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Enhancing Sensory Attributes and Shelf Stability of Restructured Beef Steaks for Meat Value Addition

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ABSTRACT

Achieving optimal tenderness and marketability of meat derived from aged animals with suboptimal characteristics is pivotal for enhancing meat value in the industry. Meat restructuring is a potent approach to attain this goal, primarily by formulating restructured beef steaks. These steaks are developed through a blend of type II (round and chuck in 1:1 ratio) and type III quality cuts (brisket, plate, and flank in a 1:1:2 ratio), acknowledged for their toughness. This study focused on standardising chunk size and the proportion of type II and type III quality cuts in restructured beef production, with a primary focus on assessing the shelf stability of the standardised product stored at deep freezer conditions for 60 days. The evaluation covers a broad spectrum of physico-chemical characteristics, TBARS, tyrosine, colour values, microbiological parameters, and sensory attributes. Our key findings unveil significant improvements in sensory attributes, increased pH, consistent cooking yield, and subtle variations in colour values and microbial counts, with the Restructured Beef Steaks (SRBS) consistently surpassing the control. Proximate composition, texture analysis, and shear force values further distinguish SRBS from the control. This research reinforces the feasibility of producing shelf-stable restructured beef steaks with superior sensory attributes, with a focus on a combination of 50% type II and 50% type III quality cuts. Vacuum tumbling is highlighted for its role in enhancing texture, tenderness, and cohesiveness, ensuring extended deep freezer storage without compromising sensory qualities. Additionally, the integration of microbial transglutaminase and mincing contributes to enhancement of sensory attributes.

Keywords: Restructured beef steaks, Microbial transglutaminase, Type II quality chunks, Type III quality minced cuts, Shelf-life study

Introduction

Meat, an indispensable component of the modern human diet, offers a concentrated source of vital nutrients and exceptional culinary experiences. With a surge in global meat production and consumption, beef, celebrated for its high-quality proteins, vitamins, and minerals, has gained prominence. Notably, research by (Hilton *et al.* 1998) highlights the inherent challenges associated with meat quality. Palatability, sensory tenderness, connective tissue content and flavour scores tend to deteriorate as carcass maturity increases. (Stika *et al.* 2007) further observed decreased tenderness in beef steaks from mature cows (>10 years of age) compared to their younger counterparts (<4 years). (Cho *et al.* 2009) emphasize the

consumer's preference for tenderness when evaluating meat quality, a characteristic that remains challenging to assess before purchase due to its wide range of variability, including the amount and type of connective tissue and muscle fiber properties (Kim *et al.,* 2010). To address these challenges, the application of restructuring techniques in meat processing emerges as a promising approach. (Smith, 1984) provides a comprehensive definition of restructuring as the utilisation of manufacturing steps to create consumer ready to use products closely resembling intact muscles, diverging from traditional ground meat. This technology not only allows for the enhancement of tenderness and juiciness but also offers control over various product attributes such as shape, colour, texture and flavour. Furthermore, restructuring presents an opportunity to upgrade the value of meat trimmings, which might otherwise be considered less valuable. Traditional restructured beef products maintain the integrity of meat chunks through myofibrillar protein extraction, employing mechanical action and ionic strength (Trout and Schmidt, 1986). These techniques extend their applicability beyond beef to encompass other meat types, including poultry, fish and seafood, facilitated by commercially available cold-set binders (Moreno *et al.,* 2008). A diverse range of cold binding systems has been developed, featuring calcium alginate, microbial enzyme-based binders, such as ActivaTM transglutaminase products, blood-based binders like Fibrimex®, FX Technology and Products, and protein/chemical binders exemplified by Pearl Meat Binders and Chiba Flour Mills. Notably, transglutaminase offers a method for cold gelification of muscle protein, reducing the need for additives such as NaCl and phosphate (Wijngaards and Paardekooper, 1988). Intriguingly, the consideration of meat quality extends to the shelf-life of restructured meat products. Gupta and Sharma (2016) explored the sensory quality of control and functional restructured spent hen meat blocks during storage and revealed that while all sensory attributes remained excellent for up to 45 days, product acceptability diminished on the 60th day due to off-flavour development and an elevated microbial load. This study provided valuable insights into the safe storage of functional restructured meat products under specific conditions. Additional insights into the impact of storage on meat quality come from (Kandeepan and Biswas, 2007), who suggest that the tyrosine value may increase with longer storage times, reflecting the influence of inherent and microbiological meat deterioration. Furthermore, research by (Stika *et al.* 2007) highlights that lipid oxidation, measured through TBARS values, is not significantly affected by the age of the animal but increases during prolonged storage. Their findings underline the importance of understanding the oxidative changes that occur during storage. In addition, research by (Gadekar *et al.* 2014a) reveals that the aerobic mesophilic count displayed a notable decrease on day 30 but subsequently increased on day 60, illustrating the dynamic nature of microbial populations during storage. The presence of psychrophiles and coliforms in restructured meat products is also highlighted, emphasizing the importance of storage conditions in microbial control.

In light of these considerations, this comprehensive study is undertaken with the specific aim of standardising a restructured beef from low-valued cuts, specifically Type II and Type III (Kim *et al.* 2010) meat cuts. A key focus of this research is the rigorous evaluation of the shelf-life of the standardised product during 60 days of storage under deep freezing conditions. The objectives encompass estimating the product's durability over time and evaluating its quality. This study aims to contribute to the advancement of meat processing techniques, aligning meat products with modern consumer preferences and addressing the critical issue of product stability during storage.

