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Volatile Flavour Compounds in the Tenderloin of the Donkey, Pork, Mutton and Beef and their Meat Flavour Nucleotide Contents

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Abstract: ***The value of donkey meat in terms of dietary nutrition, flavor and health care efficacy is gradually accepted and recognized by consumers. Research on donkey meat globally mainly focuses on the composition and function of donkey meat fatty acids and donkey meat products. There are few studies on the flavor characteristics and taste of donkey meat, and the chemical substances that form the unique flavor and taste of donkey meat are infrequently studied. The basics have not yet been demystified, which greatly restricts the development of the donkey meat and its products as well as the development of its market. In order to make better use of the donkey meat resources and improve the practical value of the donkey, we compare the tenderloin of donkey with the other three kinds of daily tenderloin meat (pork, mutton and beef), and then analyze the difference in their flavor substances. The experimental research mainly includes the use of Heracles II fast gas phase electronic nose combined with headspace solid phase microextraction- gas chromatography-mass spectrometry to determine the main volatile odor substances in the donkey tenderloin, and compared with other tenderloin meat. Nucleotide results showed that the main taste nucleotide in the donkey tenderloin is 5'-inosinic acid, and inosinic acid is the main component of fleshy umami taste, which is an important indicator to measure the umami taste. To a certain extent, it shows that the meat of the donkey meat is delicious. The donkey meat is also rich in nutrients, delicious in taste and high in food value. From a nutritional point of view, the donkey meat is an ideal animal food raw material. It can provide high quality animal protein resources to the body. Heracles II nose, headspace solid phase microextraction, gas chromatography and mass spectrometry analysis, showed a clear difference between donkey meat volatile components as compared to the meat from the other animals, and lead to differences in the contribution of the primary component. The electronic nose (electronic nose, EN) is an effective means of rapid determination of meat flavor, which can be used for quality grading, adulteration identification, identification of freshness, but this is influenced by environmental factors to a larger extend, and not accurate analysis of a certain amounts of flavor and other shortcomings, therefore combined with gas chromatography - mass spectrometry (GC-MS), to achieve efficient and accurate qualitative and quantitative detection of flavor compounds. For flavor nucleotides, high performance liquid chromatography (HPLC), high pressure infusion system, the liquid sample to be tested as a mobile phase, To be loaded with the column stationary phase, the components in the separation column, and further into the detector detects realize analysis of samples.***

Keywords: volatile flavor substances, meat flavor, flavor nucleotides and meat tenderloin

1. Introduction

Meat is an important part of human life which serves as a major source of protein, the donkey is of equal status as most other meat sources and their intake is determined by socio-economic factors, ethical issues, religious beliefs and tradition. The donkey meat and its products such as the donkey skin (donkey-hide gelatin), and milk also has some irreplaceable advantages, whether in terms of nutritional effect, medicinal value as well as taste and flavour. In recent years, with the continuous improvement of people's diet structure and improvement of people's meat requirements, in terms of taste, flavour and nutritional importance, the donkey meat is becoming more recognized as oppose to the previous struggle of the development and acceptability of donkey meat. Donkey meat is characterized by lean meat, less fat, high-protein, high-essential amino acids, low fat, low cholesterol and low in calories. It contains high levels of unsaturated fatty acids, which can greatly reduce the adverse effects of saturated fatty acids such as the problems associated with the human cardiovascular system, and it is becoming very popular with the market, especially in China, some parts of Africa and South America.

The significance of this research is to unmask the importance of donkey meat. There is very little research and knowledge on the flavor, taste, basic chemical composition and physical properties of this meat, which greatly restricts its market development as well as its products. Therefore, to carry out research on donkey meat flavor components and to establish its relevance has an important role in promoting the development of donkey meat and its products.

Juandeng [1] Studies have shown that, from a nutritional point of view, the donkey is an ideal animal food ingredient. Analysis and comparison of donkey meat and other livestock major nutrients content showed that, in the donkey meat, there is 23.5% protein, 5.0% fat content, and 65mg / 100g cholesterol. Beef, mutton and pork have lower protein content than the protein content of donkey; the donkey meat has a lower fat content than beef, mutton and pork. The donkey meat has a higher amino acid composition than pork, beef, mutton and chicken as well as polyunsaturated fatty acids and linoleic acid. It has up to 10.1% linoleic acid and mineral elements; its Fe content is also significantly higher than other poultry meat.

