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The Distribution of Mineral Elements and Protein in Soft Rice was Determined and Analyzed by SEM-EDS Technology

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Abstract

Soft rice is a kind of high-quality rice between glutinous rice and sticky rice. It has low amylose content, crystal clear grains, sweet taste, soft glutinous, and is suitable for cooking and porridge. Chalkiness in soft rice is a white opaque part formed by loose endosperm. It is an important character that affects the appearance quality, processing, and cooking quality of rice, and also an important limiting factor that restricts the standard rate of high-quality rice in China. The combination of scanning electron microscope and energy dispersive spectrometer (SEM-EDS) can be used for in-depth analysis of rice, visualization, and quantitative analysis of element distribution in rice. The results showed that there were many kinds of mineral elements in soft rice seeds, among which C and O were the most abundant, followed by N and P, and Mg, Al, P, S, K, Ca, Mn, and Zn were less. The contents of C, N, P, and S in the non-chalky area were significantly higher than those in the chalky area. Especially N and S were the best indicators of protein, and the contents in the chalky area were higher than those in the non-chalky area. It means that the protein content in the chalky part of soft rice seed is less than that in the non-chalky part, which affects the nutritional quality of soft rice. Therefore, the results of this study laid a solid foundation for the in-depth analysis of the distribution of mineral elements and protein in soft rice and their effects on the quality of soft rice, which also provided important information for the cultivation of new high-quality rice varieties in the future.

Keywords: Soft rice; Chalkiness trait; Element content; Protein distribution; SEM-EDS.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. It is the staple food for more than 3 billion people in more than 100 countries and regions in the world [1, 2]. With the continuous improvement of people's living standards, high-quality rice is more and more favored by consumers [3, 4]. Generally speaking, the eating quality of rice with amylose content > 20% is worse, while that of rice with amylose content < 15% is better. Soft rice is a new type of rice with amylose content between 2% and 15% [5]. Soft rice has many advantages, such as soft but not rotten, good expansibility, elastic, hard after cold, little retrogradation and so on. It is a high-quality raw material for instant food such as instant rice and rice snacks [6]. Revealing the distribution of elements and protein in soft rice will provide theoretical support for the cultivation of new varieties of high-quality soft rice, and has important theoretical significance and potential reference value.

Generally, rice quality can be divided into appearance quality, nutrition quality, milling quality, cooking and eating quality [7, 8]. Chalkiness is an important factor affecting rice appearance quality [9]. Previous studies have shown that chalkiness is closely related to starch content and structure in rice endosperm [10, 11]. Chalkiness area of rice is a kind of optical property caused by some cavities caused by loose arrangement of regional starch grains in endosperm, which is easy to be broken during processing [12]. The higher the chalkiness rate and the larger the chalkiness area, the lower the amylose content and protein content in endosperm, the worse the milling quality, the lower the transparency and head rice rate, and the lower the yield and eating quality of rice [2, 13-16]. Therefore, chalkiness is closely related to appearance quality, eating quality and yield [11, 17, 18]. Chalkiness is one of the most important quality traits in rice.

Modern nutrition medicine and clinical medicine research show that Fe, Zn, Se, Cu, Mn, Ca and Mg play an extremely important physiological and biochemical function in the body, in the formation of bone tissue, nerve impulse conduction, enzyme formation and activation, synthesis of some hormones and vitamins and a series of processes. It plays an irreplaceable role in the prevention and treatment of diseases, improving health and preventing aging [19], but these mineral elements must be taken from food. In recent years, the change of people's dietary pattern has led to the increasingly serious imbalance of mineral elements, which has become an important factor affecting human health. With the help of health care products, food additives or drugs, trace elements that human body lacks can be quickly replenished, but there are problems such as increasing investment, increasing cost and small coverage. Based on the above reasons, domestic and foreign scholars believe that bioaugmentation may be a safe and effective way to increase the content of trace elements in edible parts of crops [20]. Therefore, it is an effective way to solve the problem of global mineral nutrition imbalance to strengthen the breeding research of mineral rich rice varieties and improve the content of mineral elements in rice.

Plant is the main source of protein for human beings. Compared with other cereal crops, rice seed protein has relatively balanced amino acid composition, high content of lysine and threonine, and good performance in solubility, biological value and energy absorption. Therefore, rice seed protein has high nutritional value [20]. Therefore, the study of protein content in soft rice is one of the important directions to improve the nutritional quality of soft rice, which is of great significance to promote the healthy development of human body.

In this study, the chalkiness characters of endosperm, including white center, white belly and white back, were observed by SEM, and the contents of mineral elements and protein in the chalkiness and non-chalkiness parts of two soft rice varieties, Baxidabaigu and Erkuaigu, were detected by SEM-EDS The distribution of protein in soft rice can provide reference for breeding new varieties of soft rice with high quality in the future.

2. Materials and Methods

2.1. Test Materials

The main varieties of soft rice in Southern Henan were Baxidabaigu and Erkuaigu, respectively.

2.2. Experimental Design
2.2.1. Field Planting

According to the different growth stages of the two soft rice germplasm materials, they will be sown in batches in the same experimental field of Xinyang Normal University in 2020 to ensure that these Yunnan soft rice germplasm materials will bloom in the middle of August, and the growth conditions of their filling maturity period will be relatively consistent after flowering. Each soft rice variety was planted with 3 rows and 12 plants in each row, with the spacing of 16.5cm × 26.4cm. The two varieties of soft rice were cultivated and managed in common field from sowing, transplanting to final seed maturity. The seeds of soft rice were dried naturally and threshed by (5TS-150A) thresher. The seeds were stored at room temperature for 3 months.

2.2.2. Scanning Electron Microscope Observation

Ten dry seeds of each variety were selected. After removing the husk, four of them was selected, which was high-quality rice, heart chalkiness rice, belly white rice and back chalkiness rice. The selected brown rice was fixed with tweezers, and gently knocked with a knife back near the middle of the rice grain to make it break naturally under pressure. The broken part was cut off with a knife to make a sample about 2mm thick, which was glued to the copper sample table with conductive adhesive, coating (Platinum) on Eiko-IB5 Ion Sputter. The back, abdomen and middle part of the cross section of the rice grain were observed under S-4800 scanning electron microscope (Hitachi, Japan). The SEM images were obtained under high vacuum with 30kV accelerating voltage.

3. Results and Analysis
3.1. Element Distribution of Soft Rice

SEM-EDS can be used for in-depth analysis of rice, visualization and quantitative analysis of elements. The main nutrients in rice seeds are starch, storage protein, amino acids and lipids. Most of them are starch and protein, and the sum of their weight accounts for more than 90% of the dry weight of rice seeds [21, 22]. The results of scanning electron microscope (SEM) of the cross sections of soft rice Baxidabaigu and soft rice Erkuaigu are shown in Table 1 and Table 2, including the cross section of heart chalkiness rice, belly white rice and back chalkiness rice. The two abdomen and the center of rice seed of each cross section are selected to detect the element content, including the chalkiness and non-chalkiness parts of soft rice. According to the data in Table 1 and Table 2, in addition to a large amount of C, H, O elements, rice also contains a small amount of N, S, Mg, Al, P, K, Ca and other elements, which is consistent with the main substances in rice including carbohydrate, protein, lipid and water.

