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Quality and shelf life of cooked chicken sausages at chilling and super chilling storage

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Abstract
The present study evaluated the effects of chilling and super chilling on the physicochemical, microbiological and sensory quality of cooked chicken sausages during storage. Significant ($P < 0.01$) changes in pH, moisture, free fatty acids, thiobarbituric acid, and tyrosine values were observed with increasing storage time with super chilling showing greater stability and slower deterioration than chilling. Microbiological analysis revealed significant ($P < 0.01$) increases in total plate counts, psychrophilic and yeasts and molds, though super chilling effectively delayed proliferation and extended microbial acceptability by 1-2 weeks compared with chilling. Sensory attributes including appearance, flavor, juiciness, texture and overall acceptability scores declined progressively during storage. However, super chilled sausages maintained significantly higher scores and remained acceptable up to 28 days whereas chilled sausages were acceptable only up to 14 days. The findings demonstrate that super chilling is superior to conventional chilling, effectively retarding physicochemical and microbial deterioration while preserving sensory quality and extending the shelf life of chicken sausage.

Keywords: Chicken, meat, sausages, chilling, super chilling, quality, shelf life

Introduction
Sausages can be prepared from various meats by combining comminuted skeletal muscle with salt, fat, spices, condiments, binders and extenders to achieve desirable sensory properties and shelf life. Proper formulation enhances taste, flavor and juiciness. Sausages are among the most popular processed meat products due to their affordability, nutritional value, sensory acceptability and convenience. However, chicken sausages, despite their nutritional advantages, are more susceptible to lipid oxidation due to their high polyunsaturated fatty acid content, as well as to microbial spoilage.

Low-temperature preservation is widely used to minimize spoilage and biochemical degradation in poultry meat. Refrigerated storage (4 ± 1 °C) slows deterioration but is limited to short-term use and unsuitable for long-distance transport. Frozen storage (−20 to −18 °C) enables long-term preservation but may cause undesirable changes in odor, color, flavor, and texture. Super chilled storage (−3 to −1 °C), also referred to as deep refrigeration or partial freezing, reduces the product temperature just below its initial freezing point. Poultry meat stored under super chilling has been shown to remain stable for up to 20 days under aerobic packaging without adverse quality effects (Rathod *et al.*, 2022) [13]. Compared with conventional freezing, super chilled products contain less frozen water (5-30%), resulting in reduced structural damage and lower protein denaturation. Consequently, super chilling extends shelf life while maintaining freshness and color stability (Choe *et al.*, 2016) [4].

In this context, the present study was undertaken to evaluate the physicochemical, microbiological, and sensory acceptability of cooked chicken sausages stored under chilling and super chilling conditions.

Materials and methods
Chicken meat: Fresh broiler meat was procured from local retail stalls. The boneless meat was cut into small chunks, frozen for 1-2 hr to facilitate mincing, and minced twice through a meat mincer (Mado, Germany) using a 5 mm plate. The minced meat was used for cooked sausage preparation.

Casings
Cellulose casings (25 mm diameter; Viskase, USA) were obtained from a local meat processing equipment dealer and soaked in warm water (32 ± 2 °C) for 30 min prior to use.

Cooked sausage formulation
The standard formulation consisted of 73.0% minced chicken meat with sodium tripolyphosphate (0.5%), salt (1.5%), refined vegetable oil (7.5%), refined wheat flour (3.0%), condiment mix (3.0%; onion and garlic 2:1 ratio), spice mix (1.5%) and ice flakes (10.0%).

Product preparation
Minced chicken, sodium tripolyphosphate and salt were chopped in a bowl chopper (Scharffen, Germany) for 1 min. Fat was added and chopped for 2 min, followed by spice mix, condiments and ice flakes. Refined wheat flour were then incorporated and chopping continued for 1.5 min to obtain a stable emulsion. The emulsion was stuffed into 25 mm cellulose casings using a hydraulic sausage stuffer (Dadaux, France) and manually linked. Sausages were conditioned at 4 ± 2 °C for 1 hr, then cooked in a water bath at 80 ± 2 °C for 15 min until a core temperature of 75 ± 2 °C was reached. Six batches were prepared. Cooked sausages were packed in polyethylene bags subjected to storage studies at refrigeration (4±1°C) and super chilling (-2±1°C) temperatures. Samples were analyzed on 0, 7, 14, 21, 28, 35 and 42nd day for sensory attributes, physico-chemical parameters and microbial quality.