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2. Background

Precursors of volatile compounds in meat

Raw meat is characterized by a very weak odor; however it constitutes a matrix rich in non-volatile precursors of volatile compounds responsible for the development of meat products flavor (Table 1). It contains amino acids, peptides, saccharides, inorganic salts and inorganic acid [15].

Table 1: Precursors of selected volatile compounds in meat products (Huang & Ho, 2001; Balagiannis et al., 2009)

Precursors	Volatile compounds formed
Cysteine, Monosaccharides	2-acetyl-2-thiazoline
Proline, reducing sugars	2-acetyl-1-pyrroline
Methionine, Monosaccharides	Methional
Cysteine, ribose	2-furanmethanothiol
Leucine, isoleucine, glucose	3-methylbutanal, 2-methylbutanal
Thiamine, cysteine/ribose	2-methyl-3-furanthiol, bis (2-methyl-3-furyl)disulfide
Fatty acids	(E)-2-nonenal, (E, E)-2, 4-decadienal, 1-okten-3-on

Odor detection thresholds, flavor notes and odor activity values of selected volatile compounds.

3. Materials and Methods

3.1 Materials and Reagents

Sample collection

Donkey tenderloin was supplied by Shandong Dong-E E-Jiao Co., Ltd. Shandong China

Fresh pork, beef, lamb meat (tenderloin): Wuxi China Resources Vanguard Supermarket.

Dichlorobenzene and N-heptane AR AR, Aladdin Reagent (Shanghai) Co., Ltd, perchloric acid, Potassium hydroxide and Petroleum ether AR AR, Shanghai and Shanghai test, Hypoxanthine 98%, Inosine monophosphate 99%, Guanylate 98%, AMP 98%, and Inosine 99% US Sigma company.

Instruments and equipment

AR224CN Electronic balance, Ohaus Instrument Co. Ltd, China,); HeraclesII fast GC electronic nose, Alpha MOS SA Limited (France), triple quadrupole GC-MS, TSQ Quantum XLS, Thermo Fisher Scientific Ltd. (United States), HPLC analysis Shimadzu LC20A Shimadzu Japan, HH-3A digital thermostat water bath, Changzhou Guohua Electric Co., Ltd. (China), 5804R Centrifuge, , Eppendorf Eppendorf, Germany, PH meter OHAUS, United States, 20mL vial, Agilent Technologies Inc. (USA), M8-1-B010-8022 Mincerand C12095302636 SA Gas Chromatograph.

3.2 Methods

HeraclesII Speed Gas Electronic Nose

(1) Sample preparation

Volume of donkey, pork, beef and mutton tenderloin, was minced with a meat grinder after, each sample was weighed 5.000 g in 20 mL vial, the rear cover and seal into a thermostat water bath of 80 degrees Celsius water for 30 Determination to room temperature min, cooled.

(2) Detecting parameters

Column two different polarities: DB-5-FID1 and DB-1701-FID2; column diameter: 1 mm; column length: 2 m; temperature program rate: 10 ℃ / s; Detector: Hydrogen flame ionization detector (the FID); instrument equipped with auto sampler. A sample (1) Preparation of the test, the injection volume was 2000 μL; feed rate of 125 μL / s; inlet temperature of 200 ℃, Injection duration is 13 s; detector temperature was 260 ℃.

3.3 SPME-GC-MS detection

(1) Preparation of internal standard solution

Formulated with highly purified water at a concentration of 0.018 μg / μL solution of 1, 4-dichlorobenzene as an internal standard substance solution.

(2) Sample preparation

Volume of pork, beef, mutton and donkey was minced with a meat grinder, each sample was weighed 5 g into 20 mL headspace vials, capped sealed after placed in a thermostatic water bath of 80 deg.] C 30 min cook measured after cooling to room temperature.

(3) Headspace Solid Phase Microextraction - GC Analysis

Headspace Solid phase extraction: extraction head Model 50/30 μm DVB / CAR / PDMS; extraction head through 250 deg.] C, aged for 30 min, placed in a heating tray 80 deg.] C headspace extraction temperature in 50 min. After the extraction, the extraction needle is inserted and removed GC inlet, 2 min to complete the test.

Gas chromatography-Mass Spectrometry Conditions: DB-5 capillary column (Agilent J & W GC 30 m × 0.25 mm × 0.25 μm); carrier gas: high-purity He, flow rate 1.0 mL / min; split less injection; Inlet Temperature: 250 ° C; column initial temperature: 40 ° C, held 3 min; programmed temperature (10 ° C / min) to 240 ° C, holding 5 min.

