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ORIGINAL ARTICLE

Favorable Conditions for Fermentation of Acerola Wine

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ABSTRACT
Acerola is characterised by a high vitamin C content, which is many times higher than that of other fruits that are considered good sources of this vitamin, such as guava, cashew apple, orange or lemon. In this research, we focus on investigation the favourable conditions for acerola fermentation. We draw out conclusion the best enzyme ratio 0.15%, incubate at 45^{0}C at 1.5h; proliferation at 24 hours; water addition 20%; yeast ratio 9%; sugar supplementation 10% so that the dry matter 21^0 Bx; fermentation during 7 days.
Keywords: *Acerola, fermentation, yeast, wine*

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INTRODUCTION

The Acerola (*Malpighia glabra*), also popularly known as the cherry or cherry-antilles-of Barbados, has its origins in the Caribbean, Central America and northern South America belongs to the family Malpighiaceae. A fruit, when ripe, has a color variation that goes from orange to wine, passing through the red. This coloration is the result of the presence of anthocyanins. Acerola fruit is drupaceous, whose form can vary from round to conic. When ripe, it can be red, purple or yellow. The fruit weight varies between (3 and 16) g.

Six fermented acerola ice creams were produced, containing different starter cultures (*Bifidobacterium longum*, *Bi.lactis*, and traditional yogurt starter culture—*Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*) and final pH (5 and 4.5). The ice creams were evaluated for probiotic culture viability, vitamin C stability, and sensory acceptance. Mix fermentations were stopped when pH 5.0 and 5.5 were attained. However, after the addition of acerola pulp the determined pH were 4.5 and 5, respectively. Mixes were frozen and stored for 15 wk at −18 °C. The viable counts for probiotic cultures remained above the recommended minimum limit of10^6 cfu/g during 15 week of storage even in products with pH 4.5. Vitamin C concentration remained around 140 mg/100 g of product [3].

Carotenoid composition has been investigated in acerola fruits (*Malpighia emarginata* DC. syn.*Malpighia glabra* L.) and derived products. In the ripe fruit, four major carotenoids were identified (β-carotene, β-cryptoxanthin, lutein, and violaxanthin) together with other minor carotenoids (neoxanthin, antheraxanthin, neochrome, luteoxanthin, auroxanthin, β-cryptoxanthin-5,6-epoxide, β-cryptoxanthin-5,8-epoxide, *cis*-β-carotene, and *cis*-lutein). An average composition for the ripe fruit has been estimated as follows: β-carotene (536.55 μg/100 g fw), β-cryptoxanthin (417.46 μg/100 g fw), lutein (99.21 μg/100 g fw), violaxanthin (395.33 μg/100 g fw), and total minor carotenoids (197.33 μg/100 g fw). Vitamin A values are similar to those described in tomatoes and some tropical fruits such as guava and papaya. After juice-making, including a pasteurization stage as thermal processing, decreases in carotenoid content were observed as well as progress of *cis*-isomers and structural rearrangement of xanthophylls containing 5,6 epoxide groups. The occurrence of such modifications affected the nutritional value of fruits as well as their antioxidant capability, properties that could be used as a measurement of processing quality [8].

They made the sensorial analysis of Barbados cherry. A standardized questionnaire was used to evaluate the effect of soluble solids (°Brix) and the concentration of fruit pulp on sensorial quality attributes (color, flavor and aroma) of wines; which were measured on hedonic scale, to obtain the best condition

for manufacturing wine from Barbados cherry. *Saccharomyces cerevisiae* yeast was used for fermentation [7].

They studied the stability of a beverage formulated with acerola fruit juice and green coconut water with added caffeine [1]. The beverage was prepared with 25% acerola pulp, 75% green coconut water and sugar up to 12°Brix, and caffeine (125 mg L^{-1}), heat processed at 90 °C for 30 s and packed in 250-mL glass bottles. Chemical, physicochemical, microbiological and sensory analyses of the beverage were performed just after processing and during 6 months of storage at room temperature (27 °C). The vitamin C content decreased significantly throughout storage, from 399.5 to 189.6 mg 100 mL^{-1}, although it has remained relatively high. The anthocyanins initially present (0.025 mg 100 mL^{-1}) were completely lost during the storage at a mean rate of 4 μg 100 mL^{-1} $month^{-1}$. The product was microbiologically stable during storage. Colour changes were also observed with absorbance at 420 nm, with average values ranging from 0.19 to 0.24. However, according to the sensory analyses the product was acceptable during the 6 months of storage, presenting sensory scores (colour, taste and global acceptance) from 6.5 to 5.5, which suggests its potential for market.

