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Microbial Effects on Sesame-Flavor Liquor Accumulation

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Physico-chemical indexes in the accumulation process of sesame-flavor liquor were investigated in this work. The results indicated that ALA, LEU were showed 40% (w%) in aliphatic amino acids, and glucose 60% in reducing sugars, respectively. The activities of functional microorganisms (e.g., mould, bacteria) and hydrolases (e.g., sugar enzyme) were increasingly improved in the range of 4-16 h. Bacillus was the dominant bacteria reaching to 80%. Mould amount was lower than that of bacteria and saccharomycetes, keeping a relatively stable stage, with a maximum of 4×10^4, 5×10^7 and 4×10^7 counts/g at 16 h, respectively. Moreover, saccharifying enzyme vitality was not influenced obviously in sesame-flavor liquor brewing. Nutriments and aroma-producing precursors produced in the preparation phase are beneficial for microorganism.

Key words: Microorganism; Sesame-flavor liquor; Accumulation; Amino acid; Reducing sugar; Enzyme vitality.

Sesame-flavor liquor possesses three natures (i.e., great, pure, sauce flavor), while it is special in relish [1] with the specific fragrance of fry sesame. The accumulation is the preparing phase before putting raw materials in pool for fermentation in high temperature[2].In the phase, environment is comparatively open that benefits for microorganism growth duo to air circulation. Therefore, this phase is appropriate for microorganism in Jiuqu to propagate by large and in net environment[3]. However, it has not been found that a better effect occurred duo to longer time or more raw materials to completely depredate [4]. Amounts of reducing sugars (RSs) can be produced, posing a problem of controlling the ferment speed later. Minor molecule enzyme and sphere protein in auxiliary materials would be utilized by microorganism in acid environment, which leads to aroma-producing precusors fade away[5]. The total content of pyrazine group has been found to reach to 34.7% [6] and fragrant materials (e.g., pyrazine) can be generated by Maillard reaction occurred between depredated RSs and amino acids (AAs).

As new type of liquor, its production technology has a long way to be perfect duo to the instability of the output of high quality liquor. Different indexes were analyzed in this work, aiming at elaborating the importance of accumulation before fermentation, and further avoiding the blindness of traditional experiments to improve the technology for steady production of high quality sesame liquor.

MATERIAL AND METHODS

Three groups materials (fermented grains) were tested and sampled 0, 4, 16, 24 and 40 h. AA and RS was determined using amino acid automatic analyzer (L-8900, Japan) and high performance liquid chromatography (LC-20A, Japan), and vitality of saccharifying enzyme[7] was measured as reported, respectively. The count was conducted

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with the method of dilution plate coated separation. Saccharomycete and mould was fostered with YEPD and czapek's medium at a constant temperature of 30°C, while bacteria were fostered with LB agar nutrient medium at 37°C, respectively.

RESULTS AND DISCUSSIONS

Variation of AA

As Table 1 shown, aliphatic series AAs (e.g., ALA, GLY, ILE, VAL, LEU), accounting for about 40% (w%), were the main acids during the accumulation of AAs. In addition, acidic AAs (e.g., ASP, GLU), basic AAs (ARG, LYS) and hydroxyl AAs (e.g., SER, THR) held nearly 20, 10 and 10%, and aromatic AAs (PHE, TRY), imino acid (PRO), sulfur-containing AAs (CYS, MET) held approximately 8, 7 and 3%, respectively.

It indicated that the total amount of AA increased in a low rate during the first 4 hours. While it accumulated in a high speed between 4 and 16 h, and increased slightly from 16 to 24 h. Finally, it went through a slow fall process beyond 24 h to the end. Variation of AAs showed that microorganisms produced proteases are likely to tend to die with the increase of temperature, simultaneously, vitality of proteases could be also influenced by a increasingly high temperature. Whereas, a favorable condition can be provided by a comparative high temperature for the formation of empyreumatique materials of sesame-flavor liquor[8], which consumed some amino acids.

