Study on the Amino Acids in different Varieties of Osmanthus Fragrans Using Grey Pattern Recognition and Grey Hierarchical Clustering Analysis

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Abstract. The research aimed to comprehensively evaluate 17 kinds of amino acids in different varieties of Osmanthus fragrans. The grey pattern recognition and grey hierarchical clustering analysis methods were used to analyze the data of amino acids and find out the relationship among 17 kinds of themselves including aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine. Combined with MATLAB2013 and SPSS softwares, the gray metrology methods were made on the amino acids of Osmanthus fragrans and the efficacy of traditional Chinese medicine. The first gray correlation coefficient factor had greater grey correlation coefficient on the indexes of $X_1$, $X_2$, $X_5$, $X_6$, $X_7$, $X_8$, $X_9$, $X_{10}$, $X_{11}$, $X_{12}$, $X_{13}$, $X_{14}$, $X_{15}$, $X_{16}$, and the second gray correlation coefficient factor had greater grey correlation coefficient on the indexes of $X_4$, $X_{15}$, and the third gray correlation coefficient factor had greater grey correlation coefficient on the index of $X_8$. 17 kinds of amino acids in Osmanthus fragrans in the order were Gold Osmanthus fragrans > Silver Osmanthus fragrans > the Four Seasons Osmanthus fragrans > Dan Osmanthus fragrans. Based on grey pattern recognition and grey hierarchical clustering analysis, a recognition model for evaluating traditional Chinese medicine quality was constructed. These studies provided the scientific base and theoretical foundation for the future large-scale rational relation development of Osmanthus fragrans resources as well as the relationship between the amino acids and traditional Chinese medicine efficacy for the human.

1. Introduction

Osmanthus (Osmanthus fragrans LOUT.) was one of the ten traditional flowers in China, mainly divided into four varieties including Gold Osmanthus fragrans, Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans, native to Asia, mostly in China and Japan, while China was in the majority, especially in Guangxi, Hubei, Guizhou, Jiangsu, Zhejiang, Anhui and other places. The resources of Osmanthus fragrans in China are rich, which contains rich amino acids, especially 8 kinds of amino acids necessary for human body. Proteins were the material basis of life, life was a form of existence of proteins. Amino acids were the basic units of proteins, basic materials to repair tissue and construct the cells, the most basic materials composed of biological proteins and related to the life activities. The lack of any kind of essential amino acids in human body would lead to abnormal physiological function, affect the metabolism of antibody, eventually leading
to disease. Therefore, the research[1-3] on the relationship between human health and amino acids in Osmanthus fragrans was of great significance.

2. Materials and methods

2.1 Materials
This paper selected 17 kinds of amino acids including aspartic acid $X_1$, serine $X_2$, glutamic acid $X_3$, glycine $X_4$, histidine $X_5$, arginine $X_6$, alanine $X_7$, proline acid $X_8$, cystine $X_9$, tyrosine $X_{10}$, threonine $X_{11}$, valine $X_{12}$, methionine $X_{13}$, methionine $X_{14}$, isoleucine $X_{15}$, leucine $X_{16}$, phenylalanine $X_{17}$ in four varieties of Osmanthus fragrans including Gold Osmanthus fragrans, Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans as an analysis sample, the original data from the literature[4].

2.2 Methods

2.2.1 Grey pattern recognition
Grey pattern recognition was one of the most commonly used grey metrology methods for each scheme and the correlation coefficient of the ideal scheme consisting of the best indicators. The grey correlation degree was obtained by the correlation coefficient, then according to the size of the correlation degree to sort, analyze to come to the conclusion. This method was superior to the classical mathematical method, through the intention, views and requirements of conceptualization, model, so that the study of the grey system from structure, model, relationship gradually changed from black to white, made uncertain factors gradually clear.

Grey pattern recognition analysis provided us with a way to analyze the relationship between the analytical factors, the sample size and the number of samples with and without rules were equally applicable, data and samples might not have statistical significance, which would make up for the use of mathematical statistical methods (principal component analysis, factor analysis, etc.) as a result of the analysis system defects, and the amount of calculation was small, very convenient.

2.2.2 Grey system clustering analysis
Grey system clustering analysis was to classify the research object, put all the case classification in different classes, so that individuals of the same class had greater similarity, individuals of different categories had greater differences.

3. Results and discussion

3.1 Grey pattern recognition process

3.1.1 Select reference sequence
With n samples, each sample had m evaluation indexes, which was composed of the evaluation unit sequence $\{X_{ij}\}$, $i=1,2,\ldots,n$; $j=1,2,\ldots,m$. Using Grey pattern recognition method the reference sequence was firstly selected as the evaluation measure. The optimal reference sequence was $\{X_{sj}\}$, each index of the optimal reference sequence was the optimal value or maximum of n samples corresponding indicators.

