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Impact of vacuum packaging on color and odor in correlation to physicochemical and microbial characteristics of chilled beef during 50 days of refrigerated storage

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ABSTRACT

Although the production of vacuum-packed chilled beef has been increased significantly, many suppliers are still unfamiliar with how storage affects its quality. Sixty vacuum-packed refrigerated beef Longissimus dorsi samples were collected on the same day of packaging from a large meat processing facility in Egypt, and kept at 4°C for 10, 30, and 50 days post-packaging. The purpose of this study was to examine the changes in both color and odor in correlation with microbiological, physicochemical, and sensory qualities to determine the storage stability of vacuum-packed chilled beef. The results showed that all sensory characteristics were significantly impacted during storage. Unacceptable odors were reported, and the redness (a* values) dropped to 14.04. Furthermore, all the microbial populations under investigation increased, and the concentrations of volatile organic chemicals were elevated. Additionally, the purge loss increased, resulting in the loss of nutrients and the release of meat pigments in the package, which generally impacted on its visual impression. Overall, it has been concluded that the quality of vacuum-packed chilled beef needs to be improved, especially the color and odor problems, as well as the shelf life.

Introduction

The demand for chilled beef is anticipated to grow worldwide, and extending shelf-life has become essential for beef-producing and exporting countries, as consumers increasingly favor meat chilled to near-freezing temperatures (Gonzalez *et al*., 2022). This preference reflects trends in consumer expectations for freshness and quality, making it essential for suppliers to adapt and meet these demands. As the market evolves, proper storage and packaging methods will play a crucial role in ensuring that the beef maintains its safety and quality throughout its shelf life (Nethra *et al*., 2023).

The shelf life of beef refers to the period from when the product is packaged until it is consumed, during which the beef maintains its safety and experiences minimal loss of quality. The shelf life is determined by the point at which a microbiological limit is reached or when there is a noticeable and unacceptable change in the organoleptic properties, e.g., appearance, texture, flavor, and color, as well as the nutritional value (Frank *et al*., 2019).

Vacuum packing is the most used method for preserving fresh meat, which extends its shelf life without the need for freezing or adding preservatives (Nethra *et al*., 2023). To achieve an optimized shelf life for vacuum-packed chilled beef, it is essential to combine low initial microbial counts on the meat with a barrier packaging film that has low oxygen permeability, as well as excellent temperature control between −2 and −1°C during storage. Generally, vacuum-packed chilled beef can have a shelf life of 60 to 90 days post-slaughter, depending on the initial microbial counts and the effectiveness of storage temperature (Sumner *et al*., 2021). Vacuum packaging eliminates oxygen, which inhibits the growth of spoilage bacteria that thrive in oxygen-rich environments, reduces weight loss, maintains color, and allows certain intrinsic enzymes to enhance the tenderness (Jaspal *et al*., 2021). The shelf life of vacuum-packaged chilled beef is limited due to various issues during storage, e.g., deterioration of color and flavor once the package is opened, microbial growth and oxidation phenomena, as well as increased purge loss (Frank *et al*., 2019).

Meat color is a decisive quality attribute that significantly influences consumers' purchasing decisions at the retail level (Ramanathan *et al*., 2022). Both freshness and wholesomeness are correlated with the bright cherry-red color in beef, therefore, any deviation from this desired color can result in waste and economic losses for the meat industry (King *et al*., 2023). The odor of raw meat upon opening the package, referred to as "confinement odor," can serve as an initial indicator of beef freshness. However, the strength and character of this odor may change over the storage period, and this change may not always be strongly related to bacterial growth or meat quality.

Therefore, the primary objective of the current study was to investigate the changes in color and odor of vacuum-packed chilled beef during storage of vacuum-packed beef for 50 days at 4°C, and to correlate the changes in both criteria with the changes in the physicochemical and microbial characteristics.

