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Effects of Various Supplements on Riboflavin Production by *Ashbya gossypii* in Whey

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Abstract

Fermentation studies on the production of riboflavin were carried out with whey and whey supplemented with various supplements in Erlenmayer flasks and a laboratory fermenter using *Ashbya gossypii.* Riboflavin production started to increase most rapidly after 2 days of fermentation and continued to increase until the 5th day. The quantities of riboflavin produced by *A. gossypii* in whey after 8 days of Erlenmayer flask fermentation with bran, soybean flour, glycine + peptone and sucrose as supplements; were 389.5, 120.7, 101.7 and 87.5 mg/L, respectively. The lowest yields were obtained using peptone and soybean oil (23.2 and 17.5 mg/L, respectively) and supplements. Riboflavin produced in whey by *A. gossypii* during 8 days of fermentation in a fermenter were found to be four times higher than in Erlenmayer flask studies.

Key Words: *Ashbya gossypii*, whey, riboflavin, fermentation.

Değişik Katkıların *Ashbya gossypii* ile Peynir Altı Suyunda Riboflavin retimine Etkileri

Özet

Riboflavin üretimi fermentasyon çalışmaları ile *A. gossypii* kullanarak sallamalı Erlenmayer ve laboratuvar fermentörü kullanarak katkısız peynir altı suyu ve ıeitli katkı maddelerinin eklendiği peynir altı suyu ile yapılmıştır. Riboflavin üretimi fermentasyonun ikinci günü sonunda daha hızlı artmaya balam ve beşinci güne kadar artı devam etmiştir. Peynir altı suyuna eklenen kepek, soya unu, glisin + peptonla ve sukroz sallamalı Erlenmayer flask fermentasyonuyla 8 gün sonra *A. gossypii*'nin üretmiş olduğu riboflavin miktarları sırasıyla 389.5, 120.7 ve 101.7 mg/L olarak tespit edilmiştir. Peynir altı suyuna eklenen pepton ve soya yağı ile daha düşük miktarda riboflavin üretilmiştir (sırasıyla 23.2 ve 17.5 mg/L). Peynir altı suyunda ve peynir altı suyuna eklenen katkı maddeleri ile, *A. gossypii* tarafından fermentörde 8 gün sonra üretilen riboflavin miktarları sallamalı Erlenmayer flask çalışmalarına göre yaklaşık dört kat daha yüksek bulunmuştur.

Anahtar Sözcükler: *Ashbya gossypii*, peynir altı suyu, riboflavin, fermentasyon.

Introduction

Whey is produced in very large quantities by the dairy industry and often becomes an environmental threat. Several processes have been proposed for whey utilization, largely based on fermentation by microorganisms (*Kluyveromyces* spp., *Candida* spp., *Lactobacillus* spp., etc.) that utilize lactose naturally (Porro et.al., 1992). Riboflavin is produced by many microorganisms, including bacteria, yeasts, and molds (Crueger and Crueger, 1989). Commercial fermentation processes for the production of riboflavin or riboflavin concentrates are relatively recent, having been developed in the last 45 years (Perlman, 1979). Riboflavin is synthesized by many microorganisms including bacteria, yeasts, and fungi. The first microorganism reported to be capable of producing riboflavin was *Clostridium acetobutylicum* and subsequently two other microorganisms, ascomycetes, namely, *Eremothecium ashbyii* and *Ashbya gossypii* have been reported by Prabhakar et.al. (1993). The fungs *A. gossypii* produces a huge amount of this vitamin and is therefore used for most of the microbial production processes. About 30% of the world industrial riboflavin output is produced by direct fermentation with *A. gossypii* and up to 15 g/L is reported to be the maximum yield (Stahmann et.al., 1994).

In the present study, the effects of various supplements on the riboflavin production rate by *A. gossypii* in whey were investigated with Erlenmayer flask and laboratory fermenter studies. The specific riboflavin production rate during the fermentation period was also determined.