MS conditions: transfer line temperature: 240 ° C; Ion source temperature: 240 ° C; scan range: 35-500 m / z; Ionization voltage: 70 eV.

3.4 Analysis of flavor nucleotides

(1) Standard curve

An appropriate amount of water with high guanosine monophosphate (5'-GMP), inosine monophosphate (5'-IMP), hypoxanthine (Hx), adenosine monophosphate (AMP), inosine (HxR) five kinds of standard samples nucleotides formulated to 1000 μmol / L of the mother liquor was diluted to a gradient 100μmol / L, 40μmol / L, 20μmol / L, 10μmol / L, 5 μmol / L of solution, injection volume was 20 μL.

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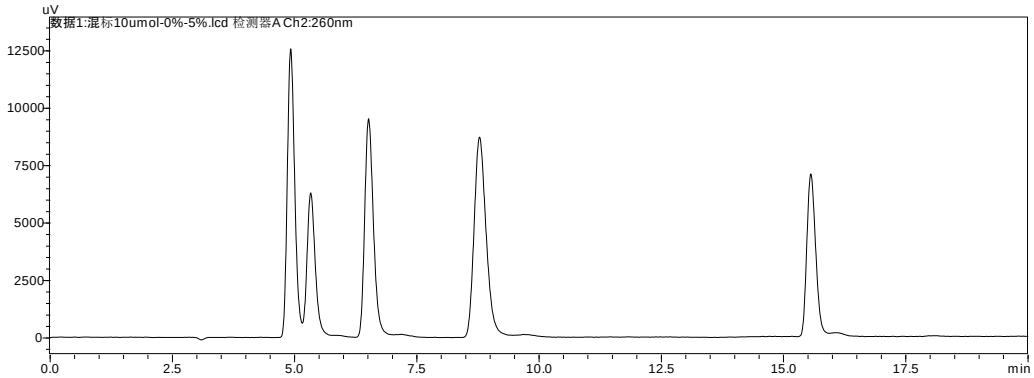

Figure 2-1: FIG nucleotide HPLC standard solution

Liquid chromatography analysis of the detection results nucleotide mixed standard solution to HPLC chromatogram, shown in Figure 2-1, the order of the peak guanosine monophosphate 5'-GMP, IMP 5'-IMP, inosine purine Hx, AMP AMP, inosine HxR. The qualitative retention time, peak area as the abscissa, standard concentration as the ordinate, the standard curve, the results as shown in Table 2-3.

Table 2-3: nucleotides quantitative standard curve

Flavour nucleotides	Standard curve equation
Guanosine monophosphate 5'-GMP	y = 12636x + 4211.5
IMP 5'-IMP	y = 8147.3x - 4717.9
Hypoxanthine Hx	y = 9825.1x + 31443
AMP AMP	y = 15176x - 3953.7
Inosine HxR	y = 8825.4x + 2353.6

(2) Sample preparation

Volume of donkey, pork, beef and mutton tenderloin was minced with a meat grinder, weighed approx. 5 g of each sample in a 50 mL centrifuge tube was added 15 mL 5% perchlorate pre-chilled, with a high speed homogenizer after homogenization device, refrigerated centrifuge (4℃, 10000 r / min, 5 min) centrifugation. The supernatant was transferred in a beaker of 100 mL, was added 15 mL 5% precooling perchloric acid, centrifuged again after shaking, the above-described operation is repeated twice. The supernatant was adjusted to pH 6.5 times after centrifugation, then 1: 1 KOH to move it to 50 mL volumetric flask to volume with high purity water, after 0.45 μm micro porous membrane filter for HPLC analysis.

(3) Chromatographic conditions

Column: Galaksil EF-C18 Bio, 5 μm, 25 cm; mobile phase: A: 0.05 mol / L NaH2PO4- water, B: methanol, SPD-20A UV detector wavelength: 260 nm; injection volume 20 μL.

(4) Liquid phase conditions

0-6 min 0% methanol; 6-7 min 0% -5% methanol; 7-16 min 5% methanol; 16-17 min 100% methanol; 17-30 min 100% methanol.

