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RESEARCH ON THE TECHNOLOGICAL PARAMETERS OF ORANGE RAKIA PRODUCTION

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Abstract. This research aims to identify the technological parameters involved in the production of rakia from Ha Giang oranges. The fermentation must was prepared by adding the disinfectant and antioxidant $K_2S_2O_5$ at a concentration of 150 mg/L to ensure the sterility and stability of the orange juice for the subsequent fermentation stage. Using the native yeast strain *Saccharomyces cerevisiae* HB2 isolated from oranges, the factors influencing the fermentation process were determined, including fermentation temperature of 30°C, initial total soluble solids after adding sugar of 20 °Bx, initial yeast cell density of 8.5×10^6 CFU/mL, and pH of 4.1. At the pilot scale of 5 liters, after fractional distillation and the removal of 3% ethanol from the head and 3% from the tail fractions, the resulting heart fraction had an alcohol concentration of 53% vol. After dilution to a final alcohol concentration of 35% vol by distilled water, the finished rakia had a characteristic orange aroma. The sensory score achieved was 18.16, which is in the 'good' category.

***Keywords**:* fermentation process, fermentation factor, orange juice, native yeast, rakia.

1. Introduction

Oranges have long been considered a valuable food. They contain many essential nutrients for humans and are particularly rich in minerals and vitamins, especially vitamin C, which helps prevent diseases and strengthen immunity. In addition, oranges contain natural colorants, essential oils, and bitter compounds [1], [2]. Rakia is a high-alcohol spirit with deep cultural characteristics of the Balkan countries, produced by distilling fermented fruit juice. It contains ethanol along with various volatile and non-volatile components, such as methanol, aldehydes, esters, and higher alcohols, which contribute to its distinct sensory qualities [3]. Rakia is considered a traditional drink that is

ubiquitous in the Balkans, which is a strong fruit-based spirit with an alcohol content ranging from 40% to 50%. Rakia production technology dated back to ancient times, and the preservation of this knowledge across generations has become an integral part of Bulgarian cultural identity [4]. With the technological revolution in fruit processing, which ensured the widespread production of high-alcohol drinks in the Mediterranean from the 7th to the 12th centuries, rakia became integrated into the food system, ritual spheres, cultural exchanges, and social lives of the peoples of many countries in Southeastern Europe, such as Albanians, Greeks, Eastern Romans, and Southern Slavs [5]. The indigenous microorganisms have been emphasized as a concept originating from locally available natural materials. After isolation and selection, these microorganisms demonstrate excellent adaptability and production capacity. Apples, oranges, bananas, and other locally available fruits were used as raw materials for isolating and selecting ethanol-fermenting yeasts [6], [7]. The quality of orange wine depends on the type and the origin of yeast used in the fermentation process [8], [9]. *Saccharomyces cerevisiae var. ellipsoideus* isolated from orange juice produced moderate alcohol and was identified as the best microorganism for orange wine production [10]. A research team of the Faculty of Food Technology, University of Economic - Technology for Industries, has selected the indigenous yeast strains *Saccharomyces cerevisiae* HB2 from Hoa Binh oranges [11]. Rakia can be prepared from different fruits such as grapes, plums, apricots, quince, pears, apples, figs, and peaches. The technological process of manufacturing homemade rakia can be described as follows: Selection of raw materials (fruits), preparation of fermentation vessels, addition of yeasts, fermentation, distillation of the rakia, adjustment to the required degree, aging, and storage [4].

The acidic pH and the use of $K_2S_2O_5$ in the must (100 mg/L) contributed to the reduction of the bacterial population and consequently allowed yeast growth. Viable cell counts during the fermentation period did not detect bacteria, indicating that the metabisulfite was effective in controlling bacterial growth during the fermentative process. In a previous report, *S. cerevisiae* UFLA CA1174 was employed to produce an alcoholic beverage from oranges. The fermentation was performed with total soluble solids (TSS) in the fermented must adjusted to 16 ºBrix, and a pH of 4.5 at room temperature. As a result of 24 h of fermentation, a high ethanol concentration of 58.13 g/L was obtained. The orange spirit produced from the heart fraction had high concentrations of acetaldehyde, ethyl acetate, isoamyl alcohol, and 2-phenylethanol. The results indicated that orange juice could be a good raw material for producing orange rakia [12]. In addition to yeast strain, other fermentation factors influencing the wine quality included fermentation temperature, initial total soluble solids (TSS), initial yeast cell density, and pH [3], [8]-[10]. Using the selected indigenous yeast strain *S. cerevisiae* HB2, this study aimed to determine the technological parameters for orange rakia production employing the yeast strain isolated from the orange.

