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SHORT COMMUNICATION

OPTIMIZATION OF FERMENTATION PROCESS FOR APRICOT VINEGAR

Optimización del proceso de fermentación para producir vinagre de albaricoque

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Key words: alcoholic fermentation, acetic fermentation, single factor experiment, orthogonal experiment.

ABSTRACT

To study the optimal fermentation conditions of *Prunus armeniaca* L. (apricot), single factor experiments and orthogonal experiments were implemented. The experimental results demonstrate that the optimum alcoholic fermentation conditions are: sugar degree a 9%, inoculation quantity of yeast at 0.7%, fermentation temperature at 30 ºC. The alcohol degree could reach 7.56%; the optimum acetic fermentation conditions are: alcohol degree at 7%, inoculation quantity of acetic acid bacillus at 0.8%, fermentation temperature at 32 ºC, fermentation time at 7 days, and the total acid content could be obtained as 6.78 g/100 mL. Under such conditions, the fruit vinegar has rich *Prunus armeniaca* L. flavor and pure taste.

Palabras clave: fermentación alcohólica, fermentación acética, experimento de factor único, experimento ortogonal.

RESUMEN

Para estudiar las condiciones óptimas de fermentación de *Prunus armeniaca* L. (albaricoque), se implementaron experimentos de factor único y experimentos ortogonales. Los resultados experimentales demuestran que las condiciones óptimas de fermentación alcohólica son: grado de azúcar 9%, cantidad de inoculación de levadura 0.7%, temperatura de fermentación 30 ºC. El grado de alcohol podría alcanzar el 7.56%; las condiciones óptimas de fermentación acética son: grado de alcohol 7%, cantidad de inoculación de bacilo de ácido acético 0.8%, temperatura de fermentación 32 ºC, tiempo de fermentación 7 días; el contenido total de ácido podría ser de 6.78 g/100 mL. En tales condiciones, el vinagre de fruta tiene sabor rico y puro a *Prunus armeniaca* L.

INTRODUCTION

Prunus armeniaca L., a plant of the Rosaceae family of the prunus genus, is widely cultivated in Northeast, Northwest, and North China, providing abundant resources mainly for edible almonds. It has a 60%-80% flesh component and low sugar content. The contents of organic acid, protein, fat, VC, pectin, calcium, potassium, iron, selenium, manganese, polyphenols, and other nutritional and health ingredients are all better than many ordinary fruits. The fruit is not eaten by many due to its sour and astringent taste and cannot be stored for long because it is easily rotten, resulting in a considerable amount of waste. Making apricot vinegar is a fast process without requiring high-quality raw materials. The nutrient and flavor apricot pulp with over 15% of soluble solids is an excellent choice (Ma et al. 2021). Brewing fruit vinegar is undoubtedly the best way to make apricot pulp profitable. At present, fruit vinegar, such as apple vinegar and citrus vinegar, has been mass-produced in China and is well-received by consumers. However, apricot vinegar is rarely seen in reports. This article reports fruit vinegar production with apricot pulp as the raw material. It determines the optimal fermentation conditions for apricot vinegar through single-factor and orthogonal experiments, providing references for future processing of apricot pulp (Wang et al. 2015, Tian and Wang 2016, Ji et al. 2021).

MATERIALS AND METHODS

Test materials

Two main varieties of apricot, Youyi and Longwangmao, were selected from Baodai Township, Zhulu County, Zhangjiakou City, Hebei Province, with their fruit flesh provided as the test materials. The fruits taken were required to be well-grown, normally matured, and free from pests and diseases when harvested.

Experimental process

Prunus Armeniaca L. pulp → cleaning and pitting → milling and filtering → clarifying → adjusting sugar degree → alcoholic fermentation → acetic fermentation → original fluid → secondary filtering → apricot vinegar drinks

Operation key point

Fruit selection: Select fully ripe apricot fruits and remove the rotten ones and those infected by pests and diseases. Wash them thoroughly with running water, remove their stones, and then crush them with high-speed mill homogenizer to obtain apricot fluid.

