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Original Article

Utilization of soybean residue to produce monacolin K -cholesterol lowering agent

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Abstract

Soybean residue is a byproduct from the manufacture of soybean milk and tofu which are popular foods in Thailand. Soybean residue was used as substrate in solid state fermentation by *Monascus purpureus* IFRPD 4046 to produce monacolin K, a cholesterol lowering agent. The optimum fermentation conditions, such as temperature, pH and fermentation time, were determined. *M. purpureus* IFRPD 4046 grew well on soybean residue and reached the maximum growth at 7th day. The highest yield of monacolin K concentration was 191.88 mg/kg in the optimized conditions of fermented soybean residue at pH 4, 25% moisture content and incubation time of 7 days at 30^{o}C.

Keyword: soybean residue, *Monascus purpureus*, monacolin K, atherosclerosis

1. Introduction

Large amounts of soybean residue are generated as a byproduct from the manufacture of soybean milk and tofu, which are popular foods in Thailand. The soybean residue poses disposal problems in addition to existing pollution (Shurtleff and Aoyagi, 1979). Utilization of soybean residue by microbial fermentation has been shown to produce beneficial products such as riboflavin (Kinoshita *et al*., 1985), lipase (H - Kittikun and Tani, 1986), fructofuranosidase (Hayashi *et al*., 1992), single cell protein (Khare *et al*., 1994) and Chitosan (Suntornsuk *et al*., 2002).

The *Monascus* is an Asian traditionally fermented fungus used on food and medicine for over thousands of years known as red fermented rice (RFR) koji or anka. RFR are currently being used as health foods in the United States and many Asian countries such as Japan,.Taiwan, China, Korea, Thailand, Philippines and Indonesia (Wang and Lin, 2007). For example, a previous report recommended the use of RFR for colorants in cooking, Chinese traditional medicine and red wine, monacolin K and GABA production (Chen and Hu, 2005).

Monacolin K, commercially known as mevacor, cholestin, lovastatin and mevinolin is a secondary metabolite of *Monascus* and *Aspergillus* species with the molecular formula $C_{24}H_{36}O_5$ and molecular mass of 404.55 (Endo, 1979). Monacolin K is a more active methylated from compactin, and a potent competitive inhibitor of 3-hydroxy - 2 methylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis (Albert *et al*., 1980). Monacolin K can inhibit cholesterol biosynthesis and lower blood cholesterol levels in both humans and animals (Wang *et al*., 2004). In the clinical setting , monacolin K used as a dietary supplemented with a standardized content resulted in an the 18% decrease in total cholesterol, 23% decrease in low density lipoprotein in cholesterol, and 15% decrease in triglycerides,

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which can help in alleviating altherosclerosis (Brown *et al*., 1978).

Coronary heart disease (CHD) is the main clinical manifestation of atherosclerosis and is the major cause of death in many countries. Atherosclerosis might also affect the peripheral arteries and the cerebral circulation, leading to other debilitating or life threatening conditions. The primary risk factor for atherosclerosis is hypercholesterolemia (Endo, 2004).

The previous study on the monacolin K production by *Monascus purpureus* found that this production was be directly or indirectly affected by the cultivation conditions such as pH, temperature and moisture content (Blanc *et al*., 1995; Wang *et al*., 2004).

Solid state fermenlation always produced more monacolin K than submerged cultivation, perhaps because monacolin K was more stable and easily released from substrates under conditions of solid state cultivation while submerged cultivation resulted in accumulation of monacolin K on the mycelium (Su *et al*., 2003). So in this study, the soybean residue was chosen to be substrate in solid state fermentation by *M. purpureus.*

Table 1, shows the compositions of soybean residue obtained from soybean milk factory which contains nutrients that can be utilized for microbial growth. The composition of soybean residue which is rich in water-insoluble ingredients making it is a potentially useful substrate for microbial fermentation (Suntornsuk *et al*., 2002)**.**

This study was aimed to utilize the soybean residue as a substrate for the monacolin K productions by *M. purpureus.* The optimum conditions including moisture content, the initial value of pH and temperature were determined.

To identified the appropriate conditions (moisture, temperature and pH) for using soybean residues as a substrate in solid state fermentation for the production of monacolin K by *M. purpureus* IFRPD 4060.

