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Differences in the Content of Protein and Essential Amino Acids between Different Rice Varieties

Zhiyuan ZHANG[1], Jianzhou TANG[1*], Ling ZHOU[2], Xinghai LIU[3]

1. Department of Biological and Environmental Engineering, Changsha University, Changsha 410022, China; 2. Changsha Tiansong Biotechnology Co., Ltd., Changsha 410022, China; 3. Taoyuan Science and Technology Development Co., Ltd., Taoyuan 415705, China

Abstract Evaluation of the protein content is a major indicator of the nutritional quality of rice, and the protein quality of rice is the best among cereal crops. Essential amino acids play an irreplaceable role in human growth, development and health care. Essential amino acid is a key ingredient to measure the nutritional value of rice. Using the experimental rice processed from the rice variety "Yuzhenxiang" sprayed with plant nutrients (patent number: ZL201110103910.9), namely high essential amino acid nutritional rice, combined with five kinds of high quality rice imported by COFCO and homegrown "Wuchang rice", we send the samples of the seven kinds of rice to Hunan Food Testing Center, and adopt HPLC method to test the content of protein and eight kinds of essential amino acids. Three bags of rice are randomly selected for each kind of rice, and each bag is a replication. The test results show that there are highly significant differences in the content of essential amino acids between different kinds of rice ($F = 246.29^{**}$, $P = 5 \times 10^{-71}$), and there are also highly significant differences in the content between different kinds of essential amino acids ($F = 3937.09^{**}$, $P = 4 \times 10^{-146}$). The test results of protein content indicate that there are highly significant differences in the content of protein between different kinds of rice ($F = 3937.0973.29^{**}$, $P = 5.81 \times 10^{-11}$), and the test results of lysine content show that there are highly significant differences in the content of lysine between different kinds of rice ($F = 3937.0973.29^{**}$, $P = 5.81 \times 10^{-11}$).

Key words Rice, Essential amino acids, Protein, High essential amino acids, Nutritional rice

1 Introduction

Since Liebig developed the theory of mineral nutrition, it has long been thought that the plant can only absorb inorganic nitrogen, and the organic nitrogen in the soil can be absorbed and utilized by the plant only after the microorganisms in the soil are mineralized into inorganic nitrogen. However, a growing number of studies show that the plant can also directly use amino-acid N and actively absorb amino-acid N secreted by roots [1-4]. M. Yamagata *et al.* [5] find that the upland rice applied with organic N grows better than the upland rice applied with chemical fertilizer N; it can absorb more N elements, and compared with corn, the upland rice prefers the absorption of ammonium, amino acids and proteins. Liu Qingcheng *et al.* [6] spray the mixture of eleven kinds of amino acids with different concentration on the celery leaf, and find that amino acids can be directly absorbed by the celery stems and leaves. Under sterile culture conditions, Xu Yulan *et al.* [7] use 15 N to label glycine and leucine and prove that amino acid molecule can enter directly into the plant, and after 15 d of absorption, 70% of 15N is transferred from the culture medium to plants. Schiller *et al.* [8] find that a plant in Namibia can absorb amino acids as a nitrogen source in the absence of inorganic nitrogen, and it mainly absorbs glycine and serine. Thomton [9] studies the absorption of glycine in ryegrass, and finds that the ryegrass with sterile roots can absorb the full glycine molecule, and after more than 3 h, at least 80% of glycine still keeps intact molecules. Under sterile sand culture conditions, the studies of Mo Liangyu *et al.* [10] demonstrate that wheat can absorb glycine, glutamic acid and lysine, and during the seedling stage of wheat, the uptake of amino-acid N is equal to the uptake of ammonium N. Zhang Tianqian *et al.* [11] use sterile culture medium to cultivate rice and find that there is no aspartate transaminase activity on the rice root surface, and thus believe that the rice can not break down amino acids which enter into the rice plant in the molecular state. However, existing research only takes the amino acids as the organic molecules providing nitrogen source to the plant, but never involves whether these exogenous amino acids participate in other kinds of metabolism in plants or directly participate in protein synthesis. Especially the transfer of high-quality soy protein to rice grain is not touched upon by anyone. We use the plant nutrients produced from the fermentation of *Bacillus subtilis* YS-45 [12] in citrus for spraying on the rice leaves from initial heading stage to full heading stage, to increase the content of essential amino acids in rice by 6.82% – 26.56% [13].

