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Full Length Research Paper

Research on effect of *Penicillium* sp. in *Luzhou-flavor* liquor

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***Penicillium* sp. isolated from *Daqu* was as starting strain. In order to investigate the role of *Penicillium* sp. in *Luzhou-flavor* liquor, *Penicillium* sp. was operated to Koji seeds and then simulated solid-state fermentation for liquor testing under different conditions, which included adding different amount of Koji seeds and *Daqu*. The results showed that, when 20% of *Daqu* was added, *Penicillium* sp. koji increased from 0.5 to 2%, compared with blank control group, liquor yield reduced by 2.23% from 29.77%, total ester decreased by 1.70% from 37.25%, total acid increased by 6.57% from 12.41% and four esters had different degrees of decrease trend. While added amount of koji seeds exceeded 1.0%, ethyl lactate content was higher than ethyl caproate content. When added amount of *Penicillium* sp. koji was 1.0%, yield of liquor, total esters, total acid and the four esters were all enhanced with the increase amount of *Daqu*.**

Key words: *Penicillium* sp., *Daqu*, Koji seeds, *Luzhou-flavor* liquor.

INTRODUCTION

Quality of *Daqu* was a major factor to the *Luzhou-flavor* liquor, as it is not only with saccharification and fermentation but also with fermented flavor enhancer effect. The quality of *Daqu* directly impacted on the production, quality and style of the liquor (Yao et al., 2003). As *Daqu* is prepared by a natural inoculation of molds, yeasts and bacteria on the grains, its composition and quality depends heavily on experience, weather and geographical factors. This gives the characteristics to *Daqu* and liquors in their places of origin, and makes it difficult to reproduce these liquors in other places (Wang et al., 2008; Wang, 2008; Zhang et al., 2005). Ethyl caproate determines the main flavor and taste of *Luzhou-flavor* liquor and with significant proportion of total esters. Ethyl butyrate is another fragrant aroma component of the liquor and low content of it would make the liquor with fruit flavor, however, as the content of ethyl butyrate is higher than normal value, it would bring odor smell to liquor and finally affect the liquor quality. Liquor presents with apple flavor and banana flavor while the content of ethyl acetate is high, otherwise it presents pear incense. Ethyl lactate content should be less than ethyl caproate in *Luzhou-flavor* liquor, otherwise it would effect the flavor and taste of the liquor (Wang et al., 2008a).

Penicillium sp. is one kind of the microorganisms frequently isolated from *Daqu* and with a certain proportion in the *Daqu* microbial populations. Some reports have referred that *Penicillium* sp. was harmful bacteria in *Luzhou-flavor* liquor, but the effect of *Penicillium* sp. in *Luzhou-flavor* Liquor have rarely been studied. Therefore, in order to investigate the role of *Penicillium* sp. in *Luzhou-flavor* Liquor, *Penicillium sp.* with cellulase and amylase was made into Koji Seeds and then simulated solid-state fermentation for Liquor testing under different conditions.

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MATERIALS AND METHODS

Strains and medium

Penicillium sp. used in the experiment was isolated from *Daqu*, which was provided by Zhijiang Co., Ltd. *Penicillium* sp. was

cultured on Potato Dextrose Agar medium (PDA) (Zhou, 2006), and Koji Seeds was cultured on the solid medium containing: 20 g of *Fupi*, 18 ml of Nutrient (Mandels, 1976), *Daqu* and Raw materials used in liquor brewing were also provided by Zhijiang Co., Ltd.

Koji seeds cultured

Penicillium sp. was activated on PDA medium at 29℃ for 7 days, and then its spores were washed into the solid medium with 1 ml sterilized saline water. The solid medium was cultured at 29℃ for 5 to 6 days, followed by dried at 35℃ and then wrapped up with paper to be used later. While shook the flask after 16 h, agitated the flask when cultured after 30 h and patted the flask to ensure the medium agglomerated as pie, obliqued the medium in the flask space; medium was flipped up and down after cultured for 3 days; however, it should be ensure that the medium was not shake broken.

Liquor brewing

Liquor brewing was carried out at two different conditions and simulated solid-state fermentation for Liquor brewing. Different adding amounts of Koji seeds brewing scheme shown in Table 1, in the experiment 600 g raw materials was as inventory rating and 20% *Daqu* were adding (Li, 2009), then cultured at 30℃ for 15 to 18 days. While different adding amounts of *Daqu* brewing test scheme was shown in Table 2, 600 g raw materials was as inventory rating in the test group and *Penicillium* sp. koji adding was 1.0%, then fermented at 30℃ for 15 to 18 days.