3.1.2 The original data normalization processing
Usually evaluation index to measure was not uniform, therefore, it is necessary to normalize the raw data: $Y_{ij}=x_{ij}/\bar{x}_j$, where $Y_{ij}$ was normalized data after treatment, $x_{ij}$ for the original data, $\bar{x}_j$ for the mean value of the j-th index for n samples. After normalizing the data, the data were shown in table 1.
### Table 1: The data after the normalization of raw data

| Amino Acids | Gold Osmanthus Fragrans | Silver Osmanthus Fragrans | Dan Osmanthus Fragrans | Four Seasons Osmanthus Fragrans |
|-------------|-------------------------|---------------------------|------------------------|---------------------------------|
| Aspartic Acid X1 | 1.0970                  | 0.9556                    | 1.0905                 | 0.8569                          |
| Serine X2    | 1.3267                  | 1.1287                    | 0.6832                 | 0.8713                          |
| Glutamic Acid X3 | 0.8367                  | 1.0408                    | 0.9388                 | 1.1633                          |
| Glycine X4   | 0.6667                  | 1.3333                    | 0.6667                 | 1.0000                          |
| Histidine X5 | 1.8065                  | 0.1398                    | 0.8387                 | 1.2043                          |
| Arginine X6  | 1.7183                  | 1.4225                    | 0.7606                 | 0.0845                          |
| Alanine X7   | 1.7549                  | 1.2647                    | 0.4314                 | 0.5490                          |
| Proline Acid X8 | 1.4812                  | 0.9850                    | 0.5865                 | 0.9398                          |
| Cystine X9   | 1.4865                  | 0.8919                    | 0.6486                 | 0.9595                          |
| Tyrosine X10 | 1.6727                  | 0.5636                    | 1.2906                 | 0.4727                          |
| Threonine X11 | 1.2061                  | 1.0303                    | 0.8424                 | 0.9091                          |
| Valine X12   | 1.0720                  | 0.9920                    | 0.9360                 | 0.9840                          |
| Methionine X13 | 1.6857                  | 1.1333                    | 0.8190                 | 0.3524                          |
| Phenylalanine X14 | 1.0945                 | 0.9685                    | 0.9843                 | 0.9528                          |
| Isoleucine X15 | 1.0276                  | 1.0994                    | 0.7956                 | 1.0718                          |
| Leucine X16  | 1.4179                  | 0.9851                    | 0.7015                 | 0.8657                          |
| Phenylalanine X17 | 1.4400                  | 0.9133                    | 0.9000                 | 0.7533                          |

#### 3.1.3 Calculation of correlation coefficient
Relative to the optimal reference sequence, the correlation coefficient:

$$\xi_{i(s)} = \frac{\Delta_{\min} + \rho \Delta_{\max}}{Y_{ij} - Y_{ij}} + \rho \Delta_{\max}$$

$$\Delta_{\min} = \min \left| Y_{ij} - Y_{ij} \right|, \Delta_{\max} = \max \left| Y_{ij} - Y_{ij} \right|$$

(i=1,2,…,n;j=1,2,…,m),\(\rho\) was resolution ratio, Value of 0.5.

#### 3.1.4 Calculation of correlation degree
Relative to the optimal reference sequence, the correlation degree

$$R_i(s) = \frac{1}{m} \sum_{j=1}^{m} \xi_{i,j(s)}$$

Ranking of each evaluation unit could be given according to the correlation degree of the evaluation sequence relative to the reference sequence. The greater \(R_i(s)\) was, the more the correlation degree would become between the correlation evaluation unit sequence and the optimal reference sequence, the better evaluation unit. The correlation degree of a sequence to be evaluated was greater than the correlation degree of the reference sequence, it showed that the sequence and the reference sequence would be the most similar to the quality of the best sequence, namely the optimal quality was the quality of the best, so that the quality of Chinese herbal medicine could ultimately be comprehensive evaluation results[5-10].