(5) Calculation Method

1) Nucleotide flavor content determination

2.2.3 Using the standard curve, 5'-GMP content of the sample is obtained, and the 5'-IMP.

Formula: X = C * V * m

Wherein: X- detecting a target substance content, mg / 100g; C- standard concentration curve corresponding target substance, mg / mL

V- Volume of the sample, mL; m- sample mass, g

2) Taste activity values (TAVs) calculated

Taste activity values (TAVs) of a component can be used to flavor contribution to the overall evaluation of food samples, if TAV <1, the component does not significantly affect the taste of the food; if TAV> 1, the pair of component the taste of food significantly affected, TAV larger the value, the greater the degree of contribution to food taste.

Formula: TAV = C / T

Wherein: C- measured quality of taste substances; T- odor threshold measured substance

4. Result and Discussion

Speed Gas electronic nose analysis of volatile components

Electronic Nose can simulate the human olfactory system, the chemical signal into an electrical signal, wherein the volatile material is formed on the sensor response spectrum is performed to detect the overall flavor [5]. This technique has a short detection time, fast, good repeatability, effectively avoiding complicated pretreatment process, reduces human error, determining a quick assessment of the odor component inexpensive.

DFA analysis 3.1.1 donkeys, pork, mutton, beef tenderloin volatile substances

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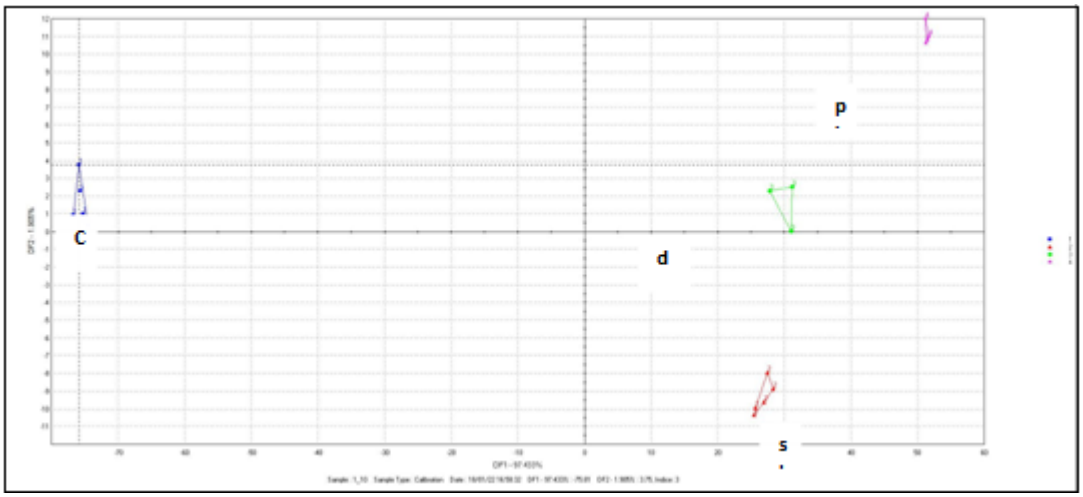
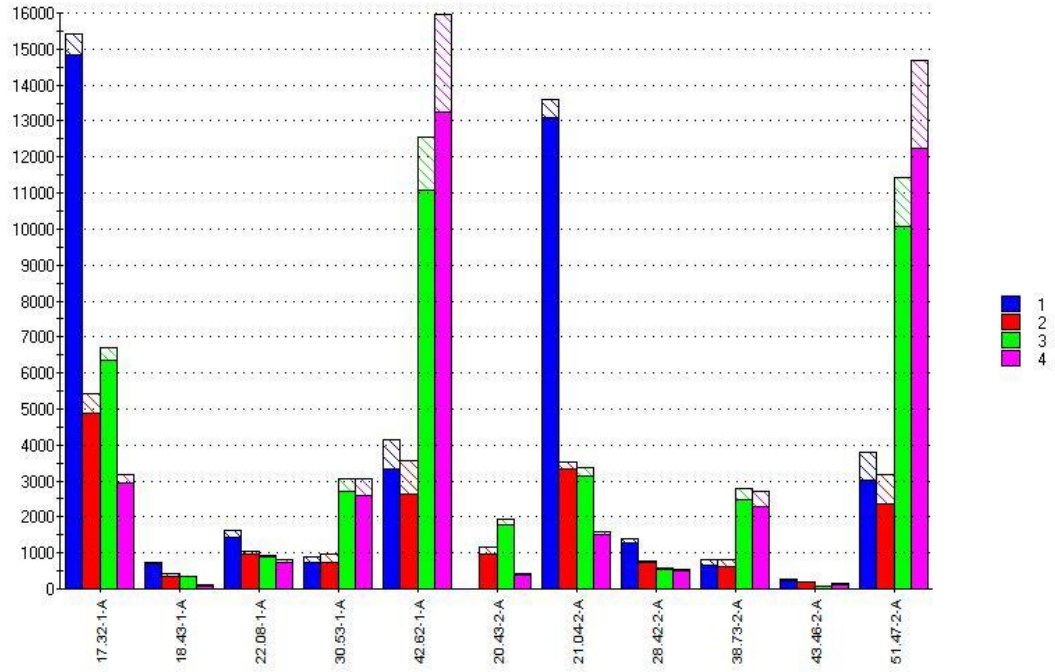