Materials And Methods

Ingredients

Beef cuts, including round, chuck (type-II meat cuts), brisket, plate and flank (type-III meat cuts) were sourced from aged cattle (five years old and above). The cattle were humanely and scientifically slaughtered, followed by the deboning process, which adhered to strict hygienic standards at the Meat Technology Unit, Mannuthy, Thrissur, kerala. The procured beef cuts underwent immediate chilling at 4±1 °C for twenty-four hours to facilitate the aging process. The deboned meat was subsequently aerobically packed in high-density polyethylene bags and stored under frozen condition (-22±1 °C). Prior to use in the preparation process, the frozen meat was thawed at 4±1 °C. The present study was conducted for estimation of shelf life of the standardised product. Additional Ingredients: Refined sunflower oil (Fortune, India) was consistently used as the cooking oil during sensory evaluation study; Spice Mixture: The spice mixture included coriander powder, beef masala powder, black pepper, red chilli powder, turmeric powder, cinnamon, clove and nutmeg; Curing Ingredients: Sodium chloride, sugar, sodium tri-polyphosphate and sodium nitrite were employed as curing agents used in the formulation was determined following an extensive series of preliminary pilot studies to achieve the desired product specification.

Product formulation

The restructured beef block formulation was standardised by conducting several trials. The standardised formulation was used for entire study (Table 1).

Table 1. Standardised formulation for restructured beef steaks

S. No.	Ingredients	Specification	In percentage
A	Type II quality	Round, Chuck (1:1)	50 %
	Type III quality	Brisket, Plate, Flank (1:1:2)	50 %
	Total meat	Round, Chuck, Brisket, Plate, Flank	**100 %**
B	Cold-set binder	Microbial trans-glutaminase	0.75 % of meat
C	Curing in-gredients	Sodium chlo-ride	1 % of meat
		Sodium tripoly-phosphate	0.3 % of meat
		Sodium nitrite	120 ppm of meat

Preparation of restructured beef block

Deboned aged beef cuts (round and chuck) were sectioned into chunks with standardised dimensions of 7.5 cm × 10 cm x 5cm, taken in a 1:1 ratio. Additionally, deboned aged beef cuts (brisket, plate and flank) were taken in a ratio of 1:1:2 and minced through a thirteen-millimeter grinder plate using a meat mincer (MADO primus Model MEW 613, Germany). The round and chuck chunks (7.5 cm × 10 cm × 5 cm) at 1:1 ratio and minced beef at 1:1:2 ratio were brushed with microbial transglutaminase slurry (MTGase one part and distil water four parts) on their surface using rubber basting brush and preblended with cured ingredients (salt, sodium tripolyphosphate, sodium nitrite) at the levels specified in table 1. After pre-blending, the chunks along with minced meat were placed in a vacuum tumbler (BIRO Vacuum Marinade Tumbler, Table Top Model: VTS-43, United States of America) for mechanical tenderisation and for fastening the curing process. The vacuum tumbler was set with eight rpm drum speed. The tumbling was done for one hour with ten minutes break after first thirty minutes. Prior to tumbling, the vacuum tumbler was chilled by adding ice flakes. Subsequently, the chunks were tightly packed in PE/Al/PA laminated pouches and vacuum-sealed using a vacuum packaging machine (Vacuum packaging machine, Model: WM-19/S/CE-OSNAVAC, Germany). The vacuum-packed meat was stored at refrigeration temperature (4±1 °C) for twelve hours for equilibration and then transferred to a deep freezer (-22±1 °C) for twenty-four hours. On the day of sensory analysis, the restructured beef blocks were thawed at refrigeration temperature (4±1 °C) until they reached a core temperature of five degree Celsius. Then, they were sliced into steaks with a thickness of five millimeter using a meat slicer (Slicer Automatic, Model: 300 VV-CE, Chennai). The steaks were subsequently seasoned with the spice mix according to the levels as specified and pan fried until they achieved a golden yellow colour.

Experimental Design

The experiment comprised three distinct phases, each meticulously designed to investigate the development and shelf life of Standardised Restructured Beef Steaks (SRBS).

Phase One: Chunk Size Evaluation

In this phase, aged beef cuts from the round and chucks (taken in 1:1 ratio) were carefully selected for segmentation. The cuts were transformed into chunks of three varying dimensions: 7.5 cm × 10 cm × 5 cm, 10 cm × 10 cm × 5 cm and 10 cm × 12.5 cm × 5 cm. Sensory evaluations were conducted to determine the optimal chunk size, chunk size 7.5 cm × 10 cm × 5 cm ultimately identified as the most favourable for next phase two study.

Phase Two: Meat Type Blending

Based upon the findings of Phase One, aged beef type-III cuts, specifically brisket, plate, and flank taken as 1:1:2 ratio and then minced. The next step involved blending this minced meat with the previously standardised type-II meat, which consisted of round and chuck cuts with a selected dimension of 7.5 cm × 10 cm × 5 cm. Three treatment combinations were created: (70% type II chunks + 30% type III minced meat), (60% type II chunks + 40% type III minced meat), (50% type II chunks + 50% type III minced meat), and the control sample, represented by 100% type II chunks. The selection of the optimal treatment combination among the four was based on criteria that included cooking yield and sensory evaluations. The combination (50% type II chunks + 50% type III minced meat) was selected as the standardised product for shelf-life study

Phase Three: Shelf Life Investigation

The final phase aimed to evaluate the shelf life of the standardised product. Both the Standardised Restructured Beef Steaks (SRBS) and control restructured beef steaks (C1), prepared in accordance with the selected treatment combination and were vacuum-packed. These samples were subsequently stored under deep freezing conditions for duration of 60 days.

