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Appropriating conditions for acquisition high-content α – amylase of germinated brown rice variety *Oryza stiva* Anhdao

Nghiên cứu điều kiện thích hợp để thu nhận gạo lứt nẩy mầm có hoạt độ α – amylase cao từ giống gạo lứt Anh Đào

Research article

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Brown rice is a food ingredient which has high nutritious values. During germination, some nutritious and functional components are increased such as lysine, vitamin E, B_1, B_6, magnesium, calcium, iron… and especially γ – amino butyric acid. Enzyme activity will also change during the germination of the grains. The α – amylase activity of ungerminated grains is very low, only 34.91 UI/g. This is because the enzyme is hibernating and not activated. During germination, enzyme activity will increase. Submerge the Anhdao brown glutinous rice for 6 hours at 30°C in solutions with different pH values (2.0, 3.0, 4.0, 5.0, 6.0). The results show that at pH 3.0 the activity of α – amylase enzyme reaches the highest value of 82.93 UI/g. After the submersion, incubate the germinated brown rice in unentirely anaerobic condition at different temperature of 25, 30, 35, 37°C. The result showed that at 35°C after 24 hours of incubation, the α – amylase activity reaches the highest value of 89.82 UI/g. Examine the dynamic of changes of α – amylase activity against time at 35°C, we can see that in the first 28 hours the α – amylase activity increased significantly. Highest α – amylase activity reaches 97.10 UI/g after 28 hours of incubation. In reality, people usually use enzyme from germinated grains for many food manufacturing industries. α – amylase activity increases during incubation, which can bring promising prospects for processing sugar syrup and prebiotics food from germinated rice.

Gạo lứt là một nguyên liệu thực phẩm có giá trị dinh dưỡng cao. Trong quá trình nảy mầm các thành phần dinh dưỡng và chức năng của hạt gạo lứt được tăng lên ví dụ như lysine, các vitamin E, B1, B6, magie, canxi, sắt... và đặc biệt là γ – amino butyric acid. Hoạt tính của hệ enzyme sẽ thay đổi trong suốt quá trình nảy mầm của hạt gạo. Hoạt tính enzyme α – amylase của hạt gạo chưa nảy mầm là rất thấp, chỉ đạt 34,91UI/g, do enzyme của hạt đang ở trạng thái ngủ chưa được kích hoạt. Trong quá trình nảy mầm thì hoạt tính của α – amylase tăng lên. Tiến hành ngâm gạo lứt giống nếp Anh Đào trong 6 tiếng, ở 30°C trong nước ngâm có pH khác nhau (pH 2.0, 3.0, 4.0, 5.0, 6.0). Kết quả cho thấy, ở pH 3.0 hoạt độ enzyme α – amylase cao nhất đạt 82,93 UI/g. Sau quá trình ngâm, tiến hành ủ nảy mầm gạo lứt yếm khí không hoàn toàn ở những nhiệt độ khác nhau 25, 30, 35, 37°C, kết quả ở 35°C sau 24 giờ ủ hoạt độ enzyme α – amylase đạt cao nhất là 89,82 UI/g. Khảo sát động học sự thay đổi của hoạt độ enzyme α – amylase theo thời gian ở nhiệt độ ủ 35°C, kết quả cho thấy,trong 28 giờ đầu hoạt độ của α – amylase tăng mạnh. Hoạt độ α – amylase cao nhất đạt 97,10 UI/g sau 28 giờ ủ. Trong thực tế người ta đã sử dụng enzyme từ hạt nảy mầm cho rất nhiều ngành sản xuất thực phẩm. Hoạt độ của α-amylase sau quá trình ủ mầm tăng lên, có thể đem lại triển vọng sử dụng để chế biến dịch đường và các sản phẩm có tính prebiotic từ gạo lứt nảy mầm.

Keywords: α-amylase, germinated brown rice , germination.

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1. Introduction

Rice is one of the major food source for half of the population in the world. Nowadays, the society is developed, life condition is improved, rice is not just a conventional food source but it can also be used as a functional food. Many studies showed germinated brown rice (GBR) has higher contents of nitritious and bio functional compounds compare to the conventional rice [1],[2],[7]. Therefore, GBR can be considered as a highly nutritious and healthy food source compare to the milled white rice and it can also supply human demands.

