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Effect of Marine Bacillus BC-2 on the Health-Beneficial Ingredients of Flavor Liquor

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Abstract

The main aroma component of Luzhou-flavor Liquor is ethyl caproate, which is combined with appropriate amount of ethyl butyrate, ethyl acetate, ethyl lactate and so on. By adding the marine bacillus BC-2 (Accession number: MK811408) to substrate sludge, the bacillus complex bacterial liquid (pit Mud Functional Bacterial liquid) has been modified. The complex bacterial liquid was used in the production of Luzhou-flavor Liquor and it dramatically promoted the content of health-beneficial ingredients in the new workshop. These results demonstrated that the marine bacillus BC-2 can effectively improve the quality and health benefit of Luzhou-flavor Liquor.

Keywords

Luzhou-Flavor Liquor, Marine Bacillus BC-2, Flavoring Components

1. Introduction

In China, Luzhou-flavor Liquor is the most widely used Luzhou-flavor Liquor in social intercourse and its taste is closely related to the quality of pit mud. Pit mud is the place where bacillus grows, propagates and metabolizes to produce function factors, whose content affects the quality and health benefit of Luzhou-flavor Liquor [1].

Trace element composition is a scientific index for evaluating Luzhou-flavor Liquor quality. Previous studies have shown that Luzhou-flavor Liquor quality and aroma are attracting more and more attention [2]. Ethanol and water are the main components of Luzhou-flavor Liquor; they account for 98% - 99.9% (e.g. vodka) of the total Luzhou-flavor Liquor [3], while the remaining 2% is essential, such as alcohols, organic acids, esters, phenols and so on [4]. These microelements have a great influence on the quality of Luzhou-flavor Liquor and determine the different aroma and flavor characteristics of different liquors. Thus

there are different flavor styles of liquor. According to the fragrance types, the common are: fragrance, sauce, strong fragrance, sesame and rice fragrance, etc. Moreover, low molecular organic acids, lactones, phenols and heterocycles are the beneficial ingredients included in liquor [5]. Among them, tetramethylpyrazine, 4-methyl guaiacol and 4-ethyl guaiacol are aromatic and odorous substances commonly contained in liquor and they have certain physiological activities. Especially, tetramethylpyrazine, which contributes sweetness, fruit and flower fragrance to wine, has physiological effects such as inhibiting platelet aggregation, improving microcirculation and reducing brain atrophy injury [6] and the phenolic compounds, 4-methyl guaiacol and 4-ethyl guaiacol are excellent free radical eliminators, which have good antioxidant, anti-tumor and enhancing human immunity [7] [8] [9]. Therefore, the study of these three beneficial components in liquor has certain guiding significance for exploring the health benefits of liquor.

In the actual production process, the degradation of pit mud will lead to the decrease of ethyl caproate content in Luzhou-flavor Liquor, which seriously affects the flavor and taste of liquor [1]. At present, researchers have adopted many methods to repair the aged pit mud, such as super-concentrated compound bacillus solution [10], solid (liquid) pit mud functional bacteria [10], artificial pit mud resistance [11] and so on, to remedy the adverse consequences of pit mud degradation.

In this study, we added marine bacillus BC-2 to pit mud and then modify the bacillus complex bacterial liquid. The modified complex was used to cultivate artificial old pit mud and to produce Luzhou-flavor Liquor. The content of 4-methyl guaiacol in liquor in which new pit mud functional bacterial liquid was greatly increased from 305.17 g/L to 567.47 g/L. Furthermore, the content of 2,3,4,5,6-Tetramethylpyrazine also increased dramatically from 974 g/L to 1400 g/L. These results showed that the marine bacillus BC-2 can be used to improve the health factor of Luzhou-flavor Liquor.

2. Material and Methods

2.1. Materials and Reagents

Bacillus: screened from Mariana trench seawater.

Seed liquid preparation: Beef extract 5 g, peptone 10 g, sodium chloride 5 g, water 1000 mL, pH = 7.0 - 7.2. The prepared seed culture liquid was separated into triangular bottles, sealed with a sealed film, wrapped in newspapers, and then sterilized in a high-pressure sterilizing pot. The sterilization conditions were 121˚C and 20 min. Activated strains were inoculated into triangular flasks containing seed culture medium. The volume of liquid was 100/250 mL, 130 r/min in oscillator and 24 h in oscillatory culture at 37˚C.