Product analysis

pH estimation: The pH of the restructured beef steak was determined using a digital pH meter according to AOAC (2016).

Cooking yield: The weight of beef steaks before and after cooking were recorded. Cooking yield was expressed in per

cent as per (Boccard et al. 1981)

Thio-Barbituric Acid Reactive Substances (TBARS) evaluation: Thio-Barbituric Acid Reactive Substances (TBARS) value of restructured beef steak was determined by the extraction method of (Witte et al. 1970).

Tyrosine value (TV) evaluation: Tyrosine Value (TV) of the samples was estimated as per the method followed by (Strange et al. 1977).

Colour evaluation: Colour of the restructured beef steak was determined objectively as per (Navneet and Kshitji, 2011)

Microbiological evaluation: Aerobic Plate Count / Total Viable Count (TVC): Total viable count of aerobic bacteria of each sample was estimated by pourplate method, as described by Morton (2001); Psychrotrophic count: Psychrotrophic count of each sample was estimated by pour plate method as described by (Cousin et al. 2001) and their counts were expressed as $\log_{10}$ CFU/g.

Sensory evaluation: Samples obtained during the course of investigation were analysed by a semi-trained panelists consisting of ten members from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy using 8-point Hedonic scale (Berry et al. 1995).

Proximate composition: Moisture, Fat content, Protein content and Total ash content of the sample determined by AOAC (2016).

Warner-Bratzler Shear Force Values (WBSF) estimation: The WBSF values of the restructured beef steaks for control and treatment were recorded as per the method outlined by (Wheeler et al. 1997).

Texture Profile analysis: The textural properties of the control and treated samples were evaluated as per (Bourne, 1978).

Statistical analysis

The experiment was replicated six times. The data obtained for the physicochemical, microbiological and sensory characteristics of the control and standardised restructured beef steaks were assessed statistically by repeated measures ANOVA, one-way ANOVA and Friedmann test. Independent sample T test done for proximate analysis, WBSF and texture profile analysis using the Statistical Package for Social Sciences (SPSS) software 24.0 version (Snedecor and Cochran, 1994).

Results And Discussion

Proximate composition

The result of the proximate composition (in per cent) of the control (C_1) and Standardised Restructured Beef Steaks (SRBS) are as follows (The values are expressed as their Mean ± Standard error; Means with different lower-case letters are significantly different between storage days).

Control (C_1): Moisture – 63.47 ^{b} ± 0.48; Protein – 17.52 ^{a} ± 0.56; Fat – 10.57 ^{a} ± 0.13; Total ash – 8.45 ^{a} ± 0.15.

SRBS: Moisture – 72.05 ^{a} ± 0.17; Protein – 13.08 ^{b} ± 0.54; Fat – 8.15 ^{b} ± 0.22; Total ash – 6.72 ^{b} ± 0.19.

The proximate analysis of SRBS and C_1 revealed notable differences. All the parameters of the proximate differed significantly ($p<0.001$) between the control and SRBS. Except for moisture, the control had higher protein, fat and total ash per cent compared to SRBS. As the type III quality beef cuts were minced, the surface area for the action of the curing ingredients increased and with the tumbling process, their action became effective leading to the increased moisture retention, decreased protein and ash content which reflected in the values of the SRBS in contrast to control. The result goes in accordance with the studies of Ahmed et al. (1989) and Dimitrakopoulou *et al.* (2005). Additionally, the restructuring technique proved effective in reducing fat content by removing connective tissues and fat during product preparation.

Warner-Bratzler Shear Force (WBSF) value estimation

The Warner-Bratzler Shear Force (WBSF) values, expressed in kg/cm², for both the control (C_1) and SRBS were 4.57 ^{a} ± 0.02 and 4.01 ^{b} ± 0.19 (The values are expressed as their Mean ± Standard error; Means with different lower-case letters are significantly different between storage days) respectively. Statistical analysis revealed a significant difference ($p<0.05$) between the control and SRBS. This reduction in shear force values in SRBS is indicative of improved tenderness; a phenomenon closely associated with the diminished particle size achieved during processing. This finding is consistent with the research conducted by (Gurikar et al. 2014) which has established that reduced particle size leads to a marked enhancement in meat tenderness. Moreover, our findings align with the study conducted by (Kim et al. 2010) which provides further support for the assertion that the standardised product, SRBS, can be classified as an exceptionally tender meat product. This classification is substantiated by the observation that the WBSF score for SRBS falls below the critical threshold of 4.09, underscoring the efficacy of the standardisation process in enhancing the tenderness of the beef steaks.

Texture profile analysis

Texture profile analysis of control (C_1) were as follows (The values are expressed as their Mean ± Standard error; Means with different lower-case letters are significantly different between storage days): Hardness (N cm^{-2}) - 72.63^{a} ±3.56, Springiness (cm) - 0.63^{b} ±0.06, Adhesiveness - 0.23^{b} ±0.02, Chewiness (N cm) - 1.26^{b} ±0.03 and of SRBS were as follows: Hardness (N cm^{-2}) - 51.89^{b} ±2.88, Springiness (cm) - 0.92 ^{a} ±0.03, Adhesiveness - 0.38 ^{a} ±0.01, Chewiness (N cm) - 2.45 ^{a} ±0.37.