Materials and methods

Samples

This study involved three independent trials to assess the changes in color and odor of vacuum-packed chilled beef in correlation with the sensory quality, microbiological load, and volatile contents. A total of sixty vacuum-packed chilled beef L. dorsi were collected from a major meat complex in Egypt. Samples for each trial were collected from the same production batch, with each sample consisting of three packages, each weighing 500 grams. All samples were packed in Low-Density vacuum bags (Vollrath, USA) and sealed using an AV 840 double chamber Henkelman vacuum packing machine (Germany) for 30 s at 90 kPa (0.9 bar). The bags had a thickness of 40 microns, an oxygen transfer rate of 3.99 cm^3/ 645 cm^2 (100 in^2)/24 hours (65% RH, 23°C), and a water vapor transfer rate of 0.54 g/ 645 cm^2 (100 in^2)/24 hours (90% RH, 38°C). Immediately after packaging, the samples were transported to the laboratory of the Food Hygiene and Control Department at the Faculty of Veterinary Medicine,

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Cairo University, using an isolated ice box to maintain aseptic conditions without unnecessary delays, and stored at 4°C with constant temperature monitoring throughout the storage period. The samples were examined next day post-packaging and then at 10, 30, and 50 days where three replicates were withdrawn for analysis in triplicate for sensory analysis, purge loss assessment, deterioration criteria evaluation, myoglobin fractionation, instrumental color analysis, microbiological examination, and volatile compounds analysis, with three readings were obtained for each parameter.

Sensory analysis

The sensory analysis was performed on raw beef samples to determine their quality throughout varying storage periods with vacuum packaging. Before opening each pack, it was visually examined for vacuum integrity and packaging quality. The sensory qualities of each sample were assessed by a semi-trained panel consisting of 23 males and 27 females, all of whom were regular consumers of vacuum-packed meat. The panelists were from the Faculty of Veterinary Medicine at Cairo University, including both staff and students, and they had diverse backgrounds to adequately represent consumer preferences. The panelists have participated in the evaluations throughout the storage period. Before the assessments, a practice training session was held to familiarize the panelists with the evaluation of samples at different shelf-life stages. The sensory analysis was conducted in a sensory laboratory equipped with partitioned cabinets and standardized lighting conditions (ISO, 2007), following the guidelines established by the American Meat Science Association (AMSA, 2016). Each selected pack was sterilized by wiping with 70% ethanol and then transferred to a sterile cutting board. The pack was carefully cut open along the seal with a sterile scalpel. Panelists were asked to evaluate the samples based on appearance, odor, consistency, and overall acceptability using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like).

Pigment chemistry" myoglobin fractionation"

The meat pigments in raw vacuum-packed beef samples were analyzed following the method described by Bekhit *et al*. (2003). Five-gram beef samples were homogenized in 25 mL ice-cold phosphate buffer (40 mM, pH 6.8) for 10 seconds. The homogenate was then allowed to stand for 1 hour at 4 °C before being centrifuged at 4500 g for 30 minutes at the same temperature. The supernatant was filtered through Whatman No. 1 filter paper, and the absorbance was measured at 572, 565, 545, and 525 nanometer using a spectrophotometer (Pye-Unicam, UK). Subsequently, the percentages of different myoglobin fractions were calculated (Krzywicki, 1982).

Instrumental color

The color of vacuum-packed fresh beef samples was measured using Croma meter (Konica Minolta, model CR 410, Japan) equipped with D-625 illuminant with a 52 mm diameter sphere size, 8 mm /11 mm aperture size, and 10° observer angle and calibrated with a white plate. A portable colorimeter was used to measure the color parameters on the surface of each sample after 30-minute bloom period. The mean values of color were obtained and expressed using the Commission International de l'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) color system (Shin *et al*., 2008).

Physico-chemical criteria

Purge loss

The purge loss for each sample was calculated by measuring the weight of the vacuum-packed steak (total weight), the weight of the meat without its packaging (meat weight), and the weight of the dry packaging (Łopacka *et al*., 2016). The purge loss% was calculated from the following formula:

Deterioration criteria

The pH value was measured by homogenizing a 5 g meat sample with 20 ml of distilled water for 30 seconds (Kandeepan *et al*., 2009). A pH meter (Lovibond Senso Direct) equipped with a probe-type electrode (Senso Direct Type 330) was used to obtain three readings for each sample, and the average was calculated. The total volatile basic nitrogen (TVBN) content (mg/100 g) was determined following the perchloric acid extraction method outlined by the European Union (1995). The thiobarbituric acid reactive substances (TBARS), expressed as mg malonaldehyde per kg of sample, were measured according to the method developed by Du and Ahn (2002).