Analytical methods

The alcohol (c_1%, V/V) in the distilled liquor was determined by standard curve of ethanol (Tang, 2010), and then the alcohol (c%, m /m) and the liquor yield (which represents the abilities of transforming the utilized raw materials to alcohol) were calculated as follows:

The alcohol (c%, m/m) = the alcohol (c_1%, V/V) × ethanol density (0.7893, g /ml)

Liquor yield (96% ethanol, %) (Shen, 2007) = the alcohol (c%, m/m) × coefficient of ethanol transformed from 96 to 100% (0.8411)/raw materials (g) × 100

Total ester content was determined by saponification method and indicated by ethyl acetate (Yi, 2008). While total acid content was measured with alkali titration and indicated by acetic acid (Yi, 2008). Four esters were determined by using a Gas Chromatograph equipped with a FID detector, and analyzed by amyl acetate internal standard (Shen, 2007; Cesar et al., 2009).

RESULTS AND DISCUSSION

Standard curve of ethanol

Figure 1 showed that linear regression equation of ethanol was as follows: $y = 1.4086x + 0.0084$, $R^2 = 0.9967$, and had well correlation. So it could be used to determined the alcohol (c_1%, V/V).

Liquor brewing results of different Koji seeds adding amount

were all decreased while the amount of *Penicillium* sp. koji increased. And compared with blank control group, liquor yield reduced by 29.77% and alcohol deceased by 25.44%, while koji seeds adding reached to 2%. Liquor yield decreased may be due to the presence of *Penicillium* sp. inhibited the growth of *Rhizopus* which was studied in earlier research (unpublished), as we all known, *Rhizopus* is one of the main microbes which could producing glucoamylase. As a result, utilization of raw starch reduced, further more liquor yield decreased.

It is shown from Figure 3 that total esters deceased with the increase of *Penicillium* sp. koji adding, however, total acid increased. And the results showed that, when amount of Daqu was 20% and *Penicillium* sp. koji adding increased from 0.5 to 2%, compared with blank control group, total ester decreased by 1.70 from 37.25% and total acid increased by 6.57 from 12.41%. Generally speaking fermenting power reduced when acidity increased (Ciu, 2007), that is to say, fermenting power of *Daqu* reduced with the increased of *Penicillium* sp. koji adding. Acidity increased may be due to the performance of *Penicillium* sp. that could promote some acid bacteria such as *Lactobacillus* producing acid. Figure 4 was ester content by gas chromatogram.

Four esters had different degrees of decrease trend when the amount of *Penicillium* sp. koji increased (Figure 5), and the result was consistent with the conclusion of Figure 3. When *Penicillium* sp. koji adding increased to 2%, compared with the blank control group, ethyl caproate of test group reduced by 47.70%, ethyl lactate reduced by 29.63%, ethyl acetate decreased by 26.96%, ethyl acetate decreased by 59.26%. The concentration change of esters would affect the flavor and taste of the liquor, thus affected liquor quality. Ethyl lactate content was higher than ethyl caproate content when ethyl caproate increased to 1%, as ethyl caproate was the main flavor of the liquor so finally affected the liquor flavor greatly. This may be because *Lactobacillus* had greater competition in the strong acidic environment, however, Caproic acid bacteria had not suited to the acidic environment, finally leading to concentration of ethyl caproate lower than ethyl lactate (Wang and Wang, 1994).

Liquor brewing results of different amounts of *Daqu*

Figures 6, 7 and 8 showed that acohol, liquor yield, total acid and total ester all enhanced when the amount of *Daqu* increased. When *Penicillium* sp. koji was added with 1.0% and the amount of *Daqu* reduced from 28 to 20%, liquor yield, total esters, total acid and the four esters were all almost the same as the blank control. But obviously it would increase the production cost if increase the amount of *Daqu*.

Conclusions

Liquor brewing test showed that *Penicillium* sp. was

Table 1. Different adding amounts of Koji seeds brewing test scheme.

Scheme	Koji seeds (%)	*Daqu* (%)	Raw materials (g)
1	0.5	20	600
2	1.0	20	600
3	1.5	20	600
4	2.0	20	600
Control	0	20	600

Table 2. Different adding amounts of *Daqu* brewing test scheme.