Amylase is one of the most common enzymes in nature. It hydrolyses starch into monosaccharides, aids the digestion of brown rice [7], supplies energy for biochemical processes, including the biosynthesis of functional compounds such as Gamma-aminobutyric acid (GABA) [13,14], and plays important roles in hydrolyzation and saccharification process in beverage industry [9]. Many studies were carried out in order to increase GABA and other nutritious content in brown rice. Many germinating conditions give different amylase contents. Submersing and germinating process are some of the most popular methods to increase the GABA and amylase content in germinated brown rice [14].

2. Materials and research method

2.1 Study sites

Studies were carried out in laboratories at Faculty of Biotechnology and Food Technology, Hanoi University of Science and Technology. In this study, we examined factors that affect the germination and enzyme activity: pH, temperature, time, to determine the most suitable condition for highest α – amylase activity.

2.2 Materials and chemicals

Anhdao glutinous rice, crossbed and selected by Dr. Tan Dao Xuan, former Dean of Faculty of Biotechnology, Hanoi Pedagogical University 2, is commonly cultured in some provinces such as Bacninh, Phutho, Ninhbinh, Thaibinh… Anhdao rice was brought at Thaibinh Seed Corporation (ThaiBinh see), this species has high yield of product (8 tonnes/ha), fine grains, sticky rice, good smell, tasty and good resistance against pathogenic worms. Anhdao brown rice was collected after the outer hull removed, preservation time of 30 days in cool refrigerator (4 - 6^{o}C).

Chemicals used: DNS (dinitrosalicylic acid), HCl, Na_2HPO_4, KH_2PO_4, $NaCH_3COO$, $Na_3C_6H_5O_7$ (sodium citrate), I_2, KI purchased from Merck company, Germany, starch and other Chinese chemicals.

2.3 Research method

2.3.1 Experimental method

Submersion and germination of Anhdao brown rice: Took 50 grams of hull removed brown rice, submerged in Sodium chloride 0.9% for 30 minutes. Then submerge in 100mL of tap water at 30^{o}C, took sample after 2, 4, 6, 8, 10 hours and determined the saturated humidity to have the suitable submerge time for the incubation process. After determination the suitable submerge time, continued determining the amylase activity with citrate submerge buffers which had different pH values of 2, 3, 4, 5, 6 at 30^{o}C. The sample was then incubated in not entirely anaerobic condition at 35^{o}C for 24 hours.

Determine the suitable temperature and time for the highest enzyme activity: To achieve high α- amylase activity, submerged brown rice with suitable time and submerge solution with suitable pH value. After that, continued to examine the suitable incubation temperature at 25, 30, 35, 37^{o}C for 24 hours and examined the suitable incubation times of 20, 24, 28 hours at the previously examined temperature. The samples at periods of times were determined for the α- amylase activity.

2.3.2 Determination of alpha amylase activity

α – amylase activity was determined by Rukhliadeva – Geriacheva (1979) method [15]. The method is based on the hydrolyzation of starch by enzymes of amylase group into dextrin with different molecular weights. Measured the color intensity at 656 nm of starch and hydrolysed products with iodine by Spectrophotometer to determine enzyme activity. An unit of enzyme activity is defined as the amount of the enzyme that produces a certain amount of enzymatic activity, that is, the amount that catalyzes the conversion of 1 micromole of substrate per minute in standard condition (30^{o}C, 10 minute).

2.3.3 Determination of nutritious compositions in Anhdao brown rice

Method to determine the ash content: The ash content was determined by combustion at 600^{o}C for 4 hours Le Thanh Mai [5]. Method of determining the humidity content: The humidity content was determined by drying until reaching constant weight at 105^{o}C [5] with Memmert UN55 oven, Germany.
Method to determine the Carbohydrate content: First hydrolyse the sample with acid then determine by DNS method, according [5].
Method to determine the total protein content: Kjeldahl method [16].
Method to determine the total lipid content: Using Soxhlet method [17].

2.3.4 Data analyzing method

Experiments were repeated 3 times. The data then were analyzed by Microsoft Office Excel 2007 with the error under 0.05.