Pectinase enzymatic hydrolysis: 4000 active units of liquid pectinase were added to the apricot fluid, accounting for 0.2% of the fluid with a temperature at 45 ºC and an enzymatic hydrolysis time of 2.5 hours. The processed fruit juice was clear and transparent.

Adjusting sugar content: Since apricot pulp has a low sugar content, sugar must be added as a prerequisite of fermentation to reach the designed alcohol content. Sucrose was added as sugar. For better fermentation results, half of the sugar was added at the beginning, and the other half after a period time of fermentation.

Alcoholic fermentation: Add the activated yeast starter culture into the fruit pulp for fermentation after the adjustment of sugar content. The temperature should be maintained at around 30 ºC for 3 days. This stage should be finished when the alcohol content no longer increases and the bacterial strain is depleted (Zhang et al., 2006).

Acetic fermentation: Add a 0.8% acetobacter amount into the mash after the alcoholic fermentation is finished. As an aerobe, acetobacter needs to be fermented with constant stirring or oxygen feeding. The temperature of the fermentation broth and the contents of alcohol and acetic acid are measured daily. When the acetic acid content no longer increases, acetic fermentation should be finished (Wang 2012, Zhao et al. 2015).

Determination of alcohol fermentation process conditions

Single factor determination of conditions in alcoholic fermentation process

The alcoholic fermentation was carried out in 250 mL conical flasks each filled by 80% with the fruit pulp. Each experiment group was repeated three times to explore the alcoholic fermentation condition, the original sugar degree: 8%, 9%, 10%, 11%, 12% (fermentation temperature fixed at 26 ºC, inoculation quantity of yeast at 7%); fermentation temperature: 24 ºC, 26 ºC, 28 ºC, 30 ºC, 32 ºC, (sugar degree fixed at 10%, inoculation quantity of yeast at 0.7%); inoculation quantity of yeast: 0.5%, 0.6%, 0.7%, 0.8%, 0.9% (fermentation temperature fixed at 26 ºC, sugar degree at 10%) (Xu, 2010; Zhang, 2010; Qi et al., 2021).

Optimization of alcoholic fermentation conditions

As shown in **Table I**, the three factors, the original fermentation temperature, sugar degree, and inoculation

TABLE I. FACTORS AND LEVELS OF ORTHOGONAL EXPERIMENT FOR OPTIMIZING THE ALCOHOLIC FERMENTATION.

Level	Factors		
	A sugar degree (%)	B inoculation quantity of yeast (%)	C fermentation temperature (ºC)
1	9	0.6	28
2	10	0.7	30
3	11	0.8	32

quantity of yeast, were taken as levels ($L_9(3^3)$) for the orthogonal experiment (He 2021). Alcohol degree was taken as the index to determine the optimal conditions for the alcoholic fermentation (Tan 2019).

Determination of acetic fermentation process conditions

Single factor determination of conditions in acetic fermentation process

The acetic fermentation was carried out in 250 mL conical flasks each filled by 80% with the fruit wine obtained from the previous experiment. Each experiment group was repeated three times to explore acetic fermentation condition, fermentation temperature: 26 ºC, 28 ºC, 30 ºC, 32 ºC, 34 ºC, 36 ºC, (inoculation quantity of acetic acid bacillus at 0.8%, alcohol degree at 7%, 6 days); alcohol degree: 4%, 5%, 6%, 7%, 8%, 9% (fermentation temperature at 30 ºC, inoculation quantity of acetic acid bacillus at 0.8%, 6 days); inoculation quantity of acetic acid bacillus: 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1% (fermentation temperature at 30ºC, alcohol degree at 7%, 6 days); fermentation time: 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, (fermentation temperature at 30 ºC, alcohol degree at 7%, inoculation quantity of acetic acid bacillus at 0.8%).