According to the grey pattern recognition methods, the grey correlation coefficient and correlation degree were calculated by using the grey measurement methods[11-17] and the MATLAB2013 and SPSS softwares. Correlation coefficient, correlation degree and ranking with relative to the reference sequence of different varieties of Osmanthus fragrans were listed in Table 2.
Table 2: Correlation coefficient, correlation degree and ranking with relative to the reference sequence of different varieties of Osmanthus fragrans

| projects | Gold Osmanthus fragrans | Silver Osmanthus fragrans | Dan Osmanthus fragrans | the Four Seasons Osmanthus fragrans |
|----------|-------------------------|---------------------------|------------------------|------------------------------------|
| Correlation coefficient | aspartic acid X₁ | 1.0000 | 0.8549 | 0.9923 | 0.7763 |
| | serine X₂ | 1.0000 | 0.8080 | 0.5643 | 0.6466 |
| | glutamic acid X₃ | 0.7184 | 0.8718 | 0.7878 | 1.0000 |
| | glycine X₄ | 0.5556 | 1.0000 | 0.5556 | 0.7143 |
| | histidine X₅ | 1.0000 | 0.3333 | 0.4627 | 0.5805 |
| | arginine X₆ | 1.0000 | 0.7380 | 0.4653 | 0.3378 |
| | alanine X₇ | 1.0000 | 0.6296 | 0.3864 | 0.4087 |
| | proline acid X₈ | 1.0000 | 0.6268 | 0.4822 | 0.6062 |
| | cystine X₉ | 1.0000 | 0.5836 | 0.4986 | 0.6126 |
| | tyrosine X₁₀ | 1.0000 | 0.4290 | 0.6858 | 0.4098 |
| | threonine X₁₁ | 1.0000 | 0.8258 | 0.6962 | 0.7372 |
| | valine X₁₂ | 1.0000 | 0.9124 | 0.8597 | 0.9045 |
| | methionine X₁₃ | 1.0000 | 0.6014 | 0.4902 | 0.3846 |
| | methionine X₁₄ | 1.0000 | 0.8687 | 0.8832 | 0.8547 |
| | isoleucine X₁₅ | 0.9207 | 1.0000 | 0.7328 | 0.9679 |
| | leucine X₁₆ | 1.0000 | 0.6582 | 0.5377 | 0.6015 |
| | phenylalanine X₁₇ | 1.0000 | 0.6127 | 0.6068 | 0.5482 |
| correlation degree Ri(s) | 0.9526 | 0.7267 | 0.6287 | 0.6524 |
| ranking | 1 | 2 | 4 | 3 |

The first gray correlation coefficient factor had greater grey correlation coefficient on the indexes of X₁, X₂, X₅, X₆, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₆, X₁₇, as shown in table 2. X₁ had a protective effect on the muscle, a protective effect against myocardial infarction and could increase the flavor and promote appetite. X₂ was a component of the serine phospholipids in the brain and other tissues that could treat lung disease. X₅ could be involved in blood cell protein synthesis, promote the generation of blood, produce ammonia, promote dilation of blood vessels, increase the permeability of the vascular wall and glandular secretion that had the effect on allergic disease: cure stomach and duodenum effects in treating heart function, angina pectoris, lower blood pressure, which had the effect of asthma and rheumatoid arthritis. X₈ could reduce blood ammonia, increase muscle activity, maintain sexual function, had certain effect to the treatment of sperm. X₉ could promote the metabolism of alcohol in the blood, had hepatoprotective effect. X₁₀ could increase appetite, promote metabolism, prevent and cure Alzheimer's disease and there was remarkable effect on chronic diseases such as treatment of neurogenic inflammation, ulcer and stunting. X₁₁ had the function of transforming certain amino acids to balance, lack of it would cause weight loss, make people thin, and even death. X₁₂ could promote nervous system function normal, if the lack of it, would cause the tactile sensitivity improved, muscle ataxia disorders. X₁₃ involved in the synthesis of choline could participate in the formation of hemoglobin, tissue and serum, promote the function of the pancreas, spleen and lymph, promote the synthesis of skin protein and insulin, improve the activity of muscle, had the function of fat to the prevention and treatment of atherosclerosis of the hyperlipemia. X₁₄ was the composition of the liver and gallbladder, could promote brain development, fat metabolism and the pepsin secretion, regulate the pineal gland, mammary gland, corpus luteum and ovarian to prevent degeneration of the cells, enhance immunity and improve growth retardation and calcium absorption to promote bone growth. Tips could be from Osmanthus fragrans in continuous uptake of amino acids. X₁₆ could promote skin, wound and bone healing and had certain
effect on reducing blood sugar levels in the treatment of dizziness, if the lack of it, would stop the growth, weight loss.

$X_{17}$ could be converted to tyrosine in the body, promote the synthesis of thyroid hormone and adrenaline, participate in the elimination of loss of kidney and bladder function. Table 2 showed, the second gray correlation coefficient factor had greater gray correlation coefficient on the indexes of $X_4$, $X_{15}$. $X_4$ could reduce the blood sugar levels and cholesterol levels in the blood, prevent and treat diabetes, blood clots, blood clots, high blood pressure, excessive gastric acid, improve muscle vitality. $X_{15}$ could maintain the balance of the body, promote the increase of appetite and anemia and treatment of mental disorders, also to participate in the spleen, brain and thymus gland metabolism and regulation, if the lack of it, there would be physical failure, coma and other symptoms. The third gray correlation coefficient factor had greater grey correlation coefficient on the index of $X_3$, as shown in table 2. $X_3$ that had curative and therapeutic effect on acid poisoning, epilepsy and mental schizophrenia, neurasthenia treatment, etc. could protect the skin moist, prevent chapped, improve the central nervous system activity, promote oxidation, participate in brain protein and glucose metabolism, maintain and promote brain cell function, promote the increase of intelligence, boost the body's immune function[24-28].