3. Result and discussion

3.1 Determination of nutritious compositions in Anhdao brown rice

Nutritious compositions in Anhdao brown rice are showed in Table 1 (Humidity of brown rice 11,6± 0,31). The result shows that, this strain has similar nutritious contents compare to others glutinous rice strain in Vietnam.

Table 1. Nutritious compositions in Anhdao brown rice

Nutrition information	Content, % (by dry weight)
Starch	86.11 ± 0.46
Total protein	8.57 ± 0.21
Total fat	2.72 ± 0.15
Ash	1.53 ± 0.07

3.2 Determine suitable saturated water absorption time for Anhdao brown rice

Anhdao brown rice was submerged with distilled water to gain saturated state for the best germination. According to the study result, the humidity of the grains from 2 to 4 hours increased quickly, then the grains began to absorb water slowly. This is maybe because the starch compounds is swelled. The gaps between compounds decrease, the grain begin to absorb water slowly. After 6 hours, the humidity reached saturated value. After that, moisture content increased insignificantly (Figure 1).

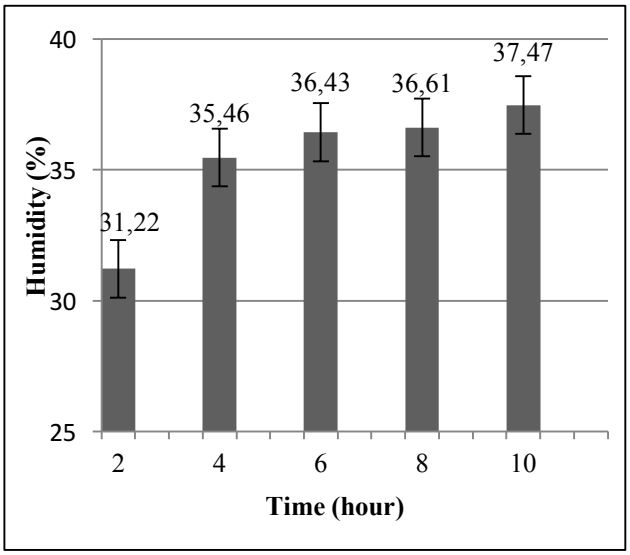

Figure 1. Effect of submerge time on humidity

According to Bello et al. [3], rice grains absorb water and reach saturated state after 5 hours of submersion and Banchuen et al. [2] 5 to 7 hours of submersion. Besides those, some studies of Duy Nguyen Le Doan [10] on 2 different species of brown rice IR 50404 and Jasmine 85 showed that brown rice reaches saturated state after 6 hours. These results fit the experiments on the Anhdao brown rice despite of different strains of rice.

3.3 Effect of pH value of submerge solution on α-amylase activity

Effect of pH value on grains is showed in Figure 2. The results showed that the sprout sizes are not so different. Sprouts which were submerged at pH 3.0 and pH 4.0 grow finest. Result shows that highest enzyme activity was reached at pH 3.0 then decreased from pH 4.0 to 6.0. At pH 2.0, enzyme activity had lowest value (Figure 3).

