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# COMPARISON OF LABORATORY PROCESSES FOR PRODUCING GLUCOMANNAN FLOURS FROM AMORPHOPHALLUS PLANT IN VIETNAM AND THEIR CHARACTERIZATION - PART 1

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#### ABSTRACT

In this study, two lab-scale frequently used (traditional dry method and two-stage wet-milling method using water-miscible medium ethanol/water: 50/50 v/v containing 1-2  $^{0}$ /<sub>00</sub> natribilsulfite) and for the first time one modified traditional process using cold-drying technique and one modified two stage processes using wet-milling, centrifuging, cold-drying technique to manufacture glucomannan (GM) flour from *Amorphophallus* species in Vietnam were carried out. Main GM flour parameters were determined for GM production process comparison.

Keywords: Amorphophallus sp, glucomannan, dry method, modified wet-milling method, cold drying method

#### 1. INTRODUCTION

Recently in Vietnam, some Amorphophallus species belonging to Araceae family and containing high glucomannan content namely A. krausei, A. yuloensis, A. konjac; A. corrugatus have been propagating, cultivating for producing glucomannan (GM) [1]. The main product - glucomannan have effective treatment and prevention of high cholesterol, obesity [2, 3], constipation [4], diabetes [5] and other modern diseases of civilization [6].

Using various sources of glucomannan rich Amorphophallus species, traditional method for the extraction and purification of KGM have been dry process (milling of dried konjac chips then purifuing via wind-sifting. Recently, the low-cost two-stage technique using water-miscible medium (methanol/water: 50/50 v/v) containing 1- 2  $^{\circ}/_{00}$  natri bilsulfite [7] was developped and concluded that this combined wet - dry process has more advantage over traditional one. In this process, the drying techniques used were hot air dry at 125  $^{\circ}$ C and vaccuum drying between 85 - 130  $^{\circ}$ C. The hot air dry could lead to colour change of the GM flour without SO<sub>2</sub> addition while the vaccuum drying seemed to be expensive. Based on our trial on less than 1 hour natural cold wind drying of thin Amorphophallus slices, we could observe the semi-dried, colour unchanging slices, we intend to use this cold air drying technique to dry the

glucomannan flour without using SO<sub>2</sub>. This cold air drying technique have been used to treat many agricultural products [8].

In Vietnam, few scientific researchs in GM isolation and their characterization have been done [9, 10]. In this study, we will compare two above mentionned glucomannan preparation methods with modified cold air drying technique lab-scale process for producing konjac flour from Amorphophallus species planted in Vietnam. The first modified cold air drying technique comprised the milling of cold dried chips into GM powder and then wind-sifting for further purification. The second cold air drying glucomannan production method modified the two-stage technique using water-miscible medium wet milling of fresh chips or tubers into GM paste and then centrifuging, cold-drying the paste into GM flour. The wet milling technique has the advantage of GM purification.

#### 2. MATERIALS AND METHODS

#### 2.1. Materials

The fresh tubers of A. krausei planted in Dak Nong and Lam Dong provinces were were newly havested and used for this study.

All chemicals and reagents used in experiments were of analytical grade.

### 2.2. Preparation laboratory methos for producing GM flour from A. krausei

#### 2.2.1 Traditional hot air dry process (method 1)

This traditional process starts by slicing and hot air SO<sub>2</sub> addition drying krausei flakes. The dried slices are ground into powder. The dried powder should contain the same glucomannan content as in the original krausei tubers. Then it will be sifted to separate from impurities. The dried purified flour is called "common GM fine flour" and has high sulphur content.

#### 2.2.2 Two stage hot air - wet milling process (method 2)

Wet processing uses anti-swelling agent solution (water - ethanol) to prevent the glucomannan gel formation in water and anti-browning (normally sulphite compound) agent solution to prevent polyphenoloxidases and tannins easily browned reaction for dipping of freshly milling krausei corns. This method comprises crushing, centrifugation and hot air drying. The disvantage of hot air drying step is that the flour can become sticked togther and hard. Thus, second step involves grinding and sifting of starch from the surface of GM granules. The resulted dried and purified flour is called "GM fine flour" and has low sulphur content.

