การผลิตเอนไซม์และคุณสมบัติของโคจิกากงาดำ โดยเชื้อ *Aspergillus oryzae* KU 2011

Enzyme production and properties of black sesame seed cake koji by *Aspergillus oryzae* KU 2011

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บทคัดย่อ

การศึกษาการใช้กากเมล็ดงาดำอัด ร่วมกับแป้งมันสำปะหลังคั่วในการทำโคจิโดยใช้เชื้อ *Aspergillus oryzae* KU 2011 ในการหมัก แล้วทำการศึกษาคุณสมบัติ ทางกายภาพ และ วิเคราะห์ชนิดและระดับปฏิกิริยาของเอนไซม์ ในโคจิตามช่วงระยะเวลา พบว่า อุณหภูมิของ โคจิ จะเพิ่มขึ้นอย่างรวดเร็วถึงระดับสูงสุดที่ 35 ºC ภายใน 24 ชั่วโมง และพบสปอร์สีเขียวได้ใน 48 ชั่วโมง ความเป็นกรด-ด่าง เปลี่ยนแปลงเล็กน้อยตลอดช่วงเวลาการหมัก แต่ความชื้นค่อยๆลดลง จาก 44.35% เป็น 22.55% ระดับสูงสุดของเอนไซม์ โปรติเอส อไมเลส ไลเปส และ เซลลูเลส คือ 7.56, 33.33, 218.49 และ 10.13 unit/ kg dry weight ที่เวลา 24, 18, 30, และ18 ชั่วโมง ตามลำดับ เอนไซม์ อัลคาไลน์ โปรตีเอส เป็น โปรติเอส ที่เด่นในโคจิ

Abstracts

Black sesame seed cake and roasted cassava starch was used as subtrate for koji production by *Aspergillus oryzae* KU 2011.The serial physical properties and enzymatic profiles and activities of koji were analyzed. The koji temperature rapidly increased to the maximum at 35 °C within 24 hours and the green spore could be observed at 48 hours. The koji pH slightly changed during process but the moisture gradually decreased from 44.35% to 22.55% at the end. The peak level of protease, amylase, lipase, and cellulase were 7.56, 33.33, 218.49 and 10.13 unit/ kg dry weight, at 24, 18, 30, and 18 hours, respectively. Alkaline protease was dominant protease activity in the koji.

key words; black sesame , *Aspergillus*, koji, amylase, protease, lipase, cellulase

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Introduction

Black sesame seed cake was by product from sesame oil production plant. Black sesame seed (*Sesamum indicum* L.) contains potent antioxidant sesamin. Sesamin is the most abundant lignan in sesame seed (Ahmad, 2006, Choi and Kim,1985, El-Shafei,1990, Kato *et al*,1998). Sesamin has various health benefit properties such as decrease blood pressure , significantly decrease serum

and liver cholesterol (Ahmad, 2006,Chang *et al*, 2002,Sugano *et al*,1990). *Aspergillus oryzae* is beneficial food fungi because it produces protease, amylase, lipase, and cellulase and others (Biesebeke *et al*,2002, Chutmanop *et al*,2008, Garcia *et al*, 2009, Hui *et al,* 2004, Nakadai *et al,*1972). In order to utilize of the black sesame seed cake as high value ingredient for left over sesamin source. The biological extraction by using fungal enzymes is the appropriate way in form of sesame sauce which contains sesamin. Koji is crucial step of sauce making (Narahara *et al*,1982, Pandey,2003,Sanchez *et al*,2000,Yokotsuka,1986). This study was conducted to get the basic that needed to know on the koji characteristic before going to further fermentation step.

Materials and methods

1. Koji preparation: screw pressed sesame seed cake was obtained from sesame oil production plant in Chonburi province. Added water to the seed cake at 40% and autoclaved at 121ºC for 40 minutes. After that mixed with sterile roasted cassava starch at 20 %. Incubated at 30 ºC and took sample every 6 hours for 96 hours and kept at -20 ºC until analysis.

2. Enzyme analysis: the specimens were analyzed for enzyme production; protease was measured in terms of international units (IU), which is defined as the amount of enzyme which releases 1 mmol of tyrosin per minute from casein as substrate (Garcia *et al*,2009),; lipase activity was determined by the enzymatic capacity to hydrolyze the substrate 4-nitrophenyl laurate (pNPL), which liberates p-nitrophenol (pNP) that is absorbed at 400 nm at pH 7(Narahara,1982.); amylase activity was measured with the dinitrosalicylic acid method using soluble starch as substrate, one amylase unit (U) is defined as the amount of amylase releasing 1mol reducing sugar as maltose per minute (Narahara *et al*,1982), and cellulase activity was assayed as described by Suganuma *et al* (2007),which was expressed in terms of microgram of glucose equivalents per gram per 30 min.