They assessed the impact of processing parameters (inlet temperature, 170–200C; drying aid-to-acerola ratio, 2:1–5:1; and percent replacement of maltodextrin by cashew tree gum as drying aid, 0–100%) on degrees of retention of ascorbic acid (AA) and anthocyanins (AC) during spray drying of acerola pomace extract [4].

They evaluated the effect of the processing and long-term storage on the antioxidant potential and activity of antioxidant enzymes of frozen purées from six acerola clones [6].

Seven wine samples were prepared varying the amount of pulp of *acerola* fruits and the sugar content using the simulated annealing technique to obtain the optimal sensory qualities and cost for the wine produced. *S. cerevisiae* yeast was used in the fermentation process and the sensory attributes were evaluated using a hedonic scale. *Acerola* wines were classified as sweet, with 11°GL of alcohol concentration and with aroma, taste, and color characteristics of the *acerola* fruit. The simulated annealing experiments showed that the best conditions were found at mass ratio between 1/7.5-1/6 and total soluble solids between 28.6-29.0 °Brix, from which the sensory acceptance scores of 6.9, 6.8, and 8.8 were obtained for color, aroma, and flavor, respectively, with a production cost 43-45% lower than the cost of traditional wines commercialized in Brazil [2].

They investigated the extraction of rutin from acerola waste using alcohol-salt-based aqueous two-phase systems [5]. Initially, the partitioning was studied using model systems with pure and commercial rutin. The impact of the ATPS constituents and composition, initial amount of rutin, temperature and addition of electrolytes was evaluated. Rutin can be recovered either in the alcohol-or-salt-rich phase depending on the salt used. To validate the optimization process, rutin extraction from acerola waste was carried out further. The results obtained with the real samples are in close agreement with the model systems and validate the optimization tests and support their applicability in bioresourcerelated processes.

The main purpose of this research is to investigate different conditions affecting to the fermentation such as enzyme ratio, enzyme incubation, yeast proliferation, water addition, yeast ratio, sugar addition, fermentation time so that the best quality wine can be acchieved.

MATERIAL & METHOD

Material

Acerola is collected in Mekong River Delta, Vietnam. Saccharose, citric acid, pectinase, *Saccharomyces cerevisiae* are originated from Can Tho Univeristy, Vietnam.

Figure 1. Ripen acerola

Research method

In this research, we examine composition in ripen acerola and its wine, both weight percentage and chemical elements. Moreover, we must determine the suitable conditions for fermentation such as pH, temperature, time, yeast ratio, pectinase and sugar supplementation.

Determine pectinase supplementation

To determine the pectinase supplementation, we use the minced acerola and divide into 5 samples, each sample 200ml. We investigate different enzyme ratios: 0%, 0.10%, 0.15%, 0.20%, and 0.25%. Then we

incubate them at 45^0C in 1.5 hours; press the fluid to examine the juice recovery, reduced sugar and sensory characteristics such as color and turbidity.

Determine the water supplementation

Prepare 4 samples of minced acerola, each sample 100 gram. Collect the juice and add water with different water ratio 15%, 20%, 25%, 30%. Press the fluid and analyse the dry matter by Brix meter. Then we adjust the composition in juice before fermentation. After fermentation, we verify color, taste and flavour.

Determine the yeast proliferation time

We count number of yeast cell in various proliferation times (20, 24, 28, 32, 36, 40 hours).

Determine the yeast ratio in supplementation

Conduct the experiment with 4 samples, add sugar 19g/ sample (each sample 200ml), adjust pH=4, supplement some elements 0.2ml/l (amoni sunfat 100g/l, vitamin B_1 0.25 g/l, canxi pantotenat 0.25g/l, biotin 0.002 g/l). We examine different yeast ratio 5%, 7%, 9%, 11%. After fermentation, we verify ethanol concentration, residual sugar and sensory score to draw out the best yeast ratio.

Determine the sugar supplementation

Conduct the experiment with 4 samples, each sample 200 ml, adjust pH 4, and supplement some elements 0.2ml/l (amoni sunfat 100g/l, vitamin B1 0.25 g/l, canxi pantotenat 0.25g/l, biotin 0.002 g/l). We examine different sugar supplementation 6%, 8%, 10%, 12%. After fermentation, we define ethanol concentration, residual sugar and sensory score to draw out the best sugar supplementation.