Table 1. The variation trend of AA in No.1 group

AA content	Time (h)				
(μg/g)	0	4	16	24	40
ASP	18.97	29.88	147.99	181.01	191.67
THR	30.97	47.79	136.75	161.00	181.14
SER	42.90	68.52	180.9	191.72	181.87
GLU	89.17	132.41	462.70	473.55	409.66
GLY	35.86	54.70	151.09	158.11	133.44
ALA	105.62	175.14	429.89	569.44	517.12
CYS	13.05	15.09	32.01	38.91	41.97
VAL	39.11	75.01	199.83	218.73	181.41
MET	20.02	23.67	50.10	66.71	50.44
ILE	29.16	38.09	132.72	141.14	122.97
LEU	41.34	78.17	279.10	261.10	251.79
TYR	23.87	42.19	109.06	125.54	132.87
PHE	28.02	50.01	139.17	155.09	159.03
LYS	34.08	46.93	102.68	123.23	131.00
HIS	27.92	33.68	86.39	102.34	93.11
ARG	33.11	40.91	151.74	195.43	212.46
PRO	36.09	80.15	299.91	259.94	219.20
Sum	649.26	1032.34	3091.32	3422.99	3211.15

Variation of RS

RS generated in accumulation process mainly contained glucose (about 60%) that can provide nutrition for microorganism, and variation of glucose exhibited significantly with the time going onas shown in Table 2. This indicates that accumulation is an important preparation process for sesame-flavor liquor brewing. Moreover, pentose (such as xylose, arabinose) just occupied small part, and was found to vary not significantly during the whole accumulation process. However, from the beginning to 16 h, the total amount of pentose increased slightly, and then decreased beyond 16 h to the end. This suggested that the amount of RS produced was slightly bigger than that consumed in the early 16 h, while the reverse in the later process. However, pentose can not be directly consumed by microorganisms[9], which implies that pentose is primarily consumed to participate in the aroma-producing with AA and

other materials produced in accumulation. This point could also be proved with the decrease of total amount of AAs from 24 h. Moreover, RS variation exhibited significantly for the time, which indicated that accumulation is an important preparation process for sesame-flavor liquor brewing.

Variation of micropopulation

Bacteria

The amount of live bacteria increased slowly in the early 4 h of the accumulation and stayed in demurrage phase, as demonstrated in Fig.1a, while it increased significantly beyond it, reaching up to 5×10^7 counts/g at the end. Meanwhile, the amount of live bacteria in No.2 material was slightly lower than that in No.1 and No.3 samples, which is related to the low concentration of original RS in No.2 material.

Bacillus accounted for about 40% in the initial 16 h before fermentation and the percentage of bacillus increased from 16 h, with a maximum of 80% in the end. Simultaneously, this period is the same time when the hydrolysis rate of starch speeds up[10]. Therefore, hot-like bacillus plays an important role in the hydrolysis process of starch that is the content of raw material during the fermentation of sesame-flavor liquor[11]. Compared with the decrease of pentose and AA in the late period of accumulation, it can be concluded that bacillus has the potential to promote the Maillard reaction between pentose and amino acid.

Table 2. The RS variation trend in No.1 sample

RS content	Time (h)				
(mg/g)	0	4	16	24	40
xylose	1.32	1.42	1.49	1.53	1.51
arabinose	0.95	0.98	1.03	1.12	1.15
fructose	4.01	4.13	4.56	4.07	3.72
mannose	0.54	0.61	0.66	0.59	0.63
glucose	10.49	12.66	18.33	10.58	8.18
Sum	17.31	19.8	26.07	17.89	15.19

Saccharomycetes

The growth curve of saccharomycetes was similar to that of bacteria as shown in Fig.1b, representing continuous multiplication stage. While the count of live saccharomycetes was slightly less than the amount of live bacteria (Fig.1a), with a maximum of 4×10^7 counts/g. This indicated that nutrients and aroma producing precursors can be accumulated in this phase for the multiplication of bacteria, which play a significant role in the later successful fermentation and the formation of flavor liquor materials.

Mould

The total amount of mould (mainly included Hanoi Bai Qu) was far lower than that of bacteria and saccharomycetes with a maximum of 4×10^4 counts/g at 16 h, as illustrated in Fig.1c. It went through a fast accumulation process during the demurrage after 4 h. The internal environment tended to change duo to the consumption of oxygen, which could result in dying to mould. However, as the main product of mould metabolism, the total amount of AAs decreased not significantly with the dying of mould, keeping a relatively stable trend,, which maybe connect with autocytolysis duo to the increasing temperature and decreasing of pH. However, as the main product of mould metabolism, amino acid decreased not significantly with the dying of mould, maintaining a comparative stable stage. This connect with autocytolysis, which results from the raising temperature and decreasing of pH [12].