Analytical procedures
pH was determined using a digital pH meter (Century Instruments Ltd., India). Moisture content of the product was determined as per the procedure of AOAC (1995) [1]. Free fatty acid (FFA) percentage was determined following the method of Koniecko (1979) [9]. The procedure of Witte *et al* (1970)[20] was followed to estimate thiobarbituric acid value (TBA). Trichloroacetic acid extract of each sample was used for measuring the absorbance at 532 nm. TBA value was calculated as mg malonaldehyde per kg meat sample by referring to a standard graph prepared using known concentration of malonaldehyde. Tyrosine value of stored samples was determined based on the procedure of Strange *et al* (1977) [15].
Total plate count, psychrophlic count, coliforms, yeast and mold, and staphylococcal counts were determined according to APHA (1984) [2]. Ready-made media (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) were used. Serial dilutions were prepared aseptically by blending 10 g of sample with 90 ml of 0.1% sterile peptone water in a pre-sterilized blender. Plate count agar (23.5 g/L, pH 7.0 ± 0.2) was autoclaved (15 lb, 15 min) before use. Plates were incubated at 30 ± 1 °C for 48 h (total plate count) and at 4 ± 1 °C for 14 days (psychrophilic). Coliforms were enumerated on Violet Red Bile Agar incubated at 37 ± 1 °C for 48 hr, while yeast and mold counts were obtained on Potato Dextrose Agar incubated at 25 ± 1 °C for 5 days. Results were expressed as log10 cfu/gm sample.

Sensory evaluation
Cooked sausage slices were evaluated by a panel of trained scientists and postgraduate students in Livestock Products Technology. Attributes including appearance and color, flavor, juiciness, tenderness, binding, and overall acceptability were assessed using a 9-point descriptive scale (Keeton, 1983) [8], where 9 = extremely desirable and 1 = extremely undesirable.

Statistical analysis
Data from six trials were analyzed according to Snedecor and Cochran (1989) [14] to compare treatment means and determine the effects of treatments and storage.

Results and discussion
Changes in physico - chemical characteristics
Storage significantly ($P < 0.01$) affected the physicochemical parameters of chicken sausages (Table 1). pH declined gradually up to 14 days under both chilling and super chilling, followed by a significant increase. The initial decrease may be attributed to cross-linking reactions that remove amino groups from proteins (Ockonkwo *et al.*, 1992) [11], whereas the later increase likely resulted from protein degradation, collagen hydrolysis and microbial production of alkaline metabolites (Webster *et al.*, 1982) [18]. Super chilling maintained greater pH stability, indicating slower microbial and enzymatic proteolysis.
Moisture content declined progressively during storage, with significant ($P < 0.01$) losses after day 14. Chilling resulted in sharper reductions (65.92 ± 0.05% to 58.78 ± 0.68%), while super chilling minimized drip loss, retaining significantly higher values (58.96 ± 0.40% at day 28). These findings agree with Xia *et al.* (2019) [2], who reported better water retention under lower storage temperatures.
Free fatty acid (FFA) values, markers of lipolysis, increased significantly ($P < 0.01$) with storage. In chilled samples, FFA rose from 0.31 ± 0.07% on day 0 to 0.91 ± 0.05% by day 14, whereas superchilled samples showed slower increases, reaching 0.88 ± 0.05% by day 28. This suggests inhibition of microbial lipase activity under super chilling.
Thiobarbituric acid (TBA) values, indicators of lipid oxidation, also increased significantly ($P < 0.01$). Chilled sausages reached 1.29 ± 0.03 mg malonaldehyde / kg by day 14, while super chilled samples reached only 1.00 ± 0.05 mg malonaldehyde /kg by day 28. Although values remained below the threshold limit of 1-2 mg malonaldehyde /kg (Pearson, 1967) [12], the slower oxidation under super chilling confirmed its effectiveness in retarding rancidity, consistent with Jay (1996) [7]. Tyrosine values, reflecting proteolysis, increased significantly ($P < 0.01$). Chilled sausages reached 1.25 ± 0.05 mg/100 gm by day 14, while super chilled samples recorded 1.21 ± 0.01 mg/100 gm only by day 28. This trend reflects protein denaturation and enzymatic breakdown (Daly *et al.*, 1976) [5], with super chilling retarding protease activity. The present findings indicated that, chilling accelerated deterioration in terms of pH instability, moisture loss, lipid hydrolysis, rancidity and proteolysis, whereas super chilling effectively delayed these changes, extending product stability by 1-2 weeks.