Microbiological analyses

The ISO/6887-1 (2017) method was used to prepare the tenfold decimal dilutions used for enumeration of the different bacterial groups. The assessment of the microbial quality includes the enumeration of the total aerobic mesophilic bacteria using plate count agar (Oxoid CM0325) and incubation at 35°C for 48 hr for the enumeration of aerobic mesophilic bacteria (Dale Morton, 2001), and enumeration of the psychrotrophic bacteria (4°C for 7 days) (Vasavada and Critzer, 2015). Double sets of Glutamate Starch Phenol-red agar plates (GSP, 50875, Merk, Darmstadt, Germany) supplemented with 100 IU/ml penicillin G (Cat. No. 13752) incubated at 25°C for 3 days were used for enumeration of *Pseudomonas* (Kielwein, 1969). Reinforced Clostridial Agar "RCM" (Oxoid CM0149) and incubation under anaerobic conditions at 35ºC for 48 h (Scott *et al*., 2001) was used for the enumeration of mesophilic anaerobic bacteria. Moreover, DeMan Rogosa Sharpe agar plates (Oxoid CM 1153) were used to enumerate lactic acid bacteria after incubation for three days at 30ºC under microaerophilic conditions with 5% CO2 (De Man *et al*., 1960). Total yeast and mold counts were determined by incubating a Sabouraud dextrose agar plate (M063 HIMEDIA, Germany) for five days at 25ºC (Beuchat and Cousin, 2001). The violet red bile glucose agar plates (Oxoid CM 1082) incubated at 37ºC for 24 h were used for counting *Enterobacteriaceae* (ISO, 1979). The average count for each sample was reported as $\log_{10}$ Colony-Forming Units/g sample ($\log_{10}$ CFU/g).

Volatile compounds analysis

Volatile compounds were extracted using the HS-SPME method, and the analysis was conducted on a Headspace-solid phase microextraction-gas chromatography-mass spectrometry as the method developed by Acquaticci *et al*. (2024). Following optimization of the HS-SPME conditions, a 3 g sample was combined with 5 ml saturated NaCl solution in a 20 mL tightly capped vial. The sample was then incubated at 40°C for 40 min with continuous stirring at 250 rpm. Fiber coating was performed using a Divinylbenzene/Carbon-Wide Range/Polydimethylsiloxane DVB/C- WR/PDMS (80 µm) fiber. The fibers were sited inside the headspace of the sample at a speed of 20 mm/s and a penetration depth of 35 mm after being conditioned for 10 minutes at 250°C. The extraction was performed, and then the fiber was inserted into the injector port. The desorption occurred at 250°C for 2 min, and the fiber was conditioned at 250°C for 5 min.

The volatile compounds were identified using a gas chromatography-mass-spectrometry system (Santa Clara, CA, USA) equipped with a PAL RTC 120 auto-sampler device through the comparison of their mass spectra with those of the US National Institute of Standards and Technology in combination with the calculation of their experimental linear

retention indexes. The relative percentage of the area (%) of each peak was used to calculate the abundances of the compounds. MSD Chem Station Software (Agilent, Version G1701DA D.01.00, Santa Clara, CA, USA) was used to manage the data, where three analyses of the samples were performed, and the relative standard deviation (% RSD) was set up below 20%

Statistical analysis

The results were carried out in triplicate and expressed as mean ±S.E using one-way Analysis of Variance (ANOVA) using the SPSS program for Windows (SPSS Inc., Chicago, IL, USA), and the p-value was observed to be statistically significant ($p < 0.05$). The same software was used to calculate the correlation coefficients between sensory panel score of color, instrumental color indexes, and myoglobin fractions, as well as the deterioration criteria nd the microbiological load.