The results indicate significant differences ($p<0.05$) between

the control and SRBS in all examined texture parameters. Notably, SRBS exhibited higher values for springiness, adhesiveness and chewiness when compared to the control, while the hardness values differed. The observed reduction in hardness in SRBS can be attributed to the reduced particle size resulting from the mincing process, which led to increased moisture retention and more breaking of connective tissue. This finding aligns with the research conducted by (Reddy, 2011). Additionally, the effective removal of connective tissue through mincing contributed to the lower hardness values, consistent with the study of (Strange and Whiting, 1990). Furthermore, the salt and sodium tripolyphosphate employed in SRBS formulation had an impact on the springiness and adhesiveness values, which were higher than those in the control group. This outcome is consistent with the findings of (Cardello et al.1983). The introduction of a low-value ingredient also increased chewiness, as corroborated by Reddy's study in 2011. Consequently, the standardised SRBS product demonstrated desirable springiness, adhesiveness and chewiness compared to the control group, confirming the effectiveness of the standardised formulation and processing techniques.

Shelf-life analysis

To evaluate the shelf life of the Standardised Restructured Beef Steaks (SRBS), which comprised 50% type II and 50% type III quality meat, alongside the control (C_1) restructured beef steaks composed entirely of type II meat, both products were vacuum-sealed in laminated pouches and stored at a constant temperature of -22±1°C for a total duration of 60 days. Over the course of the storage period, various physico-chemical characteristics, colour values, microbiological parameters and sensory attributes were assessed on the 0th, 15th, 30th, 45th and 60th days.

pH estimation

The physico-chemical characteristics, specifically pH for C_1 and SRBS on different evaluation days are as follows. For **Control (C_1):** 0th day - 5.95^{aA} ± 0.01; 15th day - 5.96^{bA} ± 0.01; 30th day - 5.98^{cA} ± 0.01; 45th day - 5.99^{dA} ± 0.01; 60th day - 6.01^{eA} ± 0.01 and for **SRBS:** 0th day - 5.86^{aB} ± 0.01; 15th day - 5.87^{bB} ± 0.01; 30th day - 5.87^{bB} ± 0.01; 45th day - 5.90^{cB} ± 0.01; 60th day - 5.92^{dB} ± 0.01 (The values are expressed as their Mean ± Standard error; Means with different upper-case letters as superscripts are significantly different between control and SBRS; Means with different lower-case letters are significantly different between storage days).

The pH values of the control sample experienced a significant ($p<0.001$) increase from the initial (0th) day to the 60th day. Similarly, the pH of SRBS also exhibited a significant ($p<0.001$) increase over the storage period, except for deviations noted on the 15th and 30th days. It is noteworthy that significant ($p<0.001$) differences were observed between the control and SRBS on all five evaluation days, with the control consistently displaying a higher pH value compared to SRBS during the entire shelf life study. The utilisation of microbial transglutaminase as a cold-set binder was found to increase the pH in the restructured beef steaks, which is consistent with findings in the studies conducted by (Means et al. 1987) who employed alginates as cold-set binders, noted progressive rise in pH with the increase in duration of storage of beef steaks and (Ensor et al. 1989) in restructured turkey meat. Furthermore, our observation of increasing pH during frozen storage aligns with the findings of (Esguerra, 1994) in restructured beef steaks.

Cooking yield evaluation

The physico-chemical characteristics, specifically cooking yield (in per cent) for C_1 and SRBS on different evaluation days are as follows. For **Control (C_1):** 0th day - 59.00^{B} ± 0.5; 15th day - 59.33 ± 0.46; 30th day - 59.33 ± 0.46; 45th day - 59.17^{B} ± 0.45; 60th day - 59.00^{B} ± 0.43 and for **SRBS**: 0th day - 61.17^{A} ± 0.53; 15th day - 60.67 ± 0.46; 30th day - 60.67 ± 0.46; 45th day - 60.67^{A} ± 0.45; 60th day - 60.83^{A} ± 0.43 (The values are expressed as their Mean ± Standard error; Means with different upper-case letters as superscripts are significantly different between control and SBRS; Means with different lower-case letters are significantly different between storage days).

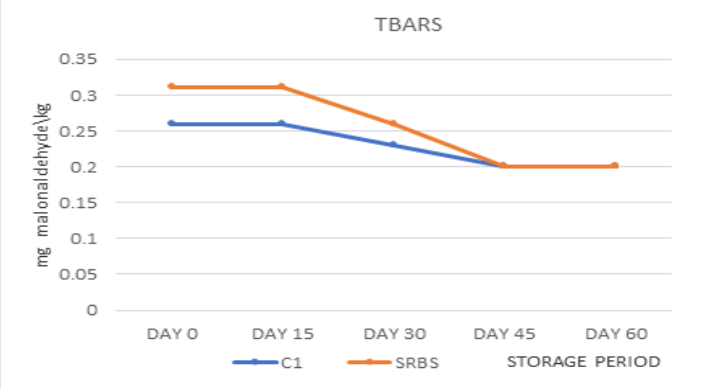
The cooking yield of both samples remained relatively stable throughout the storage period, with no significant differences noted. Notably, the mean cooking yield values exhibited a significant difference ($p<0.001$) between C_1 and SRBS on the 0th, 45th and 60th days, with SRBS consistently demonstrating a higher cooking yield percentage compared to the control. After eight weeks of frozen storage under vacuum packaging, cooking yield values exhibited no significant fluctuations, in agreement with the study by (Bhattacharya et al. 1988).