Table 1: Changes in physico-chemical parameters of cooked sausages during storage at refrigeration (4±1°C) and super chilling (-2±1°C) temperature

Treatments	Period (days)					Treatment Means ± SE
	0	**7**	**14**	21	28	
pH						
Chilling	6.47 ± 0.08^{a}	6.78 ± 0.07^{b}	6.91 ± 0.05^{c}	NP	NP	6.72 ± 0.07
Super chilling	6.47 ± 0.08^{a}	6.58 ± 0.13^{b}	6.61 ± 0.08^{b}	6.70 ± 0.10^{c}	6.89 ± 0.04^{c}	6.65 ± 0.09
Days Means ± SE	6.47 ± 0.08^{a}	6.68 ± 0.09^{b}	6.76 ± 0.07^{c}	6.70 ± 0.10^{bc}	6.89 ± 0.04^{c}	-
Moisture (%)						
Chilling	65.92 ± 0.05^{a}	62.95 ± 0.45^{b}	58.78 ± 0.68^{c}	NP	NP	62.55 ± 0.39
Super chilling	65.92 ± 0.05^{a}	63.98 ± 0.72^{b}	62.98 ± 0.22^{c}	60.91 ± 0.50^{d}	58.96 ± 0.40^{e}	62.35 ± 0.38
Days Means ± SE	65.92 ± 0.05^{a}	63.47 ± 0.59^{b}	60.88 ± 0.45^{c}	60.91 ± 0.50^{c}	58.96 ± 0.40^{d}	-
Free fatty acid(% oleic acid)						
Chilling	0.31 ± 0.07^{a}	0.62 ± 0.06^{b}	0.91 ± 0.05^{c}	NP	NP	0.61 ± 0.06
Super chilling	0.31 ± 0.07^{a}	0.46 ± 0.06^{b}	0.60 ± 0.05^{c}	0.75 ± 0.06^{d}	0.88 ± 0.05^{e}	0.60 ± 0.06
Days Means ± SE	0.31 ± 0.07^{a}	0.54 ± 0.06^{b}	0.76 ± 0.05^{c}	0.75 ± 0.06^{c}	0.88 ± 0.05^{d}	-
TBA (mg malonaldehyde/kg)						
Chilling	0.25 ± 0.03^{a}	0.70 ± 0.04^{b}	1.29 ± 0.03^{c}	NP	NP	0.75 ± 0.03
Super chilling	0.25 ± 0.03^{a}	0.40 ± 0.05^{b}	0.74 ± 0.04^{c}	0.94 ± 0.03^{d}	1.00 ± 0.05^{d}	0.67 ± 0.04
Days Means ± SE	0.25 ± 0.03^{a}	0.55 ± 0.05^{b}	1.02 ± 0.04^{c}	0.94 ± 0.03^{c}	1.00 ± 0.05^{c}	-
Tyrosine (mg /100 g)						
Chilling	0.42 ± 0.08^{a}	0.71 ± 0.11^{b}	1.25 ± 0.05^{c}	NP	NP	0.79 ± 0.08
Super chilling	0.42 ± 0.08^{a}	0.67 ± 0.08^{b}	0.91 ± 0.04^{c}	1.03 ± 0.05^{d}	1.21 ± 0.01^{d}	0.85 ± 0.05
Days Means ± SE	0.42 ± 0.08^{a}	0.69 ± 0.10^{b}	1.08 ± 0.05^{c}	1.03 ± 0.05^{c}	1.21 ± 0.01^{c}	-

Number of observations: 6

Means with common superscripts in a row (Lowercase letters) and in a column (uppercase) did not differ significantly (P<0.01).