Optimization of acetic fermentation conditions

As shown in **Table II**, the four factors, fermentation temperature, alcohol degree, inoculation quantity of acetic acid bacillus and fermentation time, were taken as levels ($L_9(3^3)$) for the orthogonal experiment (Hu 2021). Total acid content was taken as the index to determine the optimal conditions (Yang 2020).

Determination method

Alcohol determination: alcohol counting estimation; total acid content determination: acid-base titration counting by acetic acid.

RESULTS AND ANALYSIS

Optimization of conditions in alcoholic fermentation process

The effect of fermentation temperature on alcoholic fermentation

As **figure 1** shows, alcohol degree displays a trend from rise to decline as temperature rises. The lowest alcohol degree was 6.56% appearing at 24 ºC. The alcohol degree rose quickly in 24-26 ºC, and the rise gradually slowed down at above 26 ºC. When temperature reached above 30 ºC, the alcohol degree went down as temperature continued to rise. An environment of high temperature over 30 ºC might inactivate the yeast. Therefore 30 ºC was fixed as the fermentation temperature for alcoholic fermentation.

The effect of initial sugar degree on alcoholic fermentation

As **figure 2** shows, alcohol degree dislays a roughly rising trend as sugar degree rises. Alcohol degree increased quickly when sugar degree ranged from 8% to 10%, while the increased slowed down when sugar degree was from 10% to 12%. The alcohol degree valley was 5.91% at the point of 8% sugar degree, and it peaked at 7.14% when sugar degree

TABLE II. FACTORS AND LEVELS OF ORTHOGONAL EXPERIMENT FOR OPTIMIZING THE ACETIC FERMENTATION.

Level	Factors			
	A alcohol degree (%)	B inoculation quantity of acetic acid bacillus (%)	C fermentation temperature (ºC)	D fermentation time (d)
1	6	0.7	28	6
2	7	0.8	30	7
3	8	0.9	32	8

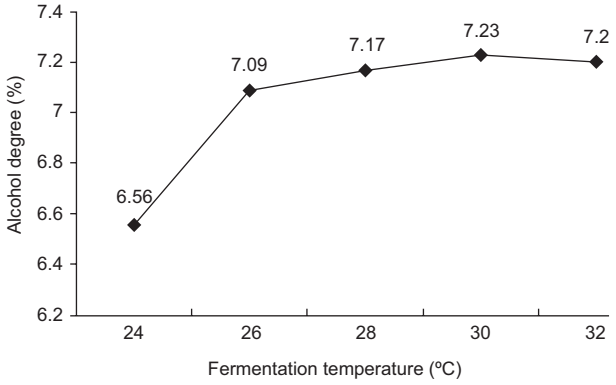

Fig. 1. Effect of fermentation temperature on alcohol fermentation.

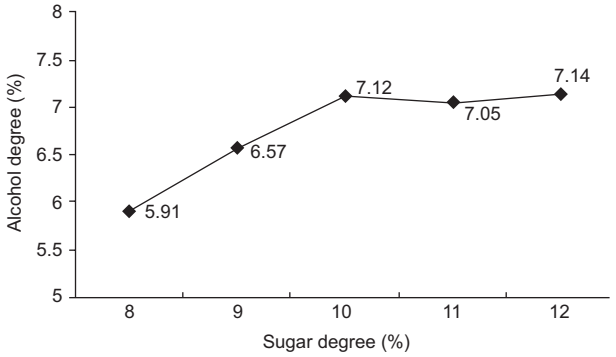

Fig. 2. Effect of sugar degree on alcohol fermentation.

was 12%. The alcohol degree did not continue to increase at higher sugar degrees. When sugar degree reached a level too high, yeast growing would be largely restrained, causing a waste of raw materials. Therefore, 10% was selected as the sugar degree for the alcoholic fermentation.