Materials and Methods

Whey Preparation

One lot (25 L) of Turkish White cheese whey was obtained from a local cheese factory and autoclaved for 20 min at 121°C. After cooling, it was stored at 4°C until used in the experiments. The approximate components of the whey from cheese are total solids (6.350%), water (93.700%), fat (0.500%), protein (0.800%), lactose (4.850%), ash (0.500%) and lactic acid (0.005%) (Ertşrk, 1996).

Cultures

Ashbya gossypii NRRL Y-1056, known to produce riboflavin, was kindly supplied by USDA, Agricultural Research Service, Nat. Center for Agricultural Utilization and Research, University St. Peoria, USA. The culture was stored frozen at −30°C on malt extract (ME) agar (Difco laboratories, Detroit, MI) slants. When needed, the culture was thawed and incubated in ME broth (Difco) at 28°C for 3 days. It was subcultured twice for 3 days at 28°C before use in vitamin production.

Lactobacillus casei ATCC 7469, obtained from USDA, Agricultural Research Service, Nat. Center for Agricultural Utilization and Research, University St. Peoria, USA, was used in the bioassay tests. Stock cultures were maintained at 4°C on lactobacillus agar (Difco) slants. When needed, the culture was thawed and incubated in lactobacillus broth (Difco) at 37°C for 24 h and stored in a refrigerator. It was subcultured twice at 37°C for 24 h before use.

Erlenmayer Flask Studies

About 150 ml of the supernatant of sterile cheese whey stored in a refrigerator was placed in 250 ml sterile Erlenmayer flasks. They were inoculated with 2% of 3-day old *A. gossypii* culture and allowed to grow in the dark, in a bench type water bath shaker ST-402 (Nşve, Sanayi ve Malzemeleri malat ve Ticaret A.., stanbul) with a 55 rpm wrist-shaking action at 28°C ± 2 (Wickerham et.al., 1946). Samples were removed daily under aseptic conditions and analyzed for riboflavin content. Fermentations were carried out for each of the following substrate conditions: whey, and whey supplemented with soybean oil, yeast extract, glycine, sucrose, peptone, glycine+peptone, soybean flour and bran. All the supplements were added to the whey in 10g/L (w/v) amounts except glycine which was added at 1 g/L (w/v). All fermentation media were sterilized at 121°C for 30 min.

Fermenter Studies

After Erlenmayer flask fermentation studies, plain whey, and whey with supplements soybean flour and wheat bran were selected and studied in a 1.5 L of bench scale jacketed jar fermenter (EYELA Jar Fermenter MBF, Tokyo Rikakikai Co., Ltd.). Aeration was provided by means of a gas sparger and maintained at 0.1 NL/min. The fermentation medium was agitated using a stirrer at a speed of 300 rpm. The sampling was carried out using a sterile pipet under aseptic conditions. Foaming was prevented by using 10 ml/L soybean oil.

Analysis of Riboflavin

Riboflavin was determined using a microbiological assay (Difco, 1984; Helrich, 1990). *Lactobacillus casei* ATCC 7469 and the turbidimetric method of measuring growth response to riboflavin was used. Procedures for the preparation of standard solutions, standard curves, assay tubes, inoculum, and culture maintenance were carried out according to the method of Difco (1984). The media used were those recommended by Difco (1984).

Ten ml aliquots of fermented medium were put in sterile test tubes and heated for one h at 120°C in order to liberate the bound vitamin in the cells into the culture medium (Crueger and Crueger, 1989). Two samples were analyzed from each heated fermented medium. The cells were separated by centrifugation (Hattich Roto Silenta II) for 15 min at 8000 rpm. Light transmission was measured using a spectrophotometer (Pharmacia LKB Biotech, Novaspec II, Cambridge, England) at a 600 nm wavelength.

Results and Discussion

Whey was selected as a nutrient source in this study for its high rate of production throughout the world (Porro et.al., 1992; Liao et.al., 1993). For each 100 kg of cheese produced, 900 kg of whey is obtained and huge amounts of it has to be discarded, constituting an environmental threat (Liao et.al., 1993). In Turkey 229,000 tons of different types of cheese (together with about 2,000,000 tons of whey) were manufactured in 1990 (Gşne and Albayrak, 1994). The riboflavin content of the fermented media during the 8 days was determined using *A. gossypii* with the microbiological assay method.