2 High essential amino acid nutritional rice

Through breeding, spraying plant nutrients, exogenous amino acid nutrient solution and other technologies, the content of essential amino acids in raw grain is improved, and the raw grain is processed into the high essential amino acid nutritional rice with total content of eight kinds of essential amino acids (histidine, threonine, methionine, vlaine, phenylalanine, isoleucine, leucine, and lysine) in the range of 3000 – 4ooo mg/100 g. The rice with

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* Corresponding author. E-mail: 49071398@qq.com

total content of eight kinds of essential amino acids more than 4000 mg/100 g is super nutritional rice. The essential amino acids play an irreplaceable role in human growth and development and health care. The daily requirement is 714 mg per kg of weight and 352 mg per kg of weight in infancy and childhood, respectively. The main way for people to take in essential amino acids is animal protein and vegetable protein, but excessive dependence on meat will bring high blood pressure, high cholesterol, coronary heart disease and other diseases. Therefore, it is particularly important to improving the essential amino acid content of rice. Currently, there are two main methods to improve the content of essential amino acids in rice: one is to make up the nutrient supplement using lysine and vitamins, and then make the rice absorb it, or supplement the ground rice with nutrient enhancer, and then carry out granulation and drying; the other is to keep the nutrients in rice outer layer or germ. However, there are shortcomings in both methods. The indicators in National Standard for High Quality Rice (GB/T17891) implemented from April 1, 2000, are mainly concentrated in processing quality, appearance quality, cooking and eating quality and shapes. For the inherent quality of high quality rice, it only touches upon the amylose content, gel consistency and taste, but the key part-content of essential amino acids having a real impact on rice nutritional value, is not mentioned. The content of essential amino acids is a principal part of rice nutrition, and the differences in rice nutritional value depends on the differences in the content of essential amino acids. In order to improve the content of essential amino acids in rice, it is necessary to conduct some studies on how to enhance the nutrition and health function of rice.

3 The importance of the eight kinds of essential amino acids

Human proteins contain twenty kinds of amino acids, eight of which can not be synthesized in the human body (lysine, tryptophan, phenylalanine, methionine, isoleucine, vlaine, leucine and histidine). The human diet must be supplemented with these amino acids which are called essential amino acids for human nutrition. Essential amino acids play an irreplaceable role in human growth and development and health care. Lysine can promote metabolism, regulate the pineal gland, mammary gland, corpora lutea and ovary, and prevent cell degradation. It can also improve appetite, promote the secretion of pepsin, enhance immune function, improve growth retardation, prevent tooth decay, promote children's growth, enhance the absorption of calcium, and promote bone growth. It participates in the formation of microvascular epithelial mesenchymal cells of connective tissue, and keeps the normal osmotic pressure; if without lysine, people's sensitivity will decline, women will menopause, and there will be anemia, dizziness, nausea and other symptoms. Tryptophan plays a major role in promoting gastric and pancreatic juice as well as the synthesis of hemoglobin, preventing pellagra and improving appetite. Phenylalanine is mainly involved in the elimination of the loss of renal and bladder function, and is converted into tyrosine in the body to promote the synthesis of thyroid hormone and epinephrine. Methionine is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group, an α-carboxylic acid group, and an S-methyl thioether side chain, classifying it as a non-polar, aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it and thus it must be obtained from the diet. It participates in the synthesis of choline, and can prevent arteriosclerosis hyperlipidemia, increase muscle activity and promote the synthesis of skin proteins and insulin. The main function of threonine is to transfer some amino acids so as to achieve balance. The lack of threonine can lead to body weight loss, and even death. Isoleucine is anα-amino acid that is used in the biosynthesis of proteins. It is essential in humans, meaning the body cannot synthesize it, and must be ingested in our diet. Isoleucine is mainly involved in the regulation and metabolism of thymus, spleen and pituitary gland, and can maintain the balance of the body, treat mental disorders, enhance appetite and anti-anemia. The lack of isoleucine will lead to physical failure, coma and other symptoms. Valine mainly affects corpus luteum, breast and ovary, and keeps the normal function of the nervous system. The deficiency of it will result in particularly high tactile sensitivity and muscle movement disorders. Histidine plays a role in regulating metabolism, participates in the synthesis of blood cells, promotes the formation of blood, produces histamine, promotes blood vessel dilation, increases the blood vessel wall permeability, promotes glandular secretion, and effectively treats stomach and duodenum diseases, heart failure, angina, high blood pressure, asthma and rheumatoid arthritis.