2. Content

2.1. Materials and methods

2.1.1. Materials

Oranges and yeast strain: Undamaged, unbruised Ha Giang oranges were purchased in Hanoi. Sucrose (refined sugar) was a product of Lam Son Sugarcane Group, Thanh Hoa. Indigenous yeast strain *Saccharomyces cerevisiae* HB2 was provided by the research team of the Faculty of Food Technology, University of Economic Technology for Industries.

Chemicals: $K_2S_2O_5$ and other materials such as agar, ethanol 96%, barley malt, phenolphthalein, NaOH, $NaHSO_3$, $NaHCO_3$, HCl, starch, H_2SO_4, I_2, $CuSO_4.5H_2O$, $KMnO_4$, $Fe_2(SO_4)_3$ were of technical grade.

2.1.2. Methods

Preparation of orange juice: After being washed, the oranges were squeezed out using a handheld juicer. The orange juice was then filtered through a sieve to remove the orange pulp residue. The orange pulp was washed with distilled water to recover the remaining nutrients.

Determining the $K_2S_2O_5$ concentration: The microbial culture medium used for examining the $K_2S_2O_5$ ratio was prepared through the saccharification process of barley malt and 2% agar. The appropriate concentration of $K_2S_2O_5$ was determined by adding 50, 100, 150, 200, or 250 mg/L to the orange juice. Then, 100 µL of the orange juice was taken and spread on the surface of agar plates made from barley malt. The obtained colonies after 48 hours of incubation at 30 °C were observed.

Determining the influence of fermentation factors: The impact of initial TSS of 18, 20, 22, and 24 °C after adding sucrose was examined at 30 °C, an initial yeast cell density of 8 - 9×10^6 CFU/mL, and a pH of 4.1. The effect of fermentation temperature was investigated with the range of 18, 22, 26, 30, 34 °C using an initial TSS of 20 °Bx, initial yeast cell density of 8 - 9×10^6 CFU/mL, and pH of 4.1. Initial yeast cell density was evaluated in the range of 2.5 – 16.3×10^6 CFU/mL with an initial TSS of 20 °Bx, at 30 °C, and a pH of 4.1. pH range of 4.0 – 4.4 was examined using initial TSS of 20ºBx, fermentation temperature of 30 °C, and initial yeast cell density of 8.5×10^6 CFU/mL. The control sample has a pH of 4.1. Orange juice was fermented on a pilot scale of 5 liters/batch, and the first distillation was produced until all alcohol was recovered. The second distillation was conducted to remove 3% of ethanol in the head and 3% in the tail fractions, and the resulting heart fraction had an alcohol concentration of 53% vol. The finished rakia was obtained by diluting the heart fraction with distilled water to an alcohol concentration of 35% vol.

Biochemical analysis: The reducing sugar and vitamin C content in orange juice were determined according to the standard method of Le TM et al. [13]. Total acid content in orange juice was assayed according to the process described in TCVN 4589:1988 [14]. The apparent concentration in the fermentation must was measured

using a refractometer. The alcohol concentration in the fermented must was determined by distillation followed by measurement of the density D(20/20 °C) with a pycnometer as described in TCVN 5562:1991 [15]. Finished total soluble solids in the fermented must were determined in the same manner according to TCVN 5565:1991 [16]. Initial yeast cell density was counted using a Neubauer chamber. pH was determined using a LaMotte handheld pH meter. Total acid content and ester contents in fermented must were determined by the titration method with NaOH 0.1N according to Le TM et al. [13]. Aldehyde content in fermented must was determined according to TCVN 8009:2009 [17].

Sensory evaluation: The quality of the obtained alcohol was evaluated according to TCVN 3217:1979 [18].

Statistical analysis: The experimental data were processed by Microsoft Excel 2016 and Minitab 20 software. The fermentation experiments were conducted in two replicates.

2.2. Results and discussion

2.2.1. Determination of the orange juice composition

The chemical composition of orange juice was analyzed according to standard laboratory methods, and the results were presented in Table 1.