Effect of Fermentation Time

The riboflavin production rate (mg/L) during the 8 days of fermentation by *A. gossypii* in whey, and whey supplemented with different ingredients in Erlenmayer flask studies are shown in Figures 1, 2 and 3. Riboflavin production increased most rapidly after 2 days of fermentation and continued to increase until the 5th day of fermentation in whey supplemented with bran (342.2 mg/L), and the quantities of riboflavin produced with plain whey was 26.3 mg/L. Riboflavin increased to 60.4, 37.6, 28.0, 53.7 and 61.3 mg/L with yeast extract, sucrose, glycine, glycine+peptone, and soybean flour respectively after 5 days of fermentation.

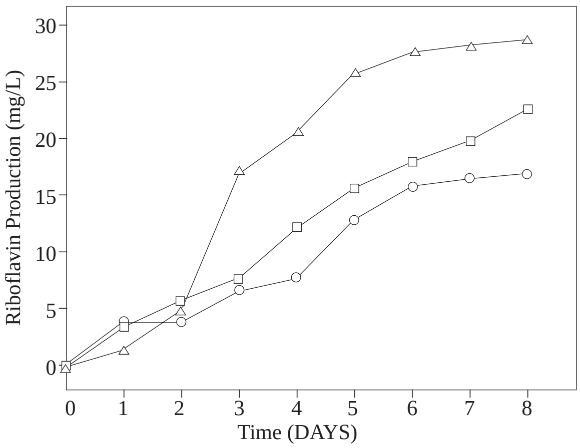

Figure 1. Production of riboflavin by *Ashbya gossypii* from whey, and whey supplemented with soybean oil and peptone in shake flask studies (O = whey + soybean oil; □ = whey + peptone; △ = whey).

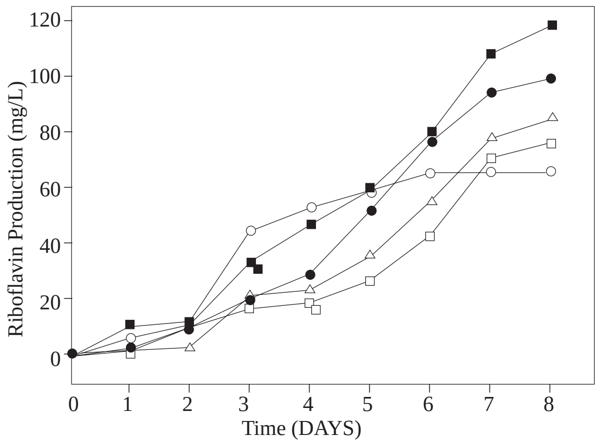

Figure 2. Production of riboflavin by *Ashbya gossypii* from whey supplemented with yeast extract, glycine, sucrose, glycine + peptone, and soybean flour in shake flask studies (O = whey + yeast extract; □ = whey + glycine; △ = whey+sucrose; • = whey+glycine+peptone; ■ = whey + soybean flour).

The riboflavin production rate (mg/L) during the 8 days of fermentation by *A. gossypii* in plain whey, and whey supplemented with bran and glycine + peptone in fermenter studies are shown in Figures 4, 5 and 6 respectively. Riboflavin production increased most rapidly after 1 day of fermentation and continued to increase until the 5th day. The quantity of riboflavin produced with plain whey was 84.5

mg/L, and the values were 220.7, and 722.7 mg/L for whey supplemented with peptone+glycine, and bran respectively.

There was a time lag in the riboflavin production consistent with the literature (Prabhakar et.al., 1993). Product formation started during the first two days and attained its maximum level after about five days.