Thio-barbituric Acid Reactive Substances (TBARS) evaluation

TBARS values, expressed as mg malonaldehyde/kg, were measured for C_1 and SRBS during the storage period (figure 1). The TBARS values for both samples remained stable on the 0th and 15th days, followed by a decreasing trend during the 30th and 45th days, with stability reestablished on the 60th day of the storage study. A significant decrease ($p<0.05$) in the mean TBARS values was observed during the storage days for both SRBS and control, with significant differences ($p<0.05$) noted between the samples on the 0^{th,} 15th and 30th days. The lower TBARS values in both SRBS and control can be attributed to the effect of vacuum packaging, which acts as an oxygen barrier (as per the findings of Smiddy et al. 2002), thus limiting the lipid oxidation process. This reduction in TBARS values is consistent with the gradual increase in

redness (a*) values, as per Sanchez-Alonso et al. (2008) and odour values, following (Ockerman and Organisciak, 1979) in the restructured samples.

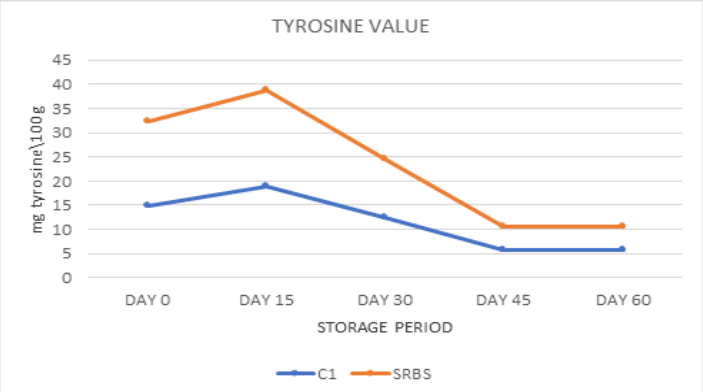
Fig. 1: Effect of storage days on TBARS value of control and standardised restructured beef steaks



Tyrosine value evaluation

Tyrosine values, expressed as mg tyrosine/100g, for both C_1 and SRBS throughout the 60-day storage period are presented in Figure 2. Significant differences in tyrosine values were observed between the two samples, with SRBS consistently displaying higher values, indicating a greater degree of protein degradation in SRBS. As the storage period progressed, a significant increase ($p<0.001$) in tyrosine values was noted for both C_1 and SRBS on the 15th day, indicating an initial phase of proteolysis. Subsequently, both samples exhibited a decreasing trend in tyrosine values until the 45th day, aligning with the work of (Ziauddin et al. 1993), who observed decreased tyrosine values in frozen meat cuts and minced meat during storage, possibly due to reduced proteolytic activity or the formation of less soluble protein compounds. Notably, both C_1 and SRBS reached a stable phase in tyrosine values by the 60th day of storage, indicating a potential equilibrium in protein degradation and proteolysis activity. A decreased tyrosine value also reflects a low bacterial count, supported by the findings of (Strange et al. 1977).

Fig. 2: Effect of storage days on Tyrosine value of control and standardised restructured beef steaks



Colour evaluation

The impact of storage duration on Hunter L*a*b* colour values for both the control (C_1) and Standardised Restructured Beef Steaks (SRBS) are summarised. (The values are expressed as their Mean ± Standard error; Means with different upper-case letters as superscripts are significantly different between control and SBRS; Means with different lower-case letters are significantly different between storage days). **Hunter L* colour values** - for **Control (C_1)**: 0th day - 25.43^{aA} ± 0.32; 15th day - 24.73^{bA} ± 0.40; 30th day - 23.83^{cA} ± 0.31; 45th day - 22.95^{dA} ± 0.34; 60th day - 21.85^{eA} ± 0.39 and for **SRBS:** 0th day - 26.88^{aB} ± 0.32; 15th day - 26.32^{bB} ± 0.40; 30th day - 25.65^{cB} ± 0.31; 45th day - 25.03^{dB} ± 0.34; 60th day - 24.38^{eB} ± 0.39. **Hunter a* colour values** – for **Control (C_1):** 0th day - 11.82^{a} ± 0.20; 15th day - 13.78^{bA} ± 0.30; 30th day - 14.73^{cA} ± 0.15; 45th day - 15.43^{dA} ± 0.11; 60th day - 16.23^{eA} ± 0.04 and for **SRBS:** 0th day - 11.42^{a} ± 0.20; 15th day - 12.70^{bB} ± 0.30; 30th day - 13.90^{cB} ± 0.15; 45th day – 15.00^{dB} ± 0.11; 60th day - 16.07^{eB} ± 0.04. **Hunter b* colour values** – for **Control (C_1):** 0th day - 7.47 ^{a} ± 0.09; 15th day - 8.47 ^{b} ± 0.05; 30th day - 9.65 ^{c} ± 0.04; 45th day - 10.72^{d} ± 0.08; 60th day - 11.45 ^{e} ± 0.07 and for **SRBS:** 0th day - 7.48 ^{a} ± 0.09; 15th day - 8.42 ^{b} ± 0.05; 30th day - 9.63 ^{c} ± 0.04; 45th day - 10.62^{d} ± 0.08; 60th day - 11.65 ^{e} ± 0.07.

A significant decrease ($p<0.05$) in lightness value (L*) was observed during the storage period for both C_1 and SRBS. The gradual decline in lightness, which continued up to eight weeks of frozen storage, is consistent with the findings of (Chen and Trout, 1991). A reduction in lightness values signifies an increase in the darkness of the meat during frozen storage, aligning with the study by (Ockerman and Organisciak, 1979).