Vitality of saccharifying enzyme

We can conclude that the greatest vitality of saccharifying enzyme showed approximately 550U/g between 16 and 24 h, observed in Fig.1d. The vitality of saccharifying enzyme enhanced comparatively slow duo to microorganisms were in demurrage stage during the early 4 h of accumulation and their multiplication appeared at logarithmic phase between 4 and 16 h, From 16 h,

saccharomycetes and bacteria were still in logarithmic phase, but the the vitality variation of saccharifying enzyme exhibited not distinctly. Meanwhile, the vitality of saccharifying enzyme was not influenced significantly by saccharomycetes and bacteria, which indicated that mould is a main saccharifying agent in sesame-flavor liquor brewing.

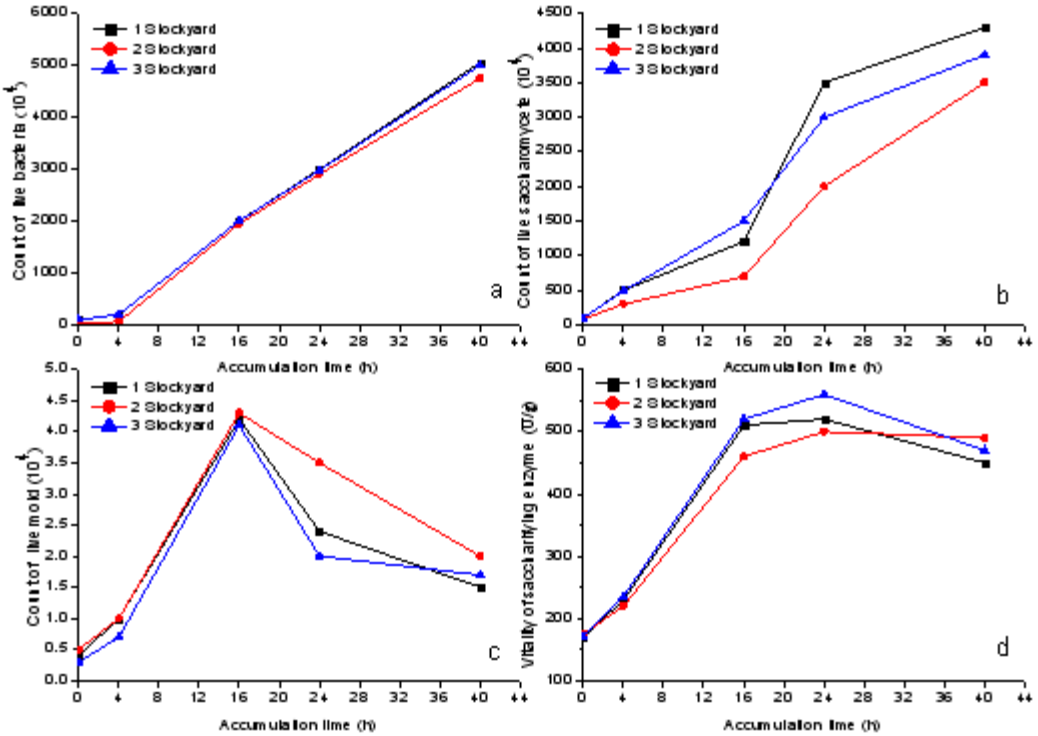

Fig.1. Effect of temperature on variation of micropopulation: live bacteria (a), saccharomycete (b), mould (c) and the vitality of saccharifying enzyme (d) in 3 groups

CONCLUSION

The accumulation was an important preparation phase in sesame-flavor liquor brewing. During the accumulation, the increase of nutriments and aroma-producing precursors such as amino acids and reducing sugars makes sufficient preparation for the production of sesame-flavor liquor brewing by microorganisms. Aliphatic series amino acids and glucose were the main content in amino acids (40%, w%) and reducing sugars (60%), respectively, and reducing sugars varied significantly for the time. The total amount of reducing sugars decreased after16 h duo to the continuous multiplication of bacteria and saccharomycetes consuming reducing sugars that related to the internal environment changes (e.g., temperature, pH and oxygen situation) which influenced the vitality of saccharifying enzyme. Pentose cannot be used by microorganisms while it is an important precursor material to form representative aroma of sesame-flavor liquor brewing at 16 h.

Moreover, in the aspect of micropopulation, the amount of bacillus accounted for about 40% during the initial16 h and reached to 80% in the end. Mould amount keeping a stable stage was lower than that of bacillus and saccharomycetes with a maximum of 4×10^4, 5×10^7 and 4×10^7 counts/g at the time of 16 h, respectively. Moreover, the vitality of saccharifying enzyme was not influenced significantly by saccharomycetes and bacteria, which indicated that mould is a main saccharifying agent in sesame-flavor liquor brewing and reached up to 550U/g between 16 and 24 h.

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