2. Materials and Methods

2.1 Microorganism and inoculation

M. purpureus IFRPD 4060 was obtained from IFRPD collection. The stock culture was maintained on SDA (Sabouraud - 2% dextrose agar) slant. After inoculation from the original slant, the cultures were incubated at 30°C for 5 to 7 days and stored at 4°C.

Table 1. The compositions of soybean residue

Composition (%)	Soybean residue[a]
Moisture	76.5 ± 0.1
Crude fiber	1.6 ± 0.1
Fat	5.5 ± 0.3
Ash	0.5 ± 0.0
Carbohydrate	7.0 ± 0.1

[a]Values are the means of three replicated measured ± SD

2.2 The soybean residue preparation

Soybean residue was obtained from soybean milk factory (IFRPD) and dried by air flow oven at 60°C for 6 h before autoclaving to prevent spoilage. In small scale cultivation, 40 g of soybean residue was placed in 500 ml conical flask and autoclaved at 121°C, for 15 min.

2.3 The stock culture preparation

To the culture from SDA (Sabouraud - 2% dextrose agar) slant 10 ml of sterile water was added, and the culture on the surface of the slant was scraped with an inoculating needle and agitated thoroughly, Two milliliters of culture suspension was transferred 2 ml to an erlenmeyer flasks containing 100ml. of SDB ,0.3% rice powder and then incubated at 30°C in replicated shake flask (500 ml) held on a shaker (250 rpm). The cultures were grown for 3 days and the initial colony concentration (cfu) determined by spread plate method.

2.4 Solid state fermentation

40 g of dried soybean residue was placed in a 500 ml erlenmeyer flask and distilled water was added to adjust the moisture content and then autoclave at 121°C for 15 min. After cooling, the substrate was inoculated with 2 ml stock culture, mixed well and incubated at 30°C for 3 to13 days.

2.5 Determination of optimized condition for monacolin K production

Soybean residue was fermented with *M. purpureus* by solid state fermentation. The effect of moisture content (20%, 25%, 30%, 35%, 40%) was adjusted by adding sterile water and temperature was adjusted to 30°C, 35°C. The initial pH value (3, 4, 4.5, 5, 5.5) was adjusted by 0.5% lactic acid .

2.6 Determination of monacolin K

The concentration of monacolin K was determinated by HPLC method. Before determination, the fermented soybean residue was extracted as described by Chen and Hu (2005)**.**

Briefly, 1 g of fermented soybean residue powder was extracted with 5 ml of 75% ethanol at 30°C for 1 h. with constant shaking at 250 rpm. The suspension was centrifuged for 15 min at 4,500 rpm and then filtered through 0.45 mm filter paper and analyzed by HPLC method. Chromatographic separation was conducted on a Backman Ultrasphere ODS column (150 mm 4.6 min id). Acetonitrile at 0.1% trifluoroacetate (65 : 35, v/v) were used as the mobile phase. The eluent was pumped at a flow rate of 1 ml/min with UV

detection at 238 nm.

3. Result and Discussion

The stock culture was prepared by culturing *M. purpureus* in SDB at 30°C with shaking at 250 rpm. There was a problem in mycelium pellet which disturbed the growth and the separation of *M. purpureus* mycelium. This problem was solved by adding 0.3% rice powder which decreased the pellet of mycelium and increase the growth of *M. purpureus* .

After soybean residue was fermented by *M. purpureus*, the color of soybean residue changed to a red color as shown in Figure 1. After drying, the fermented soybean residues were extracted and injected to HPLC analyzer. The chromatograms are shown in Figure 2. The peak of monacolin K was released at 10.802 min (Figure 2b).

As shown in Table 2, *M. purpureus* grew well at pH ranging from 3.5 to 6.78. However, the maximum monacolin K production was 109.33 mg/kg at pH 4. Therefore, pH 4 was chosen for further experiments.

The growth stages of *M. purpureus* was shown in Figure 3: the lag phase, exponential phase, stationary phase and death phase were at the 1st-2nd day, the 3th -5th day, the 5th-7th day and after the 7th day, respectively. At the 7th day, the optimum growth of *M. purpureus* was 5.85 log cfu/g.

a b

Figure 1. Changing color of soybean residue by *Monascus purpureus* a. before b. after fermentated by *Monascus purpureus*.

However after the 7th day of cultivation, the growth of *M. purpureus* declined.