# 2.2.3 Modified cold air - dry milling process (method 3)

The described traditional process has disadvantaged of using hot air SO<sub>2</sub> addition in drying krausei flakes. In order to prevent GM flour quality lowering by krausei polyphenoloxidases and tannins easily returning brown and to avoid using anti-brown SO<sub>2</sub> addition, we will try to dry krausei flakes in cold air equipment (designed and manufactured in our project). The tubers are sliced and then cold dried. Dried slices are ground into powder and sifted. The resulted dried and purified flour is called "common GM fine flour" and has low sulphur content.

# 2.2.4 Modified two stage wet milling - cold drying process (method 4)

In this modified wet processing, krausei tubers are peeled, sliced and dipped into antiswelling (water - ethanol) and anti-browning (NaHSO<sub>3</sub>) agent solution. These slices are crushed in same solution to form GM paste and to wash impurities. The GM paste is centrifuged to separate GM wet solid. Last step involves cold drying GM wet granules. The resulted dried and purified flour is called "GM fine flour" and has low sulphur content.

#### 2.3. Analysis of Glucomannan flours

The content of glucomannan is determined by the DNS method and carried out in our laboratory [11]. Sulphur content is measured in a laboratory of Institute of Environment Technology – VAST. Colour of GM flour is observed by eyes under normal light.

#### 3. RESULTS AND DISCUSSION

#### 3.1. Preparation processes of GM flours

# 3.1.1 Result of traditional hot air dry GM flour production process (method 1)

This method has been carried out to manufacture GM flour in 4 steps. In step 1, the skin and small roots of newly harvested, fresh 250 – 300 g tubers from planted A. krausei were peeled, discarded and then sliced. The slices were immersed in water-miscible medium (ethanol/water: 1.5/1.0 v/v) containing 0,2  $^{0}/_{00}$  natribilsulfite in 3 minutes, followed by ovendrying at 120°C in sulphur-burning flow for 40 min in Heraus dryer. This drying process (step 2) was continued at 60°C until a constant weight was obtained. The dried slices were ground to less than 0.125 mm in average size in step 3. Step 4 started when wind-shifting by a fan for 10 minutes was used to separate the impurities mainly the starch particles of less than 0.01mm diameter out of the coarse krausei flour to get common Glucomannan fine flours as GM-CM-HD-1 samples. Three parameters were determined for obtained samples.

# 3.1.2 Result of two stage wet milling - hot air GM flour production process (method 2)

Two stage wet milling - hot air processing method is carried out in our laboratory by 5 steps. In step 1, chosen planted A. krausei 250 – 300 g tubers were peeled and their skin, small roots and sub-epidermal tissues were removed. Peeled tubers were sliced into 6□8 mm thick pieces and put immediately into water-miscible medium (ethanol/water: 1.5/1.0 v/v) containing 0,2  $^{0}$ /<sub>00</sub> natribilsulfite. In step 2, all the peeled krausei slices and ethanol/water/ natribilsulfite solution were brought to a commercial fruit miller for pulverization for 20 minutes. Resulted solid - solution mixture consisted of coarse krausei particles of 0.02-2 mm diameter and the impurity powder of particle diameter of 0.001 mm or less. The mixture was centrifuged at 2.500 rpm to separate GM wet solid. In step 3, the wet solids were hot-air dried at 120°C. Resulted GM powder was grilled in step 4 and fan sifted for 10 minutes to get Glucomannan fine flour (GM-HW-2) samples in step 5. These samples were used for analysis.

# 3.1.3 Result of modified cold air - dry milling GM flour production process (method 3)

Method 3 process passed same 4 steps as in method 1 to produce GM flour. The only change was the use of cold air drier without the use of sulphur burning instead of hot air drier.

Same quantity of krausei slices obtained and immersed in water-miscible medium (ethanol/water: 1.5/1.0 v/v) containing  $0.2 \, ^0/_{00}$  natribilsulfite in 3 minutes, as in method 1 were cold-air dried at  $10 \, ^\circ\text{C}$  for 10 hours in our homemade cold air dryer. The dried slices were ground to less than  $0.125 \, \text{mm}$  in average size. The impurities mainly the starch particles of less than  $0.01 \, \text{mm}$  diameter were separated from GM flour by fan-wind sifting for 10 minutes. Final products were common Glucomannan fine flours named GM-CM-CD-3 and were analyzed.

