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Effect of different protein levels in diets on the quality of yak meat based on electronic nose and sensory evaluation

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Abstract

This study investigates the impact of varying protein levels in yak diets on the sensory quality and volatile profiles of yak meat. The experiment utilized electronic nose technology to analyze volatile organic compounds (VOCs) in meat samples, alongside sensory evaluation to assess attributes like color, texture, and odor. Three groups of yaks were fed diets with low (12%), medium (14%), and high (16%) protein levels. The results indicated that the low-protein group scored highest in terms of sensory attributes, particularly color and texture, while the medium-protein group had the lowest scores. The high-protein group had higher odor scores but presented drier meat with less fat content. Electronic nose analysis revealed significant differences in the volatile odors associated with different protein levels, particularly aromatic alkanes, inorganic sulfur compounds, and long-chain alkanes. The study highlights how dietary protein levels influence both the sensory quality and volatile composition of yak meat, providing insights into improving meat production processes for better market competitiveness.

Keywords: Yak meat; Protein levels; Sensory quality; Volatile organic compounds (VOCs); Electronic nose.

1 | Introduction

The yak (Bos mutus) is a mammal belonging to the bovine family and is a domesticated animal unique to the Qinghai-Tibet Plateau. Yak farming is concentrated in the Qinghai-Tibet Plateau and surrounding provinces (such as Tibet, Gansu, Sichuan, etc.)[1]. As an important livestock species in the Qinghai-Tibet Plateau region, yaks provide local herders with abundant milk, meat, fuel resources, and economic benefits, making them essential to the daily life of pastoral areas. Yak meat holds unique market value due to its distinctive flavor, meat quality, and nutritional content, and the protein level in the diet is considered one of the key factors influencing meat quality[2].

Electronic nose (e-nose) technology offers a method for mimicking the mammalian olfactory system to detect and distinguish odors[3]. In meat quality assessment, electronic nose technology has found diverse applications[4]. It can be used to determine meat freshness by detecting VOCs produced during spoilage[5]. E-noses can also aid in identifying meat type and origin, as different species and production systems can result in distinct volatile profiles . Furthermore, this technology can be employed to analyze the flavor profiles of meat, capturing the complex mixture of volatile compounds that contribute to its characteristic aroma . Compared to traditional methods like gas chromatography-mass spectrometry (GC-MS), electronic noses offer a rapid, non-destructive, and potentially less expensive approach for meat quality analysis[6]. Several studies have utilized electronic nose technology to investigate yak meat flavor. These studies have explored the volatile compounds in yak meat influenced by factors such as breed, feeding method, and muscle type . However, the application of this technology to specifically assess the impact of varying dietary protein levels on yak meat volatile profiles appears to be an area with limited existing research[7].

Sensory evaluation relies on the use of human panelists to assess the quality attributes of meat through their senses[8]. These panelists, who can be either trained or untrained consumers, evaluate attributes such as tenderness (ease of cutting and chewing), juiciness (moisture release during chewing), flavor (taste and aroma), and overall acceptability[9]. Sensory evaluation directly measures human perception, which is a primary driver of consumer satisfaction and thus holds significant importance in the meat industry[10]. Various methods are employed in sensory analysis for meat research. Scaling involves using numerical or verbal scales to rate the intensity of perceived attributes . Classification involves assigning samples to descriptive categories (e.g., tender, tough) . Grading systems categorize meat based on overall quality[11]. Descriptive analysis, such as Quantitative Descriptive Analysis (QDA), uses trained panels to provide detailed profiles of sensory attributes using specific terminology . Consumer preference tests involve untrained consumers indicating their liking or preference for different meat samples . The choice of method depends on the specific research objectives, with trained panels offering detailed attribute profiling and consumer panels providing insights into overall liking and market potential . Sensory evaluation has been applied to assess the quality of yak meat in several studies. These investigations have examined the sensory attributes of yak meat in relation to factors like breed, muscle type, and the impact of cooking methods . However, similar to electronic nose studies, research specifically focusing on how different dietary protein levels affect the sensory characteristics of yak meat appears to be limited[12].