Results

Vacuum-packed fresh beef L. dorsi muscle was stored at 4°C for up to 50 days to monitor the changes in its quality attributes using sensory, physicochemical, and microbial assessment on the next day post-packaging and then at 10, 30, and 50 days of storage. According to the sensory assessment, there was a modest change in color, odor, consistency, and overall acceptability in the first 30 days of refrigerated storage; however, by the 50th day, all of the sensory traits had been significantly affected. In the first ten days of storage, all samples had the highest sensory scores, but after that, they somewhat decreased. Although the overall sensory quality was just slightly acceptable at the end of the storage period, all samples had reportedly unacceptable odor scores (Table 1).

Table 1. Changes in sensory panel characteristics, myoglobin fractions and instrumental color of vacuum-packaged chilled beef at different storage times.

	Storage time/days			
	0	10	30	50
Color	$7.67^{a}\pm0.57$	$7.33^{ab}\pm0.37$	$7.00^{b}\pm0.19$	$5.33^{c}\pm0.26$
Odor	$7.67^{a}\pm0.38$	$7.00^{b}\pm0.19$	$6.67^{c}\pm0.33$	$4.33^{d}\pm0.38$
Consistency	$7.67^{a}\pm0.48$	$6.67^{b}\pm1.15$	$6.33^{b}\pm0.28$	$5.55^{c}\pm1.00$
Overall acceptability	$7.67^{a}\pm0.76$	$7.00^{b}\pm0.48$	$6.85^{c}\pm0.43$	$5.22^{d}\pm0.19$
Myoglobin (mg/g)				
Deoxy myoglobin	$27.35^{a}\pm2.86$	$30.01^{b}\pm3.04$	$32.21^{b}\pm1.45$	$34.73^{c}\pm4.22$
Oxymyoglobin	$65.80^{a}\pm3.45$	$61.74^{b}\pm4.07$	$55.29^{c}\pm2.30$	$43.44^{d}\pm3.45$
Met myoglobin	$-19.47^{a}\pm2.67$	$-18.44^{a}\pm4.05$	$-2.87^{b}\pm0.18$	$1.53^{c}\pm0.54$
Instrumental color				
*L**	$38.37^{a}\pm1.17$	$37.71^{ab}\pm2.37$	$35.21^{b}\pm1.65$	$32.26^{c}\pm1.73$
*a**	$22.10^{a}\pm2.16$	$14.89^{b}\pm2.43$	$14.52^{b}\pm1.00$	$14.04^{b}\pm1.17$
*b**	$1.90^{a}\pm0.06$	$2.61^{b}\pm0.7$	$4.51^{c}\pm0.56$	$8.10^{d}\pm0.28$

$^{a-d}$Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

Both deoxymyoglobin and metmyoglobin levels were continuously increased, while oxymyoglobin levels significantly decreased during storage (Table 1). While the metmyoglobin level increased from -19.47 mg/g immediately after vacuum packing to -18.44, -2.87, and 1.53 mg/g at the 10th, 30th, and 50th days of storage respectively, the level of oxymyoglobin decreased from 65.8 mg/g after packaging to 61.74 mg/g on the 10th day, 55.29 mg/g on the 30th day, and finally 43.44 mg/g by the 50th day.

Table 1 also displayed variations in the instrumental surface color evaluation in terms of changes in L*, a*, and b* values over the storage period. The interaction between packing and storage duration had a significant effect on the lightness (L* values), which dropped throughout the storage period before hitting its lowest on the 50th day. Furthermore, by the end of vacuum packing storage, the redness (a* values) decreased to 14.04 (Table 1). Samples at 50 days of storge had the greatest b* values.

The purge loss of vacuum-packed beef L. dorsi muscle did not significantly increase until the 30th day of storage. On the other hand, the values at the 50th day of storage showed a substantial increase (Table 2). The mean pH value of vacuum-packed fresh L. dorsi beef muscle increased from 5.80 to 5.92 in ten days (Table 2).

The pH then dropped to 5.61 on the 30th day. After 50 days of storage, the pH finally increased to 6.72 (Table 2).

Table 2. Physicochemical criteria and microbiological quality of vacuum-packaged chilled beef at different storage times.