Scheme	Koji seeds (%)	*Daqu* (%)	Raw materials (g)
1	1.0	22	600
2	1.0	26	600
3	1.0	28	600
4	1.0	30	600
Control	0	20	600

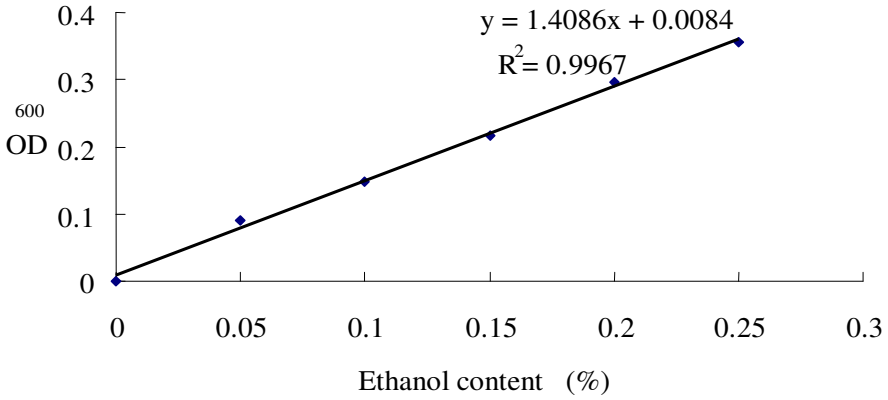

Figure 1. Standard curve of ethanol.

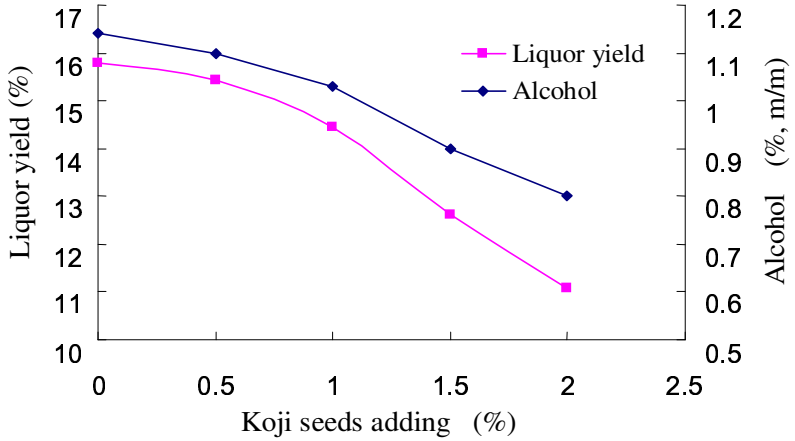

Figure 2. Alcohol and liquor yield of different *Penicillium* sp. Koji seeds adding amount.