This study uses electronic nose technology to analyze the changes in volatile compounds in yak meat with different protein levels and, in combination with sensory evaluation, explores the specific effects of protein levels on sensory characteristics such as flavor, aroma, and texture. Electronic nose technology efficiently and objectively captures the odor components in meat, providing scientific data support for sensory evaluation. This, in turn, offers new technological methods and theoretical foundations for optimizing the yak meat production process and improving meat quality.

2 | Materials and methods

2.1 Animals, slaughter, and meat samples

The experiment was conducted at the Jinyintan Standardized Cattle and Sheep Breeding Demonstration Farm Co., Ltd. in Haiyan County, Qinghai Province (Longitude: 100°23'–101°20', Latitude: 36°44'–37°39'). The region has an altitude of 3100 m and an average annual temperature of 0.2–3.4°C. Fifteen healthy adult male yaks, aged 2.5–3 years and with similar body weight, were randomly divided into three groups: low protein (LP: 12%), medium protein (MP: 14%), and high protein (HP: 16%). The experiment followed a completely randomized design with three levels of protein as the sole variable. The trial was divided into a 14-day pre-trial period and a 180-day formal experimental period, with all yaks housed in barns. The diet composition and nutritional levels are shown in Table 1, with crude protein (CP) levels formulated according to references[13] and NY/T 815-2004 "Feeding Standards for Beef Cattle"[14]. The entire experiment was conducted following the guidelines outlined in DB65/T 2257-2005 "Yak Feeding and Management Regulations"[15]. At the end of the feeding trial, all experimental yaks were transported to a nearby commercial slaughterhouse, where they were slaughtered in strict accordance with animal welfare requirements, with feeding ceasing 24 hours before slaughter and water restriction starting 2 hours prior to slaughter. Upon arrival at the slaughterhouse, the yaks were weighed, and slaughter was carried out according to GB/T 19477-2004 "Beef Slaughtering Operations"[16]. The slaughter and sampling procedures were performed by professional personnel following standardized protocols. The longest dorsal muscle was collected from the space between the 12th and 13th ribs of each yak as the experimental sample. Place the sample in a -20°C freezer for 24 hours. After thawing at 4°C, weigh 2g of the chopped meat sample into a 20mL headspace vial. Seal the vial with a cap and proceed with the detection.

2.2 Measurement indicators

2.2.1 Sensory evaluation

According to the identification standards of GB/T 22210-2008 "National Standards for Sensory Evaluation of Meat and Meat Products"[17], 45 food science students were randomly selected to form an evaluation panel. They underwent sensory evaluation training, which included meat color identification, meat surface viscosity identification, meat odor identification, and meat cross-sectional tissue identification. The samples were then evaluated in a professional sensory evaluation laboratory. The scoring criteria are shown in Table 2.

Table 2 Sensory Evaluation Criteria

Category	Criteria	1-5 Points	6-15 Points	16-25 Points	Max Points
Smell	Is the meat's smell fresh and natural?	Has off-flavors or odor	Smell is average	No off-flavor, fresh and natural aroma	25
Texture and Elasticity	Is the texture of the meat clear, and how is the elasticity and firmness when pressed?	Texture is unclear, does not rebound after pressing	Texture is average, slightly rebounds after pressing	Texture is clear, rebounds quickly after pressing	25
Surface Stickiness	Is the surface of the meat sticky, and does it have a certain level of moisture?	Surface is too dry, poor stickiness	Surface is slightly dry, average stickiness	Surface is moderately moist, good stickiness	25

2.2.2 Electronic nose analysis

2.2.2.1 Electronic Nose Analysis Parameters.

See Table 3.