The effect of yeast inoculation on alcoholic fermentation

As **figure 3** shows, alcohol degree displays a trend from rise to decline as inoculation quantity of yeast rises. Alcohol degree got a stable uplift when inoculation quantity of yeast ranged from 0.5% to 0.7%, while it went down steadily at the 0.7-0.9% range of inoculation quantity of yeast. The lowest alcohol degree was 6.48% at the point of 0.5% inoculation quantity of yeast. The summit alcohol degree was 7.21% when inoculation quantity of yeast was 0.7%. Not enough yeast inoculation quantity would put off the alcoholic fermentation, resulting in longer fermentation period. While too much yeast would lead to accelerated bacterial reproduction of the nutrients in

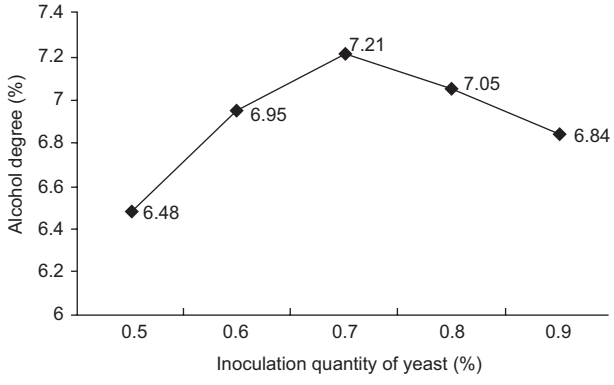

Fig. 3. Effect of inoculation quantity of yeast on alcoholic fermentation.

fermentation broths, thus alcoholic fermentation was reduced. Inoculation quantity of yeast was selected at 0.7% for the alcoholic fermentation.

Orthogonal optimization of alcoholic fermentation conditions

The experimental results are presented in **table III** with $A_1B_2C_2$ as the best combination in the orthogonal experiment: sugar degree at 9%, inoculation quantity of yeast at 0.7%, and fermentation temperature at 30ºC. Under such conditions, alcohol degree was 7.56%. Based on range analysis of this orthogonal experiment, $R_B > R_C > R_A$, which means in alcoholic fermentation, inoculation quantity of yeast has the biggest impact on alcohol degree, followed by fermentation temperature and then sugar degree. The order of the influencing factors for alcoholic fermentation was B > C > A (inoculation quantity of yeast > fermentation temperature > sugar degree).

Optimization of conditions in acetic fermentation process

The effect of alcohol content on acetic fermentation

As **figure 4** shows, total acid content displays a trend from rise to decline as alcohol degree rises. Total acid content went up averagely when alcohol degree ranged from 4% to 7%, while it fell evenly when alcohol degree was at the range of 7-9%. The bottom total acid content was 5.92 g/100 mL at the point of 4% alcohol degree. The highest total acid content was 6.67 g/100 mL when alcohol degree was 7%. The total acid content gradually reduced when alcohol degree exceeded 7%. It is possible that acetic fermentation could be restrained from too high alcohol degree. Therefore 7% was selected as the alcohol degree for acetic acid fermentation.

TABLE II. RESULTS AND ANALYSIS OF ORTHOGONAL EXPERIMENT FOR THE ALCOHOLIC FERMENTATION.

Experiment number	A sugar degree (%)	B inoculation quantity of yeast (%)	C fermentation temperature (ºC)	Alcohol degree (%)
1	9	0.6	28	6.76
2	9	0.7	30	6.88
3	9	0.8	32	7.17
4	10	0.6	30	7.32
5	10	0.7	32	6.59
6	10	0.8	28	6.96
7	11	0.6	32	6.54
8	11	0.7	28	7.05
9	11	0.8	30	7.12
K_1	6.937	6.873	6.923	-
K_2	6.597	6.840	7.107	-
K_3	6.903	7.083	6.767	-
R	0.054	0.243	0.340	-