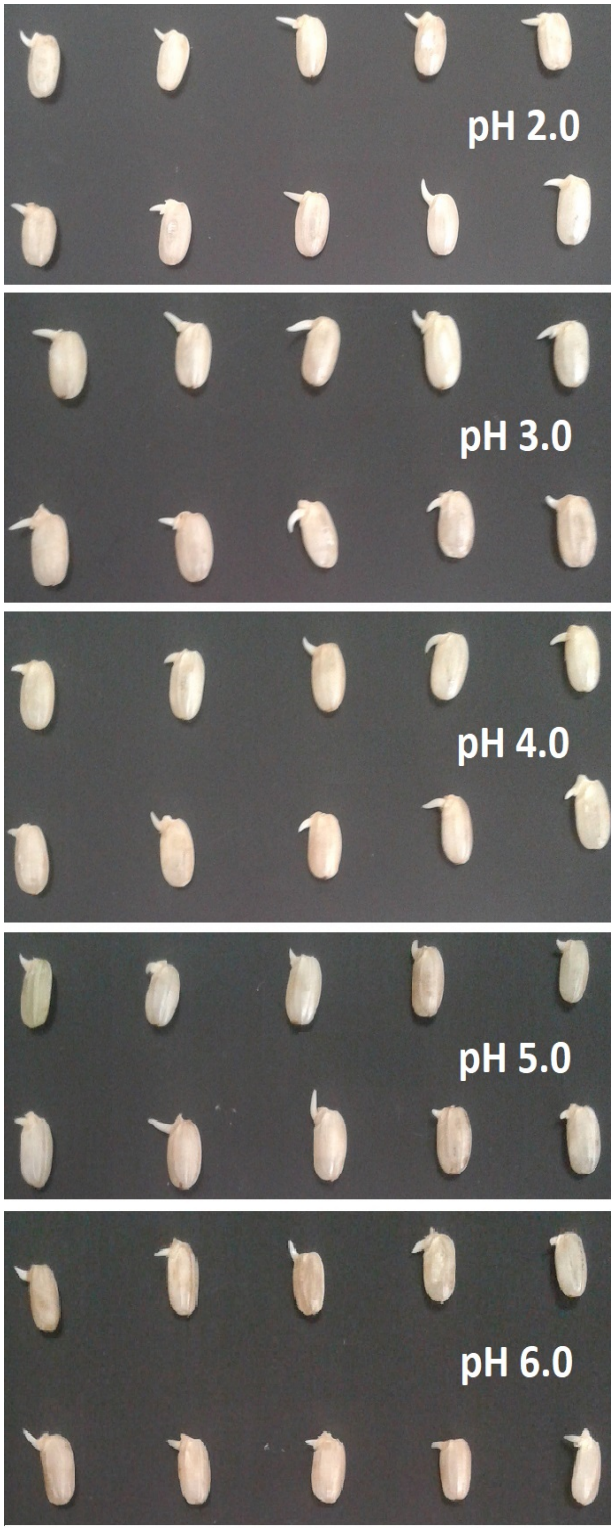

Figure 2. Grains with different germinating conditions

As we know, suitable pH range for formation and activation of α – amylase in many strains is different. During germintation, the changing of many sugars, starch in many strains depends on α-amylase activity in the grain. In this study, at pH 3.0 Anhdao brown rice had highest enzyme activity of 82.93 UI/g. According to the result of Le Nguyen Duy Doan el. al. [10], suitable pH of the submerge solution for high enzyme activity is 4.0 with MBĐ strain and 3.0 with IR50404 strain. So, pH 3.0 was chosen for the next studies.

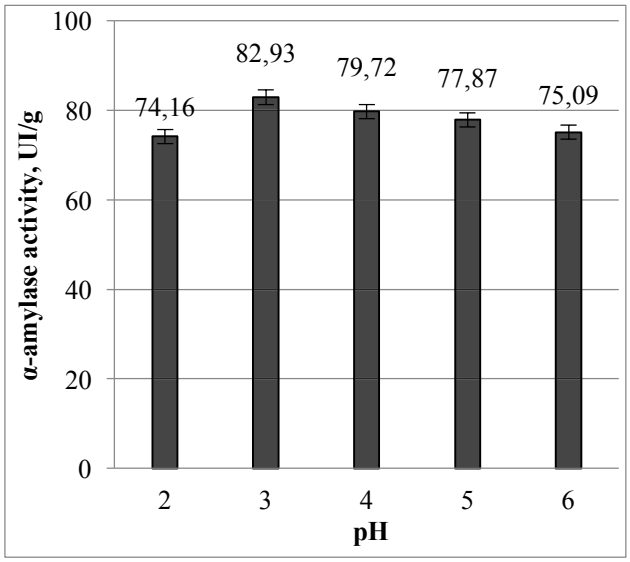

Figure 3. Effect of pH of submerge solution on α - amylase activity

3.4 Effect of incubation temperature on α - amylase activity

The result is presented on Figure 4. that the enzyme activity increased during germination. Temperature had huge effect on α – amylase activity during germination of brown rice. At different incubation temperatures, α – amylase activity changes. α – amylase activity in brown rice before germination was quite low, only reached 34.91 UI/g. After germination, α – amylase activity increased 3 times. Incubation at 35^{o}C gave the highest α – amylase activity of 89.82 UI/g. This result fits the study result of Le Nguyen Duy Doan et al. [10].