Figure 3-1: pork, donkeys, mutton, beef DFA FIG electronic nose ridge

Pigs, beef, mutton and donkeys' four kinds of electronic nose DFA animal test data analysis results shown in Figure 3-1. Illustrates horizontal and vertical coordinates represent the first index to distinguish (DF1) and a second of the DFA converted distinguishing index (DF2), DF1 contribution rate of 97.433% DF2 1.905% 99.338% total of both, DF1 and DF2 have been described contains a wealth of information, it can reflect differences in the overall sample information. Can be seen from FIGS. 3-1 to four loin volatile odor breeders size difference, wherein the distance between the abscissa of the minimum sheep and donkeys ridges, both described more similar flavor, while the distance between the ridges maximum beef and donkeys, show both flavor quite different.

Bar Figure 3.1.2 four kinds of meat volatile substances analysis



Note: Blue: Red Cow Back: Sheep Ridge Green: Donkey purple ridge: Ridge Pig
Figure 3-2: Cattle, sheep, donkeys, pigs, four samples electronic nose ridge Bar FIG.

Chromatogram corresponding to different polarities in the two columns, the retention time can identify differences in flavor four loin three animals, respectively, and 22.08-1-A 28.42-2-A, 30.53-1-A and 38.73 -2-A, 42.62-1-A and 51.47-2-A. Preliminary material thereby identify differences exist four samples, and qualitative analysis of substances using a difference with the instrument AroChemBase databases showed possible flavor as shown in Table 3-1.

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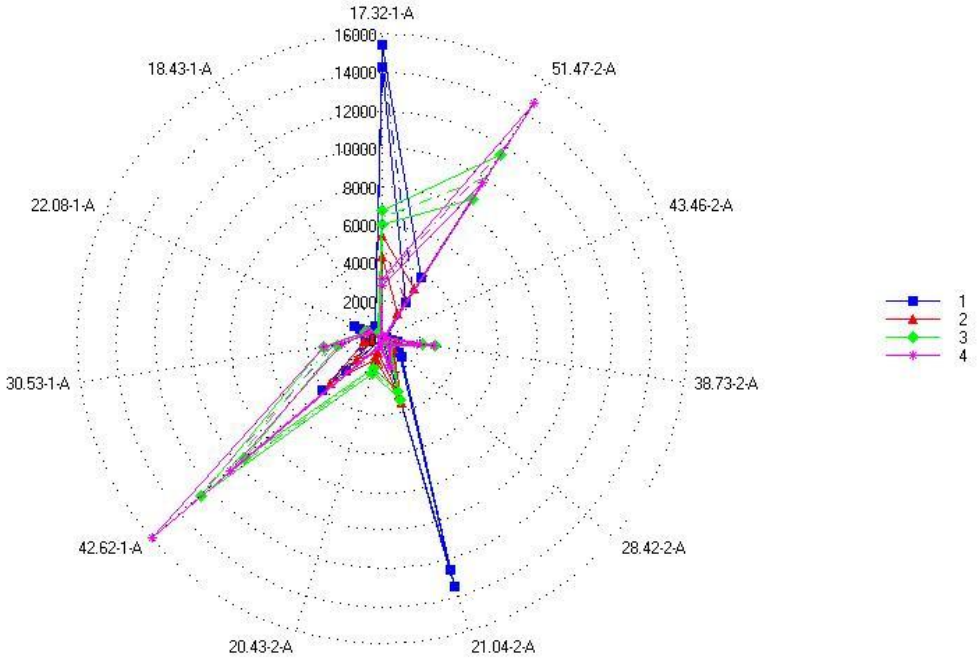
Table 3-1: Four animals' tenderloin possible differences in flavor ingredient information

