International Journal of Multidisciplinary Research and Development

IJMRD 2014; 1(5): 90-93
www.allsubjectjournal.com
Received: 09-09-2014
Accepted: 22-09-2014
e-ISSN: 2349-4182
p-ISSN: 2349-5979

Nguyen Phuoc Minh
Tra Vinh University, Vietnam

Biosynthesis of amylase from *Aspergillus oryzae* using purple fragrant rice ST as growth substrate

Nguyen Phuoc Minh

Abstract
Purple fragrant rice ST is an heirloom variety of rice that contains high levels of pigments, which make each grain appear dark purple to black in color. Consuming purple fragrant rice provides our body with a number of nutrients, including some compounds not found in white or brown rice. *Aspergillus* species have attracted attention for their role in fermentation of oriental food products or industrial application of hydrolytic enzymes. Within the range of carbohydrolases produced by this species, the majority has been considered as α -amylase and glucoamylase. Purpose of our research is to utilization of purple fragrant rice ST as growth substrate for *Aspergillus oryzae* fermentation to biosynthesize amylase. This approach is to comprehensively exploit nutrient elements inside purple fragrant rice ST, contributing to its value-added enhancement. We have examined moisture content in raw material (purple fragrant rice ST) for fermentation, culturing temperature and time for *Aspergillus oryzae* proliferation and growth to biosythesize amylase. Further more, we also investigate the drying temperature to keep *Aspergillus oryzae* living and maintain amylase enzyme activity. Our results show moisture content in raw material 60%; culturing temperature 30 ^{o}c in 2 days, drying temperature for finished product 50 ^{o}c, 16 - 18h.

Keywords: *Aspergillus oryzae*, purple fragrant rice ST, biosynthesis, amylase.

1. Introduction

Purple fragrant rice ST contains a lot of nutritious elements such as amino acids, minerals and antioxidants. This purple fragrant rice contains about 75% of starch, with a very high mineral content, rich in amino acids that specifically contain large amounts of anthocyanins with the antioxidant effect good for the health of users. When purple rice is cooked, the bran gives it a nutty flavor and adds to its dietary fiber. Purple rice can be found in many health food stores and organic food stores. According to analysis results from CASE (Vietnam), the main components in the purple fragant rice ST include calcium (81.8 mg/kg), iron (11.3 mg/kg), potassium (2470 mg/kg), dietary fiber (6.13%), anthocyanin (1,296 mg/kg).

Studies on fungal amylase especially in the developing countries have concentrated mainly on *Aspergillus* species probably because of ubiquitous nature and non-fastidious nutritional requirement of this organism (Abu et al 2005 and Gomes *et al*., 2005). The exclusive production of glucoamylases is achieved by Aspergillus Niger (Wang *et al*., 2006) and *Aspergillus oryzae* in enzyme industry (Biesbeke *et al*., 2005). Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Spectrum of application of amylases has widened in many sectors such as clinical, medicinal and analytical chemistry. Besides, their use in starch saccharification, they also find applications in baking, brewing, detergent, textile, paper industries and distilleries (Ramachandran *et al*., 2004). Glucoamylases are industrially important hydrolytic enzymes of biotechnological significance and are currently used for dextrose production, confectionary, baking and in pharmaceuticals (Pandey *et al*., 2000).

There are a large number of studies on the α-amylases of *Aspergillus* sp. Two kinds of acid stable α-amylase from *Aspergillus kawachii* have been reported (Mikami *et al*., 1987; I. Akpan *et al*., 2004). There has also been reported an acid-stable and an unstable α-amylase from *Aspergillus niger* (Arai *et al*., 1968; Adekunle Odunayo Adejuwon *et al*., 2012), crystals of *Aspergillus oryzae* enzyme (Fischer and Montmollin, 1951; Akabori *et al*., 1954; Makiko Kariya *et al*., 2003; Mohamed Abdel Fattah Farid *et al*., 2011), and some properties of *A. Awamori* α-amylase (Bhella and Altosaar, 1984; Takayoshi Matsubara *et al*., 2004). *A. Awamori* produces several potent amylolytic enzymes for bioindustry, and the mold also

Correspondence:
Nguyen Phuoc Minh
Tra Vinh University, Vietnam

produces three forms of α-glucosidases (Yamazaki *et al.*, 1977) and two types of glucoamylases (Shruti Puri *et al.*, 2013). These are glucoamylase I that can be adsorbed onto raw starch, and glucoamylase II that can not be adsorbed (Ueda, 1981). There are several microorganisms that have been found to produce raw starch digesting amylolytic enzymes. *A. Awamori* (Ueda *et al.*, 1974) and *A. Oryzae* (Miah and Ueda, 1977) produced glucoamylases having activity for adsorption and digestion of raw starch.