There was a significant increase ($p<0.05$) in redness value (a*) during the storage period for both C_1 and SRBS, with significant differences ($p<0.001$) maintained between the samples throughout the entire study. The gradual rise in redness (a*) values from the 0th to the 60th day of storage suggests the absence of oxygen- mediated or haemoglobin (Hb)-mediated lipid oxidation in both samples. This observation is supported by the work of (Sanchez-Alonso et al. 2008), who discussed indirect methods of monitoring Hb-mediated lipid oxidation through decreases in redness (a*) values.

No significant difference was observed in yellowness value (b*) between control and SRBS during the storage period which was as per the findings of (Chen and Trout, 1991). However, there was significant increase in the yellowness value (b*) for both samples throughout the frozen storage, consistent with the study by (Sanchez-Alonso et al. 2008).

Microbiological evaluation

The microbiological quality of the developed products was rigorously assessed through the enumeration of aerobic plate count and psychrotrophic count on the 0th, 15th, 30th, 45th and

60th days of the storage period. The results are expressed as log_{10} CFU/g and are presented here. For **Control (C_1)**: 0th day - $5.88^{B} \pm 0.03$; 15th day - $5.88^{B} \pm 0.03$; 30th day - $5.87^{B} \pm 0.02$; 45th day - $5.89^{B} \pm 0.03$; 60th day - $5.89^{B} \pm 0.03$ and for **SRBS:** 0th day - $6.80^{A} \pm 0.03$; 15th day - $6.80^{A} \pm 0.03$; 30th day - $6.81^{A} \pm 0.02$; 45th day - $6.81^{A} \pm 0.03$; 60th day - $6.81^{A} \pm 0.03$ (The values are expressed as their Mean ± Standard error; Means with different upper-case letters as superscripts are significantly different between control and SBRS).

This critical analysis is instrumental in evaluating the microbial quality and safety of the meat products. No significant ($p>0.05$) changes in aerobic plate counts were observed for either C_1 or SRBS on the 15th, 30th, 45th or 60th day. Both samples maintained stable microbial counts throughout the storage period, indicating a lack of microbial proliferation. Remarkably, psychrotrophic counts were not detected throughout the entire 60-day storage period for both C_1 and SRBS. The absence of psychrotrophic bacteria over the storage duration can be attributed to a potential retardation of the log phase due to a reduction in the metabolic rate, arising from the abrupt change in the physical environment. This phenomenon is in line with the findings of (Thomas et al. 2006). It is worth noting that the microbiological count for SRBS consistently exceeded that of the control throughout the storage study. This variation may be attributed to the extensive proteolysis that occurred during the mincing process, as reflected in the tyrosine value in accordance with (Strange et al. 1977). Furthermore, the mincing process is likely to have disrupted the bacterial colony, dispersing it throughout the product. Importantly, the aerobic plate counts for both C_1 and SRBS remained consistently below the maximum acceptable levels (log_{10} 7 CFU/g), suggesting an absence of microbial spoilage in the products. This aligns with the findings of (Jay, 1996), confirming the microbial safety of the developed meat products.

Sensory evaluation

The results concerning the sensory attributes of both the control and Standardised Restructured Beef Steaks (SRBS) during their storage at -22±1°C on days 0, 15, 30, 45 and 60 are presented in table 2 (raw) and table 3 (cooked).

Raw steaks sensory evaluation

Sensory attributes for the raw restructured beef samples, encompassing appearance and colour, texture and cohesiveness, exhibited no significant differences over the storage period for both control and SRBS. However, the odour values consistently increased for both control and SRBS, stabilising by the end of the storage study. The overall acceptability of the control samples did not exhibit significant ($p<0.05$) differences over time, with only an initial difference observed between days 0 and 15 for SRBS samples. The absence of significant changes in appearance and colour, texture and cohesiveness are attributed to vacuum packaging, which effectively prevented surface dehydration and maintained these sensory attributes. The consistent cohesiveness scores suggest no significant breakdown of gelation due to microbial actions, a finding supported by (Gupta and Sharma, 2016). The gradual increase in odour values suggests reduced oxidative rancidity as the storage time increased, aligning with the work of (Ockerman and Organisciak, 1979) in restructured meat samples. The initial dip in the overall acceptability of SRBS might be related to the initial dip in odour values, which subsequently increased as the storage time progressed due to consistently acceptable scores in appearance and colour, texture and cohesiveness, along with increasing odour scores. Day-wise comparisons indicate that SRBS outperformed control samples in all sensory attributes except for odour scores, indicating a preference for SRBS over the storage period.