From table 2 showed, different varieties of Osmanthus fragrans including Gold Osmanthus fragrans, Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans contained the contents of 17 kinds of amino acids in the order from high to low: Gold Osmanthus fragrans > Silver Osmanthus fragrans > the Four Seasons Osmanthus fragrans > Dan Osmanthus fragrans. In terms of content of amino acids, the best quality Gold Osmanthus fragrans medicinal materials, followed by Silver Osmanthus fragrans medicinal materials, Dan Osmanthus fragrans medicinal materials worst.

3.2 Grey system clustering analysis
Grey system clustering analysis was a method of mathematical statistics, the original data were normalized, and then 17 kinds of amino acids including aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine of different cultivars of Osmanthus fragrans in China were evaluated and classified by the methods of quantitative analysis of the gray factors in grey system clustering analysis. Using MATLAB2013 and SPSS softwares the data were used to analyze by grey system clustering analysis. After extracting the gray factors, the gray factor data were analyzed using hierarchical clustering method of grey system clustering analysis. Based on the gray factor clustering analysis, each two samples with Average linkage linked with the method of euclidean distance measurement, the sequence was plotted in figure 1. The tree graph of grey system clustering analysis was shown in figure 1.

![Fig. 1: Tree diagram of grey system clustering analysis](image)

Figure 1 showed that according to the results of grey system clustering analysis four main varieties of Osmanthus fragrans including Gold Osmanthus fragrans, Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans were divided into two categories, a
class of Gold Osmanthus fragrans, another kind of Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans, indicating that Gold Osmanthus fragrans and other Osmanthus fragrans in nature existed obvious differences, which indicated that Osmanthus fragrans as medicinal plants in China, in terms of content of amino acids, the best quality Gold Osmanthus fragrans, followed by Silver Osmanthus fragrans. Through the grey system clustering analysis, Figure 1 showed similar extent and nature of the genetic relationship of the different varieties of Osmanthus fragrans would be found out, which would help us study them.

Figure 1 showed amino acids in Osmanthus fragrans there were similarity and provided the basis for further pharmacological study of Osmanthus fragrans medicinal materials in China. On the basis of grey pattern recognition, it was feasible to classify the different varieties of Osmanthus fragrans in grey system, and the result was the same with the grey pattern recognition analysis. The conclusion was objective, credible and persuasive[29-32].

4. Conclusions
From grey pattern recognition and grey system clustering analysis, the results showed that the contents of amino acids in Gold Osmanthus fragrans were the highest, which indicated that Gold Osmanthus fragrans as traditional Chinese medicinal materials, the best quality Gold Osmanthus fragrans, followed by Silver Osmanthus fragrans, the Four Seasons Osmanthus fragrans, Dan Osmanthus fragrans worst.

In this paper, the content of 17 kinds of amino acids in the four main varieties of Osmanthus fragrans was elucidated from two aspects of high and low by means of grey pattern recognition and grey system cluster analysis, so as to reflect the quality of corresponding medicinal materials, to dig out the implied relationship between amino acids and traditional Chinese medicine of Osmanthus fragrans, to make up for gaps in previous studies of Osmanthus fragrans medicinal materials in this field.

Using the world's most advanced MATLAB and SPSS softwares, gray metrology methods of grey pattern recognition and grey system clustering analysis method on China's four main varieties of Osmanthus fragrans including Gold Osmanthus fragrans, Silver Osmanthus fragrans, Dan Osmanthus fragrans and the Four Seasons Osmanthus fragrans were used to comprehensively analyze the data of amino acids and evaluate 17 kinds of themselves and find out the relationship among the 17 kinds of themselves including aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine of different cultivars of Osmanthus fragrans in China, which dig out hidden information between the amino acids of Osmanthus fragrans resource and traditional Chinese medicine efficacy. 17 kinds of amino acids in Osmanthus fragrans in the order were Gold Osmanthus fragrans > Silver Osmanthus fragrans > the Four Seasons Osmanthus fragrans > Dan Osmanthus fragrans. These studies provided the scientific base and theoretical foundation for the future large-scale rational relation development of Osmanthus fragrans resources as well as the relationship between the amino acids and traditional Chinese medicine efficacy for the human.

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