Purpose of our research is to comprehensively exploit nutrient elements inside purple fragrant rice ST, contributing to its value-added enhancement. We have examined moisture content in raw material (purple fragrant rice ST) for fermentation, culturing temperature and time for *Aspergillus oryzae* proliferation and growth to biosythesize amylase. Further more, we also investigate the drying temperature to keep *Aspergillus oryzae* living and maintain amylase enzyme activity.

2. Material & Method

2.1 Material

Purple fragrant rice ST is originated from Tra Vinh province, Vietnam. Its specifications are as follows: maximum moisture 15%; broken ration 4%; aflatxin B1, B2, G1, G2 < 0.2 µg/kg; packed into 5 kg PP bags with PE inside. *Aspergillus oryzae* is supplied from Pasteur Institute, HCM City, Vietnam.

Fig 1: Purple fragrant rice ST

2.2 Research method

2.2.1 Experiement 1: Effect of moisture content, temperature and time in culturing medium for *Aspergillus oryzae* fermentation

Purple fragrant rice is well dipped in water to discard foreign matter and damaged grain, absorb water into its kernel. Then it is cooked, adjusted moisture and sterilized steaming. Following that, it is cooled, mixed and cultured at different moisture contents, temperatures and times.

Factor A: temperature (A1: 27 ^{o}c; A2: 30 ^{o}c; A3: 35 ^{o}c). Factor B: moisture (B1: 50%; B2: 60%; B3: 70%). Factor C: time (C1: 1 day; C2: 2 days; C3: 3 days). Total testings: 3x3x3=27. Total samples: 27x3=81

2.2.2 Experiment 2: Effect of drying condition to amylase activity

Aspergillus oryzae is cultured at suitable conditions, vacuum dried to maintain enzyme activity. Drying is performed in three temperatures: 40 ^{o}C, 45 ^{o}C, 50 ^{o}C. Total testings: 3; total samples: 3. Amlyase activity is checked again one more time.

2.3 Testing method

Aspergillus oryzae is cultured on starch gelatination at different moisture contents and times. Amylase activity from *Aspergillus oryzae* is periodically checked by measuring optical density after being prepared with iodine.

2.4 Statistical analysis

All data are processeed by Excel 2003.

3. Result & Discussion

3.1 Effect of moisture content, temperature and time in culturing medium *for Aspergillus oryzae* fermentation

Temperature (^{o}C)	Moisture content (%)	Time (day)	Activity (unit/g)
27	50	1	4.382
27	50	2	6.808
27	50	3	7.963
27	60	1	5.381
27	60	2	6.354
27	60	3	8.459
27	70	1	1.926
27	70	2	4.646
27	70	3	4.574
30	50	1	5.934
30	50	2	7.078
30	50	3	6.273
30	60	3	7.328
30	70	1	5.014
30	70	2	6.372
30	70	3	6.247
35	50	1	2.759
35	50	2	6.495
35	50	3	6.052
35	60	1	3.268
35	60	2	6.349
35	60	3	5.054
35	70	1	4.705
35	70	2	2.546
35	70	3	2.259

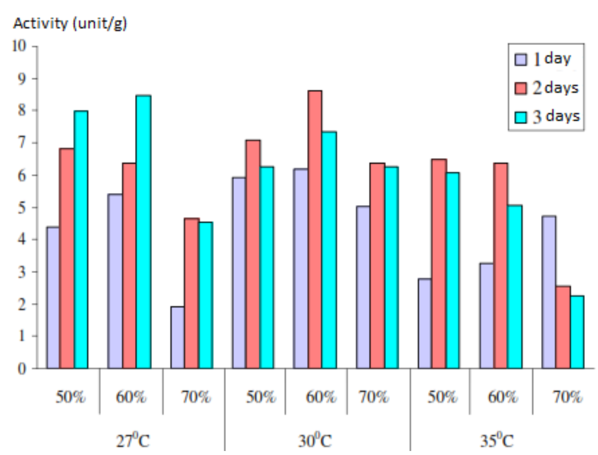

Fig 2: Amylase activity

From figure 2, we can see the highest activity of amylase after 2 days of culture at temperature 30 ^oc, 60% moisture. Amylase is also strongly produced after 3 days of culture at temperature 27 ^oc and 60% moisture. At 35 ^oC, *Aspergillus oryzae* is prohibited to grow to produce amylase, especially at high moisture content (70%). At 27 ^oC, amylase activity increases slightly day by day, the amylase activity in 3^{rd} day is higher than the first two days. At 30 ^oC, the amylase activity is higher than at 27 ^oc and 35 ^oc. So we can conclude the optimal condition for *Aspergillus oryzae* growth is performed well at 30 ^oc, 60% moisture in the 2^{nd} day.