Temperature and moisture content are also important for the growth of *M. purpureus*, and substrate which is excessively dry or wet is not suitable for the growth of *M. purpureus* and production of monacolin K. Therefore, temperature and moisture content of fermented soybean residue should be adjusted to obtain the optimum conditions for maximum production of monacolin K. The effects of temperature and moisture on the production of monacolin K

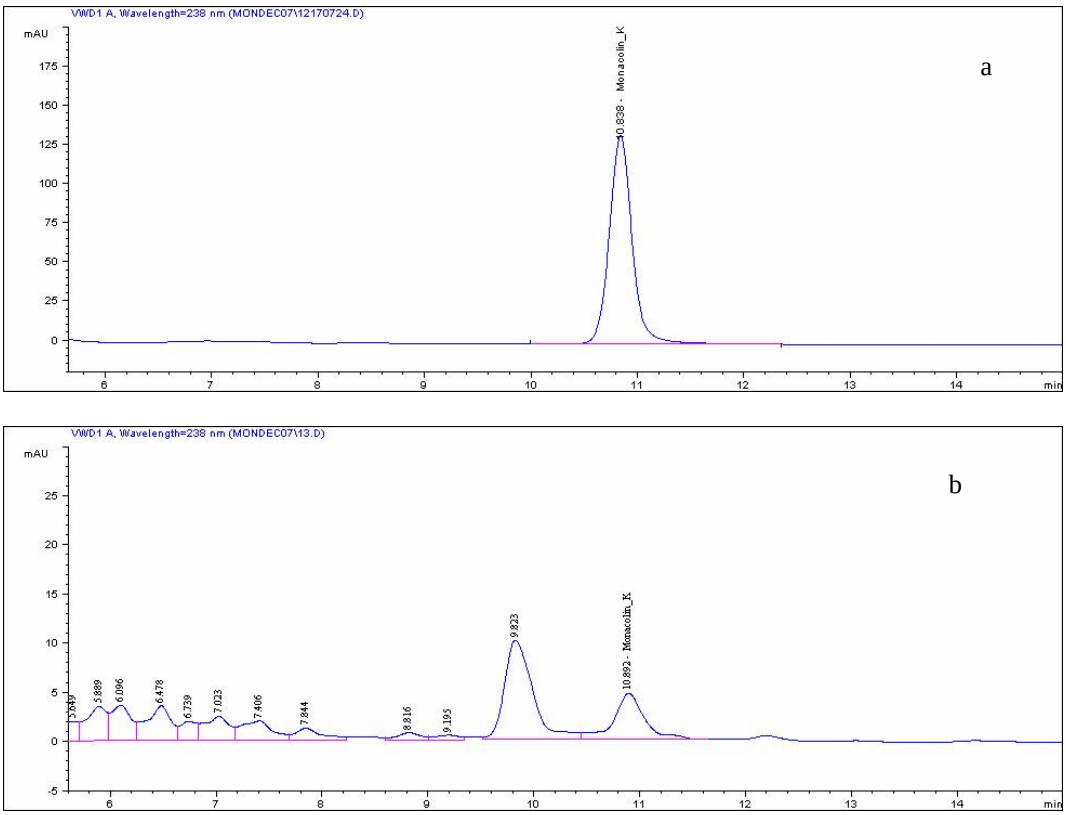

Figure 2. HPLC chromatograms of monacolin K which selected peaks at 238 nm and the peak of monacolin K showed in 10.802 min. a. standard b. *Monascus purpureus* fermented soybean residue

Table 2. The effect of initial pH value for the growth of *M. purpureus* and the production of monacolin K (substrate : soybean residue, culture condition 30°C , moisture content 30%, fermentation time : 5 days)

Initial pH value	Growth of *M. purpureus* (log CFU/g)	Concentration of monacolin K (mg/kg)
3	4.11	84.96
3.5	5.29	98.88
4	5.43	109.23
4.5	5.67	95.33
5	5.62	97.87
5.5	5.57	94.50
6.78*	5.50	90.63

*Soybean residue which un-adjusted pH

and shown in Figure 4. The concentration of monacolin K at temperature 30°C was higher than that at 35°C in every percentage of moisture content (5, 10, 15, 20, 25, 30). The maximum concentration of monacolin K was 191.88 mg/kg at 30°C with 25% moisture content.

Monacolin K is a secondary metabolite derived from the polypeptide pathway, therefore, the monacolin K was double produced on the 5th to the 9th day as shown in Figure 5. During the exponential phase, monacolin K reached maximum production on 7th day and started to decline after 7th day .