Determine the ferementation time

Conduct the experiment with 4 samples, each sample 200 ml. We investigate different fermentation time 5, 7, 9 and 11 days. After fermentation, we define ethanol concentration, residual sugar and sensory score to draw out the fermentation time.

Testing

Composition of raw material and finished product

We check raw material: weight composition (edible part, total part), chemical composition (moisture, acidity, reduced sugar) and finished product: ethanol, residual sugar.

Quality of acerola wine

There are several criteria to check for acerola wine such as sensory characteristics: TCVN 3215-79; microorganism: TPC, *E.Coli, Coliform, Cl.perfrigen, S.aureus,* yeast and mold.

Statistical analysis

All data are processeed by Statgraphics.

RESULT & DISCUSSION

Composition of raw material

Weight percentage of raw material

Table 1. Weight percentage of raw material

Sample	Weight M (kg)	Edible part m (kg)	Non-edible part (kg)	% edible part	% non-edible part
1	1.0	0.71	0.29	71.00	29.00
2	1.5	1.07	0.43	71.33	28.67
3	2.0	1.45	0.55	72.50	27.50
			Average (%)	**71.61**	28.39

From this table, we see the edible part of acerola is quite high so it's ideal for wine fermentation.

Chemical composition of raw material

- *Moisture contents in the ripen acerola*

Table 2. Moisture content in raw acerola

Criteria Sample	Weight of beaker (g)	Weight of beaker and sample before drying (g)	Weight of beaker and sample after drying (g)	Moisture (%)
1	29.10	35.76	30.02	86.19
2	33.52	40.98	34.51	86.73
3	33.54	41.55	34.75	84.89
				Average: 85.94

From the able table, we notice that the high mosture content in raw acerola is 85.94% which is suitable for wine fermentation.

- *Acidity in the ripen acerola*

Table 3. Acidity in raw acerola

Criteria Sample	Sample volumn (ml)	NaOH 0.1N titratrable (ml)	Acidity (%)
1	25	2.15	**0.28**
2	25	2.05	**0.26**
3	25	2.00	**0.26**
		Average	**0.27**

❖ *Sugar content in the ripen acerola*

Table 4. Sugar content in acerola

Criteria Sample	Weight of sample (g)	Volumn of $KMnO_4$ (ml)	Glucose (g/l)	Saccharose (g/l)
1	10	6.60	138.6	**131.67**
2	10	6.40	128	**121.60**
3	10	6.90	151.8	**144.21**
	Average		**139.47**	**132.3**

The sugar content in ripen acerola is rather high so we need a little bit sugar supplementation during wine fermentation.

Favorable conditions for wine fermentation

Effect of enzyme supplementation

Table 5. Sensory score of samples by adding enzyme

Sample	Pectinase (%)	Sensory score
1	0	Juice has light yellow, turbid, special taste
2	0.10	Juice has light yellow, turbid
3	0.15	Juice has light yellow, clear
4	0.20	Juice has dark yellow, clear
5	0.25	Juice has dark yellow, clear

On the action of pectinase with the present of ion Ca^{2+}, pectinnic acid and pectin will create two salt forms: calcium galacturonate and calcium polygalacturonate. Owing to this, the juice will be clear. Among 5 samples, sample #4 & #5 have the dark color.

❖ *Juice extraction recovery*

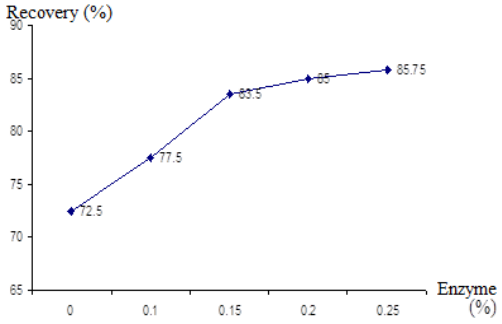

Figure 2. Juice extraction by enzyme ratio

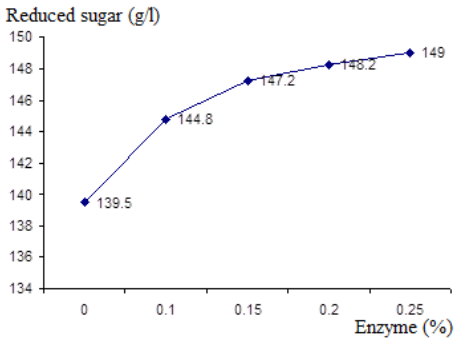

Figure 3. Reduced sugar by enzyme ratio

In sample without enzyme pectinase, samples have lot of pectin, pectinnic acid so they are turbid. The high molecular linkage prevents juice separation so the extraction recovery will be less. We use enzyme pectinaza to enhance the juice recovery. On the action of pectinase, while incubating at 45^0C with the present of ion Ca^{2+} available in fruit, this enzyme will act thoroughly and efficiently to reduce viscosity at