3. Moisture content: the moisture content of the koji was checked by using oven dry according to AOAC (AOAC, 1984).

4. Temperature and humidity record: the temperatures and humidities were checked by digital probe thermometer and hygrometer model Testo 106-T1

5. pH of koji was checked by using pH meter model Hanna HI 4222.

6. Mycelium growth characteristics: koji was sampling and observed grossly and under microscope model Nikon Labophot 2 to follow the growth stages of the mold.

Results and discussions

1. Enzyme production: there were dynamic changed of enzymes level in koji . The various types of proteases, amylases, lipases, and cellulase were produced during fermentation process. The alkaline proteases was dominate and more activity than neutral proteases in sesame seed cake koji. The

alkaline protease was peak at 24 hour and slowly dropped then began increasing until got the new second peak at 96 hours post inoculation (Figure 2).

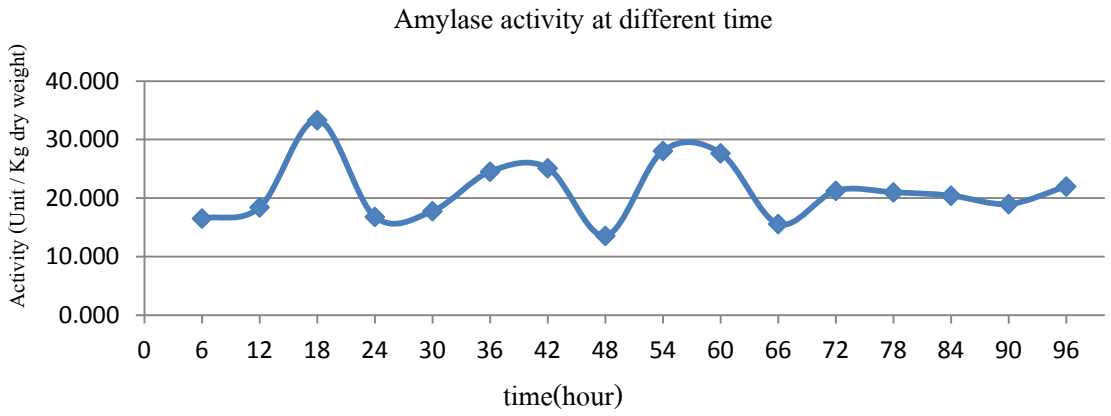

Figure 1. Amylase activity at different time.

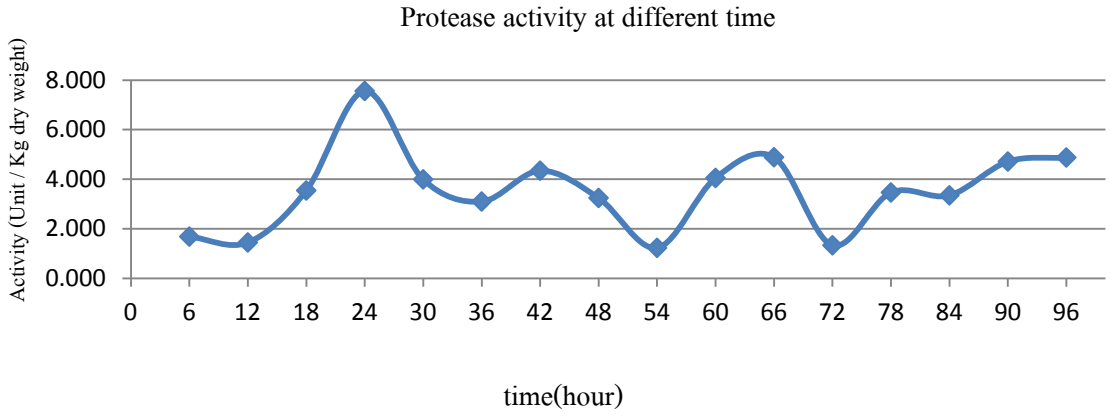

Figure 2. Protease activity at different time.

The dynamic activity of amylase was peak at 18 hour at 200 unit then dropped and slowly began to get the second higher peaks at 78 hours post inoculation at 250 unit (Figure 1).The activity of lipases was peak at 30 hours post inoculation at 1100 unit (Figure 3) .The cellulase activity was peak at18 hours post inoculation at 60 unit (Figure 4). The enzyme dynamic change and profiles were influenced by the substrate composition, strain of koji mold and ferment condition (Chancharoonpong *et al,* 2012, Chutmanop *et al*, 2008, Biesebeke *et al*, 2002) These might varied the profile of enzymes in sesame seed cake koji from soybean koji (Narahara *et al,*1982). According to soybean koji in previous report by Chancharoonpong (2012), the sesame seed koji had higher level of the enzymes in koji but the time of peak level was different. The neutral protease was higher activity than alkaline protease in soybean koji.(Hui *et al*, 2004, Liang et al, 2009, Yong *et al*,1976,).