Molecular formula	Name	RT DB-5-FID1	RT DB-1701-FID2	Possible material	Characteristic odor
C4H8O	Butanal	550	696	Butyral	Pungent odor
C5H8O2	2, 3-Pentanedione	699	789	2, 3-pentanedione	Having a sweet butter, cream, caramel aroma, with nuts and base notes. Quinone slightly sweet smell, odor diluted cream
C6H12O	Hexanal	802	895	Hexanal	Low concentration, fragrance, fruit flavor, concentration ≥4.5μg / kg, grass and rancid flavor[6]

Radar Figure 3.1.3 four kinds of meat volatile substances analysis



Note: Blue: Red Cow Back: Sheep Ridge Green: Donkey purple ridge: Ridge Pig

Figure 3-3: Four animals tenderloin flavor radar chart

FIG. 3-3 shows that four animals fillet shape different retention times in the presence of significant difference between different content of each sample i.e. the main volatile flavor substances, a large peak corresponding to a retention time of 42.62-1- A and 51.47-2-A.

Each animal tenderloin compared in terms of flavor nucleotides content

Nucleotides are the largest contributor to the flavor of meat in addition to amino acids, such as 5'-inosinic acid (Imp), 5'-guanosine monophosphate (GMP) and related derivatives, which are abundant in the meat, not only by fresh role, but also has some effect on the changes in the taste of things[17]. Therefore, this experimental determination of various meat flavor by HPLC nucleotides: hypoxanthine, inosine, inosinic acid, adenylic acid, guanylic acid content, in order to better study the donkey flavor.

Comparing donkey, pork, mutton and beef ridge total amount of flavor nucleotides found pork tenderloin> of mutton>beef>tenderloin. Tenderloin in four breeding stock, mainly flavor nucleotides inosine monophosphate and inosine 5'-IMP HxR mainly, it may be because when the animals are slaughted and stored, decomposing into adenosine triphosphate inosinic acid in a series of enzymatic reactions, inosinic acid part is formed by the action of phosphatase inosine, in general, the meat content of inosinic acid with inosine and meat related to storage conditions.

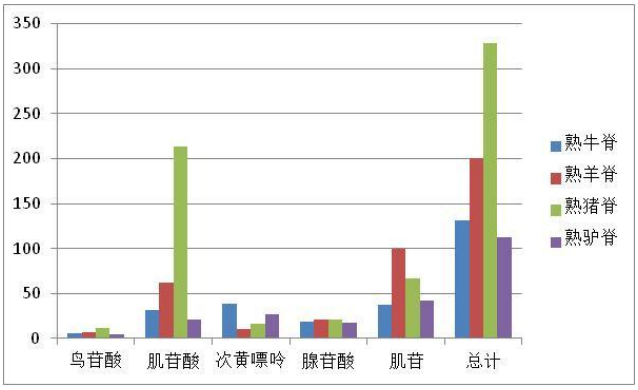


Figure 3-9: of Four animals fillet flavor nucleotides content

3.7.2 Comparison of values for each flavor nucleotides TAV meat in the form of

5'-GMP and 5'-IMP was the most representative nucleotide flavor, the flavor threshold values of 12.50 mg / 100g, 25.00 mg / 100g, the calculated activity value four loin breeder nucleotide taste (TAV), results are shown in table 3-10.

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Table 3-10: pork, beef, mutton, donkeys in the form of the flavor nucleotides value TAV

TAV value	5'-GMP	5'-IMP
Donkey ridge	0.35	0.85
Pig Ridge	0.91	8.54
Sheep Ridge	0.52	2.50
Cow Back	0.46	1.27

5'-IMP beef, pork, mutton ridges main flavor nucleotides, which are greater than a value of TAV, donkey ridges contribute less taste. 5'-GMP tenderloin taste the four contributions are small extent.

5. Conclusion

Donkey, as a domestic animal, could be reared not just for recreation exercises or for functioning as a worker animal, but additionally for donkey meat production as well as its products, as this sort of meat is very well-known nourishment in China, and some parts of Africa, Europe as well as South America.

The nourishing qualities of donkey meat show intriguing angles with regards to correlation with the standard red meat; when identified with the human wellbeing parameters, this sort of meat can be positively acknowledged by the buyers. Furthermore donkey meat has low contents of lipids and cholesterol, and demonstrates a beneficial connection between the diverse unsaturated fats. The investigations performed till now confirmed that donkey meat is a great quality meat; information acquired demonstrates that the meat is extremely high in crude protein, described by low fat substance and high in imperative minerals, for example, Potassium, Phosphorus, Iron and Zinc.

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