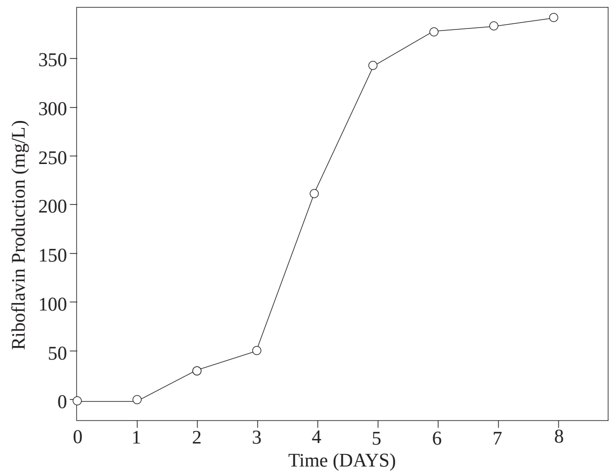

Figure 3. Production of riboflavin by *Ashbya gossypii* from whey supplemented with bran in shake flask studies.

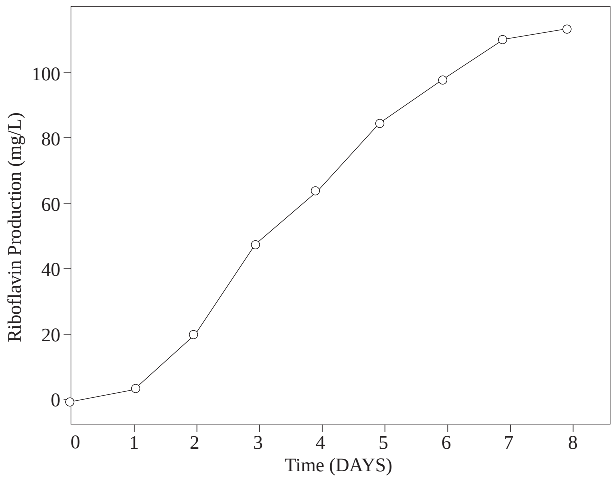

Figure 4. Production of riboflavin by *Ashbya gossypii* from whey in fermenter studies.

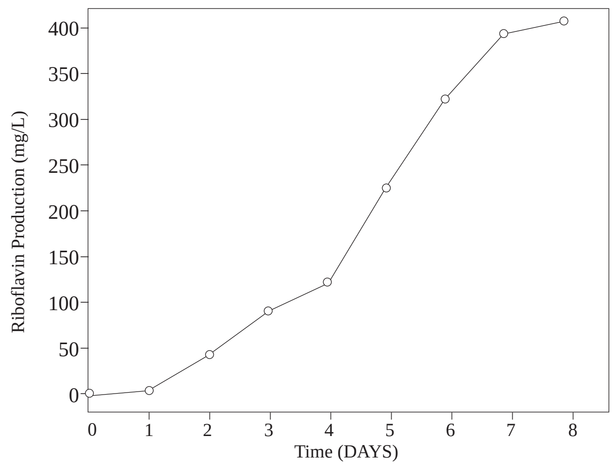

Figure 5. Production of riboflavin by *Ashbya gossypii* from whey supplemented with soybean flour in fermenter studies.

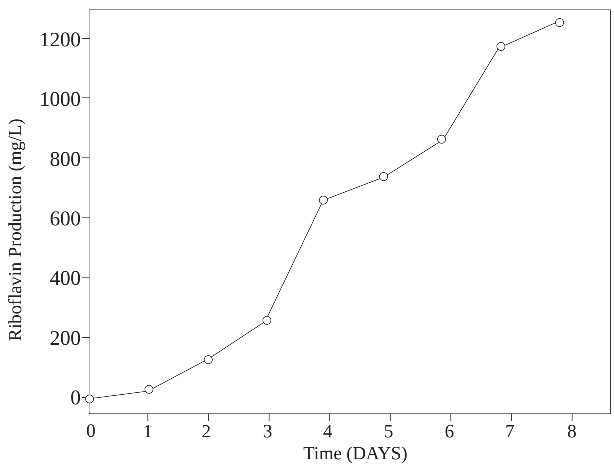

Figure 6. Production of riboflavin by *Ashbya gossypii* from whey supplemented with bran in fermenter studies.