Comparing the data of A1-3 and B1-3 in Fig. 1, it was found that the content of N element in white belly area of seeds with white belly character was higher than that in non-chalky area, the content of S element in white belly area was lower than that in central area and higher than that in contralateral abdominal non chalky area, but the content of S element in abdominal white area was 0.05% lower than the average content of S element in non-white belly area by 0.10%; The contents of Mg, Al, P, K and Ca in white belly area were lower than those in non-chalky area, while Mn was only detected in white belly area. Comparing the data of A4-6 and B4-6 in Fig. 1, it was found that the content of N and S elements in seeds with white center character in heart white area was lower than that in non-chalky area; The contents of Al and P in the white center area were also lower than those in the non-chalky area, while the contents of K and Ca in the white center area were slightly higher than those in the white belly area, but lower than those in the contralateral white belly area. However, the contents of K in the heart area were 0.19% lower than those in the non-chalky area, 0.29% lower than those in the non-chalky area, and the contents of Ca in the white center area were 0.04% lower than those in the non-chalky area, 0.12% lower than those in the non-chalky area N element was only detected in the white center area. Comparing the data of A7-9 and B7-9 in Fig. 1, it was found that the mass fraction of N in the chalky area was lower than that in the abdominal non-chalky area, and the mass fraction of s in the seeds with back chalkiness was in the order of central chalkiness > abdominal non-chalkiness > abdominal chalkiness; The mass fraction of Al was central chalky area > abdominal non chalky area > abdominal chalky area. The mass fraction of P in chalky area was lower than that in non-chalky area. The mass fraction of K and Ca in chalky area was abdominal chalky area > abdominal non-chalky area > central chalky area. However, the average mass fraction of K in chalky area was 0.285%, which was less than that in abdominal non-chalky area (0.48%) The average content of Mg and Mn was 0.075%, which was less than 0.11% of K in non-chalky area of abdomen.

Comparing the data of C1-3 and D1-3 in Fig. 1, it was found that the content of N element in the seeds of white belly character of soft rice Erkuaigu showed opposite abdominal non-chalky area > white belly area > white central area, but the average content of N element in the non-chalky area was 1.375%, which was higher than that in the white belly area by 1.14%, and the content of S element in the white belly area was lower than that in the non-chalky area; the content of K in chalkiness area was higher than that in non-chalkiness area. The content of Mg, Al and Mn in non-chalkiness area was higher than that in chalkiness area. The content of P and Ca in contralateral abdomen was non-chalkiness area > white belly area > white center area. Zn was only detected in abdomen area of soft rice Erkuaigu; Comparing C4-6 and D4-6 data in Fig. 1, it was found that N and S contents were only detected in the abdominal non-chalky area in the seeds with white center character of soft rice Erkuaigu. The contents of N and s in the abdominal non-chalky area > white center area > contralateral abdominal non-chalky area, but the mass fraction of s in the white center area was 0.21%, which was less than the average value of 0.255% in the non-chalky area; the results showed that the content of P element in the abdominal non-chalky area > white center area > contralateral abdomen non-chalky area. The content of N, Al, Ca was only detected in the abdominal non-chalky area, but not Mg in soft rice Erkuaigu. Comparing the data of C7-9 and D7-9 in Fig. 1, it was found that the mass fraction of N and S

elements in the seeds of soft rice Erkuaigu with white back was smaller in chalkiness area than in non-chalkiness area; the mass fraction of Mg, P, K, Ca and Mn in non-chalkiness area was higher than that in chalkiness area. The mass fraction of K in white center area was 0.01% higher than that in contralateral abdominal non-chalky area, but the average mass fraction of K in chalkiness region was 0.12%, which was less than that in non-chalkiness area by 0.17%, the results showed that Al was only detected in the abdominal chalky area, Ca was only detected in the contralateral abdominal non-chalky area, and Zn was only detected in the central chalky area.

Because the substances of interest are carbohydrate, protein, lipid and water, nitrogen (N) and sulfur (S) in rice samples, which are the only element characteristics, are the best indicators of protein. According to the data analysis in Table 1 and Table 2, the content of N and S elements in most of the data of soft rice Baxidabaigu and Erkuaigu was less than that in non-chalky parts.

According to the experimental data, except for C, H, O, N and S, the other elements in soft rice Baxidabaigu and soft rice Erkuaigu include P, Al, K, Ca and Mn. Although the kinds and contents of elements in different rice are different, the content of P element is higher on the whole. The content of P, Al, K, Ca and Mn is P > Al > K > Ca > Mn. Most of the data in Table 1 and Table 2 showed that the contents of other elements such as Mg, S, K and Ca in the soft rice Baxidabaigu and soft rice Erkuaigu were lower than those in the non-chalky parts. The contents of most elements in chalkiness parts were less than those in non-chalkiness parts.

3.2. Protein Distribution of Soft Rice

The content and distribution of various elements were detected by SEM-EDS, which provided a preliminary evaluation for protein identification. N and S as the only element characteristics in rice samples are the best indicators of protein [23]. The content and distribution of N and S detected by SEM-EDS provide the basis for the preliminary analysis of protein content and distribution. SEM-EDS analysis showed that the content of N and S elements in the chalkiness part of soft rice Baxidabaigu and soft rice Erkuaigu was less than that in the non-chalkiness part. Therefore, it was preliminarily inferred that the protein content in the chalkiness part of soft rice Baxidabaigu and soft rice Erkuaigu was less than that in the non-chalkiness part.

According to the table data analysis, it can also be found that the contents of elements in the abdomen of the seed section of soft rice Baxidabaigu and soft rice Erkuaigu are mostly higher than those in the center of the rice seed except C, H and O, especially N and S. The average mass fraction of N was 0.85% in the ventral region of the seed section, and 0.00% in the central region; in Erkuaogu, the average content of N in the seed abdomen was 2.08%, while the average content in the seed center was 0.48%. The average mass fraction of s in the abdominal region of the cross section of the seeds of soft rice Baxidabaigu was 0.08%, while the average mass fraction in the central region was 0.13%. In the Erkuaigu, the average mass fraction of s in the abdominal region of the seeds was 0.16%, while the average mass fraction in the central region was 0.10%. The protein concentration in aleurone layer of rice seed was higher, and the total amino acid concentration decreased gradually from outer zone to inner zone.