# 3.1.4 Result of modified wet milling - cold drying GM flour production process (method 4)

Modified wet milling - cold drying GM flour production method is done by following 3 steps. As in step 1 of all other methods, after the skin small roots and sub-epidermal tissues of fresh harvested A. krausei 250 – 300 g tubers were discarded, the tubers were sliced into small pieces and put immediately into water-miscible medium (ethanol/water: 1.5/1.0 v/v) containing 0,2  $^{0}$ /<sub>00</sub> natribilsulfite. Step 2 started when this whole mixture was put into a commercial fruit miller and was ground at highest speed. After 20 minutes, all the krausei slices were pulverized into coarse particles of 0.02-2 mm diameter with the average being about 1 mm and the impurity powder of 0.001 mm or less particle diameter. The GM wet solid was collected on the 120 mesh net after 2 – 3 minute of 2.500 rpm centrifugation by our homemade horizontal centrifuge. In step 3, the wet GM solids were cold-dried at 10°C in 10 hours to get Glucomannan fine flours named GM-CW-4 samples. Analysis was carried out for obtained GM flours.

Table 1 presented all the preparation steps of 4 processing methods. The GM flours obtained showed that all the *A. krausei* tuber processing methods led to GM flour productions. Each processing method had its own manufacturing steps and produced different quality GM flours. Modified wet milling - cold drying GM flour production (method 4) was long in preparation due to long cold-air drying of 10 hours but it has advantage of not using sulphur burning leading to low sulphur content in the final product. Other advantage was that the wet milling acted as purifying step to wash the impurities thus improving the glucomannan percentage in the GM flour. The results proved that cold air drying technology application in GM production could be feasible. Hot air dry process (method 1) even though today widely applied in large scale GM production has had disvantages of using SO<sub>2</sub> in hot air drying. By this technology as by two stage hot air - wet milling process (method 2) and modified cold air - dry milling process (method 3), low glucomannan purification capacity is obtained in the sifting classification step. The requirement for low GM quality flour decreases also leading to the replacement of this technology by other new one. The yields of GM flours were not much different between 13,1 - 14,8 % fresh krausei tubers used.

Table 1. Main	steps	in different	Α.	Krausei	CIVI	Hours	production	methods

Process	Method 1	Method 2	Method 3	Method 4		
Steps	Hot air dry process	Two stage hot air - wet milling process	Modified cold air - dry milling process	Modified wet milling - cold drying process		
1	Peeling, slicing and immersing in anti-browning water-miscible solution					
2	Hot air dry, SO2 Wet milling, addtion centrifuging		Cold air dry	Wet milling, centrifuging		
3 Grounding		Hot air dry	Grounding	Cold air dry		

4 .	Wind-sifting	Grounding	Wind-sifting	
5		Wind-sifting		
Sample	GM-CM-HD-1	GM-HW-2	GM-CM-CD-3	GM-CW-4
GM yield (% fresh tuber)	14,7	13,1	14,8	13,8

# 3.2. Analysis of GM flour samples

The results in the table 2 showed that the glucomannan contents of GM-CM-HD-1 and GM-CM-CD-3 samples were of around 60 % verse that of GM-HW-2 and GM-CW-4 samples at around 71 %. As shown in table 1, common point of GM-CM-HD-1 and GM-CM-CD-3 production processes was that there was not any wet step but just fan-sifting purification step while GM-HW-2 and GM-CW-4 production processes did have wet-milling step. It indicated that wet processes purified the GM flours better as the impurities were transferred into the milling solution and washed away in the centrifuging step. GM flours produced using traditional wind-sifting purification step were of lower purity in comparison with wet processes. In hot air dry method, the slice tubers became hard in hot air. Thus, glucomannan particles and impurities sticked firmly together. A considerable amount of impurity adhered to the glucomannan particles causing decline in the GM powder purity even after GM powder sifting to eliminate starch.