2.2. Brewing of Luzhou-Flavor Liquor

The basic control parameters Luzhou-flavor liquor brewing included grain-steeping

time, primary steaming and secondary steaming time, moisture content of grains after steaming, starter use level, and grains blending ratio. In order to drive out the odor and harmful substances in rice husk, the rice husk should be steamed for 30 - 40 minutes beforehand and then controlled the pit entry temperature is generally controlled by 15% to 17% starch concentration, acidity below 2.0, moisture 55% - 58%, temperature at 18˚C - 20˚C. 1 - 1.5 kg Daqu grains were sprinkle powder on the bottom of cellar to promote aroma generation.

2.3. Composition Determination

Take three bottles of Luzhou-flavor Liquor as sample, using the test methods of Light-color-heat-mass-meta-chemistry technology, GCMS Gas Chromatography-Mass Spectrometry, ICP-MS Inductively Coupled Plasma Mass Spectrometer, GC Gas Chromatograph, High Performance Liquid Chromatograph, Automatic Karl Fischer moisture analyzer, and SPME-HSGCMS Solid Phase Microextraction-Headspace Gas Chromatography-Mass Spectrometer.

3. Results

The modified the bacillus complex bacterial liquid optimized mixed was used to replace the traditional pit mud and then produce Luzhou-flavor Liquor under existing mature production conditions. The trace elements in Luzhou-flavor Liquor under the existing mature production conditions were determined to analyze the quality of Luzhou-flavor Liquor before and after adding marine bacillus BC-2. Changes of element contents in compound pit Luzhou-flavor Liquor of before and after added ocean bacillus were shown in Table 1. We can find that the content of element contents in flavor Luzhou-flavor Liquor with and without compound pit mud of marine bacillus BC-2 changed little. This means that the Luzhou-flavor Liquor aroma did not affect by adding of marine bacillus BC-2.

Although the addition of marine bacillus BC-2 did not affect the aroma of Luzhou-flavor Liquor, we also found that the contents of three function factors that determine Luzhou-flavor Liquor function increased to varying degrees. From Table 2, we can easily found that the quality of Luzhou-flavor Liquor produced by optimized marine bacillus BC-2 is obviously better than that produced by conventional sediment. The content of 4-methyl guaiacol in flavor Luzhou-flavor Liquor produced by functional bacteria of compound pit mud increased greatly, reaching from 305.17 μg/L to 567.47 μg/L. The 2,3,4,5,6-Tetramethylpyrazine also increased greatly from 974 μg/L to 1400 μg/L. The marine bacillus BC-2 can effectively improve the health factors of flavor Luzhou-flavor Liquor.

4. Discussion

Previous studies have shown that Luzhou-flavor Liquor quality and aroma are determined by aromatic organic compounds [3]. Results here demonstrated that

Table 1. Changes of element contents in compound pit Luzhou-flavor Liquor of before and after added ocean bacillus.

Element	Before (μg/L)	After (μg/L)	Element	Before (μg/L)	After (μg/L)
Ethyl hexanoate	205.94	206.3	Ethyl laurate	0.44	0.46
Ethyl lactate	187.4	186.3	Ethyl myristate	0.33	0.37
ethyl acetate	158.9	159.8	Ethyl palmitate	8.7	8.9
Ethyl butyrate	24.3	24.8	Ethyl oleate	2.6	2.9
Ethyl valerate	6.8	6.9	Ethyl linoleate	3.5	3.7
Ethyl palmitate	5.3	5.1	2,3-butanediol	4.2	4.1
Ethyl linoleate	2.1	1.9	Decyl Alcohol	3.11	3.2
Ethyl oleate	2.4	2.6	Beta phenylene alcohol	2.9	3.1
Ethyl decate	1.9	2.3	Lauric acid	1.1	1.2
Ethyl heptanate	2.7	2.9	Myristic acid	0.55	0.51
Ethyl caprylate	5.6	5.7	Formic acid	8.1	8.2
Ethyl formate	8.6	8.6	Acetic acid	51.4	51.1
Terbutyl acetate	4.07	4.1	propionic acid	1.3	1.6
Isoamyl acetate	0.97	1.02	Butyrate	14.11	14.6
Caproic acid propyl ester	1.55	1.59	Valerate	1.8	1.9
Isopentyl hexanoate	1.33	1.36	Caproic acid	27.3	28.5
Ethyl nonyl	4.2	4.3	Heptanic acid	0.2	0.18
Diethyl butyroacetate	6.6	6.7	Bitter	0.2	0.21
Ethyl hexanoate	204.1	206.3	Pelargonic acid	0.009	0.012
Ethyl lactate	183.3	186.3	Decic acid	0.033	0.039
Ethyl acetate	159.7	159.8	Palmitic acid	0.25	0.27
Ethyl butyrate	24.8	24.8	Linoleic acid	0.46	0.42
Ethyl valerate	6.5	6.9	Isobutyric acid	1.2	1.23
Ethyl palmitate	5.2	5.1	Isovalerate	1.5	1.42
Ethyl linoleate	1.8	1.9	Eighteen acid	0.026	0.026
Ethyl oleate	2.5	2.6	Lauric acid	0.05	0.036
Ethyl decate	2.1	2.3	Myristic acid	0.044	0.046
Ethyl heptanate	2.9	2.9	Oxalate	22.01	22.03
Ethyl caprylate	5.6	5.7	Tartaric acid	0.089	0.087
Ethyl formate	8.7	8.6	Malic acid	1.11	1.12
Terbutyl acetate	4.2	4.1	Lactic acid	2.75	2.78
Isoamyl acetate	0.98	1.02	Citric acid	2.2	2.2
Caproic acid propyl ester	1.55	1.59	Succinic acid	1	1.1
Isopentyl hexanoate	1.6	1.36	Maleic acid	0.024	0.018
Ethyl nonyl	4.2	4.3	Fumaric acid	0.18	0.2