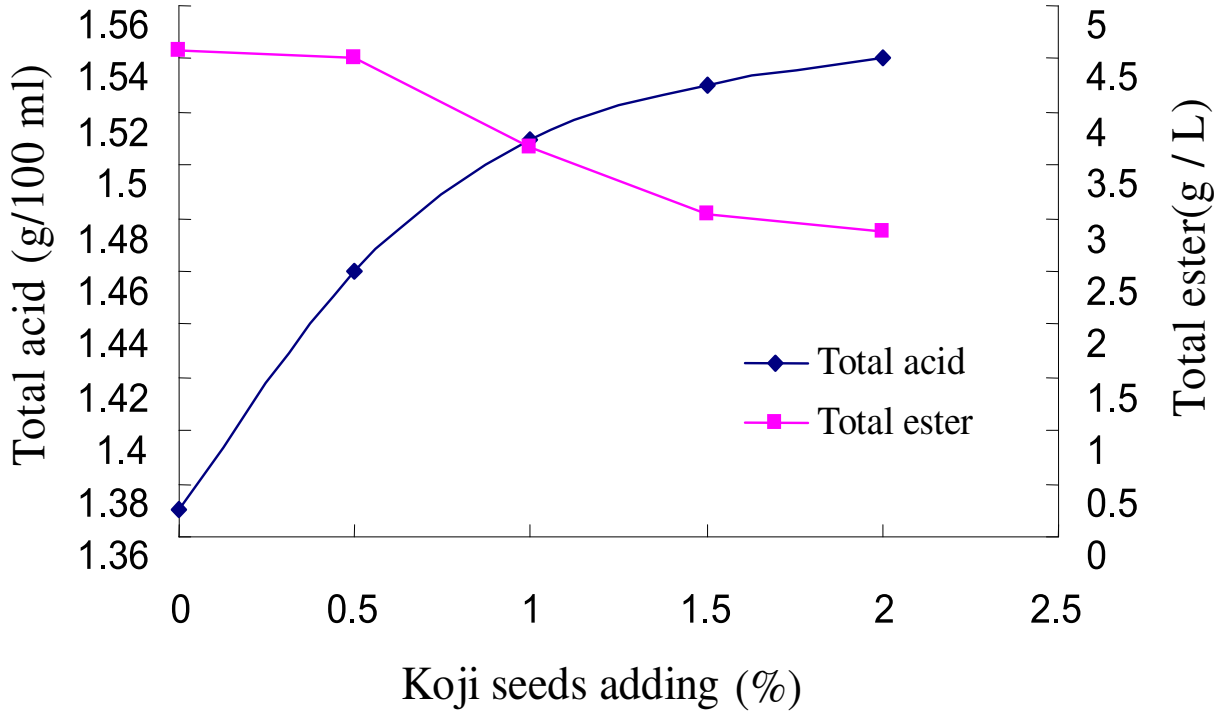

Figure 3. Total acid and total ester of different *Penicillium* sp. Koji seeds adding.