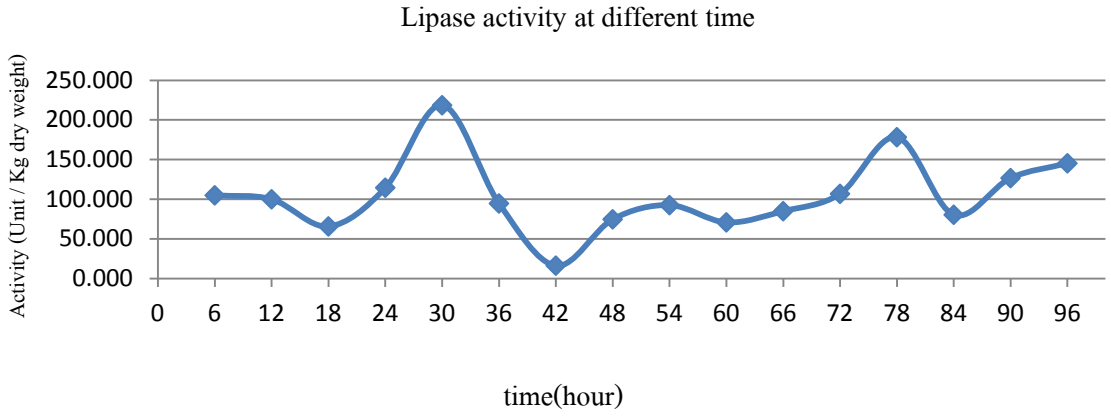

Figure 3. Lipase activity at different time.

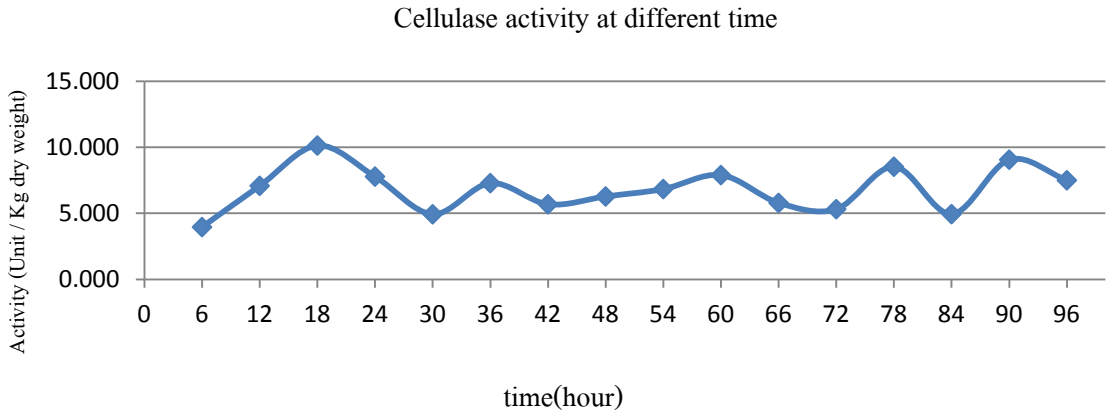

Figure 4. Cellulase activity at different time.

2. Physical and chemical properties: the pH of koji slightly decreased at the beginning from 7.10 to 6.90 and then slightly increased to 7.10 at the end. The koji pH profile of this study was in the similar trend of soybean koji (Yokotsuka,1986, Yong and Wood, 1976). The moisture of koji was gradually decreased from 44.35 % to 22.55 %. The temperature of koji rapidly increased at 24 hours to 35 degree celcius and continued in higher level to 48 hours(3-5 ºC above room temperature) and then slowly decreased to 29-30 ºC. which was 1-2 degree above room temperature (Table 1).

Table 1. Physical properties of koji at different times

Time (hour)	0	6	12	18	24	36	42	48	54	60	66	72	78	84	90	96
koji moisture (%)	44.35	42.44	41.69	41.41	40.56	39.51	37.95	37.78	35.75	33.08	30.27	27.15	25.29	23.55	22.62	22.55
pH	7.11	7.11	7.10	6.95	6.90	6.93	6.93	6.96	6.98	7.02	7.08	7.10	7.10	7.10	7.08	7.10
Ambient temperature (ºC)	32	30	27	26	30	38	26	27	29	28	27	28	29	28	26	27
Koji temperature(ºC)	30	30	31	33	35	31	33	31	30	30	29	30	31	30	29	29
Relative Humidity (%)	80	78	78	80	85	75	77	76	79	81	78	77	76	79	77	80

3. Mycelium growth: The mycelium growth of *Aspergillus oryzae* in sesame seed cake started slight whites mycelium at 24 hours and the green spores were observed on 48 hours, the seed cake was clumped by tight mycelium in the final.

Conclusions

The sesame seed cake could be used in koji production by *Aspergillus oryzae KU* 2011 fermentation and yieled good enzymatic profiles and activity. This information will support further higher value utilize of the agricultural by product sesame seed cake.

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