Table 2. Effect of storage days on sensory attributes of raw control and SRBS

Sample	Storage days					
	0th day	15th day	30th day	45th day	60th day	χ^2- value (p-value)
			Appearance and colour			
C_1	$7.09^{B}\pm0.07$	$7.09^{B}\pm0.05$	$6.96^{B}\pm0.17$	$7.14^{B}\pm0.07$	$7.14^{aB}\pm0.07$	6.517 $(0.164)^{ns}$
SRBS	$7.55^{A}\pm0.02$	$7.63^{A}\pm0.06$	$7.66^{A}\pm0.03$	$7.67^{A}\pm0.06$	$7.67^{A}\pm0.06$	5.460 $(0.243)^{ns}$
(p-value)	$(0.018)^{*}$	$(0.018)^{*}$	$(0.016)^{*}$	$(0.018)^{*}$	$(0.018)^{*}$	
			Odour			
C_1	$7.10^{bc}\pm0.03$	$7.00^{b}\pm0.04$	$7.55^{ac}\pm0.17$	$7.89^{aA}\pm0.04$	$7.89^{aA}\pm0.04$	27.631^{**} (<0.001)
SRBS	$7.10^{b}\pm0.03$	$7.09^{b}\pm0.03$	$7.46^{ab}\pm0.04$	$7.72^{aB}\pm0.05$	$7.72^{aB}\pm0.05$	27.415^{**} (<0.001)
(p-value)	$(1.000)^{ns}$	$(0.05)^{ns}$	$(0.131)^{ns}$	$(0.033)^{*}$	$(0.033)^{*}$	

			Texture			
C_1	7.17^{B}±0.07	7.17^{B}±0.06	7.16^{B}±0.06	7.11^{B}±0.06	7.11^{B}±0.06	2.667 (0.615)^{ns}
SRBS	7.67^{A}±0.05	7.76^{A}±0.06	7.80^{A}±0.03	7.71^{A} ±0.07	7.71^{A}±0.07	1.397 (0.845)^{ns}
(p-value)	(0.018)*	(0.018)*	(0.018)*	(0.018)*	(0.018)*	
			Cohesiveness			
C_1	7.20^{B}±0.05	7.24^{B}±0.05	7.23^{B}±0.05	7.21^{B}±0.09	7.21^{B}±0.09	0.881 (0.927)^{ns}
SRBS	7.73^{A}±0.04	7.79^{A}±0.04	7.79^{A}±0.03	7.80^{A}±0.04	7.80^{A}±0.04	1.898 (0.754)^{ns}
(p-value)	(0.018)*	(0.017)*	(0.017)*	(0.018)*	(0.018)*	
			Overall acceptability			
C_1	7.14^{B}±0.04	7.23^{B}±0.04	7.20^{B}±0.03	7.16^{B}±0.02	7.16^{B}±0.02	4.271 (0.371)^{ns}
SRBS	7.66^{cA}±0.03	7.76^{bcA}±0.02	7.80^{abA}±0.04	7.80^{abA}±0.04	7.80^{abA}±0.04	10.330* (0.035)
(p-value)	(0.018)*	(0.018)*	(0.018)*	(0.018)*	(0.018)*	

** Significant at 0.01 level; * Significant at 0.05 level; ns – non- significant at 0.05 level; Means with different upper case letters as superscripts are significantly different between control and SRBS; Means with different lower case letters are significantly different between storage days. The values are expressed as their Mean ± Standard error.

Cooked steaks sensory evaluation

The control samples did not exhibit significant differences in flavour, tenderness, saltiness, juiciness and cohesiveness, except for appearance and colour, which affected the overall acceptability over the storage period. In contrast, SRBS samples showed significant differences ($p<0.05$) in appearance and colour, flavour, tenderness, cohesiveness and overall acceptability between storage period. Scores consistently increased up to the 30th day and then decreased significantly ($p<0.05$) by the 45th day, remaining stable until the 60th day. Flavour scores displayed a different trend, showing a significant increase up to the 30th day, followed by a decrease, contrary to findings by Kumar (2002) and Kumar and Sharma (2004). These earlier studies reported decreasing flavour scores with an increase in storage period for vacuum-packaged samples of restructured meat blocks and patties. Juiciness scores did not exhibit significant differences over the storage period, contrasting with the study by Gupta and Sharma (2016), which reported a consistent decrease in juiciness scores with advancing storage duration in functional restructured spent hen meat blocks. The cohesiveness scores displayed no significant differences up to the 30th day, followed by a significant ($p<0.05$) decrease by the 45th day, in agreement with aspects of cohesiveness in our study. The overall acceptability scores increased gradually, peaking at the 30th day, followed by a significant ($p<0.05$) decrease on the 45th day, influenced by all sensory attributes. Despite a dip after the 45th day, SRBS consistently outperformed the control throughout the 60 day storage study. Both SRBS and control maintained overall acceptability scores rated as 'good.' This trend aligns with the study by Malav et al. (2013), they observed a similar score trend for overall acceptability. Consistent with the results from raw sensory evaluation, the cooked sensory attributes also indicated that SRBS samples significantly outperformed control samples across all sensory parameters, demonstrating the panelist's preference for Standardised Restructured Beef Steaks (SRBS) compared to the control.

Table 3. Effect of storage days on sensory attributes of cooked control and SRBS

Sample	**Storage days**					
	0th day	**15th day**	**30th day**	**45th day**	**60th day**	**χ^2 value (p-value)**
			Appearance and colour			
C_1	7.3^{a}±0.05	7.08^{bB}±0.04	7.07^{abB}±0.03	7.20^{abB}±0.08	7.20^{abB}±0.08	10.129* (0.038)
SRBS	7.4^{c}±0.07	7.68^{abA}±0.03	7.76^{abA}±0.04	7.60^{bcA}±0.06	7.60^{bcA}±0.06	12.189* (0.016)