The sulphur content of traditional  $60 - 120^{\circ}\text{C}$  drying temperature process to produce sample GM-CM-HD-1 was 27 g/kg highest due to the use of SO<sub>2</sub> anti-browning agent in the hot air dry step. All other processes did not use this agent and the krausei slices were just dipped into  $1^{-0}/_{00}$  natribilsulfite anti-browning ethanol/water 50/50 v/v solution. The sulphur residue was less than in the first method lying between 16 - 19 g/kg. In the wet-milling method, KGM granules were purified by removing alkaloids, tannins, starch, sulfur and other impurities in spent liquor.

Although the color of GM-CM-HD-1, GM-CM-CD-3 and GM-CW-4 samples were white, only GM-HW-2 sample has gray white color. It happened because part of the GM-HW-2 powder became brown while dried in hot air without  $SO_2$  addition. GM-CM-HD-1 powder was not brown as it was dried in the  $SO_2$  addition hot air drier

Sample	GM-CM-HD-1	GM-HW-2	GM-CM-CD-3	GM-CW-4
Glucomannan (%)	59,8 - 61,1	70,8 - 71,6	58,7 - 60,8	70,3 - 71,8
Sulphur content (g/kg)	26,9 - 28,1	16 - 17	18 - 19	15,9 - 16,3
Powder colour	White	Gray white	White	White

Table 2: Main parameters of A. krausei GM flours

#### 4. CONCLUSIONS

The presented data have demonstrated that using the wet-milling methods could get higher qualities of GM flour. Newly used cold air drying technique could be effective step in producing glucomannan. New modified wet milling - cold drying GM flour production process was studied to establish new route to economically produce high grade GM flour.

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#### REFERENCES

- 1. Le Thi Loan, Nguyen Phung Ha, Nguyen Thanh Hung, Nguyen Thi Ngoc Hue, Le Ngoc Hung, Le Huy Tuan. Assessement of some possible *Amorphophallus* species planted in Central Highland provinces, to be published
- 2. Vuksan V, Jenkins DJ, Spadafora P, Sievenpiper JL, Owen R, Vidgen E, Brighenti F, Josse R, Leiter LA, Bruce-Thompson C. (1999), "Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes. A randomized controlled metabolic trial.", Diabetes Care., Vol.22(6), p.913-9.
- 3. Li, B., Xia, J., Wang, Y. & Xie, B. J. (2005). Grain-size effect on the structure and antiobesity activity of konjac flour. Journal of Agricultural Food Chemistry, 53, 7404–7407
- 4. Zhang, Y. Q., Xie, B. J. & Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives. Carbohydrate Polymers, 60, 27–31.
- 5. Chen HL, Sheu WH, Tai TS, Liaw YP, Chen YC (2003), Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects--a randomized double-blind trial, J Am Coll Nutr., Vol. 22(1), p.36-42
- 6. Chua, M., Baldwin, T. C., Hocking, T. J. & Chan, K. (2010). Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex N.E. Br. Journal of Ethnopharmacology, 128, 268–278.
- 7. Zhao, J., Zhang, D., Srzednicki, G., Kanlayanarat, S. and Borompichaichartkul, C. (2010), "Development of a low-cost two-stage technique for production of low-sulphur purified konjac flour", International Food Research Journal 17: 1113-1124.
- 8. Walter J. Schaetzle (1985). Cascade desiccant air-conditioning/air drying process and apparatus with cold thermal energy storage, US Patent 4527398 A.
- 9. Nguyen Van Du & N.K. Khoi, 2004. Bổ sung ba loài thuộc chi Nưa-Amorphophallus Blume ex Decne (họ Ráy-Araceae in Viet Nam. Biotechnology Magazine, **26 (4A)**: 57-60
- 10. Nguyen Tien An, Do Truong Thien, Nguyen Thi Dong, Pham Le Dung, Nguyen Van Du, (2011). Isolation and characteristic of polysaccharide from Amorphophallus corrugatus in Vietnam. *Carbonhydrade Polymers* 84(1): 64-68
- 11. Liu, P. Y., Zhang, S. L., Zhu, G. H., Chen, Y., Ouyang, H. X., & Han, M. (2002). Professional standard for the classification, requirements and test methods of konjac flour; Technical Report NY/T 494 [online]. Sichuan: Chinese Ministry of Agriculture.