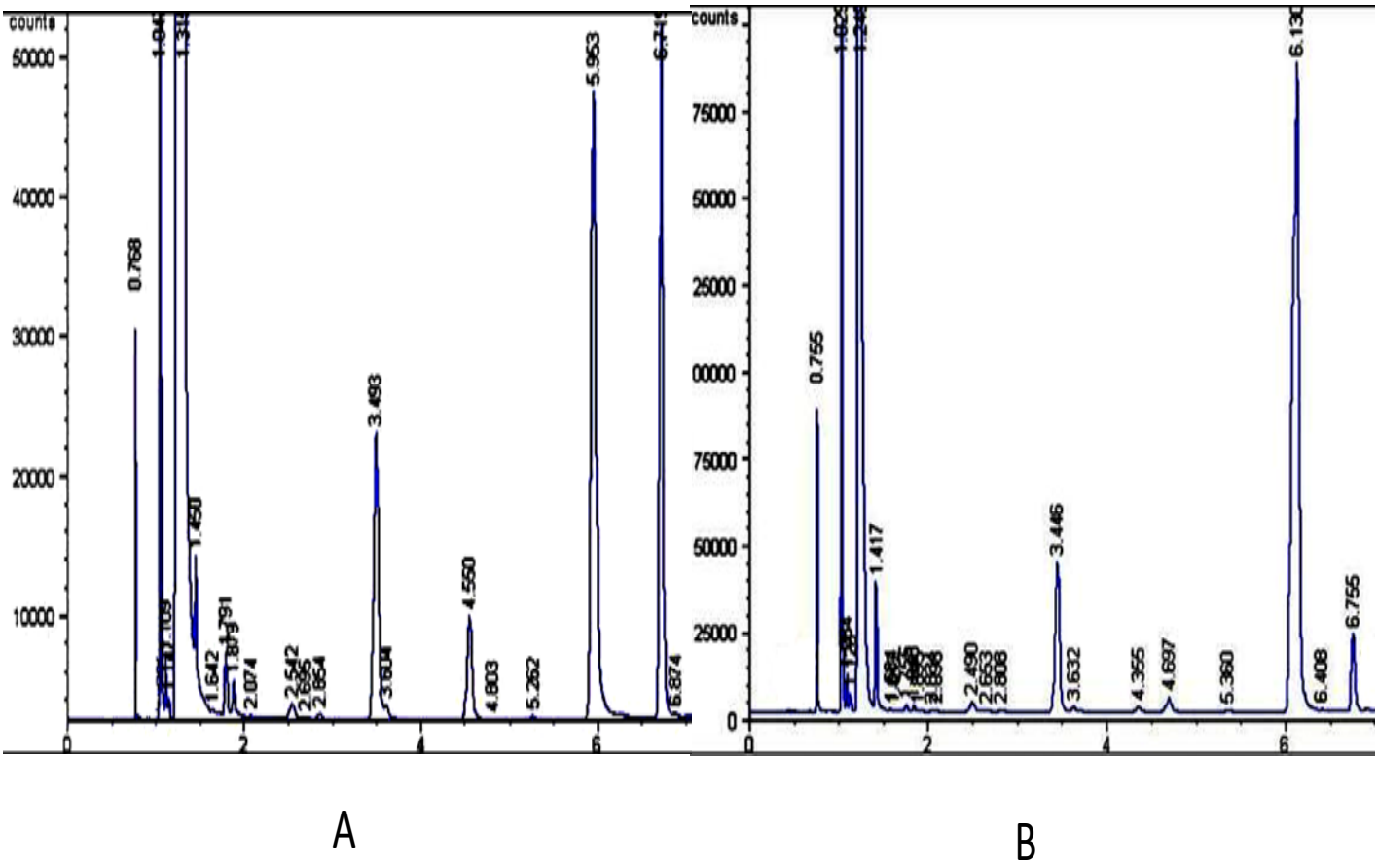

Figure 4. Ester content by gas chromatogram. A represents blank control group; B represents test group.

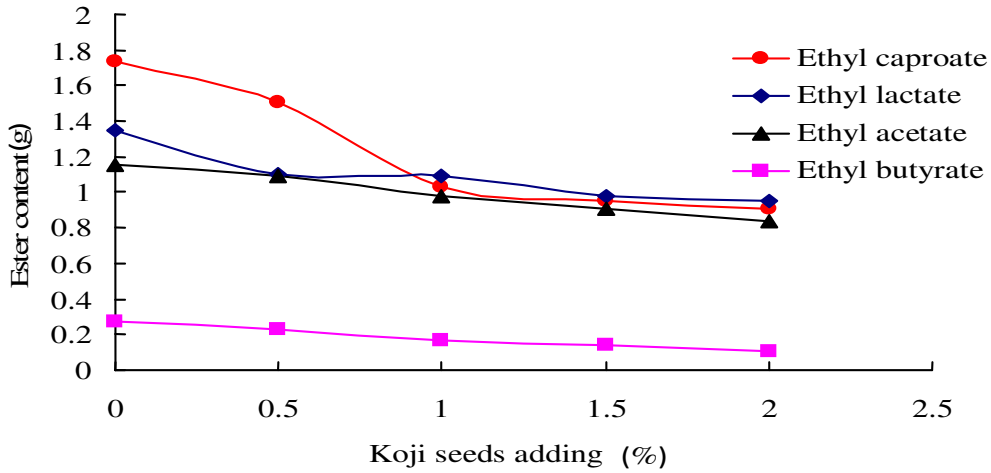

Figure 5. Content of four esters of different *Penicillium* sp. Koji seeds adding.

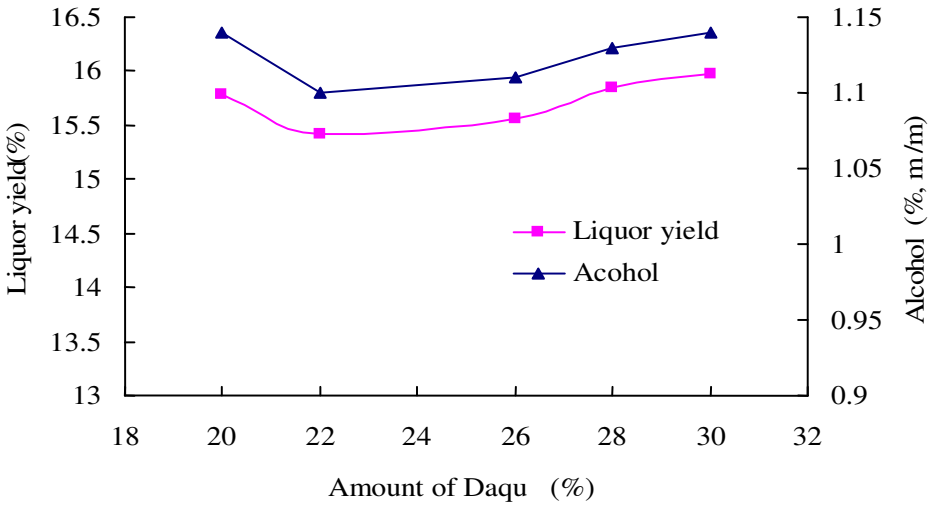

Figure 6. Alcohol and liquor yield of different amounts of *Daqu*.