4. Discussion
4.1. Element Distribution of Soft Rice

Rice contains mineral elements which are very important to human physiological and biochemical functions [19]. There are many research results on rice elements. Xue, et al. [24] pointed out that the contents of Fe in rice bran, husk, brown rice and milled rice were 8.6% ~ 43.0%, 3.9% ~ 9.5%, 0.2% ~ 5.2% and 0.2% ~ 2.8%, respectively. After de roughening, the content of Fe in rice decreased significantly, with a loss rate of 60%. Zhou, et al. [25] suggested that the order of Mn, Cu, Fe and Zn contents among glume, brown rice and milled rice was glume > brown rice > milled rice. The contents of Mn, Cu, Fe and Zn in glume were 7.0, 2.0, 7.0 and 1.4 times higher than those in milled rice. Wang, et al. [26] compared and analyzed the contents of six mineral elements (Ca, Mn, Zn, Fe, Cu and Se) in milled rice and rice bran of 36 rice varieties and Germplasm Resources in different environments (seasons and locations). The results showed that the average contents of six mineral elements in milled rice were Ca > Mn > Zn > Fe > Cu > Se, and those in rice bran were Ca > Mn > Fe > Zn > Cu > Se. Shao, et al. [27] and other researchers believe that the contents of nine elements in brown rice of different subspecies and varieties are different, but the order of contents is N > P > K > Mg > Ca > Zn > Fe > Cu > Mn; Fe content in non-glutinous rice, soft rice and glutinous rice was significantly different, and the ability of Fe absorption and accumulation in glutinous rice was significantly higher than that in non-glutinous rice and soft rice. Most of these studies on the elements in rice grains are related to the degree of grain grinding, and chalkiness is an important factor affecting the quality of rice, not only the appearance processing quality of rice, but also the cooking and eating quality [11, 17, 18, 28]. However, studies on the relationship between chalkiness and mineral elements are rare. The study of chalkiness and non-chalkiness elements in soft rice can lay a foundation for the cultivation of new soft rice varieties with good quality.

In this study, the contents of mineral elements in the cross section of Baxidabaigu and Erkuaigu soft rice grains with three different chalkiness characters, white center, white belly and back white, were detected by scanning electron microscope and energy dispersive spectroscopy (SEM-EDS). The results showed that there were a lot of C, H and O elements in Baxidabaigu and Erkuaigu soft rice, which were consistent with the main substances in rice, including carbohydrate, protein, lipid and water. The data in the table show that there are other elements such as N, S, Mn, Al, P, K and Ca in the soft rice Baxidabaigu and Erkuaigu soft rice. In addition to N and S, Mg, Al, K and Ca are also very important mineral elements for human body, and the content is Al > K > Ca > Mn. The contents of most elements in chalkiness parts of soft rice Baxidabaigu and Erkuaigu soft rice were less than those in non-chalkiness parts. It is different from the previous research results and needs further research.

4.2. Protein Distribution of Soft Rice

Compared with other cereal crops, the amino acid composition of rice seed protein is relatively balanced, and the protein shows good performance in solubility, biological value and energy absorption, with high nutritional value [20]. The protein content of rice is different from different parts. Generally speaking, the protein content in the bran layer and rice embryo is higher, and the deeper into the endosperm, the lower the protein content [29-32]. Resurrection, et al. [32] pointed out that 79.0-85.6% of the protein was mainly distributed in endosperm milk. Zhou, et al. [33] found that about 85% of the protein in brown rice of three different grain types was distributed in endosperm layer. With the increase of milling degree, the protein content gradually decreased, and it decreased to about half of the bran layer at the core layer, but the decreasing trend of different types of rice varieties was different. Chalkiness is one of the most important factors affecting the protein content in rice. It is generally believed that chalkiness is due to the loose arrangement of starch and protein particles in endosperm due to insufficient grain filling of rice seeds, which affects the light transmission [34-37].

In this study, the cross sections of rice grains with three chalkiness characters, namely, heart chalkiness, abdomen chalkiness and back chalkiness, of two soft rice varieties, Baxidabaigu and Erkuaigu, were scanned and observed. The content of N and s in different parts of rice was detected by SEM-EDS, and the protein content in corresponding parts was preliminarily inferred. The results showed that the contents of N and S elements in the non-chalky parts of Baxidabaigu and soft rice Erkuaigu were higher than those in the chalky parts, so it was inferred that the protein content in the chalky parts was less than that in the non-chalky parts. It is consistent with the previous research results of chalkiness. By analyzing the contents of N and S elements in the abdomen and the center of soft rice Baxidabaigu and soft rice Erkuaigu in the table data, we also found that, the content of N and S elements in the abdomen region was higher than that in the center region, which was consistent with the previous research conclusion that the protein content in aleurone layer was higher in rice seeds.

5. Conclusion

In addition to a large amount of C, H, O elements, soft rice seeds also contain a small amount of N, S, Mg, Al, P, K, Ca and other mineral elements. The element content of chalky parts is less than that of non-chalky parts. At the same time, the protein content of chalky part of soft rice seed was significantly less than that of non-chalky part, and the total amino acid concentration gradually decreased from the outer part to the inner part of the seed. In the area without chalkiness, the content of mineral elements is higher, and the content of protein is also higher. Therefore, reducing chalkiness in soft rice is beneficial to improve the quality of soft rice.

Acknowledgments

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Fig. 1 SEM-EDS test results of soft rice Baxidabaigu and Erkuaigu

A and B in Fig. 1 are the SEM-EDS results of morphological structure and elements of soft rice Baxidabaigu, respectively. C and D in Fig. 1 are the SEM-EDS results of morphological structure and elements of soft rice Erkuaigu, respectively.