Continued

Diethyl butyroacetate	6.5	6.7	Isoamyl alcohol	35.4	35.2
Phenylethyl acetate	0.41	0.43	Propyl alcohol	5.6	5.3
methanol	23.5	23.6	Isovaleraldehyde	0.9	0.8
Isobutanol	27.1	27.2	Propionic aldehyde	1.1	1.2
N-butanol	34.2	34.2	Isobutyral	3.95	4.1
SEC butyl alcohol	3.6	3.7	Furfural	0.15	0.16
Hexanol	8.9	8.7	N-butyral	0.11	0.13
2,3-butanediol	1.3	1.2	Acetone	0.06	0.05
Amyl alcohol	6.3	6.2	Butanone	0.18	0.17
SEC pentyl alcohol	2.4	2.3	Formaldehyde	0.99	0.98
Acetal	63.4	63.2	Benzaldehyde	5.2	5.3
Acetaldehyde	40.1	39.7			

Table 2. Changes of function factors content in compound pit Luzhou-flavor Liquor of before and after added ocean bacillus.

Function factor (μg/L)	Before (μg/L)	After (μg/L)
4-methyl guaiacol	305.17	567.47
4-ethylphenol	19.64	20.05
2,3,4,5,6-Tetramethylpyrazine	974	1400

the addition of marine bacillus BC-2 did not affect the Luzhou-flavor Liquor aroma. Meanwhile, adding the marine bacillus BC-2 can dramatically improve the content of function factor 4-methyl guaiacol and 2,3,4,5,6-Tetramethylpyrazine. Function factor of tetramethylpyrazine can be used for cancer, autoimmune, inflammatory, neuro- and cardiovascular diseases, and is believed to attenuate platelet aggregation, prevent thrombus formation and improve microcirculation as well [12] [13] [14] [15]. Moreover, previous researches proved that function factor 4-methyl guaiacol can against abnormal oxidative stress [16]. It is reasonable to presume that the Luzhou-flavor Liquor produced with the new modified bacillus complex bacterial liquid may have functions of improving microcirculation, anti-tumor and enhancing human immunity and so on.

5. Conclusion

Marine bacillus BC-2 is derived from seawater of Mariana Trench and has the characteristics of high-temperature resistance. We add it to pit mud and optimize it. The optimized pit mud used in the production of flavor liquor and it can significantly increase the contents of 4-methyl guaiacol and 2,3,4,5,6-Tetramethylpyrazine in liquor. It demonstrated the marine bacillus BC-2 with high production value. Liquor produced by optimized pit mud has health function.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Li, F., Wu, X., Wang, X. and Qiu, S. (2014) Application of Microbiological Technology in Aroma Enhancement of Luzhou-Flavor Liquor. *China Brewing*, **33**, 9-13.

[2] Liang, J., Xiao, Q., Chen, D., Wan, Y. and Zeng, Y. (2015) Study on the Distribution of Trace Elements in Liquors with Brand Based on the Principal Component Analysis. *Science and Technology of Food Industry*, **36**, 67-70.