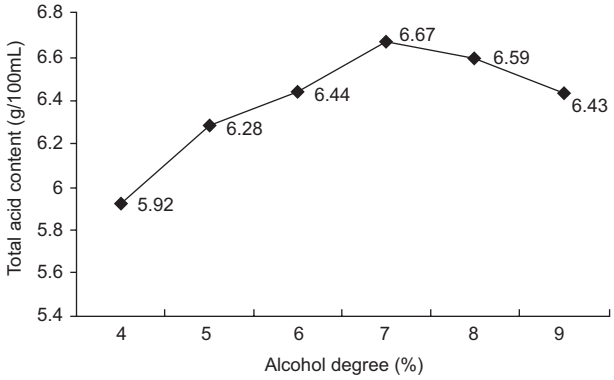

Fig. 4. Effect of alcohol degree on acetic fermentation.

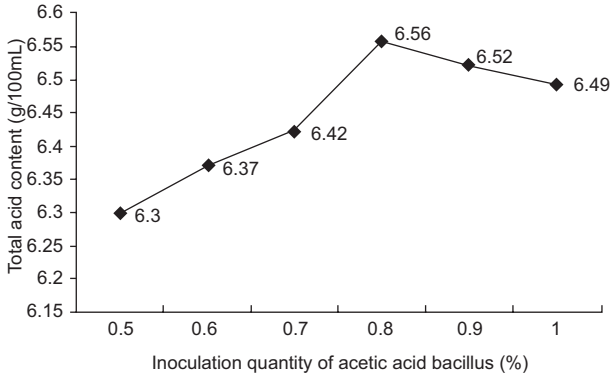

Fig. 5. Effect of inoculation quantity of acetic acid bacillus on acetic fermentation.

The effect of acetobacillus inoculation amount on acetic fermentation

As **figure 5** shows, total acid content displays a trend from rise to decline as inoculation quantity of acetic acid bacillus rises. Total acid content grew healthily when inoculation quantity of acetic acid bacillus was at 0.5-0.8%, while it declined evenly at the range of 0.8-0.9%. The minimum total acid content was 6.3 g/100 mL at the point of 0.5% inoculation quantity of acetic acid bacillus. The maximum total acid content reached 6.56 g/100 mL when inoculation quantity of acetic acid bacillus was 0.8%. Therefore 0.8% was selected as the inoculation quantity for acetic acid fermentation.

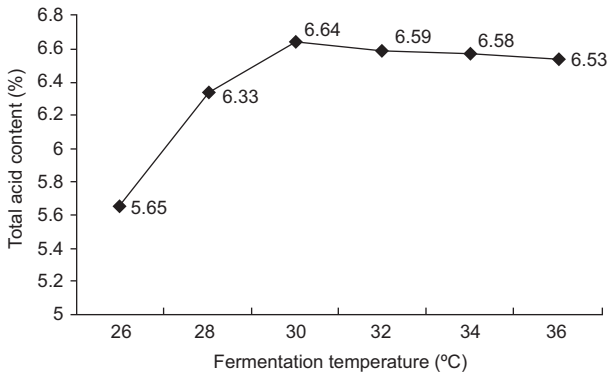

Fig. 6. Effect of fermentation temperature on acetic fermentation

The effect of fermentation temperature on acetic fermentation

As **figure 6** shows, total acid content displays a rising trend as fermentation temperature rises and peaks at 30 ºC. The total acid content was 5.56 g/100 mL at the point of 26 ºC fermentation temperature. It maximized at 6.64 g/100 mL when the fermentation

temperature reached 30 ºC. It barely changed when fermentation temperature exceeded 30 ºC. Yet if fermentation temperature is too high, the taste and fragrant of fruit vinegar could be impaired. Therefore 30 ºC was selected as the fermentation temperature for acetic fermentation (Wang 2020).