Fig. A1-A3 and B1-B3 respectively correspond to the central area, white belly area and contralateral abdominal non-chalky area of soft rice Baxidabaigu chalky seeds with white belly in Table 1; Fig. A4-A6 and B4-B6 respectively correspond to the white center area, abdominal area and contralateral abdominal area of soft rice Baxidabaigu chalky seeds with white center in Table 1; Fig. A7-A9 and B7-B9 correspond to the white center area, white belly area and contralateral ventral non-chalkiness area of soft rice Baxidabaigu chalky seeds with white back in Table 1.Corresponding to Table 1.
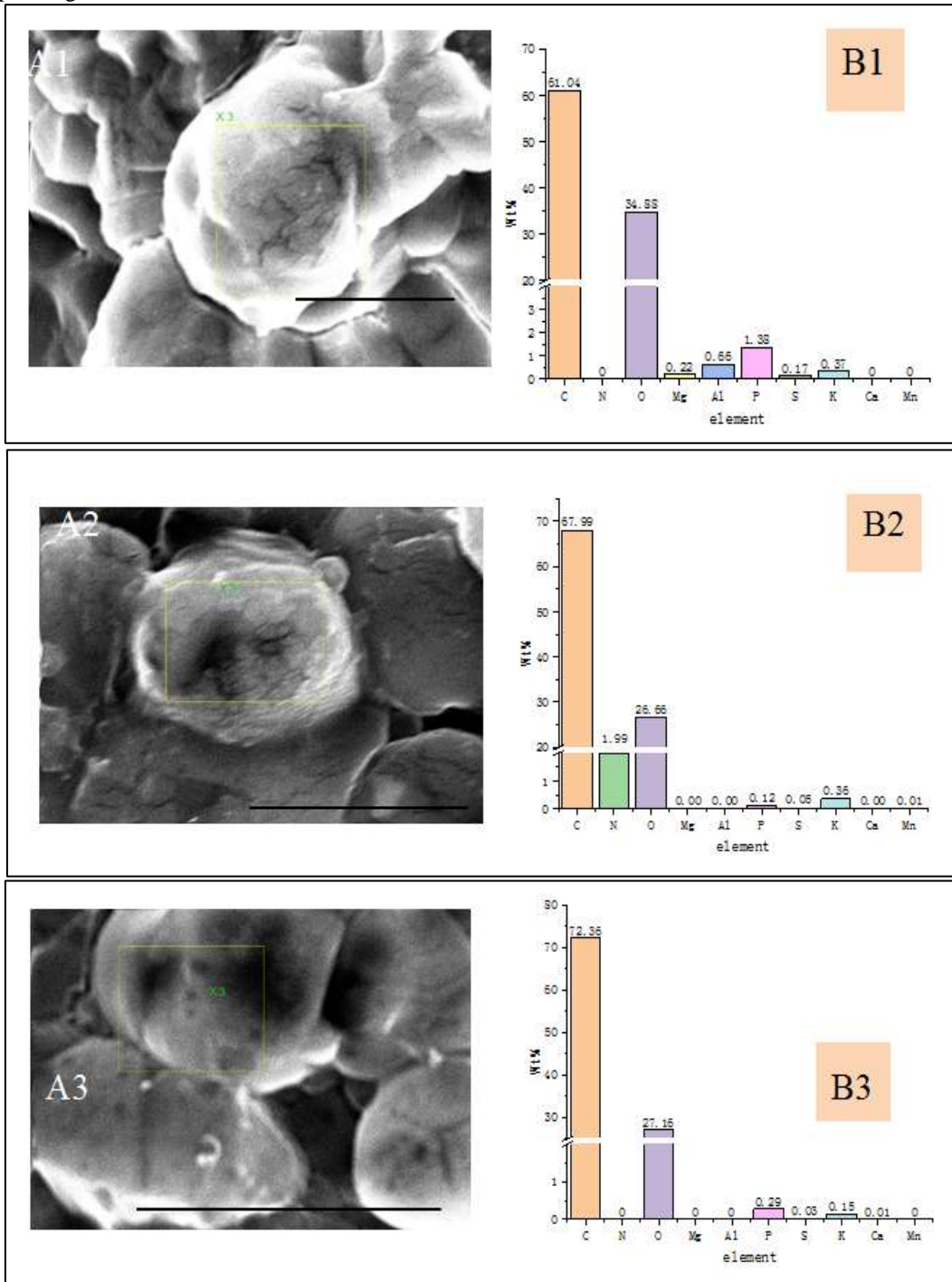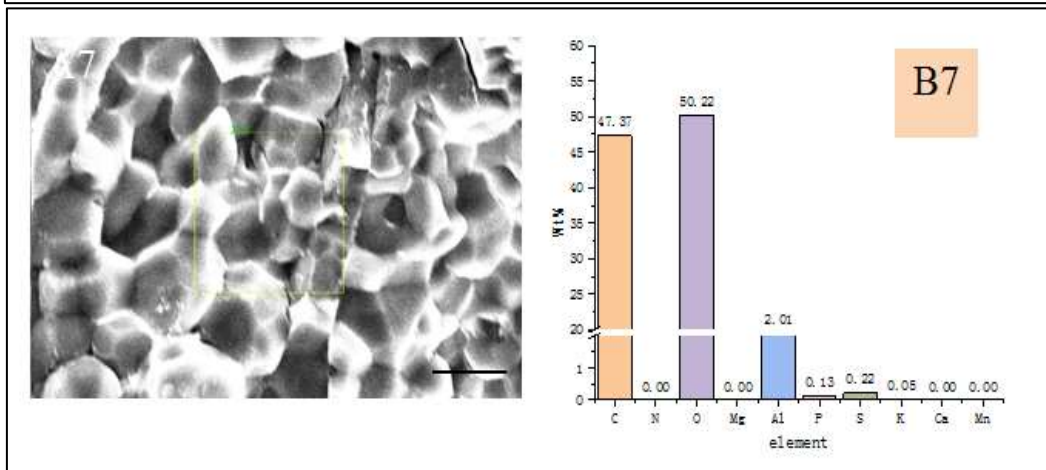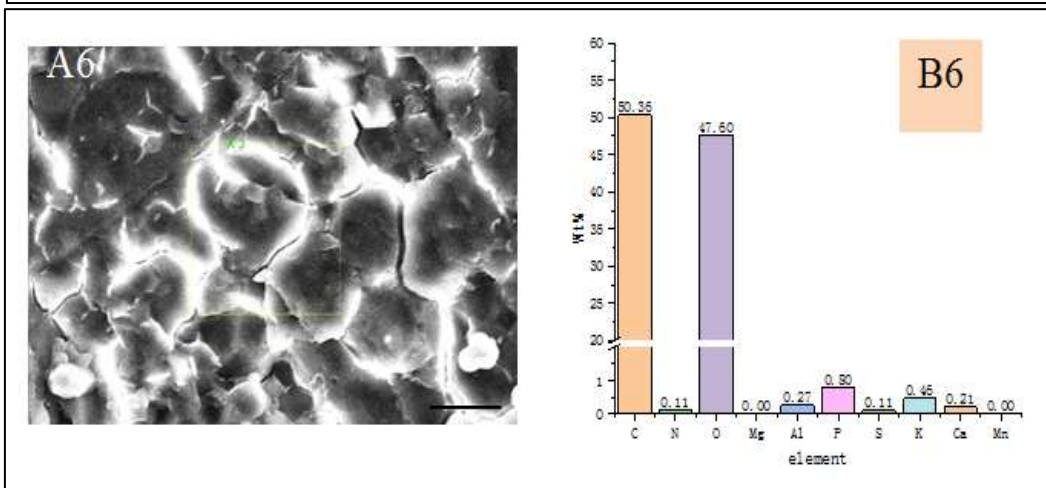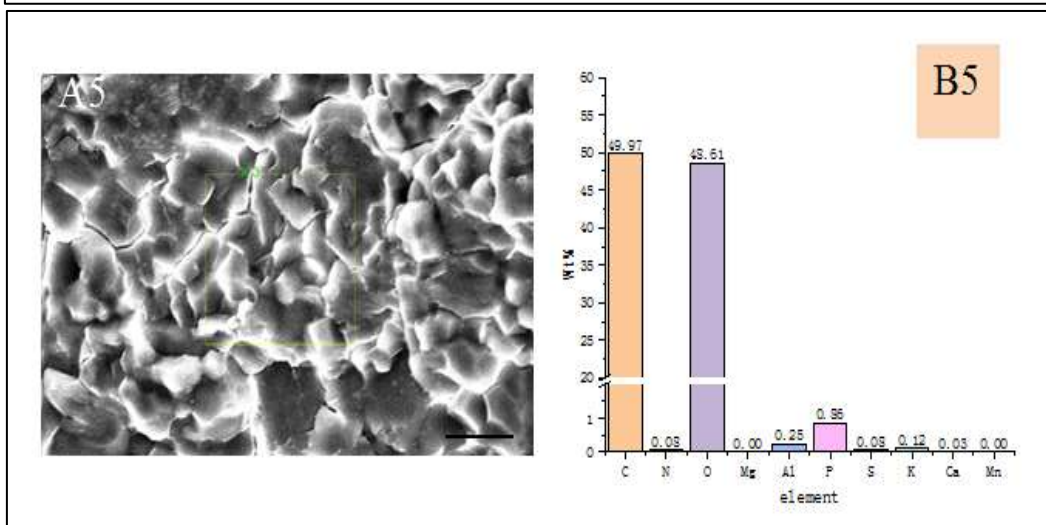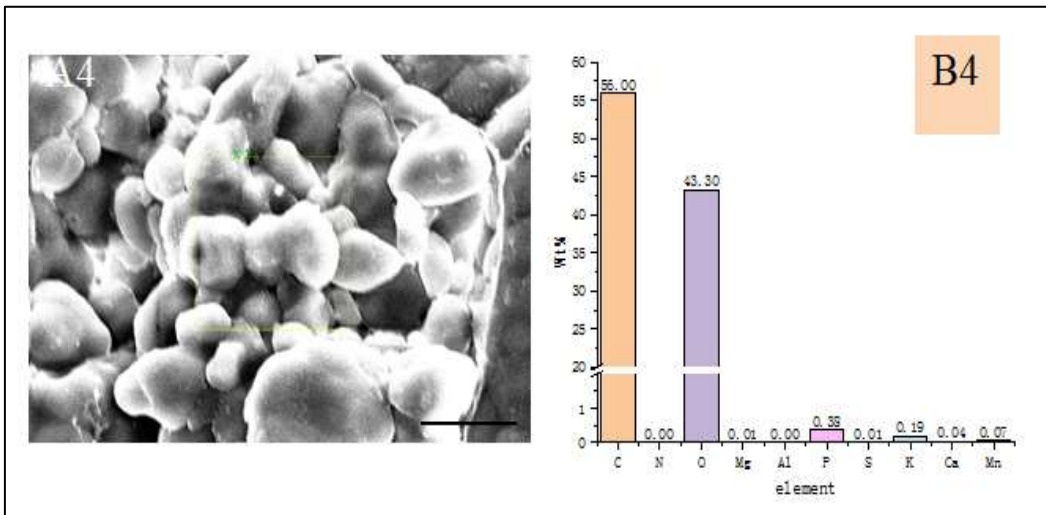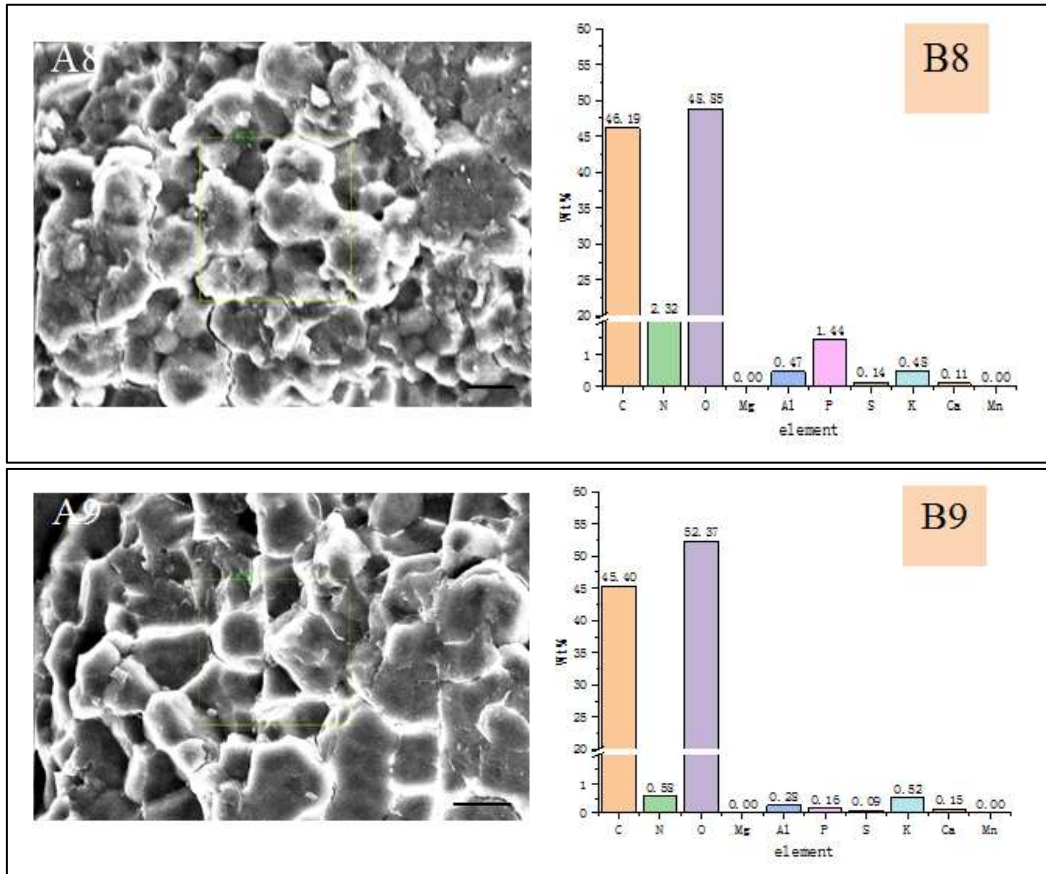
Fig. C1-C3 and D1-D3 correspond to the central area, white belly area and contralateral ventral non-chalky area of white belly seeds of soft rice Erkuaigu in Table 2; Fig. C4-C6 and D4-D6 respectively correspond to the white center area, non-chalkiness area in the abdomen and non-chalkiness area in the contralateral abdomen of white center seeds of soft rice Erkuaigu in Table 2; Fig. C7-C9 and D7-D9 respectively