Table 1. Chemical composition of Ha Giang orange juice

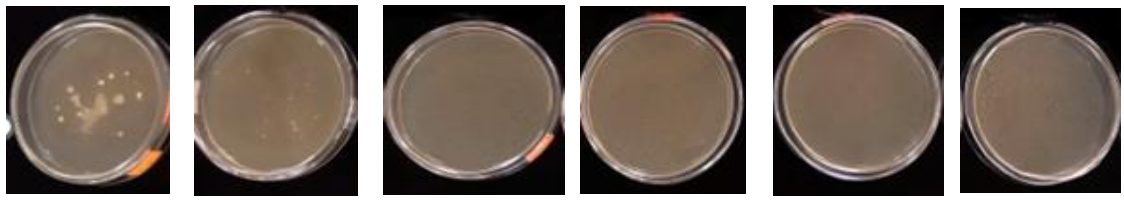
Indicator	Unit	Result
Total acid (converted to acetic acid)	g/L	0.63
Total soluble solids	^{o}Bx	6.5
Vitamin C	%	0.54
Reducing sugar	%	4.85

Data in Table 1 showed that Ha Giang orange juice had a similar composition to that reported in the Vietnam Food Composition Table [2]. *S. cerevisiae* HB2 has been isolated from oranges, and it can be hypothesized that the nutritional components in this orange juice meet the nutritional requirements for yeast growth.

2.2.2. Sterilization of fermentation must

By studying the diversity of yeast species on fresh orange peels and rotten oranges, Obasi et al. found that the fermentation process of orange juice was characterized by many different yeast species, including *Candida, Rhodotorula, Kodamaea, and Geotrichum*. The fermented product must be ensured to be free from microorganisms that could harm the fermentation process or cause disease in humans [19]. The fermentation medium in the fermentation technology must be sterile.

The sterilized fermentation medium was obtained by adding the disinfectant $K_2S_2O_5$. The appropriate concentration of $K_2S_2O_5$ for sterilizing orange juice was examined to ensure biosafety of the fermentation medium while having a negligible effect on the cultivated yeast strain. The images of the agar plates are shown in Figure 1.



0 mg/L	50 mg/L	100 mg/L	150 mg/L	200 mg/L	250 mg/L
Many colonies	Few colonies	Few colonies	No colonies	No colonies	No colonies

Figure 1. Fermentation must be performed with the treatment of $K_2S_2O_5$ at various concentrations (0-250 mg/L)

In the control without $K_2S_2O_5$, after 48 hours of incubation at 30 °C on agar medium from barley malt, many colonies with different shapes were observed. The obtained colonies had the cultural characteristics of microorganisms such as bacteria, yeasts, and molds.

The addition of $K_2S_2O_5$ at various concentrations from 50 mg/L to 100 mg/L showed an antibacterial effect, with the number of obtained colonies being reduced. When the concentration reached 150 - 250 mg/L, no colonies were observed on the agar plate, indicating that the sample treated with $K_2S_2O_5$ likely did not contain any aerobic or facultative anaerobic microorganisms.

Based on the results, the concentration of $K_2S_2O_5$ for subsequent experiments was determined as 150 mg/L. The use of $K_2S_2O_5$ in fruit juice sterilization has been popular in practice [3]. According to Vu VC, the concentration of $K_2S_2O_5$ for sterilizing lychee juice in lychee brandy production was 80-100 mg/L [20].

2.2.3. Fermentation factors

**** Effect of initial total soluble solids***

The fermentation was carried out by adding *S. cerevisiae* HB2 obtained after 24 hours of cultivation. The influence of the initial total soluble solids on the apparent extract (measured by refractometer) in the fermented must during the fermentation process is presented in Table 2.

Table 2. Apparent extract concentration in fermented must

Initial TSS (°Bx)	Fermentation time (hours)						
	24	***48***	***72***	***96***	***120***	***144***	***168***
18	16	11	8	6	5	5	5
20	17	12	9	7	5	5	5
22	18	14	8	7	6	6	6
24	21	16	11	8	7	7	7

The fermentation process included main and secondary fermentation, which was monitored by determining the apparent extract using a refractometer. After 7 days, the apparent concentration remained unchanged, and the fermentation time was established. Distillation was carried out, and the alcohol and residual dry content (real extract) were

exactly determined using a pycnometer according to Vietnamese Standards. The aldehyde, ester, and total acid content were analyzed and expressed per unit of absolute anhydrous alcohol (Table 3).