The effect of fermentation time on acetic fermentation

As **figure 7** shows, total acid content displays a rising trend as fermentation time rises and peaks at seven days. The lowest total acid content was 3.67 g/100 mL at four days. When the fermentation time reached seven days, total acid content maximized at 6.46 g/100 mL. Total acid content did not shown a significant rise when fermentation time exceeded seven days. Therefore seven days was selected as the fermentation time for acetic fermentation (Wang et al. 2019).

Orthogonal optimization of acetic acid fermentation conditions

The experimental results are shown in **table IV**, with the best combination in the orthogonal experiment as $A_2B_2C_3D_2$: alcohol degree at 7%, inoculation quantity of acetic acid bacillus at 0.8%, fermentation temperature at 32 ºC, fermentation time at seven days. Under such conditions, total acid content could reach 6.78 g/100 mL. Based on the range analysis in this orthogonal experiment, it could be concluded that $R_A > R_C > R_B > R_D$, meaning that in acetic fermentation, alcohol degree is the key factor, followed by fermentation temperature and inoculation quantity of acetic acid bacillus, with fermentation time as the least influencing factor. The order of the influencing factors for alcohol fermentation was A > C > B > D (alcohol degree > fermentation temperature > inoculation quantity of acetic acid bacillus > fermentation time).

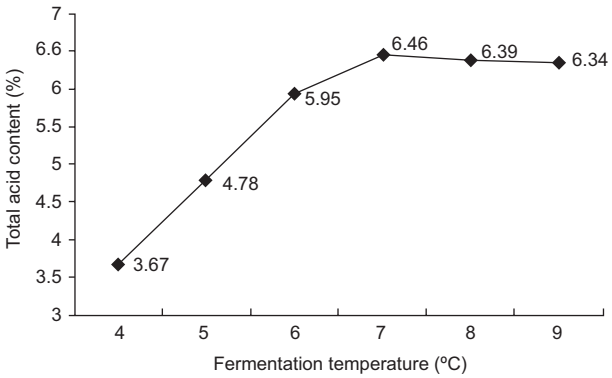

Fig. 7. Effect of fermentation time on acetic fermentation

DISCUSSION AND CONCLUSIONS

This study aimed at making fruit vinegar with apricot pulp as the raw material. Based on the single factor and orthogonal experiments carried out in this study, the optimal fermentation conditions for the alcoholic fermentation are determined as follows: when sugar content is at 9%, yeast inoculation amount at 0.7%, and fermentation temperature at 30 ºC, the alcohol content reaches 7.56%. The optimal fermentation conditions for the acetic fermentation

TABLE IV. RESULTS AND ANALYSIS OF ORTHOGONAL EXPERIMENT FOR THE ACETIC ACID FERMENTATION.

Experiment number	A alcohol degree (%)	B inoculation quantity of acetic acid bacillus (%)	C fermentation temperature (ºC)	D fermentation time (d)	Total acid content
1	1	1	1	1	4.12
2	1	2	2	2	4.32
3	1	3	3	3	4.71
4	2	1	2	3	5.01
5	2	2	3	1	6.74
6	2	3	1	2	5.88
7	3	1	3	2	6.43
8	3	2	1	3	5.67
9	3	3	2	1	4.53
k_1	4.383	5.187	5.223	5.13	-
k_2	5.877	5.577	4.62	5.543	-
k_3	5.543	5.04	5.96	5.13	-
R	1.494	0.537	1.34	0.413	-

are: when the alcohol degree is 7%, inoculation quantity of acetic acid bacillus is 0.8%, fermentation temperature is 32 ºC, fermentation time is 7 days, the total acid content reaches 6.78 g/100 mL. The fruit vinegar produced under these conditions has a rich fruit flavor and a pure taste.

This study explored a mature apricot vinegar brewing process, which could assist the further development of apricot healthy vinegar beverages to organically combine the nutrition and the health care values of apricot pulp and its vinegar. A series of natural, pollution-free, green and healthy food could be developed to achieve large-scale, rapid and comprehensive processing of apricot pulp.

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