Table 3 Electronic Nose Analysis Parameters

Parameter	Set Value
Electronic Nose Device	Heracles II Electronic Nose System

Heating Oscillation Temperature	60°C
Heating Oscillation Time	400 seconds
Sample Gas Volume	5000 μL
Gas Sampling Speed	500 μL/second
Injection Port Temperature	200°C
Initial Column Temperature	50°C, hold for 5 seconds
Column Temperature Ramp Rate	3°C/second, ramp up to 250°C, hold for 30 seconds
Data Collection Time	110 seconds
Detector Temperature	260°C

2.2.2.2 Sensor Signal Analysis

Odor substances are separated using MXT-5 (weak polarity) and MXT-7 (medium polarity) chromatographic columns. The system is equipped with 18 metal oxide sensors that are specially designed to respond sensitively to one or more substances [18]. Chromatographic data is analyzed using a hydrogen ion detector [19], and then the yak meat samples are tested. After the detection, the device's built-in database can be used for qualitative and quantitative analysis to detect volatile substances in the sample. Detailed information about the electronic nose sensor performance is shown in Table 4.

Table 4 Performance of different sensors in the electronic nose.

No.	Sensor Name	Performance Description
1	W1C	Aromatic components, benzene compounds
2	W5S	High sensitivity, very sensitive to nitrogen oxides
3	W3C	Ammonia, sensitive to aromatic components
4	W6S	Primarily selective to hydrogen gas
5	W5C	Alkanes, aromatic components

6	W1S	Sensitive to methane and other short-chain alkanes
7	W1W	Sensitive to inorganic sulfides
8	W2S	Sensitive to alcohols, ethers, aldehydes, and ketones
9	W2W	Aromatic components, sensitive to organic sulfides
10	W3S	Sensitive to alkanes, long-chain alkanes

2.3 Statistics analysis

The experimental data were first organized using Excel 2013, and one-way analysis of variance (ANOVA) was performed on the data using SPSS software (version 22.0), where $P<0.05$ indicates a significant difference. Multivariate statistical analysis plots, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), orthogonal partial least squares discriminant analysis (OPLS-DA), and a 200-time permutation test plot for OPLS-DA, were created using SIMCA software (version 14.0). A radar chart was generated using the smsB package in R software (version 4.0).

3 | Results and Analysis

3.1 Sensory Evaluation of Yak Meat

The sensory evaluation results (Table 5) indicate that the low-protein group > high-protein group > medium-protein group. The low-protein group had the highest scores, mainly due to its high color intensity and marble-like patterns on the cross-section. The high-protein group scored second, with less fat content on the cross-section but the highest smell score. The medium-protein group had the lowest scores, primarily due to the low smell score, as well as poor color and muscle texture with no fat tissue. It is worth noting that the standard deviation for the smell evaluation of taste in the sensory evaluation was relatively large, which was mainly due to the significant differences in individual smell scores within the same group.

Sample Name	Cross-Section	Sensory Description	Score Results

Low Protein Group（LP: 12%）		Color: The visible color is bright, with a high gloss. Surface: High moisture, non-sticky, smooth. Odor: No smell of mutton. Tissue: Loose cross-section, tissue fibers are fine and sink slightly under pressure, with more fat tissue.	94±4.43
Medium Protein Group（MP: 14%）		Color: The visible color is dull, with low gloss. Surface: Non-sticky, smooth, relatively dry. Odor: Slight smell of mutton. Tissue: Delicate cross-section, tissue fibers are fine and sink slightly under pressure, no fat tissue.	91±5.71
High Protein Group（HP: 16%）		Color: The visible color is dark red, with low gloss. Surface: More moisture, non-sticky, smooth. Odor: No smell of mutton. Tissue: Delicate	92±5.13

cross-section, tissue fibers are fine and sink slightly under pressure, with a small amount of fat tissue.