Table 3. Effect of initial total soluble solids on fermentation must quality

Initial TSS (°Bx)	Alcohol content (% Vol)	Alcohol content (% mass)	Residual dry content (% mass)	Aldehyde content (mg/L)	Ester content (mg/L)	Total acid content (mg/L)	Sensory score
18	9.26^{c}	7.42^{b}	2.08^{a}	45.64^{d}	1544.97^{d}	305.44^{a}	14.66^{c}
20	**11.09^{ab}**	**8.90^{a}**	**2.12^{a}**	**47.57^{c}**	**1569.78^{c}**	**323.01^{b}**	**17.88^{a}**
22	11.34^{a}	9.10^{a}	2.39^{b}	56.02^{b}	1597.35^{a}	342.74^{c}	15.36^{b}
24	10.92^{b}	8.76^{a}	3.98^{c}	58.14^{a}	1594.25^{a}	384.21^{d}	14.14^{c}

Numbers in the same column with different letters are significantly different at α = 0.05.

Using three initial TSS levels of 18, 20, 22 °Bx, the alcohol concentration of the fermented increased from 9.26 to 11.34 % vol. However, at the initial TTS level of 24 °Bx, the alcohol concentration reduced to 10.92% vol. It can be hypothesized that high initial TSS could inhibit the fermentation process.

Similar results were also reported by Ly et al., the appropriate TTS was 22 °Bx [9]. Yeast produced ethanol, CO_2, and secondary products such as organic acids, aldehyde, and ester, that play an important role as flavoring agents and sensory properties [3], [21]. At the initial TSS of 20 °Bx, the residual dry content reached the lowest value, the sensory evaluation score achieved the highest, and this TSS was selected for the following experiments.

**** Effect of fermentation temperature***

Experimental samples were prepared using the same aseptic method for fruit juice. The initial TSS was selected as 20 °Bx, and the fermentation time was 7 days. The effect of fermentation temperature on the fermented must quality was presented in Table 4.

Table 4. Effect of fermentation temperature on fermentation must quality

Ferment. Temp. (°C)	Alcohol content (% Vol)	Alcohol content (% mass)	Residual dry content (% mass)	Aldehyde content (mg/L)	Ester content (mg/L)	Total acid content (mg/L)	Sensory score
18	9.18^{d}	7.35^{d}	3.86^{a}	53.21^{a}	1540.86^{e}	418.86^{b}	14.37^{c}
22	9.83^{c}	7.96^{cd}	3.55^{a}	51.31^{b}	1469.22^{c}	406.72^{a}	$15.88b^{c}$
26	10.76^{ab}	8.42^{bc}	2.42^{bc}	51.26^{b}	1455.59^{b}	384.75^{c}	17.56^{b}
30	**10.85^{a}**	**8.63^{b}**	**2.26^{b}**	**47.65^{c}**	**1498.67^{d}**	**404.02^{e}**	**18.27^{a}**
34	10.77^{ab}	8.45^{bc}	2.53^{bc}	45.21^{d}	1449.36^{a}	311.72^{d}	15.19^{b}

Numbers in the same column with different letters are significantly different at α = 0.05.

With the increase of fermentation temperature from 18 to 34 °C, the aldehyde content decreased from 53.21 to 45.21 mg/L. According to Marinov's theory, at higher fermentation temperatures, more aldehydes are synthesized in the first 2-3 days. However, after reaching the peak, the reduction of carbonyls such as acetaldehyde occurs strongly, so the concentration of obtained aldehydes during the fermentation must be gradually decreased [3].

The fermentation temperature of 30 °C was chosen for the subsequent experiments because this sample had the highest alcohol concentration of 8.63% mass (10.85% vol), the lowest residual dry content concentration of 2.26%, and the highest sensory evaluation score of 18.27.

Research on the fermentation of orange juice by Nguyen PT and Nguyen VT also showed a suitable fermentation temperature of 30 °C [8].

**** Effect of initial yeast cell density***

Experimental samples were prepared using the same aseptic method for fruit juice. The yeast cell density was determined by a Neubauer counter. The effect of initial yeast cell density on the quality of the fermentation must after 7 days of fermentation is presented in Table 5.

Table 5. Effect of initial yeast cell density on fermentation must quality

Initial yeast cell density (CFU/ml)	Alcohol content (% Vol)	Alcohol content (% mass)	Residual dry content (% mass)	Aldehyde content (mg/L)	Ester content (mg/L)	Total acid content (mg/L)	Sensory score
2.5×10^6	8.20^b	6.56^d	4.23^a	53.75^a	1494.25^a	379.74^c	15.19^{bc}
8.5×10^6	**11.68^a**	**9.38^b**	**1.62^c**	**52.42^b**	**1444.97^b**	**366.44^a**	**17.70^a**
13.5×10^6	11.09^a	11.68^a	2.01^{bc}	47.34^c	1427.78^c	342.01^b	15.62^b
16.3×10^6	10.92^a	8.76^c	2.33^b	45.12^d	1393.35^d	304.21^d	15.09^c

Numbers in the same column with different letters are significantly different at $\alpha = 0.05$.