	Storage time (Days)			
	0	10	30	50
Purge loss	$2.90^{a}\pm0.10$	$3.51^{b}\pm0.08$	$3.77^{b}\pm0.13$	$7.53^{c}\pm0.36$
pH	$5.80^{a}\pm0.11$	$5.92^{b}\pm0.22$	$5.61^{c}\pm0.25$	$6.72^{d}\pm0.14$
TBARS	$0.10^{a}\pm0.01$	$0.18^{a}\pm0.01$	$0.34^{b}\pm0.01$	$0.52^{c}\pm0.02$
TVBN	$9.99^{a}\pm0.23$	$12.01^{b}\pm0.15$	$12.35^{b}\pm0.24$	$18.72^{c}\pm0.34$
Microbiological quality				
APC	$3.90^{a}\pm0.40$	$4.20^{a}\pm0.31$	$5.07^{b}\pm0.72$	$7.08^{c}\pm0.15$
Psychotrophic	$3.00^{a}\pm0.20$	$3.32^{a}\pm0.25$	$3.42^{a}\pm0.20$	$7.53^{b}\pm0.25$
Enterobacteriaceae	$<2.00^{a}$	$<2.00^{a}$	$4.59^{b}\pm0.46$	$6.77^{c}\pm0.42$
Pseudomonas	$<2.00^{a}$	$<2.00^{a}$	$3.30^{b}\pm0.36$	$6.20^{c}\pm0.36$
Anaerobes	$<2.00^{a}$	$<2.00^{a}$	$3.30^{b}\pm0.28$	$4.63^{c}\pm0.45$
LAB	$<2.00^{a}$	$<2.00^{a}$	$2.31^{b}\pm0.19$	$4.46^{c}\pm0.45$
Yeast	$<2.00^{a}$	$<2.00^{a}$	$2.33^{b}\pm0.35$	$3.40^{c}\pm0.25$
Mould	$<2.00^{a}$	$<2.00^{a}$	$2.30^{b}\pm0.30$	$2.33^{b}\pm0.37$

a–d Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

The oxidative stability of fresh L. dorsi beef muscle showed an initial TBARS value of 0.10 mg/kg immediately after vacuum packing, which increased gradually to reach 0.52 mg/kg by the end of the storage period. Moreover, the TVBN progressively increased from 9.99 mg/g after packaging to 12.72 mg/g after 50 (Table 2). The results of microbiological examination indicated gradual and significant changes in the microbial populations during storage as influenced by vacuum packing and storage time. Immediately after vacuum packaging, the total mesophilic and psychrotrophic bacterial counts were 3.90 and 3 log CFU/g, respectively. By the end of the storage period, these counts reached their peak at 7.08 and 7.53 log CFU/g, respectively (Table 2). However, the counts for other microbial groups remained below the detectable levels of plate counts (2 log CFU/g) after 10 days of storage, then gradually increased without exceeding the acceptable dangerous limits at the end of 50 days (Table 2). Noticeably, the lactic acid bacteria comprise a significant proportion of the microbial community and reached 4.46 log CFU/g at the end of storage time, which corresponds to an increase in pH to reach 6.72.

This study assessed the relative concentrations of volatile organic compounds in raw meat throughout a 50-day storage period. A total of 37 different kinds of volatile chemicals were found in the headspace of the raw beef samples over the storage period, including eight alcohols, eleven aldehydes, three ketones, one sulfur-containing compound, five acids, two alkanes, and seven esters (Table 3). The types and concentrations of detected volatile organic compounds showed significant increases during storage times, where minimal changes were detected at the 10th day of storage, while the establishment of real confinement odor would probably require a longer storage duration.

There was a noticeable increase in the level of ethanol between 10 and 50 days, Ketones like 2-butanone and heptanone were significantly increased during storage.

Aldehydes, easters and acids also showed increases with increase in storage times.

Table 3. Semi-quantitative (µg/kg) adjusted means for volatile organic compounds of vacuum-packaged chilled beef at different storage times.