correspond to the central chalkiness area, abdominal chalkiness area and contralateral abdominal non-chalkiness area of soft rice Erkuaigu chalky seeds with white back in Table 2. Corresponding to Table 2. Bars=10μm
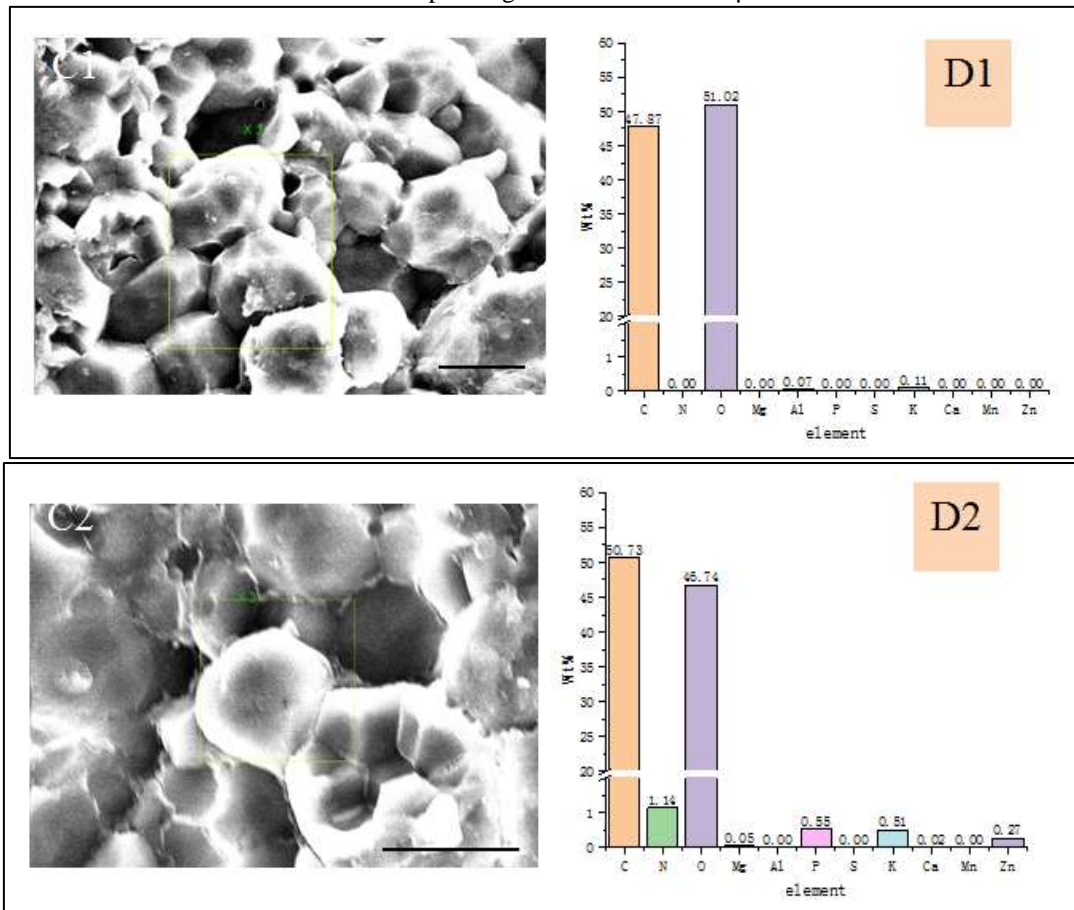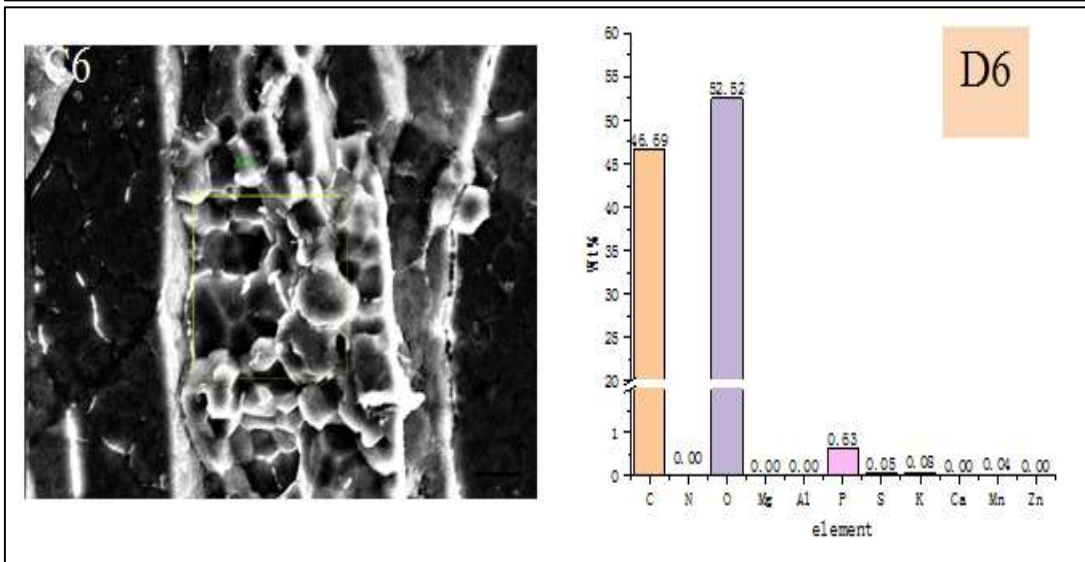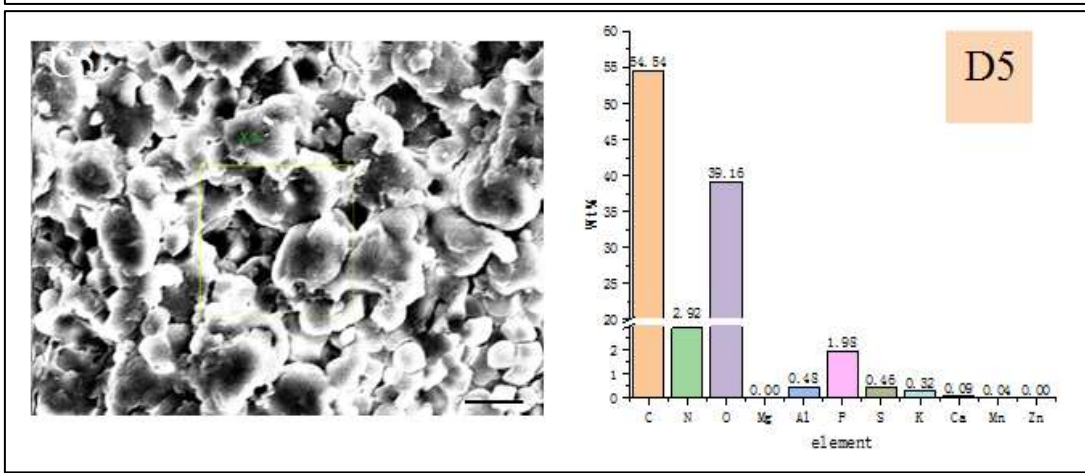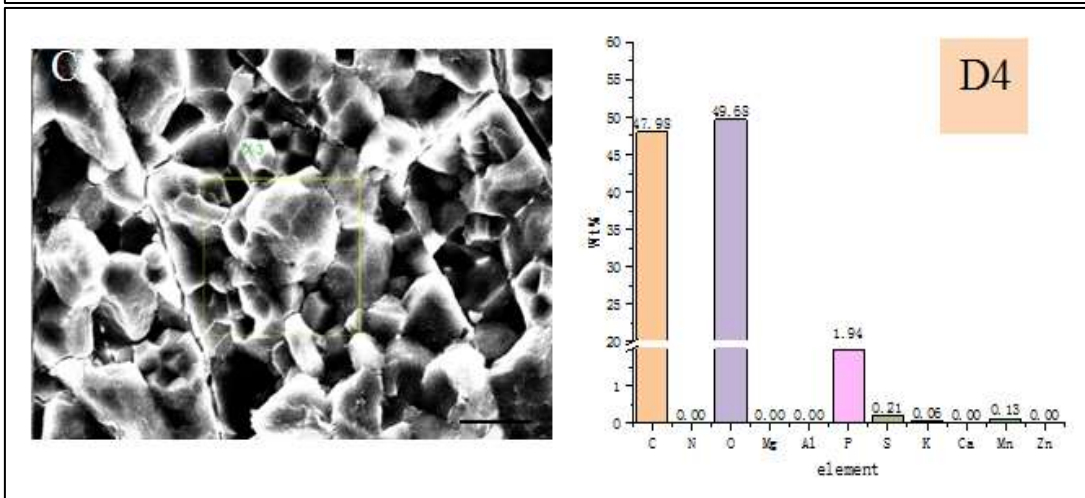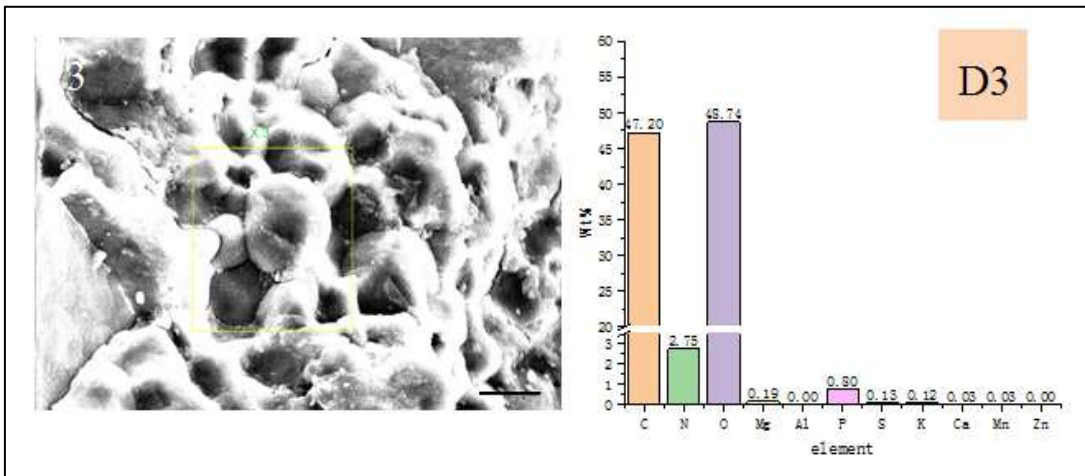
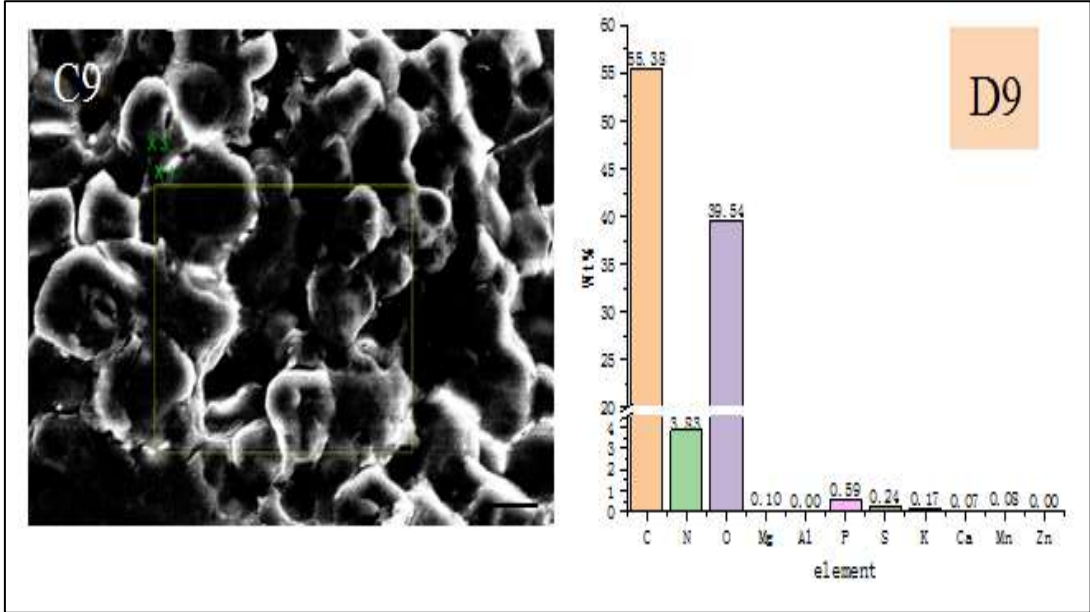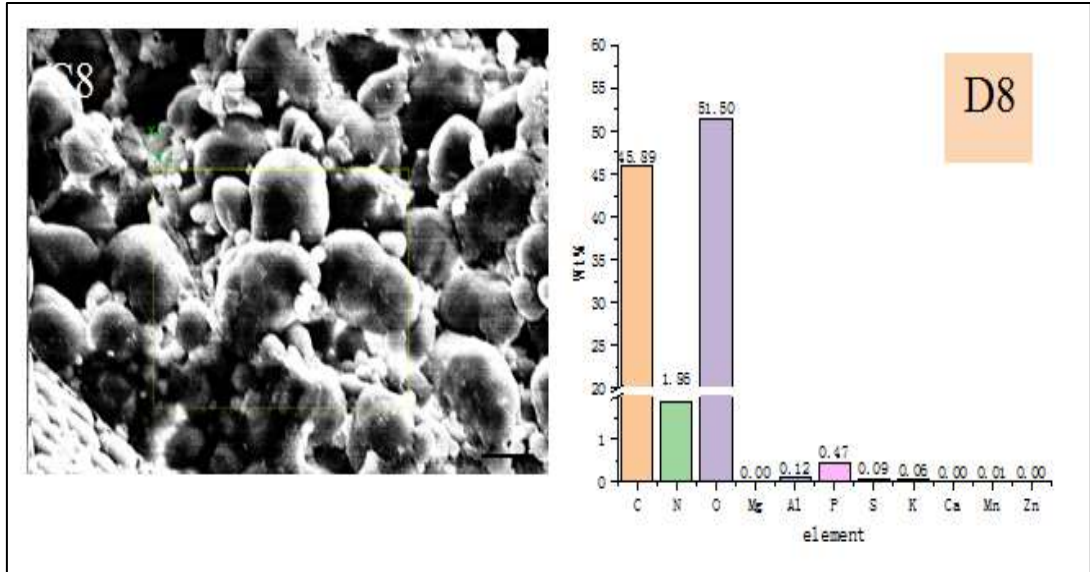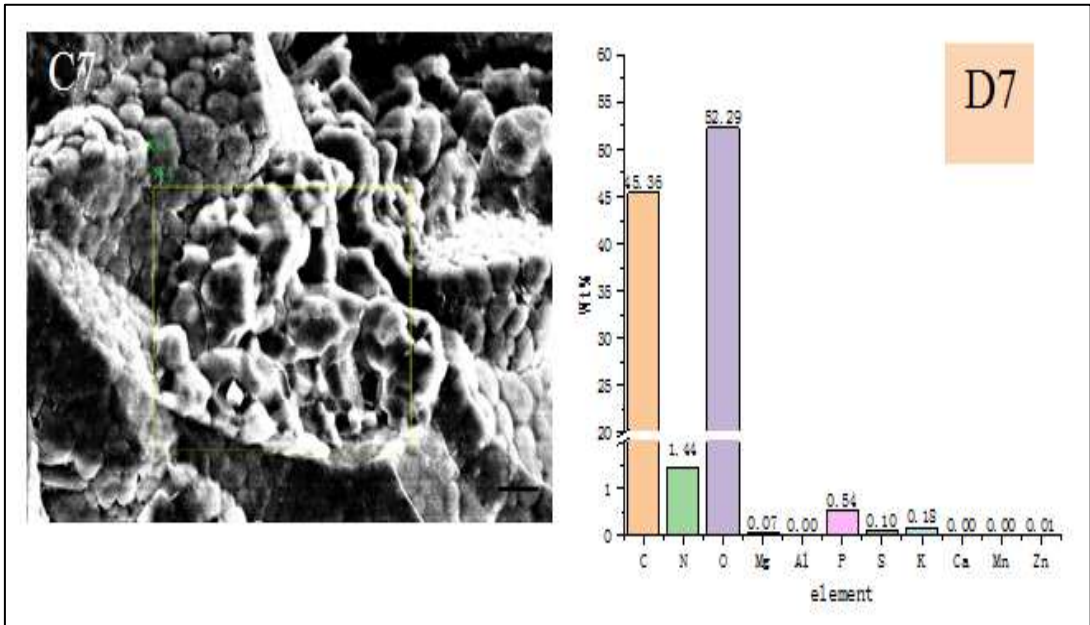**Table-1.** Element distribution of different chalkiness characters in soft rice Baxidabaigu