3.2 The electronic nose analysis of volatile odors in yak meat.

3.2.1 PCA Analysis (Principal Component Analysis)

The electronic nose system analyzes the response signals of metal sensors to the volatile odors of different samples to obtain the raw data (multidimensional matrix) of the flavor. In order to explore which volatile odor causes significant differences in individual sniffing scores within the same group of samples, the response values from each sensor of the electronic nose are combined with multivariate statistical analysis to select the differential volatile odors. Principal Component Analysis (PCA) is an unsupervised dimensionality reduction method. PCA is used to reduce the dimensionality of the volatile odors produced by these different samples to determine whether there are inter-group differences, and to clarify whether there are significant differences in the volatile odors of yak meat produced by different protein levels in the diet. As shown in Figure 1, the first principal component (PC1) contributes 58.6%, and the second principal component (PC2) contributes 12.45%, with a total of 71.05%. In PCA analysis, the relatively high contributions of PC1 and PC2 indicate that the principal components can effectively reflect the classification characteristics of the original data. However, the discrimination of volatile odors between yak meat samples produced by different protein levels in the diet is low, meaning the inter-group recognition rate is not high and the inter-group differences are large. Therefore, we further classify the samples using supervised Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA).

3.2.2 PLS-DA Analysis (Partial Least Squares Discriminant Analysis)

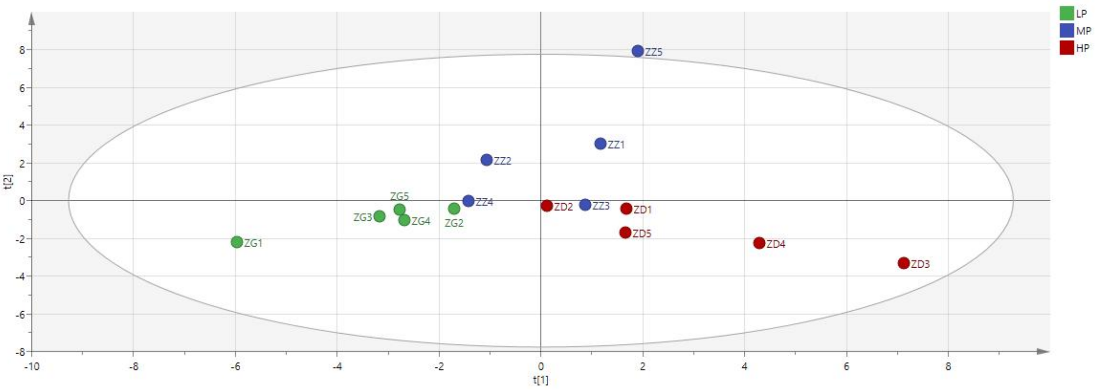

Figure 1 The PCA score plot of volatile odors in yak meat from diets

with different protein levels

In order to further differentiate the three groups of yak meat samples, a supervised Partial Least Squares Discriminant Analysis (PLS-DA) model was used to distinguish the volatile odors in the three groups of yak meat. As shown in Figure 2A, the three groups of meat samples are clearly distributed in different regions on the PLS-DA score plot, with tight clustering within groups and clear separation between groups. To avoid overfitting during the modeling process, we performed 200 rounds of permutation testing on the PLS-DA discriminant model to validate the model's effectiveness (no overfitting observed) (Figure 2B). In the 200 rounds of permutation testing for PLS-DA: $R^2Y = 0.67 > 0.5$, $Q^2Y = 0.739 > 0.5$. Generally speaking, the closer the values of R^2Y and Q^2Y are to 1, the better the model's fit and predictive ability. If R^2Y and Q^2Y values are greater than 0.5, it indicates that the model has good discriminative performance. The 200 rounds of permutation testing for PLS-DA indicate that there is no overfitting, and the volatile odors in yak meat produced by different protein levels in the diet exhibit significant differences.