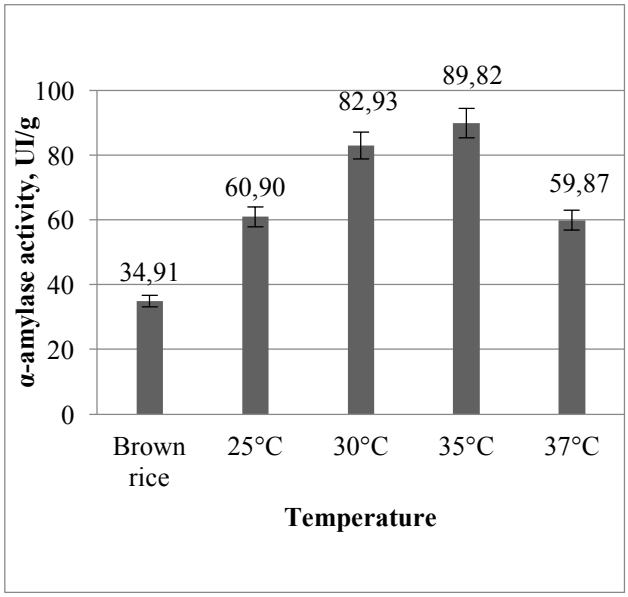

Figure 4. Effect of incubation temperature on amylase activity

3.5 Effect of incubation time on enzyme activity

Incubation time has huge effect on α- amylase content and hydrolyzied products during germination [1], [13], [14]. Experiments were carried out in 4 periods of times (20, 24, and 28 hours) at pH 3 and 35^{o}C, the results is showed in Figure 5 . It indicates that 28 hours of incubation gave the highest α – amylase activity (97.10 UI/g), almost 3 times higher than the activity in ungerminated grains. According to Premsuda [13], determination on 2 Thai strains, the highest α – amylase activity was achived after 3 days of incubation in aerobic condition, about 30 U/g. α – amylase activity differs in incubation processes maybe due to differences in strain's characters or different incubating conditions.

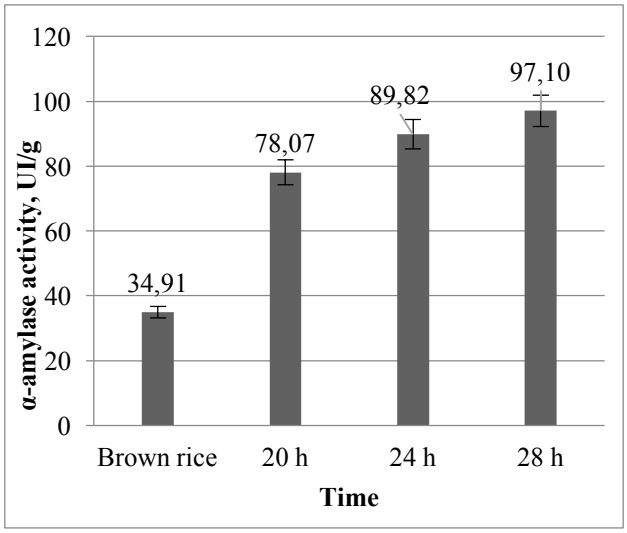

Figure 5. Effect of incubation time on alpha amylase activity

4. Conclusion

The study result shows that α-amylase activity from Anhdao brown rice depends on many factors. Time needed for the absorption of water to achieve the best condition for germination was determined at 6 hours at 30^{o}C. Incubation of Anhdao brown rice was carried out in unentirely anaerobic condition. The result shows that at pH 3.0 α-amylase activity has the highest value of 82.93 UI/g. Incubation temperature of 35^{o}C gives the highest α-amylase activity of 89.82 UI/g. α-amylase activity reaches the highest value of 97.10 UI/g after 28 hours of incubation, α-amylase activity after incubation is remarkably high, about 3 times higher compare to the activity of ungerminated grain. This is a promising sourse of enzyme for the hydrolization of starch, processing nutrtious food products from germinated brown rice.

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