Table 2: Amylase activity change by temperature, moisture and time of culturing

Temperature (^oC)	Culturing time (day)			Moisture (%)
	1	**2**	**3**	
27	4.382^{i}	6.808^{cde}	7.963^{ab}	50
27	5.381^{gh}	6.354^{ef}	8.46^{a}	60
27	1.926^{l}	4.646^{i}	4.547^{i}	70
30	5.933^{fg}	7.077^{cd}	6.273^{ef}	50
30	6.164^{ef}	8.614^{a}	7.328^{bc}	60
30	5.014^{hi}	6.372^{def}	6.25^{ef}	70
35	2.76^{jk}	6.494^{def}	6.052^{fg}	50
35	3.268^{j}	6.35^{ef}	5.054^{hi}	60
35	4.705^{hi}	2.545^{kl}	2.259^{kl}	70

From table 2, we see that the sample cultured at 30 ^oC, 60% moisture in 2 days shows the highest amylase activity 8.614 (unit/g). Other samples cultured at 30 ^oC, 50% moisture, 2 days; 30 ^oC, 60% moisture, 3 days and 27 ^oC, 50% moisture, 3 days also have high amylase activity (7.007 – 7.962 unit/g). Sample cultured at 27 ^oC, 70% moisture, 1 day has the lowest amylase activity (1926 unit/g). Se we can conclude some groups possible for culturing:

- Group #1: Temperature 30 ^oC, moisture 60% in 2 days.
- Group #2: Temperature 27 ^oC, moisture 60% in 3 days.

Between above these groups, the group #1 is mostly suitable for *Aspergillus oryzae* growth

3.2 Amylase activity in vaccum drying at different temperature

Table 3: Moisture change (%) in vaccum drying

Drying time (hour)	Drying temperature (^oc)		
	40^{o}C	**45^{o}C**	**50^{o}C**
0	62.88	64.54	61.91
2	52.95	54.69	52.78
4	44.51	47.37	39.47
6	34.02	33.76	24.15
8	25.16	14.41	10.31
10	18.06	7.608	7.436
12	14.33	4.831	4.368
14	5.787	3.615	4.20
16	4.649	3.168	3.91
18	4.231	3.062	3.764

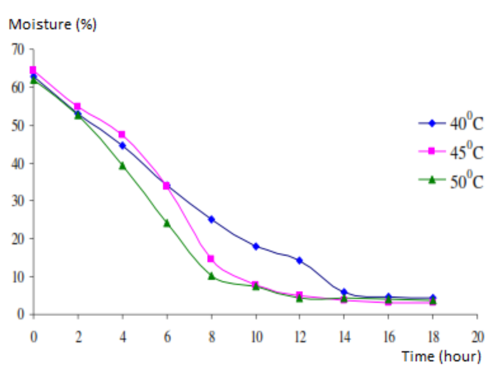

Fig 3: Moisture change (%) in vaccum drying

From table 3 and figure 3, we see the highest speed of moisture reduction at 50 ^oC and lowest at 45 ^oc. From 0-8 hours, the speed of moisture reduction is very quickly at 50 ^oC, and slowly at 45 ^oC (from 0-4 hours). After 8 hours, the speed of moisture reduction is quite better. From 8-12 hours, the moisture reduction is very slow. From next 14 hours, moisture reduction is nearly insignificant. In general, drying at 50 ºC from 16-18 hours gives the best drying speed.

Table 4: Amylase activity in drying at different temperatures

Drying time (hour)	Drying temperature (ºc)		
	40ºC	**45ºC**	**50ºC**
0	7.059	7.092	6.967
2	7.021	6.782	6.864
4	6.829	6.229	6.777
6	7.103	6.174	6.820
8	7.092	5.805	6.945
10	6.902	6.402	7.037
12	6.728	5.045	7.287
14	6.956	6.087	6.798
16	6.912	5.729	7.227
18	6.945	6.011	7.707

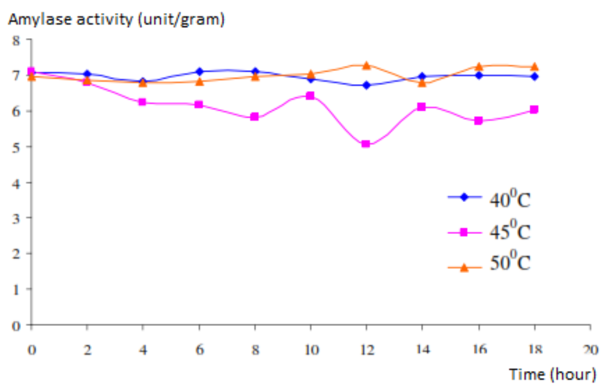

Fig 4: Amylase activity in drying at different temperatures

At 40 ºC and 50 ºC, enzyme activity is nearly stable during drying. So we choose this drying temperature 50 ºC for maintainng amylase stability.