4 Relationship between rice protein content and essential amino acids content

Protein content is a major indicator to evaluate the nutritional quality of rice, and the quality of rice protein is best among cereal crops. Its amino acid ratio is reasonable, and the amino acids can be easily digested and absorbed by the body. The biological value, digestibility and net utilization rate are all high. However, the predecessors always think that there is a certain relationship between the rice protein content and essential amino acid content, and the rice protein content is high. In a recent study, it is found that in terms of different types of essential amino acids, the rice protein content is not significantly positively correlated with the content of one single essential amino acid, and we select five kinds of rice for sampling. The sampling results are shown in Table 1. The high essential amino acid nutritional rice contains 6.6% protein and 2.69% essential amino acids, with the protein content lower than that of Gewaixiang rice, but the essential amino acid content higher than that of Gewaixiang rice. The protein content of high essential amino acid nutritional rice is lower than that of Wufengzhenzhu rice, but its essential amino acid content is higher than that of Wufengzhenzhu rice. The protein content of high essential amino acid nutritional rice is higher than that of the original Thai jasmine rice, but the essential amino acid content is lower than that of the original Thai jasmine rice. Therefore, it is necessary to further study the relationship between rice protein and essential amino acids.

Table 1 Test report of five kinds of rice

Items	Unit	High essential amino acid nutritional rice	Gewaixiang rice	Fulinmen rice	Wufengzhenzhu rice	Thai jasmine rice
His	%	0.68	0.20	0.20	0.22	0.29
Ser	%	0.47	0.24	0.28	0.30	0.37
Val	%	0.37	0.36	0.40	0.42	0.48
Met	%	0.16	0.19	0.12	0.16	0.18
Phe	%	0.30	0.40	0.32	0.36	0.44
Ile	%	0.22	0.28	0.27	0.28	0.33
Leu	%	0.40	0.58	0.49	0.54	0.66
Lys	%	0.10	0.24	0.08	0.08	0.27
Total	%	2.69	2.49	2.16	2.36	3.00
Protein	%	6.60	7.37	6.32	6.80	6.52