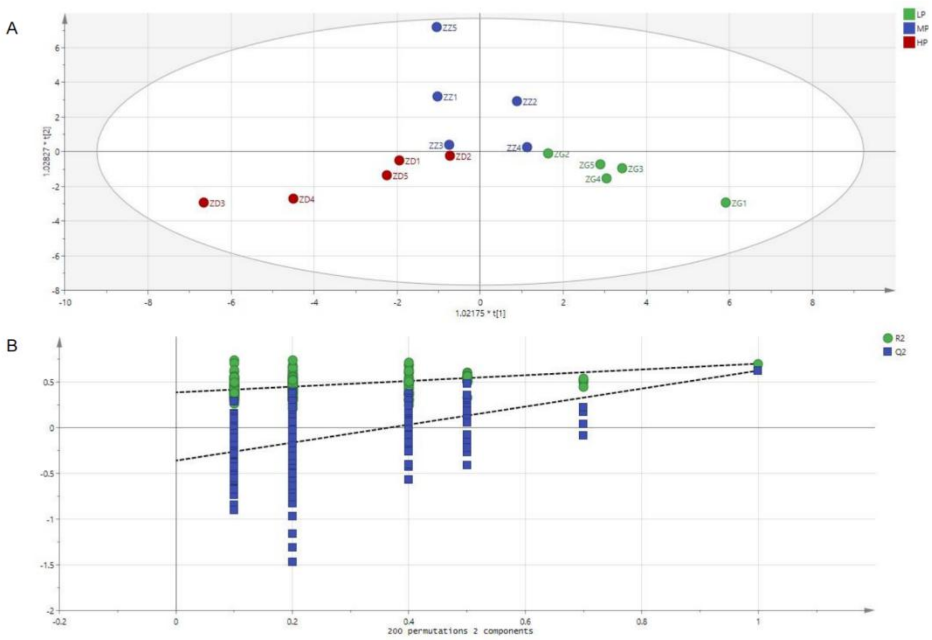

Figure 2 The PLS-DA score plot and the 200-permutation test plot of volatile odors in yak meat from diets with different protein levels

3.3 Radar Chart Analysis Method

PLS-DA analysis indicates that there are significant differences in the volatile odors of yak meat produced by different protein levels in the diet. From the electronic nose radar chart (Figure 3), it can be observed that the volatile odors in low and medium protein group yak meat have lower and similar response values on the electronic nose. Compared to the low and medium protein groups, the high protein group shows significantly higher response values on sensor W5C (alkane aromatic components), W1W (sensitive to inorganic sulfides), W3S (sensitive to alkanes, especially long-chain alkanes), and W5S (high sensitivity, particularly to nitrogen oxides) than the other two groups. Sensory evaluation shows that the high protein group has the highest sensory score for yak odor, and the standard deviation of the sensory evaluation of odor for the three groups of yak meat is the largest. In other words, the volatile odors corresponding to sensors W5C, W1W, W3S, and W5S (alkane aromatic components, inorganic sulfides, long-chain alkanes, and nitrogen oxides) may be the cause of the significant differences in individual sniffing scores.

4 | Discussion

In the study, the low-protein group of yak meat performed better in sensory scores compared to the high-protein and medium-protein groups, especially in terms of color and meat texture. The low-protein group contained more fat tissue, which made the meat softer and more delicate, with a better mouthfeel, aligning with consumer preferences. While the high-protein group had a finer meat quality, the lower fat content likely led to drier meat, affecting the texture and consumer satisfaction. The medium-protein group scored the worst, mainly due to low odor scores, poor color, and muscle texture, as well as the absence of fat tissue. However, the significant variability in odor scores within the same group resulted in a high standard deviation, which impacted the accuracy of sensory evaluations. To explore the volatile odors causing the large individual differences in odor scores within the same group, we used an electronic nose combined with multivariate statistical analysis to screen for volatile flavors in the three groups of yak meat. This aimed to enhance the flavor of yak meat and provide theoretical support for its flavor evaluation, thereby improving its market competitiveness. The results of multivariate statistical analysis of volatile odors in the three groups of yak meat indicated significant differences in volatile odors due to the different protein levels in the diets. Electronic nose analysis showed that the volatile odors in the low and medium-protein group yak meats had lower and similar response values. In contrast, the high-protein group had significantly higher response values on sensors W5C (aromatic alkanes), W1W (sensitive to inorganic sulfur compounds), W3S (sensitive to alkanes, particularly long-chain alkanes), and W5S (sensitive to nitrogen oxides) compared to the other two groups. This suggests that aromatic alkanes, inorganic sulfur compounds, long-chain alkanes, and nitrogen oxides may contribute to the variation in scores within the yak meat group.