Table 2: Changes in microbial quality of cooked sausages during storage at refrigeration (4±1°C) and super chilling (-2±1°C) temperature

Treatments	Period (days)					Treatment Means±SE
	0	**7**	**14**	**21**	**28**	
Total plate count (log_{10}cfu/gm)						
Chilling	2.94 ± 0.09^{a}	3.35 ± 0.06^{b}	4.55 ± 0.07^{c}	NP	NP	3.61 ± 0.07
Super chilling	2.94 ± 0.09^{a}	2.77 ± 0.03^{a}	3.00 ± 0.10^{b}	3.18 ± 0.15^{bc}	4.09 ± 0.26^{c}	3.20 ± 0.13
Days Means ± SE	2.94 ± 0.09^{a}	3.06 ± 0.05^{b}	3.78 ± 0.09^{c}	3.18 ± 0.15^{bc}	4.09 ± 0.26^{c}	-
Psychrophilic count (log_{10}cfu/gm)						
Chilling	ND	3.97 ± 0.07^{a}	4.22 ± 0.10^{b}	NP	NP	4.10 ± 0.09
Super chilling	ND	ND	3.72 ± 0.05^{a}	3.95 ± 0.07^{ab}	4.09 ± 0.06^{b}	3.92 ± 0.06
Days Means ± SE	-	3.97 ± 0.07^{a}	3.97 ± 0.06^{b}	3.95 ± 0.07^{b}	4.09 ± 0.06^{b}	-
Coliform count (log_{10}cfu/gm)						
Chilling	ND	ND	ND	ND	NP	
Super chilling	ND	ND	ND	ND	ND	
Days Means ± SE						
Days Means ± SE						
Yeast and Mould count (log_{10}cfu/gm)						
Chilling	ND	ND	3.29 ± 0.09^{a}	NP	NP	3.29 ± 0.09
Super chilling	ND	ND	ND	2.97 ± 0.07^{a}	3.23 ± 0.08^{b}	3.10 ± 0.08
Days Means ± SE	-	-	3.29 ± 0.09^{a}	2.97 ± 0.07^{a}	3.23 ± 0.08^{b}	-

Number of observations: 6

Means with common superscripts in a row (Lowercase letters) and in a column (uppercase) did not differ significantly (P<0.01).

Table 3: Changes in sensory attributes of cooked sausages during storage at refrigeration (4±1°C) and super chilling (-2±1°C) temperature

Treatments	Period (days)					Treatment Means±SE
	0	**7**	**14**	**21**	**28**	
Appearance						
Chilling	7.88 ± 0.51^{a}	6.88 ± 0.44^{b}	6.04 ± 0.57^{c}	NP	NP	6.94 ± 0.51

Super chilling	7.88 ± 0.51^{a}	7.47 ± 0.62^{a}	6.76 ± 0.60^{b}	6.50 ± 0.59^{bc}	6.25 ± 0.65^{c}	6.97 ± 0.59
Days Means±SE	7.88 ± 0.51	7.17 ± 0.61^{b}	6.40 ± 0.69^{c}	6.50 ± 0.59^{bc}	6.25 ± 0.65	
Flavour						
Chilling	7.67 ± 0.15^{a}	6.82 ± 0.14^{b}	5.64 ± 0.12^{c}	NP	NP	6.71 ± 0.14
Super chilling	7.67 ± 0.14^{a}	7.17 ± 0.13^{b}	6.90 ± 0.12^{b}	6.44 ± 0.11^{c}	5.94 ± 0.10^{c}	6.82 ± 0.12
Days Means±SE	7.67 ± 0.14^{a}	7.00 ± 0.13^{b}	6.27 ± 0.12^{c}	6.44 ± 0.11^{c}	5.94 ± 0.10^{c}	
Juiciness						
Chilling	8.24 ± 0.15^{a}	7.44 ± 0.18^{b}	6.10 ± 0.20^{c}	NP	NP	7.26 ± 0.18
Super chilling	8.24 ± 0.14^{a}	7.92 ± 0.16^{b}	7.40 ± 0.21^{c}	6.94 ± 0.17^{d}	6.28 ± 0.19^{e}	7.36 ± 0.17
Days Means±SE	8.24 ± 0.15^{a}	7.68 ± 0.17^{b}	6.75 ± 0.18^{c}	6.94 ± 0.17^{c}	6.28 ± 0.19^{d}	
Texure						
Chilling	7.50 ± 0.12^{a}	6.79 ± 0.15^{b}	5.60 ± 0.18^{c}	NP	NP	6.63 ± 0.15
Super chilling	7.50 ± 0.11^{a}	7.22 ± 0.14^{b}	6.97 ± 0.17^{c}	6.25 ± 0.20^{d}	5.88 ± 0.19^{e}	6.76 ± 0.16
Days Means±SE	7.50 ± 0.12^{a}	7.01 ± 0.14^{b}	6.29 ± 0.18^{c}	6.25 ± 0.20^{c}	5.88 ± 0.19^{d}	
Overall acceptability						
Chilling	7.58 ± 0.12^{a}	6.79 ± 0.16^{b}	5.57 ± 0.19^{c}	NP	NP	6.64 ± 0.16
Super chilling	7.58 ± 0.12^{a}	7.17 ± 0.15^{b}	6.99 ± 0.18^{c}	6.08 ± 0.21^{d}	5.83 ± 0.20^{d}	6.73 ± 0.17
Days Means±SE	7.58 ± 0.12^{a}	6.98 ± 0.16^{b}	6.28 ± 0.18	6.08 ± 0.21^{c}	5.83 ± 0.20^{d}	