Sample	Storage days					
	0th day	15th day	30th day	45th day	60th day	χ^2 value (p-value)
(p-value)	(0.115)^{ns}	(0.018)*	(0.018)*	(0.018)*	(0.018)*	
			Flavour			
C_1	7.14^{B}± 0.08	7.07^{B} ± 0.03	7.12^{B}±0.06	7.06^{B}±0.14	7.06^{B}±0.14	0.889 (0.926)^{ns}
SRBS	7.4^{cA}±0.05	7.53^{cA}±0.02	7.73^{abA}±0.04	7.54^{bcA}±0.08	7.54^{bcA}±0.08	6.312** (<0.001)
(p-value)	(0.018)*	(0.018)*	(0.017)*	(0.028)*	(0.028)*	
			Tenderness			
C_1	7.04^{B}±0.09	7.03^{B}±0.04	7.17^{B}±0.09	7.16^{B}±0.08	7.16^{B}±0.08	2.277 (0.0685)^{ns}
SRBS	7.62^{c} ±0.05	7.71^{bcA}±0.03	7.82^{abA}±0.02	7.63^{cA}±0.07	7.63^{cA}±0.07	13.544* (0.009)
(p-value)	(0.018)*	(0.018)*	(0.018)*	(0.018)*	(0.018)*	
			Saltiness			
C_1	6.92^{B}±0.04	6.95^{B}±0.03	7.05^{B}±0.04	7.01^{B}±0.07	7.01^{B}±0.07	4.358 (0.360)^{ns}
SRBS	7.48^{A}±0.05	7.54^{A}±0.04	7.45^{A}±0.06	7.40^{A}±0.09	7.40^{A}±0.09	2.970 (0.563)^{ns}
(p-value)	(0.018)*	(0.018)*	(0.018)*	(0.018)*	(0.018)*	
			Juiciness			
C_1	7.05^{B}±0.06	7.08^{B}±0.03	7.13^{B}±0.05	7.09^{B}±0.07	7.09^{B}±0.07	0.992 (0.911)^{ns}
SRBS	7.58^{A}±0.03	7.74^{A}±0.05	7.75^{A}±0.04	7.55^{A}±0.05	7.55^{A}±0.05	8.414 (0.078)^{ns}
(p-value)	(0.018)*	(0.018)*	(0.017)*	(0.018)*	(0.018)*	
			Cohesiveness			
C_1	7.18^{B}±0.06	7.08^{B}±0.04	7.20^{B}±0.05	7.17^{B}±0.05	7.17^{B}±0.05	4.000 (0.406)^{ns}
SRBS	7.77^{cA}±0.03	7.84^{bcA}±0.02	7.92^{abA}±0.01	7.76^{cA}±0.04	7.76^{cA}±0.04	15.111* (0.004)
(p-value)	(0.018)*	(0.017)*	(0.018)*	(0.018)*	(0.018)*	
			Overall acceptability			
C_1	7.08^{B}±0.06	7.06^{B}±0.02	7.08^{B}±0.05	7.08^{B}±0.09	7.08^{B}±0.09	1.600 (0.809)^{ns}
SRBS	7.71^{cA}±0.03	7.77^{bcA}±0.02	7.83^{abA}±0.02	7.68^{cA}±0.04	7.68^{cA}±0.04	14.579* (0.006)
(p-value)	(0.018)*	(0.018)*	(0.018)*	(0.018)*	(0.018)*	

** Significant at 0.01 level; * Significant at 0.05 level; ns – non- significant at 0.05 level; Means with different upper case letters as superscripts are significantly different between control and SRBS; Means with different lower case letters are significantly different between storage days. The values are expressed as their Mean ± Standard error.

Conclusion

The extensive investigation, which was carried out at the College of Veterinary and Animal Sciences, Mannuthy, Department of Livestock Products Technology, Meat Technology Unit, investigated the impact of storage duration on Standardised Restructured Beef Steaks (SRBS) developed using a combination of type II (round and

chuck taken in ratio 1:1) and type III quality cuts (brisket, plate and flank taken in ratio 1:1:2) which are considered as tough or less tender cuts and type II and type III used in the ratio 1:1 and compared them to a control group with respect to proximate composition, sensory attributes, and microbiological quality. The results revealed significant differences between SRBS and the control in various aspects. The control exhibited higher protein, fat, and total ash levels, while SRBS displayed enhanced moisture retention due to the removal of connective tissues and fat during the preparation process. Additionally, SRBS exhibited lower shear force values, indicating improved tenderness attributed to the reduced particle size achieved during the mincing process. The Warner-Bratzler shear force (WBSF) score confirmed SRBS as an exceptionally tender meat product.SRBS also demonstrated increased springiness, adhesiveness, and chewiness, with varying hardness values. The reduction in hardness in SRBS was linked to the effective removal of connective tissue through mincing, contributing to its desirable springiness, adhesiveness, and chewiness compared to the control. An increase in pH values was noted in SRBS, potentially due to the use of microbial transglutaminase as a cold-set binder. Lipid oxidation, as measured by TBARS values of control and SRBS, remained stable throughout the storage period, with vacuum packaging acting as an effective oxygen barrier. Tyrosine values suggested a higher degree of protein degradation in SRBS, which stabilized for both SRBS and the control by the 60th day.

In conclusion, this study provides compelling evidence that SRBS, composed of 50% type II chunks and 50% type III minced meat, is a promising meat product. It exhibits superior tenderness, desirable texture attributes, and favourable pH changes during storage. The microbiological quality remained excellent, and the sensory attributes remained stable over the 60-day storage period. These findings position SRBS as a preferred choice for consumers seeking a high-quality, stable, and sensory-pleasing meat product over extended storage duration. The research underscores the potential of SRBS as a valuable addition to the meat processing industry, with implications for product development and consumer satisfaction.

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Competing Interests

There are no conflicting interests between the authors or anybody else involved in this study project.

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