[3] Wu, T., Yang, H. and Zhang, K. (2002) The Basic Study on Liquor Aroma Related to Chromatograph Skeleton Components in Liquor. *Liquor Making*, **29**, 25-28.

[4] Cheng, L. (2013) A Brief Analysis of the Relation between Several Important Components and Quality of Liquor. *Heilongjiang Science and Technology Information*, No. 36, 62-63.

[5] Xu, Y., Fan, W., Ge, X. and Huang, Y. (2013) Scientific Recognition of Biofunctional Components in Chinese Liquors. *Liquor-Making Science & Technology*, No. 9, 1-6.

[6] Li, D. (2008) Moderate Drinking Is Helpful for Your Health. *Liquor-Making Science & Technology*, No. 10, 133-138.

[7] Wu, J., Huang, M., Sun, B., Zheng, F. and Sun, J. (2014) Analysis of Volatile Compounds in Jingzhi Baigan Liquor by Liquid-Liquid Extraction (LLE) and Gas Chromatography-Mass Spectrometry (GC-MS). *Food Science*, **35**, 72-75.

[8] Fan, H., Fan, W. and Xu, Y. (2015) Characterization of Key Odorants in Chinese Chixiang Aroma-Type Liquor by Gas Chromatography-Olfactometry, Quantitative Measurements, Aroma Recombination, and Omission Studies. *Journal of Agricultural and Food Chemistry*, **63**, 3660-3668. https://doi.org/10.1021/jf506238f

[9] Wu, T., Zhu, S., Sun, X., Zhao, W. and Cui, G. (2013) Analysis of Health Factors of Meilanchun Sesame-Flavor Liquor. *Liquor-Making Science & Technology*, No. 8, 125-130.

[10] Ren, J., Yao, W., Tang, Y., Ren, D., Liu, M., Yan, H. and Liu, X.-Y. (2014) Studies on the Cultivate Formula of Hybrid Functional Microorganism of Pit Mud. *Liquor Making*, **41**, 47-50.

[11] Zhang, X., Liu, M., Tang, Y., Zhao, K. and Gu, Y. (2017) Recent Advances in Research on the Community, Isolation, and Application of Microbes in the Pit Mud Used in Manufacture of Chinese Strong-Flavor Baijiu. *Microbiology*, **44**, 1222-1229.

[12] Zhang, M., Gao, F., Teng, F. and Zhang, C. (2014) Tetramethylpyrazine Promotes the Proliferation and Migration of Brain Endothelial Cells. *Molecular Medicine Reports*, **10**, 29-32. https://doi.org/10.3892/mmr.2014.2169

[13] Wu, Y., Xu, Z., Yang, Y., Qiu, J., Yang, M., Wu, C., Lai, Z., Tang, M., Ge, J., Yu, K.M. and Zhuang, J. (2019) Tetramethylpyrazine (TMP) Ameliorates Corneal Neovascularization via Regulating Cell Infiltration into Cornea after Alkali Burn. *Biomedicine & Pharmacotherapy*, **109**, 1041-1051. https://doi.org/10.1016/j.biopha.2018.10.091

[14] Shen, J., Zeng, L., Pan, L., Yuan, S., Wu, M. and Kong, X. (2018) Tetramethylpyrazine Regulates Breast Cancer Cell Viability, Migration, Invasion and Apoptosis by Affecting the Activity of Akt and Caspase-3. *Oncology Letters*, **15**, 4557-4563.

https://doi.org/10.3892/ol.2018.7851

[15] Mehmood, K., Zhang, H., Kashif Iqbal, M., Ur Rehman, M., Li, K., Huang, S.-C., Shahzad, M., Nabi, F., Iqbal, M. and Li, J.-K. (2018) Tetramethylpyrazine Mitigates Toxicity and Liver Oxidative Stress in Tibial Dyschondroplasia Chickens. *Pakistan Veterinary Journal*, **38**, 76-80. https://doi.org/10.29261/pakvetj/2018.015

[16] Zhao, D., Shi, D., Sun, J., Li, H., Zhao, M. and Sun B.-G. (2018) Quantification and Cytoprotection by Vanillin, 4-Methylguaiacol and 4-Ethylguaiacol against AAPH-Induced Abnormal Oxidative Stress in HepG2 Cells. *RSC Advances*, **8**, 35474-35484. https://doi.org/10.1039/C8RA06505E