Number of observations: 30

Means with common superscripts in a row (Lowercase letters) and in a column (uppercase) did not differ significantly (P<0.01).

Changes in microbial quality

Storage significantly (P < 0.01) influenced microbial counts (Table 2). Total plate count (TPC) in chilled sausages increased from 2.94 ± 0.09 log10 cfu/gm (day 0) to 4.55 ± 0.07 log10 cfu/gm (day 14). In contrast, super chilling restricted growth to 3.00 ± 0.10 log10 cfu/gm (day 14) and 3.18 ± 0.15 log10 cfu/gM (day 21), with spoilage levels (4.09 ± 0.26 log10 cfu/gm) reached only by day 28. Similar inhibitory effects of low temperature storage on microbial proliferation have been reported (Huffman *et al.,* 1975[6]; Von Holy and Holzapet, 1988) [17]. Psychrophilic were undetectable on day 0, likely due to initial cold injury (Newsome et al., 1984) [12]. In chilling, they appeared on day 7 (3.97 ± 0.07 log10 cfu/gm) and reached 4.22 ± 0.10 log10 cfu/gm by day 14. Super chilling delayed growth, with psychrotrophs detected only on day 14 (3.72 ± 0.05 log10 cfu/gm) and reaching 4.09 ± 0.06 log10 cfu/gm by day 28. Yeasts and molds followed a similar trend. Chilled samples showed fungal growth by day 14 (3.29 ± 0.09 log10 cfu/gm), whereas super chilled samples showed no growth until day 21 (2.97 ± 0.07 log10 cfu/gm). Even by day 28, counts remained lower (3.23 ± 0.08 log10 cfu/gm) than in chilled samples. Across treatments, microbial counts remained well below the spoilage limit of 6.7 log10 cfu/gm (Von Holy and Holzapet, 1988) [17], with no visible spoilage symptoms. These results confirm that super chilling significantly suppresses microbial proliferation, extending microbial acceptability by 1-2 weeks compared to chilling.

Changes in sensory quality

Sensory scores declined significantly (P < 0.01) for appearance, flavor, juiciness, texture, and overall acceptability (Table 3). Appearance declined due to surface drying and non-enzymatic browning (Chenman *et al.,* 1995) [3], but super chilled samples remained acceptable up to 28 days, whereas chilled sausages were acceptable only up to 14 days. Flavor scores declined with rising TBA values and microbial growth (Tarladgis *et al.,* 1960) [16]. Super chilling preserved flavor significantly longer, maintaining acceptability for 28 days versus 14 days for chilling. Juiciness and texture declined with storage, likely due to dehydration, drip loss, and structural protein degradation. Super chilling reduced these effects, retaining higher scores and extending sensory acceptability. Overall acceptability decreased significantly with storage, but super chilled sausages remained acceptable for 28 days, while chilled sausages fell below acceptable limits after 14 days.

Conclusion

Super chilling proved superior to conventional chilling, effectively retarding physicochemical deterioration, suppressing microbial growth, and maintaining sensory quality. By extending shelf life and consumer acceptability by up to 28 days, super chilling offers a promising storage strategy for chicken sausages, benefiting both industry and consumers by ensuring safer, higher quality products.

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