance revealed a significant difference ($P \leq 0.05$) in cohesiveness between overall storage means only. Hurdle-treated groups had lesser cohesiveness than control groups, as reported by Das *et al.* (2008) [10]. Increase in protein by way of addition of TSP may be attributed to the decrease in cohesiveness in the treatments.

Gumminess was found to be significantly lower in treatments than in controls, which is in congruence with the findings of Das *et al.* (2008) [10]. Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between overall treatment means and a significant difference between treatments at 30 hours. Gumminess, being a product of hardness and cohesiveness, is bound to decrease with a decrease in hardness and cohesiveness.

Chewiness was found to be less in treatments than in the control, in accordance with the findings of Das *et al.* (2008) [10], who reported significantly reduced chewiness in the treated sausages. Analysis of variance did not reveal any significant difference between the treatments, between the storage periods, between the overall treatment means and between the overall storage means.

Effect of Humectants on the Hunter Colour Values of Chicken Sausage

The mean ± S.E of the colour characteristics of the humectant - incorporated chicken sausages are presented in Table 4.

A constant and significant increase in L values on storage was observed, and a lower L value in the hurdle-treated groups, except for sorbitol incorporated sausages, was also noticed. Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between overall treatment means, between overall storage means and between the storage means (Control), and a significant difference ($P \leq 0.05$) between the treatments (at all periods) and between the storage means (treatment 1).

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the 'a' values between overall storage means, between the treatments (at 30 hours) and between the storage means (control), and a significant difference ($P \leq 0.05$) between the overall treatment means and between the storage means (treatment 1). The redness of the hurdle-treated products was less, and these findings are in congruence with the findings of Thomas *et al.* 2008a [25].

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the b values between overall storage means, which increased constantly. The b values were lower in the treatments, except for the sorbitol-incorporated sausages. But, an increase in b values was reported by Thomas *et al.* 2008a [25] by the addition of TSP.

Analysis of variance did not reveal any significant difference in the hue values between the treatment, overall storage treatment and overall storage means. However, the treatments had a higher value than the control group.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the chroma values between overall storage means and between the storage means. The TSP incorporated groups had lesser chroma than the control, which was observed to have lesser chroma than the sorbitol incorporated sausages.

Effect of Humectant on the Microbial Qualities of Chicken Sausage

The mean ± S.E of the microbial qualities of the humectant - incorporated chicken sausages are presented in Table 5.

Analysis of variance revealed a highly significant ($P \leq 0.01$) difference in TVC between treatments (at 0 days, 24, 30 and 36 hours) between the storage periods, and between overall storage means, and a significant difference ($P \leq 0.05$) between the treatments (at 30 hours) and between overall treatment means. A TVC of 6 log cfu/g has been reckoned as an indication of spoilage in this study, and based on that premise, it was observed that the control group was microbiologically stable only at 24 hours of storage, while that of the treatment groups was microbiologically stable at 30 hours of storage. The counts increased in storage. The T_3 group, *viz*., the Sorbitol 0.5% + TSP 1% incorporated sausages had the lowest overall treatment mean. This may be attributed to a sorbitol 0.5% + TSP 1%, probably acting synergistically in curtailing microbial proliferation.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in coliform count between the treatments, between the storage periods, between overall treatment means and between overall storage means. The coliform counts increased on storage, and were greater in the control than in the treatments throughout the study. The T_2 group, *viz*., TSP 2% incorporated sausages, had the lowest overall treatment mean.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the yeast and mould count between the treatments, between the storage periods, between overall treatment means and between overall storage means. The control group had the overall treatment mean. counts increased on storage and were greater in the control than in the treatments throughout the study. The T_3 group, *viz*., the Sorbitol 0.5% + TSP 1% incorporated sausages had the lowest overall treatment mean. Similar findings were reported by Thomas *et al.* 2008b [26] who employed texturized soy protein and skim milk powder as humectants. Humectants are substances that are capable of bringing down the water activity, which is very critical for the survival of microorganisms. The reduction in water activity in the emulsion and the sausages, consequent to the addition of humectants, is responsible for the lower microbial counts in the treatments.

Effect of Humectant on the Sensory Qualities of Chicken Sausage

The mean ± S.E of the sensory qualities of the humectant - incorporated chicken sausages are presented in Table 6.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in the appearance scores between the treatments (30 hours only), between all the storage periods, between overall treatment means and between overall storage means. The appearance scores dwindled on storage, and were greater in treatments than in controls. The T_2 group, *viz*., sausages incorporated with 2% TSP had the highest overall treatment mean, though statistically not significantly different from the other 2 treatment groups. Higher appearance scores in TSP incorporated sausages were also reported by Thomas *et al.* (2008b) [26].

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the treatments, between the storage periods, between overall treatment means and between overall storage means. The flavour scores dwindled on storage, and were greater in treatments than in the control. The treatment 2, T_2 group, *viz*., sausages incorporated with 2% TSP, had the highest overall treatment mean, though statistically not significantly different from the other 2 treatments. However, in all three storage periods, the flavour scores of the sausages incorporated with 2% TSP were the highest, and they were also statistically significant. Lower flavour scores in TSP - incorporated sausages were reported by Thomas *et al.* (2008b) [26], and this may be attributed to higher levels of TSP used by them.

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) between the treatments, between the storage periods, between overall treatment means and between overall storage means. However, the difference between the treatment means at 0 day was significant ($P \leq 0.05$). The texture scores dwindled on storage and were greater in treatments than in the control. The treatment 2, T_2 group, *viz*., sausages incorporated with 2% TSP had the highest overall treatment mean, though statistically not significantly higher than the other 2 treatments. However, on all three storage periods, the texture scores of the sausages incorporated with 2% TSP were the highest, and they were also statistically significant at 0 and 30 hrs. Higher texture scores in TSP-incorporated sausages were also reported by Thomas *et al.* (2008b) [26].

Analysis of variance revealed a highly significant difference ($P \leq 0.01$) in juiciness scores between the treatments, between the storage periods, and between overall storage means. However, the difference between the treatment means at 0 day was significant ($P \leq 0.05$). The difference between the overall treatment means was also significant ($P \leq 0.05$). The juiciness scores decreased on storage, and were greater in treatments than in the control. The T_2 group, *viz*., sausages incorporated with 2% TSP, had the highest overall treatment mean. However, on all three storage periods, the juiciness scores of the sausages incorporated with 2% TSP were the highest, and they were statistically also significant. Lower juiciness scores in TSP-incorporated sausages were reported by Thomas *et al.* (2008b) [26], and this may be attributed to higher levels of TSP.

The overall acceptability scores dwindled on storage, and were greater in treatments than in controls. The T_2 group, *viz*., sausages incorporated with 2% TSP, had the highest overall treatment mean, though statistically not significantly higher than the other 2 treatments. However, in all three storage periods, the overall acceptability scores of the sausages incorporated with 2% TSP were the highest, and they were also statistically significant at 0 and 30 hrs. Higher overall acceptability scores in TSP incorporated sausages at 3% level were reported by Thomas *et al.* (2008b) [26]. The higher overall acceptability scores are probably because of the neutral taste of TSP, so that it does not change the flavour of the sausages appreciably.

The T_2 group, *i.e.,* sausages incorporated with 2% TSP, was microbiologically more stable, and had higher sensory scores (in all aspects), and thus this (2% TSP incorporated sausages) was considered as the optimum level of addition of humectant to reduce the water activity to 0.93, without unduly affecting product quality.

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