			C	N	O	Mg	Al	P	S	K	Ca	Mn
White belly	Central area	Wt.%	61.04	-	34.88	0.22	0.66	1.38	0.17	0.37	-	-
		At.%	68.76	-	29.50	0.12	0.33	0.60	0.07	0.13	-	-
	White belly area	Wt.%	67.99	1.99	26.66	-	-	0.12	0.05	0.36	-	0.01
		At.%	75.50	1.89	22.22	-	-	0.05	0.02	0.12	-	-
	Contralateral abdominal non-chalky area	Wt.%	72.36	-	27.16	-	-	0.29	0.03	0.15	0.01	-
		At.%	77.87	-	21.95	-	-	0.12	0.01	0.05	-	-
White center	White center area	Wt.%	56.00	-	43.30	0.01	-	0.38	0.01	0.19	0.04	0.07
		At.%	63.10	-	36.63	0.01	-	0.16	-	0.07	0.01	0.02
	Abdominal area	Wt.%	49.97	0.08	48.61	-	0.25	0.86	0.08	0.12	0.03	-
		At.%	57.40	0.08	41.92	-	0.13	0.38	0.03	0.04	0.01	-
	Contralateral abdominal area	Wt.%	50.36	0.11	47.60	-	0.27	0.80	0.11	0.46	0.21	-
		At.%	57.97	0.11	41.13	-	0.14	0.36	0.05	0.16	0.07	-
White back	Central chalky area	Wt.%	47.37	-	50.22	-	2.01	0.13	0.22	0.05	-	-
		At.%	55.09	-	43.85	-	0.91	0.06	0.08	0.02	-	-
	Non-chalky area of abdomen	Wt.%	46.19	2.32	48.85	-	0.47	1.44	0.14	0.48	0.11	-
		At.%	53.80	2.32	42.71	-	0.24	0.65	0.06	0.17	0.04	-
	Chalky area in abdomen	Wt.%	45.40	0.58	52.37	-	0.28	0.16	0.09	0.52	0.15	-
		At.%	52.91	0.58	45.81	-	0.14	0.28	0.04	0.19	0.05	-