4. Conclusion

Monacolin K is formed between middle and later phase in polypeptide pathway because it is a secondary metabolite of *M. purpureus*. This study showed that the production of monacolin K by *M. purpureus* using soybean residue as substrate is produced from the 3th day to the 7th day. The pH, temperature and moisture content appropriate for monacolin K production on soybean residue were at pH 4, 30°C and 25% moisture content. Nevertheless the yield of monacolin K production on soybean residue was not very high when compared to using rice as substrate on which the monacoin K production was 481 mg/kg (Chen *et al*., 2006) which was 2.5 times higher. Perhaps, the only composition of soybean residue should not be appropriate for monacolin K production. If it was supplimented with some substrate such as rice or yam, it might help increase the monacolin K production. Using solid state fermentation under optimum conditions is one way to increase monacolin K production but the best substrate still need to be sought. There are many byproducts from food factories with potential components which are worth trying to improve the substrate for monacolin K production.

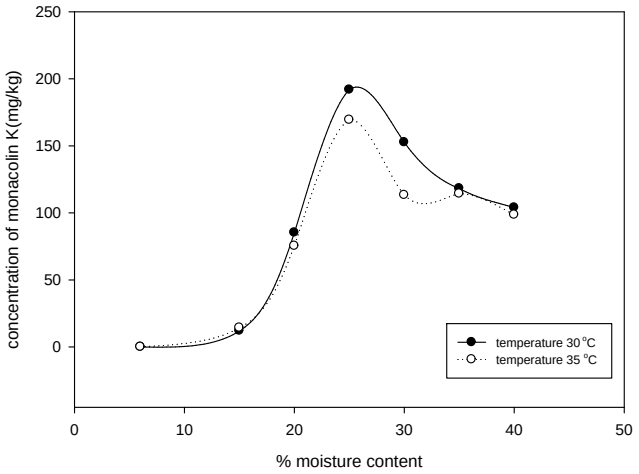

Figure 4. Effect of temperature and moisture on the production of monacolin K by *Monascus purpureus.* at 7 days, pH 4

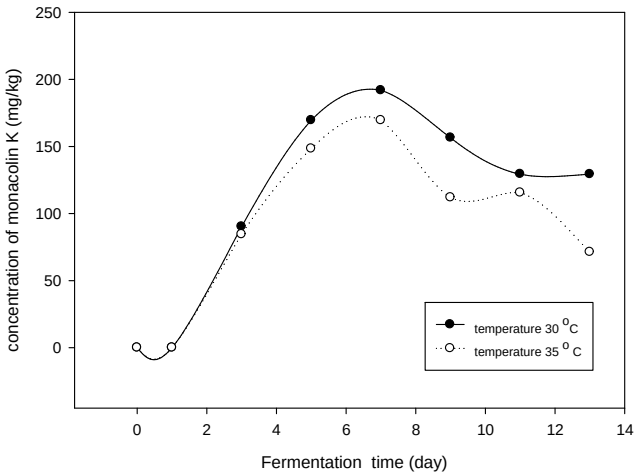

Figure 5. Effect of fermentation time on the production of monacolin K by *Monascus purpureus.* at 7 days, pH 4

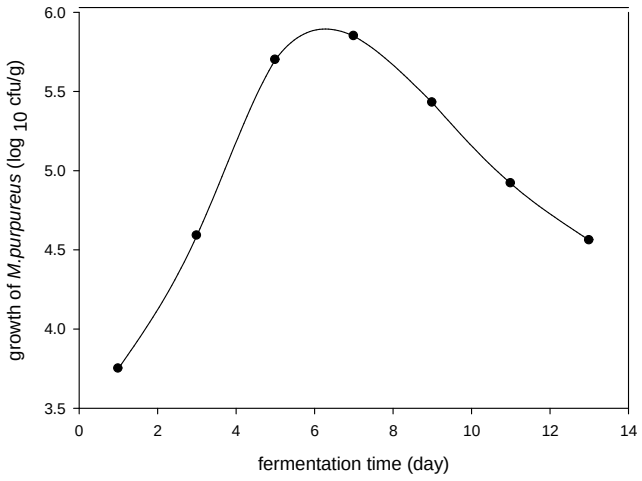

Figure 3. The growth curve of *Monascus purpureus* in solid state fermentation on 25% moisture of soybean residue at 30°C, pH 4

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