receive more juice. Recovery in sample #1 is 72.5%, sample #3 is 83.5%, sample #4 is 85%, and sample #5 is 85.75%. With the present of 0.3ml enzyme pectinase, it's enough to hydrolize 200 ml fruit pulp to get high juice extraction. After extraction, we determine the reduced sugar. Due to the hydrolysis, pectin and pectin acid will form the reduced sugar. When the enzyme ratio increase <= 0.15%, the slope of sugar increament is high and vice versa. So 0.15% pectinase is suitable for extraction. Based on the sensory evaluation, the juice extraction and the reduced sugar content, we notice the sample #3 has the good appearance. So we choose 0.15% pectinase for juice extraction.

Effect of water supplementation

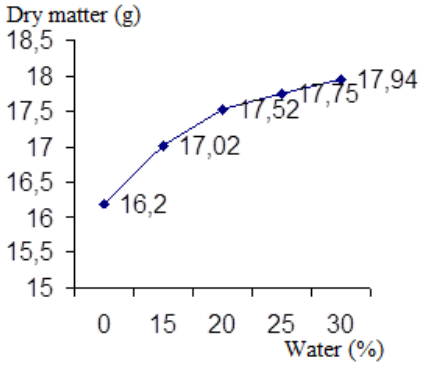

Figure 4. Dry matter in 100 g of acerola by water supplementation

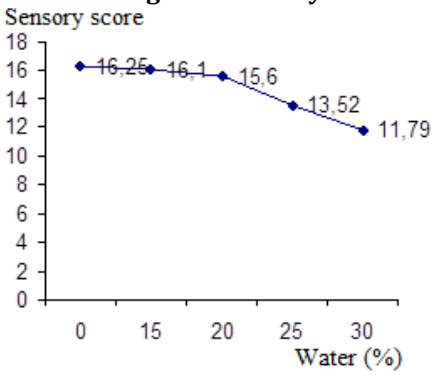

Figure 5. Sensory score of acerola wine by water supplementation

We see quite clearly that the water supplementation 20% is adaptable to get the best dry matter in juice while maintain the wine quality.

Yeast proliferation time

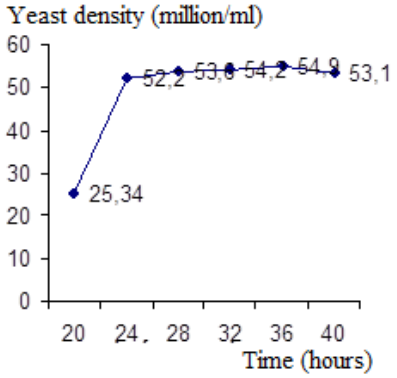

Figure 6. Number of yeast cell by proliferation time

During 24h÷36h of proliferation we can use yeast to supplement into the fermentation batch. In order to reduce the production cost, we choose the proliferation time 24h.

Effect of yeast ratio in fermentation

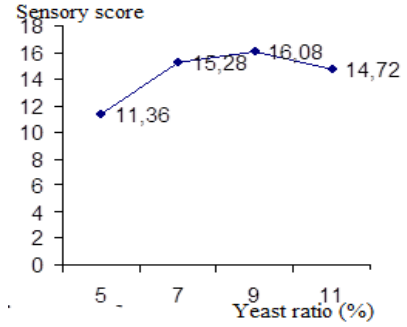

Figure 7. Sensory score of acerola wine by yeast ratio

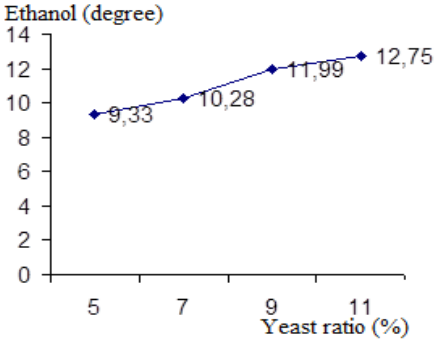

Figure 8. Ethanol of acerola wine by yeast ratio

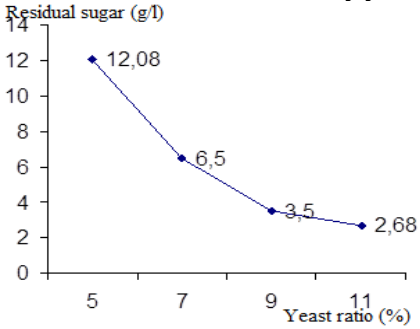

Figure 9. Residual sugar in acerola wine by yeast ratio

We see that the yeast ratio 9% is appropriated for acerola wine fermentation.