Table-2. Element distribution of different chalkiness characters in soft rice Erkuaigu

			C	N	O	Mg	Al	P	S	K	Ca	Mn	Zn
White belly	Central area	Wt.%	47.87	-	51.02	-	0.07	-	-	0.11	-	-	-
		At.%	55.44	-	44.36	-	0.04	-	-	0.04	-	-	-
	White belly area	Wt.%	50.73	1.14	46.74	0.05	-	0.55	-	0.51	0.02	-	0.27
		At.%	58.15	1.12	40.22	0.03	-	0.24	-	0.18	0.01	-	0.06
	Contralateral abdominal non-chalky area	Wt.%	47.20	2.75	48.74	0.19	-	0.80	0.13	0.12	0.03	0.03	-
		At.%	54.47	2.72	42.23	0.11	-	0.36	0.05	0.04	0.01	0.01	-
White center	White center area	Wt.%	47.98	-	49.68	-	-	1.94	0.21	0.06	-	0.13	-
		At.%	55.69	-	43.29	-	-	0.87	0.09	0.02	-	0.03	-
	Non-chalky area of abdomen	Wt.%	54.54	2.92	39.16	-	0.48	1.98	0.46	0.32	0.09	0.04	-
		At.%	62.17	2.85	33.51	-	0.24	0.88	0.19	0.11	0.03	0.01	-
	Contralateral abdominal non-chalky area	Wt.%	46.69	-	52.52	-	-	0.63	0.05	0.08	-	0.04	-
		At.%	54.03	-	45.63	-	-	0.68	0.02	0.03	-	0.01	-
White back	Central chalky area	Wt.%	45.36	1.44	52.29	0.07	-	0.54	0.10	0.18	-	-	0.01
		At.%	52.63	1.43	45.54	0.04	-	0.24	0.04	0.07	-	-	-
	Chalky area of abdomen	Wt.%	45.89	1.86	51.50	-	0.12	0.47	0.09	0.06	-	0.01	-
		At.%	53.09	1.84	44.73	-	0.06	0.21	0.04	0.02	-	-	-
	Contralateral abdominal non-chalky area	Wt.%	55.38	3.83	39.54	0.10	-	0.59	0.24	0.17	0.07	0.08	-
		At.%	62.36	3.70	33.42	0.05	-	0.26	0.10	0.06	0.02	0.02	-