Effect of Supplements on Final Yields

The quantities of riboflavin produced by *A. gossypii* (at pH 5.1, 28°C, for 8 days) in whey with different supplements; bran, soybean flour, glycine+peptone, sucrose, glycine, yeast extract, peptone and soybean oil were 389.5, 120.7, 87.5, 78.3, 68.4, 23.2, and 17.5 mg/L respectively (Figures 1, 2 and 3). The quantity of riboflavin produced with plain whey was 29.2 mg/L (Figure 1). Riboflavin increased in all fermented samples and these results confirm the results reported by Murdock and Fields (1984) that riboflavin is generally higher in fermented foods and riboflavin content is higher in corn meal fermented for 4 days.

Riboflavin produced in whey supplemented with bran was nearly four times higher than that in plain whey and in whey with other supplements. Wheat mill bran is rich in various minerals, pentosans, starch, total sugar, sucrose, and reducing sugar (Pomeranz, 1988). The increase in riboflavin production on bran supplemented whey may be explained by the mineral and sugar composition of bran. The riboflavin quantity with plain whey in fermenter studies was higher than the riboflavin produced in fermentation media supplemented with molasses and lentils (84 mg/L) by *Eremothecium ashbyii* (Prabhakar et.al., 1993).

The maximum yield of 17.5 mg/L was obtained at the end of fermentation (8 days) in whey with 10 ml/L soybean oil (Figure 1). But the yield was lower, compared with the yield of plain whey. Ghanem et.al. (1992) found that riboflavin production was appreciably enhanced with the use of corn oil by *Candida guilliermandii*, (about 13% increase), but the use of cotton seed oil, olive oil and some fatty acids showed no stimulatory effect. Crueger and Crueger (1989) have reported that soybean oil is metabolized by *A. gossypii* during fermentation and with a fermentation medium of corn steep liquor 2.25%, commercial peptone 3.5% and soybean oil 4.5%, and with a yield of 10-15 g/L.

The yield of riboflavin was higher from fermented medium supplemented with glycine+peptone (Figure 2) than the yields when glycine (Figure 2), and peptone (Figure 1) were added individually to whey. Typical data on stimulation of riboflavin production as a result of including various peptone concentrations in the media have been summarized by Perlman (1979), as has the effect of supplementation with glycine.

Riboflavin production in the laboratory fermenter (at pH 5.1, 28°C, with a stirrer speed of 300 rpm, and an aeration rate of 0.1 NL/min, for 8 days) with plain whey (Figure 4), and whey supplemented with glycine+peptone (Figure 5) and bran (Figure 6) were 113.4, 400.0, and 1227.4 mg/L respectively. Riboflavin produced by *A. gossypii* in plain whey and whey with different supplements after 8 days of fermentation in the laboratory fermenter were found to be approximately four times higher than those found in Erlenmayer flask studies. The increase in the yield of riboflavin in the fermenter studies with 300 rpm can be explained by the fact that agitation gives rise to greater oxygen transfer rates and better distribution of oxygen in the system. Prabhakar et.al. (1993) have found that riboflavin production reached high levels with 300 rpm stirrer speeds.

Total production of riboflavin on a worldwide basis is around 2000 tons per year (3). In our country riboflavin cannot be produced yet, so Turkey is importing about 25,000 kg riboflavin and the amount paid is more than $1.5 million per year (Ertşrk, 1996). Whey is produced in Turkey in large amounts (about 2,000,000 tons/year) and only a small percentage of it is used in whey powder production. The remaining part is discarded. In this study, 29.3 and 113.4 mg/L of riboflavin were produced from plain whey in Erlenmayer flask and fermenter studies by using *A. gossypii*, respectively. If all whey were used for riboflavin production, about 60,000 and 220,000 kg of riboflavin could be produced, respectively. This would be 2 and 8 times higher than the riboflavin requirement of our country. This means that about $1.5 million would be saved annually from importation expenses.

Not only the detailed growth rate including the death phase but also the effect of parameters such as temperature and pH on the fermentation of whey need further investigation. According to the results on a percentage basis, the greatest increase in riboflavin was obtained by adding bran to whey in both Erlenmayer flask and laboratory fermenter studies. *A. gossypii* NRRL Y-1056 has potential for the production of riboflavin from plain whey, and whey supplemented with natural supplements i.e. bran and soybean flour.

Acknowledgement

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