4. Conclusion

Purple fragrant rice is rich in anthocyanin antioxidants, which may be helpful in fighting cancer and heart disease. Amylase of fungal origin was found to be more of stable than the bacterial enzymes on a commercial scale. Different process conditions are successfully studied to achieve maximum yield of amylase production using various experimental designs.

5. Reference

1. Abu EA, Ado SA, James DB. Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on Sorghum pomace. Afr J Biotechnol 2005; 4:785-790.
2. Adejuwon AO, Olanike O, Adewole O. Production of amylase from *Aspergillus niger* using a defined synthetic growth medium and also rice (*Oryza sativa*) as growth substrate. Journal of Medical Research 2012; 1(7):091-094.
3. Akabori S, Ikenaka T, Hagihara B. Isolation of crystalline taka-amylase A from "Takadiastase Sankyo". J Biochem 1954; 41:577-582.
4. Akpan F, Adelaja A. Production and stabilization of amylase preparations from rice bran solid medium. World Journal of Microbiology and Biotechnology 2004; 20(1):47-50.
5. Arai M, Koyano T, Ozawa H, Minoda Y, Yamada K. Acid-stable α-amylase of black aspergilli. Part IV. Some physicochemical propreties. Agric Biol Chem 1968; 32:507-513.
6. Bhella RS, Altosaar I. Purification and some properties of the extracellular α-amylase from *Aspergillus awamori*. Can J Microbiol 1984; 31:149-153.
7. Biesbeke R, Record E, Van Biezen N, Heerikhuisen M, Franken A, Punt PJ *et al.* Branching mutants of *Aspergillus oryzae* with improved amylase and protease production on solid substrates. Appl Microbiol Biotechno 2005; 69:44-50.
8. Fischer EH, Montmollin RD. Crystallization of the α-amylase of *Aspergillus oryzae*. Nature 1951; 168:606-607.
9. Mohamed Abdel Fattah Farid, Hoda Mohamed Abdel Halim Shata. Amylase production from *Aspergillus oryzae* LS1 by solid-state fermentation and its use for the hydrolysis of wheat flour. Iranian Journal of Biotechnology 2011; 9(4):267-274.
10. Makiko K, Yoko S, Yano M, Konno H, Yukio T. Purification and properties of α-amylase from *Aspergillus oryzae* MIBA316. J Biol Macromol 2003; 3(2):57-60.
11. Miah MNN, Ueda S. Multiplicity of glucoamylase of *Aspergillus oryzae*. Starch/Starke 1977; 29:235-239.
12. Mikami S, Iwano K, Shiinoki S, Shimada T. Purification and some properties of acid-stable α-amylase from shochu koji (*Aspergillus kawachii*). Agric Biol Chem 1987; 51:2495-2501.
13. Pandey A, Nigam P, Soccol VT, Singh D, Mohan R. Advances in microbial enzymes. Biotechnol Appl Biochem 2000; 31:135-152.
14. Shruti P, Arora M, Loveleen S. Production and optimization of amylase and glucoamylase using *Aspergillus oryzae* under solid state fermentation. International Journal of Research in Pure and Applied Microbiology 2013; 3(3):83-88.
15. Takayoshi M, Youssef BA, Anindyawati T, Satoru Y, Kazuo I, Masaru I *et al.* Degradation of raw starch granules by α-amylase purified from culture of *Aspergillus awamori* KT-11. Journal of Biochemistry and Molecular Biology 37(4):422-428.
16. Ueda S. Fungal glucoamylases and raw starch digestion. Trends Biochem Sci 1981; 6:89-90.
17. Yamazaki Y, Suzuki Y, Ozawa J. Three forms of α-glucosidase and a glucoamylase form *Aspergillus awamori*. Agric Biol Chem 1977; 41:2149-2161.
18. Gomes E, Souza SR, Grandi RP, Silva ED. Production of thermostable glucoamylase by newly isolated *Aspergillus flavus* A.1.1 and Thermomyces lanuginosus A 13.37. Braz J Microbiol 2005; 36:75-82.
19. Wang XJ, Bai JG, Liang YX. Optimization of multienzyme production by two mixed strains in solid state fermentation. Appl Microbio Biotechnol 2006; 73:533-540.
20. Ramachandran S, Patel AK, Nampoothiri KM, Chandran S, Szakacs G, Soccol CR *et al.* Alpha amylase from a fungal culture grown on oil cakes and its properties. Braz Arch Biol Technol 2004; 47:309–317.