The research identified the presence of "aromatic compounds" and "hydrocarbons" through the analysis of volatile organic compounds (VOCs) in yak meat. Among specific aromatic alkanes, benzaldehyde was identified as a key compound in yak meat, often associated with almond or sweet aromas, which may contribute to the overall flavor of yak meat [20]. Studies have shown that if lipid oxidation occurs in beef, aromatic hydrocarbons could cause off-flavors [21]. It was noted that compared to housed lamb meat, the inorganic sulfur content in grass-fed lamb meat was more stable, indicating its significant role in the odors of ruminant meat. Allyl disulfide, an organic sulfur compound, has been studied in yak meat as a potential antioxidant to reduce oxidation-related off-flavors. Dimethyl trisulfide is another sulfur-containing volatile compound that has been identified in dried yak meat. Notably, methylallyl sulfide is considered a key factor in the off-flavors produced by irradiated yak meat, often described as having a rotten egg or sulfur-like odor. 2-methyl-3-(methylthio)furan, a sulfur-containing compound, has been found in dried yak meat and is associated with meaty and garlic-like flavors [22]. Hydrocarbon compounds were consistently recognized as part of the volatile organic compounds in yak meat in this study. Interestingly, grass-fed yak meat had higher levels of hydrocarbon compounds compared to artificially fed yak. In the context of rose essential oil, long-chain alkanes are considered to have the ability to fix scents, potentially influencing the persistence of other volatile compounds' aromas, although some studies suggest they have only faint scents themselves. Similarly, alkanes were also found during the early storage stages. Octane, a specific alkane, was identified as a volatile compound negatively impacting the odor of irradiated yak meat at a 3 kGy dose [23].

Studies have shown that the determination of total volatile basic nitrogen (TVB-N) is a common method for evaluating meat freshness. Elevated TVB-N levels (including ammonia and other nitrogen compounds) suggest the occurrence of spoilage in yak meat. Nitrogen-containing heterocyclic compounds, such as pyrazines, thiazoles, and pyrroles, play a crucial role in the key odor components of cooked meat, mainly produced by Maillard reactions and the degradation of thiamine. A study using GC-IMS identified an amine compound in yak meat. Notably, a nitrogen-containing heterocyclic compound was identified as an important factor in the off-flavors of irradiated yak meat. 2-acetyl-1-pyrroline, another nitrogen-containing compound, is recognized as a key flavor volatile in certain meat products, typically associated with a pleasant roasted aroma [24]. Furthermore, different cultural backgrounds and established dietary habits also influence consumers' preferences, including the perception and acceptance of meat odors. Familiarity with certain odor characteristics in traditional dishes may lead to positive associations and preferences for those odors [25].

The above discussion, combining sensory evaluation and electronic nose technology, reveals the roles of color, fat content, and flavor in the sensory quality and consumer acceptance of yak meat.

5 | Conclusion

This study combines sensory evaluation and electronic nose technology to analyze the effects of different dietary protein levels on the longissimus dorsi muscle of yaks. Sensory evaluation revealed that the low-protein group (LP: 12%) scored highest in terms of color, texture, and other attributes. The high-protein group (LP: 16%) followed, with balanced sensory scores and higher odor scores than the low and medium-protein groups. The medium-protein group (LP: 14%) had the lowest scores, mainly due to its paler color and slight gamey odor. Alkanes, aromatic compounds, inorganic sulfur compounds, long-chain alkanes, and nitrogen oxides were identified as the main factors influencing odor scores in sensory evaluation.

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