K I	Compounds	10 days	30 days	50 days	
		Alcohols /diols			
935	Ethanol	10.11^{a}±1.78	13.28^{a}±1.02	20.12^{b}±2.13	33.34^{c}±2.98
1154	1-Butanol	--	--	0.23^{a}±0.01	0.76^{b}±0.29
1337	1-hexanol	--	--	0.49^{a}±0.09	0.58^{a}±0.05
1436	1-heptanol	0.80^{a}±0.04	0.83^{a}±0.02	1.06^{b}±0.07	4.34^{c}±0.85
1533	Linalool	--	--	--	0.68^{a}±0.28
1556	1-octanol	--	--	--	0.51^{a}±0.14
1572	2,3-Butandiol	0.67^{a}±0.03	0.71^{a}±0.03	1.38^{b}±0.27	2.48^{c}±0.85
1605	Terpinen-4-ol	0.66^{a}±0.10	0.70^{a}±0.08	0.97^{b}±0.21	3.02^{c}±0.51
		Aldehyde			
906	2-Methyl butanal	--	--	0.89^{a}±0.12	1.05^{a}±0.07
921	3-Methyl butanal	--	--	3.45^{a}±0.98	9.13^{b}±1.98
1077	Hexanal	1.90^{a}±0.20	2.15^{b}±0.33	2.63^{c}±0.46	7.98^{d}±0.95
1186	Heptanal	0.76^{a}±0.10	0.82^{a}±0.22	1.26^{b}±0.38	5.42^{c}±0.41
1281	Octanal	0.89^{a}±0.11	0.95^{a}±0.28	2.37^{b}±0.23	5.87^{c}±0.87
1383	Nonanal	0.67^{a}±0.06	0.82^{a}±0.21	1.86^{b}±0.66	2.26^{c}±0.71
1485	Decanal	--	--	0.20^{a}±0.01	1.22^{b}±0.41
1519	Benzaldehyde	0.33^{a}±0.09	0.52^{a}±0.18	1.29^{b}±0.28	3.44^{c}±0.67
1715	(E,E)-2,4 Nonadienal	0.49^{a}±0.02	0.63^{a}±0.11	0.82^{a}±0.07	2.00^{b}±0.82
1838	(E,E)-2,4 Decadienal	1.02^{a}±0.29	1.42^{a}±0.30	2.31^{b}±0.69	3.53^{c}±0.59
1942	Tetradecyl aldehyde	--	--	0.91^{a}±0.08	3.80^{b}±0.75
		Ketones			
663	2-Butanone	1.50^{a}±0.23	1.89^{b}±0.76	2.46^{c}±0.86	7.73^{d}±1.28
1192	2-Heptanone	1.45^{a}±0.67	2.10^{b}±0.50	3.36^{c}±0.54	12.84^{d}±1.39
1259	3- Octanone	--	--	--	0.76^{a}±0.01
		Sulfur			
722	Dimethyl sulfide	1.78^{a}±0.35	1.99^{a}±0.24	2.45^{b}±0.52	8.58^{c}±1.11
		Acids			
1471	Acetic acid	--	--	0.45^{a}±0.11	1.27^{b}±0.37
1655	Hexanoic acid	0.39^{a}±0.01	0.44^{a}±0.05	0.50^{a}±0.03	0.58^{b}±0.07
1744	Pentanoic acid	0.67^{a}±0.05	0.85^{a}±0.12	2.83^{b}±0.98	7.56^{c}±1.23
1964	Heptanoic acid	--	--	0.45^{a}±0.03	1.27^{b}±0.81
2002	Octanoic acid	0.48^{a}±0.02	0.64^{a}±0.11	1.32^{b}±0.19	4.41^{c}±0.80
		Alkanes/alkenes			
788	Alkane RI	--	--	1.28^{a}±0.26	1.61^{a}±0.19
1037	α-Pinene	--	--	1.20^{a}±0.27	1.45^{a}±0.21
		Esters			
811	Methyl acetate	---	--	1.34^{a}±0.26	4.06^{b}±0.87
979	Methyl butyrate	1.11^{a}±0.23	1.45^{a}±0.39	2.35^{b}±0.19	3.74^{c}±0.37
1429	Ethyl octanoate	--	--	--	0.74^{a}±0.20
1629	Ethyl decanoate	1.32^{a}±0.28	1.92^{b}±0.36	2.71^{c}±0.74	7.71^{d}±0.78
1797	Methyl laurate	--	--	--	0.80^{a}±0.20
1816	Hexyl octanoate	0.76^{a}±0.21	0.88^{a}±0.30	1.24^{b}±0.88	3.12^{c}±0.23
1890	Z-oka lactone	--	--	--	0.78^{a}±0.20

$^{a-d}$Values with different superscripts in the same row indicate significant differences ($P < 0.05$).