Lower initial yeast cell density affects the budding and prolongs the fermentation time. Higher initial yeast cell density leads to low budding and growth rate, short fermentation time, and lower ester concentration. Parameters that increase the fermentation rate, such as fermentation temperature and initial yeast cell density, will reduce the formation of fatty acids [3].

The results in Table 5 showed that the appropriate yeast cell density selected for the following experiments was 8.5×10^6 CFU/mL. At this value, the product had the highest alcohol concentration and sensory score.

**** The effect of pH in the initial fermentation must***

In the experiment examining the effect of initial yeast cell density, the pH of the fermentation must be measured before the fermentation process begins at 4.1. The effect of the initial pH on the quality of fermented orange juice after 7 days of fermentation is presented in Table 6.

Table 6. Effect of pH on fermentation must quality

Initial pH	Alcohol content (% Vol)	Alcohol content (% mass)	Residual dry content (% mass)	Aldehyde content (mg/L)	Ester content (mg/L)	Total acid content (mg/L)	Sensory score
Control (pH = 4.1)	**11.43^{a}**	**9.17^{a}**	**2.23^{b}**	52.53^{a}	1463.57^{e}	346.44^{b}	**17.32^{a}**
pH = 4.0	11.09^{a}	8.90^{a}	2.56^{b}	50.14^{b}	1498.72^{d}	304.21^{e}	16.23^{b}
pH = 4.2	11.26^{a}	9.03^{a}	2.41^{b}	49.45^{c}	1564.31^{c}	342.01^{d}	16.85^{ab}
pH = 4.4	8.76^{b}	7.01^{ab}	4.58^{a}	48.64^{d}	1574.54^{b}	345.21^{c}	15.36^{c}
pH = 4.6	8.68^{b}	6.95^{d}	4.93^{a}	48.57^{e}	1596.45^{a}	379.74^{a}	14.68^{bc}

Numbers in the same column with different letters are significantly different at α = 0.05.

The results showed that the yeast grew well in the appropriate pH range of 4.0 – 4.2. A pH of 4.1 was chosen because both the sensory score and the alcohol concentration reached the highest value compared to those at other pH levels.

**** Production of orange rakia at a pilot scale***

The fermentation media were prepared according to the results of laboratory-scale studies. $K_2S_2O_5$ concentration was 150 mg/L, the initial dry matter was 20 °Bx, the density of yeast cells was 8.5×10^6 CFU/mL, the fermentation temperature was 30 °C, and the fermentation time was 7 days.

The orange juice fermentation was carried out on a scale of 5 liters/batch. The results regarding the quality of the finished alcoholic beverage after the fractional distillation process are presented in Table 7.

Table 7. Quality of finished orange rakia

Indicator	Unit	1st distillation	2nd distillation
Alcohol content	%Vol	38.0	53.0
Aldehyde content	mg/L	64.45	40.82
Ester content	mg/L	1547.54	845.67
Total acid content	mg/L	340.50	286.24
Sensory score	-	17.43	18.16

By the fractional distillation process, the aldehyde content was reduced from 64.45 to 40.82 mg/L. The alcohol concentration was increased from 38% vol to 53% vol. The total acid and ester (expressed per unit of absolute anhydrous alcohol) has contributed to improving the product flavor. After diluting with distilled water, the orange rakia with a concentration of 35% vol had a characteristic orange flavor; the sensory score reached 18.16, classified as 'good' according to TCVN 3217:1979.

3. Conclusions

From Ha Giang oranges as raw materials, the study determined some technological parameters in the production of orange rakia. The fermentation must have been microbiologically sterilized by adding 150 mg/L $K_2S_2O_5$. The technological parameters in the fermentation stage included initial total soluble solids of 20 °Bx, yeast cell density of 8.5×10^6 CFU/mL, fermentation temperature of 30 °C, pH of 4.1, and fermentation time of 7 days. At a pilot scale of 5 liters/batch of fermentation, applying the fractional distillation technique, removing 3% ethanol in the head and 3% in the tail fractions, the received heart fraction had an alcohol concentration of 53% vol. The finished rakia obtained after dilution to an alcohol concentration of 35% vol had a characteristic orange aroma, and the sensory score was in the good category.

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