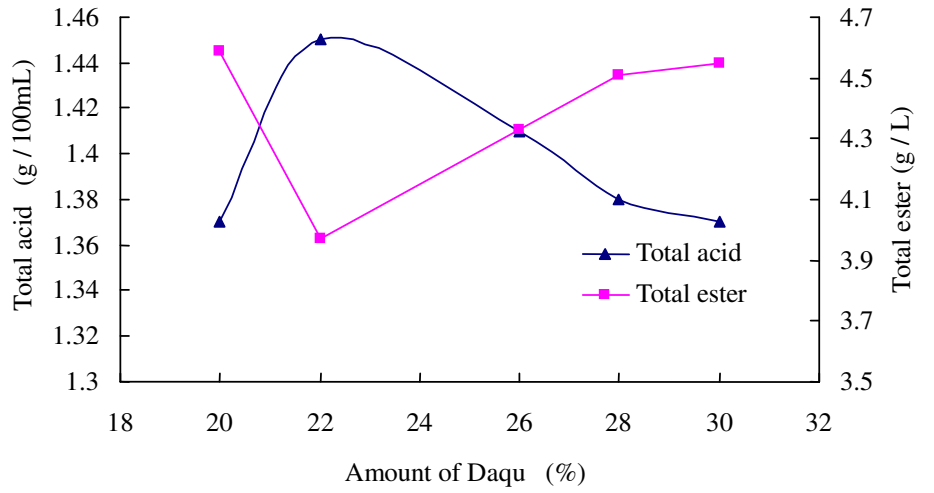

Figure 7. Total acid and total ester of different amounts of *Daqu*.

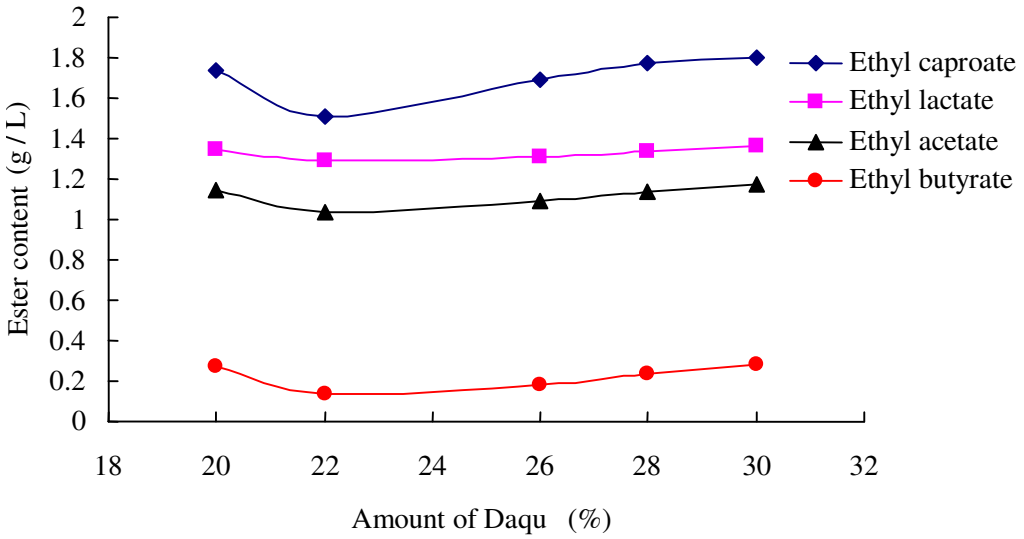

Figure 8. Content of four esters of different amounts of *Daqu*.

not only made liquor yield reduced and total ester decreased but also made total acid increased which finally effected fermentation (Ciu, 2007). And effects of *Penicillium* sp. to the liquor could reduce or disappear when the adding amount of *Daqu* increased, however, it would increase the production costs. As a result, it is necessary to control the existences of *Penicillium* sp. in *Daqu* during liquor production.

Penicillium sp. could grow well under lower temperature and damp conditions, and it had strong inhibitors to other beneficial microbes exist in *Daqu*. And heat releasing was less and temperature of environment grew slowly, as a result of the mycelium was young and tender in the early period of *Daqu* cultured. Thus it should be ensure that the temperature of early period of *Daqu* cultured was stable, as a result, *Aspergillus* could grow well so that *Daqu* quality could be stable and good. Otherwise, if the temperature was up and down it would easily infect *Penicillium* sp. So it was necessary to control the early temperature of *Daqu* cultured and the moisture of *Daqu* cultured.

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Some of the figures in figure 4 are moduled up.