Discussion

As a result of oxygen loss deoxymyoglobin and metmyoglobin levels were continuously increased, while oxymyoglobin levels significantly decreased during storage (Table 1). This alteration indicates extensive oxidation and the emergence of a brown hue. Overall, the findings showed that oxygen content, packaging technique, and storage conditions all have an impact on the variations of the fractionation of meat myoglobin content throughout storage.

The decline in L value during storage period can be attributed to the formation of metmyoglobin, which cannot be completely transformed back to oxymyoglobin or myoglobin during packaging (Avilés *et al*., 2013). Decrease in redness (a* values) was due to the oxidation of myoglobin (Table 1). The a* value was also significantly impacted by the combination of packaging and storage time. Long-term storage can adversely affect the development of blooming (Lee *et al*., 2008), which may explain the decline in a* value after 50 days. Significant variations in brownness (b* values) were also seen across the storage durations, with samples kept for 50 days exhibiting the greatest b* values due to the oxidative changes in myoglobin (Table 1). According to all of the aforementioned data, the color stability of vacuum-packed beef is negatively impacted by extended refrigerated storage for 50 days.

Additionally, sensory color assessment was strongly correlated with the results of both instrumental color and chemical changes in myoglobin. The decrease in both a* and L* values was correlated with the onset of browning. This alteration was strongly correlated with the variations in the concentration of different myoglobin fractions over the storage period, especially the oxidation of myoglobin, and the formation of considerable amounts of both metmyoglobin and deoxymyoglobin (Tables 1 & 2). Furthermore, the accumulation of pigments on the meat surface may have contributed to the color change due to water loss, as supported by high purge loss during storage (Table 2). This purge loss impacts not only on the qualities that consumers desire, like juiciness, but also results in financial losses due to a decrease in weight. The purge loss can cause a loss of nutrients and color pigments inside the packaging, which creates an unpleasant visual aspect for consumers (Lee, 2018). Additionally, the purge loss may encourage microbial growth, which could reduce the shelf life of fresh raw meat. In general, the color stability was directly affected by the oxygen content in the storage environment. The relationship between vacuum packaging and storage duration had a substantial impact on the concentration of myoglobin fractions in correlation with oxygen tension, most likely due to the conversion of oxymyoglobin to metmyoglobin (Table 4).

Table 4. Correlation coefficient between sensory panel score for color, instrumental color parameters and chemical fractions of myoglobin.

	Sensory	L*	a*	b*	Deo.	Oxy.	Met.
Sensory	1						
L*	-0.22	1					
a*	0.47	.739*	1				
b*	0.46	.737*	.998**	1			
Deo.	-.921**	.694*	-.697*	-.677*	1		
Oxy.	.984**	-.836**	.838*	.822**	-.975*	1	
Met.	-.999*	.904**	-.906**	-.894**	.935*	-.990*	1

Deo= Deoxymyoglobin, Oxy= oxymyoglobin and Met= Met myoglobin
*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

The mean pH value of beef muscle increased in first ten days of storage due to the initial slow growth of spoilage populations after packaging (Table 2). The pH then dropped because of the low oxygen content in the vacuum packaging environment, which provides a favorable condition for the growth of the anaerobic lactic acid-producing bacteria. After 50 days

of storage, the pH finally increased because of the growth of microbial spoilage populations.

The increase in TBARS values during storage are consistent with those of Murphy *et al.* (2013), who observed that lipid deterioration in vacuum-packed beef steaks increased with storage time. Moreover, TVBN progressively increased after packaging (Table 2), but it didn't reach the critical limit (20 mg/g) posted by Byun *et al.* (2003) for beef.

There was a strong, highly significant, and positive correlation between the deterioration criteria and all the investigated microbiological populations except the mold count (Table 5). With prolonged storage, the growth of all investigated microbial populations increased, especially *Pseudomonas*, causing protein breakdown, ammonia generation, and an increase in both pH and TVBN to reach unacceptable limits. According to these results, the population of microorganisms with proteolytic functions may be better understood as a result of the rise in TVBN. The obtained data further support these findings, showing that high pH is an ideal predictor for the growth of spoilage bacteria. As a result, both TVBN buildup and microbial proteolysis are correlated with higher pH values. This demonstrates that as proteolysis increases, there is a reduction in WHC and an increase in purge losses (Bond and Warner, 2007).