4.1 Technologies and measures for increasing the content of essential amino acids in rice

4.1.1 Applying the plant nutrients containing essential amino acids. In order to increase the essential amino acids in rice, many researchers have conducted many studies. Shu Xiaoli *et al.* [14] use transgenic technology, total DNA delivery, induced mutation and other methods, to transform the genetic traits of rice and obtain the amino acid-rich rice, but most people still do not want to eat the rice obtained by these methods, because people still lack adequate understanding of the rice produced by these methods, and think that there is still risk in the rice. Jin Zenghui *et al.* [15] use internal holding method and external addition method to produce fortified rice, but these methods are relatively complex, and the effect is not good. Endophytic *Bacillus subtilis* in citrus is found by us in the key technology project of the Ministry of Education in 2001—Citrus Endophytes Resources and Ecological Research. The ecological adaptation of *Bacillus subtilis* is strong, and it has diverse biological properties. It has significant antibacterial activity and strong resilience, and it is harmless to human. Currently, the study of *Bacillus subtilis* in the food area is still in its infancy. In scientific research, Wan Qi, Lu Zhaoxin *et al.* [18-19] from Nanjing Agricultural University reported the research on the use of fermentation method to produce soybean peptide for the first time, obtained a strain of *Bacillus subtilis* that can produce protease and exopeptidase in the fermentation process, and used the enzyme produced by the bacteria to hydrolyze soy protein to small peptides and amino acids. In recent years, we have conducted many field experiments and laboratory analyses, and published relevant papers. We use endophytic *Bacillus subtilis* in citrus YS-45 to produce plant nutrients, and spray the plant nutrients containing essential amino acids on rice. Results show that it can significantly increase the content of essential amino acids in rice, and the content of lysine in rice, but the growth rate of protein content is not high. And the plant nutrients can increase rice production and economic efficiency.

4.1.2 Selecting the raw grain varieties with high content of essential amino acids. Due to different genetic background, different rice varieties have different ability to synthesize amino acids. As can be seen from Table 1, the different varieties of processed raw grain have different content of essential amino acids. Therefore, we should select the rice variety with high content of essential amino acids in the production practice.

4.1.3 Application of plant nutrients and the rice variety with high content of essential amino acids. On the basis of choosing the rice variety with high content of essential amino acids, the plant nutrients are sprayed on the leaf in the late period of rice growth, which can greatly improve the content of essential amino acids in rice.

4.2 Relationship between protein and different amino acids in rice The plant nutrients (Patent No.: ZL201110103910.9) are sprayed on the rice variety "Yuzhenxiang" and the raw grain is processed into experimental rice, namely high essential amino acid nutritional rice, combined with five kinds of high quality rice imported by COFCO (original Thai jasmine rice; Taiwan Xiangqing rice; Thai jasmine rice; Akitakomachi rice; Cambodian Angkor rice) and homegrown "Wuchang rice". The samples of the seven kinds of rice are sent to Hunan Food Testing Center, and the HPLC method is used to test the content of protein and eight kinds of essential amino acids. For each kind of rice, three bags of rice are randomly selected and each bag is a replication. In Tables 2, 3, the reusable two-factor variance analysis is performed on the content of seven kinds of essential amino acids in rice. The results show that there are highly significant differences in the content of essential amino acids in various kinds of rice ($F = 246.29^{**}$, $P = 5 * 10^{-71}$), and there are also highly significant differences in the content of different kinds of essential amino acids ($F = 3937.09^{**}$, $P = 4 * 10^{-146}$). The content of leucine is highest among eight kinds of essential amino acids. The average content sorting and SSR multiple comparison results show that there are significant differences in the content of amino acids between different types of rice; the content of amino acids is highest in the experimental rice, significantly different from other groups of rice.

Table 4 performs the one-factor variance analysis on the content of protein in seven kinds of rice, and the results show that there are highly significant differences in the content of protein between different types of rice ($F = 3937.0973.29^{**}$, $P = 5.81^{*}10^{-11}$). Table 5 shows the multiple comparison of protein content of different types of rice. The results show that there are also highly significant differences in the protein content between different types of rice, and the protein content of experimental rice is highest, significantly different from other types of rice. The protein content of original Thai jasmine rice and Taiwan Xiangqing rice is ranked second, and there are no significant difference in the protein content among Thai jasmine rice, Akitakomachi rice and Cambodian Angkor rice. The protein content is lowest in Wuchang rice. Lysine is the first limiting amino acid in human body, and its content is worthy of attention. Therefore, we conduct a separate analysis of the content of this amino acid. The variance analysis results of content of lysine in different kinds of rice are shown in Table 6. It indicates that there are highly significant differences in the lysine content between different kinds of rice ($F = 3937.0973.29^{**}$, $P = 5.81^{*}10^{-11}$). The multiple comparison of the content of lysine in different types of rice is shown in Table 7, and the results show that the lysine content of experimental rice is highest, significantly different from the lysine content of other types of rice. The lysine content of original Thai jasmine rice is ranked second, and there are no significant differences in lysine content of Taiwan Xiangqing rice, Thai jasmine rice and Angkor rice, with content ranked third.