Effect of sugar supplementation

Table 6. Relationship between sugar supplementation and dry matter of juice

Sample	1	2	3	4
Sugar supplementation (%)	6	8	10	**12**
^{0}Bx	17	19	21	**23**

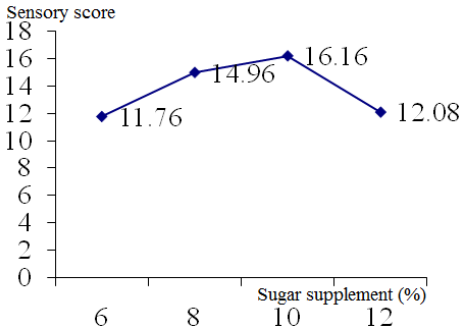

Figure 10. Sensory score of acerola wine by sugar supplementation

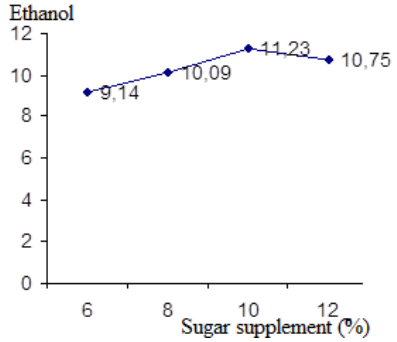

Figure 11. Ethanol of acerola wine by sugar supplementation

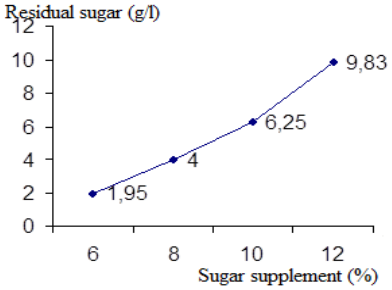

Figure 12. Residual sugar in acerola wine by sugar supplementation

When comparing sample #2 and sample #3, we choose sample #3 because of its sensory score, low residual sugar and pleasant taste.

Effect of fermentation time

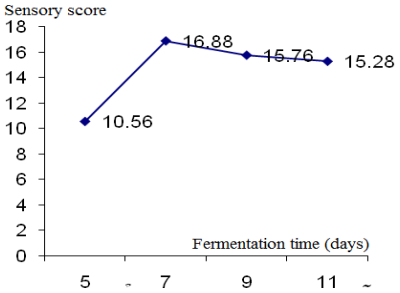

Figure 13. Sensory score of acerola wine by fermentation time

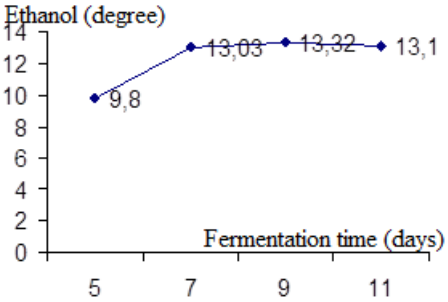

Figure 14. Ethanol of acerola wine by fermentation time

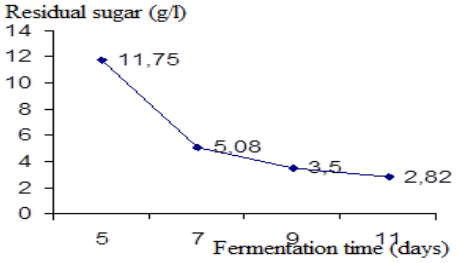

Figure 15. The residual sugar in acerola wine by fermentation time

After 7 days of fermentation, we separate yeast and store wine to get the best wine quality.

CONCLUSION

Acerola is used in the production of juice, soft drinks, gums and liqueurs. Wine can be considered as another product. The demand for acerola has increased significantly in recent years because of the relevance of vitamin C in human health, coupled with the use of ascorbic acid as an antioxidant in food and feed. Acerola fruit contains other significant components, which are likely to lead to a further increase in its production and trade all over the world.

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