Both alcohols and ketones had a discernible influence on meat flavor. Although alcohols have a minor effect on the overall flavor of meat (Baek and Cadwallader, 1997), ketones have a more substantial impact (Dajanta *et al.*, 2011). Notably, alcohols were commonly produced as a result of anaerobic bacterial activity. The noticeable increase in the level of ethanol during storage , suggesting that ethanol could be an indicator

Table 5. Correlation coefficient between deterioration criteria and microbiological counts.

	pH	TBA	TVB	APC	Psy.	Psud	An	LAB	Mold	Yeast	Entero.
pH	1										
TBAR	.761*	1									
TVBN	.737*	.978**	1								
APC	.884**	.912**	.884**	1							
Psy	.803**	.936**	.897**	.885**	1						
Psud	.900**	.898**	.846**	.904**	.894**	1					
An	.556*	.953**	.926**	.791*	.854**	.762*	1				
LAB	.981**	.865**	.844**	.930**	.875**	.941**	.702*	1			
Mold	0.12	0.41	0.36	0.35	0.35	0.40	0.35	0.16	1		
Yeast	.606*	.974**	.961**	.818**	.873**	.805**	.990**	.742*	0.42	1	
Entero.	.525*	.943**	.919**	.816**	.839**	.742*	.974**	0.67	0.50	.974**	1

*: Correlation is significant at the 0.05 level (2-tailed). **: Correlation is significant at the 0.01 level (2-tailed).

of the advanced shelf life of meat, may predict upcoming biochemical changes that correspond to changes in microbial development and a loss of both flavor and quality of vacuum-packed beef. From a sensory perspective, alcohols like 2,3-butanediol impart a creamy, buttery, and fruity odor (Casaburi *et al.*, 2015), whereas ketones, e.g., 2-butanone, provide an acetone-like, ethereal, fruity odor (Sun *et al.*, 2011), and 2-heptanone yields a creamy aroma (Casaburi *et al.*, 2015).

The majority of the volatile organic compounds included in this study have a high correlation with beef spoilage (Casaburi *et al.*, 2015). Aldehydes are crucial volatile compounds in beef, as they have lower odor thresholds and contribute significantly to overall flavor (Frank *et al.*, 2016). Aldehydes are produced through lipid degradation, such as β-oxidation or lipid auto-oxidation. The aldehydes identified included hexanal (fresh, grassy), heptanal, octanal (sweet, citrus), and nonanal (plastic, fatty). Sulfur-containing compounds are some of the most undesirable volatiles in food because of their garlic and gas-like odors (Landaud *et al.*, 2008). Dimethyl sulfide was the only sulfur volatile detected during the storage of vacuum-packed beef. These sulfur volatiles are key indicators of meat spoilage, as rapid increases typically coincide with spoilage and the development of strong objectionable odors (Mayr *et al.*, 2003).

Several acids were also detected, including pentanoic, heptanoic, acetic, octanoic, and hexanoic acids, all of which showed clear increases over time, particularly acetic acid. Multiple alkane compounds were also present, likely arising from lipid breakdown. In this study, esters were the most predominant volatiles, followed by aldehydes and alcohols, suggesting they are the primary volatiles contributing to the overall flavor of vacuum-packed chilled beef. Generally, esters are formed from short-chain acids, providing fruity aromas, or from long-chain acids, which contribute fatty flavors (Marušić *et al.*, 2014). The formation of methyl esters is consistent with anaerobic bacterial metabolism (Casaburi *et al.*, 2015).

Conclusion

It could be concluded that the safety, shelf life, and acceptability of vacuum-packed chilled beef meat can be determined based on the changes in color and odor, because once the color and odor are judged unacceptable, all other sensory attributes lose their significance to the consumers. These insights are valuable for both academic research and practical applications in the meat industry.

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Conflict of interest

The authors have no conflict of interest to declare.

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