Table 2 Variance analysis of content of different essential amino acids in rice

Source of variance	*SS*	*Df*	*MS*	*F*	*P*-value	*F* crit
Rice	219064.2	7	31295	246.29	5E-71	2.082
Amino acids	3501912	7	500273	3937.09	4E-146	2.082
Interaction	50611.44	49	1032.9	8.13	1E-21	1.453
Error	16264.56	128	127.07			
Total	3787852	191				

Table 3 Multiple comparison of content of different amino acids in rice

Types of rice	Average content mg/100 g rice	5% significant difference	Types of amino acids	Average content (mg/100 g rice)	5% significant difference
Experimental rice	324.62	a	Leucine	571.77	a
Taiwan Xiangqing rice	305.23	b	Valine	389.57	b
Original Thai jasmine rice	296.23	c	Phenylalanine	352.18	c
Cambodian Angkor rice	284.29	g	Isoleucine	263.15	d
Thai jasmine rice	274.71	e	Threonine	235.68	e
Akitakomachi rice	263.98	f	Lysine	231.25	e
Wuchang rice	228.65	g	Histidine	147.62	f
			Methionine	130.80	g

Table 4 Variance analysis results of protein content of different types of rice

Source of variance	*SS*	*Df*	*MS*	*F*	*P*-value	*F* crit
Rice	12466133	7	1780876	73.29	5.81E-11	2.657
Error	388800	16	24300			
Total	12854933	23				

Table 5 Multiple comparison of protein content of different types of rice

Types of rice	Average content mg/100 g rice)	5% significant difference
Experimental rice	7190.00	a
Taiwan Xiangqing rice	6403.33	b
Original Thai jasmine rice	6373.33	b
Cambodian Angkor rice	6066.67	c
Thai jasmine rice	5923.33	c
Akitakomachi rice	5903.33	c
Wuchang rice	560.00	d

Table 6 Variance analysis results of lysine content of different types of rice

Source of variance	*SS*	*Df*	*MS*	*F*	*P*-value	*F* crit
Rice	18362.83	7	2623.26	119.36	1.33E-12	2.657
Error	351.63	16	21.98			
Total	18714.46	23				

Table 7 Multiple comparison of lysine content of different types of rice

Types of rice	Average content	5% significant difference
Experimental rice	257.40	a
Taiwan Xiangqing rice	243.90	b
Original Thai jasmine rice	230.93	c
Cambodian Angkor rice	226.43	c
Thai jasmine rice	225.97	c
Akitakomachi rice	208.23	d
Wuchang rice	180.20	e

5 Conclusions

Protein content is a major indicator to evaluate the nutritional quality of rice, and the protein quality of rice is the best among cereal crops. Its amino acid ratio is reasonable, and the amino acids can be easily digested and absorbed by the body. The biological value, digestibility and net utilization rate are all high. Human proteins contain twenty kinds of amino acids, eight of which can not be synthesized in the human body (lysine, tryptophan, phenylalanine, methionine, isoleucine, vlaine, leucine and histidine). The human diet must be supplemented with these amino acids which are called essential amino acids for human nutrition. Essential amino acids play an irreplaceable role in human growth and development and health care. Essential amino acid is a key component to measure the nutritional value of rice. The plant nutrient containing essential amino acid is sprayed on the rice variety "Yuzhenxiang". The testing results show that it can significantly increase the content of essential amino acids in rice, and the content of lysine as the first limiting amino acid in rice is also improved significantly. "Yuzhenxiang" has the highest amino acid content, significantly different from other groups of rice, making it become the world's leading high-quality rice.

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