Application Of Natural Fermentation To Ferment Mulberry Juice Into Alcoholic Beverage

Nguyen Thi Duyen, Le Thanh Hai, Nguyen Phuoc Minh, Dong Thi Anh Dao

Abstract: Mulberry (Morus nigra) is a fruit not known only for its nutritional qualities and its flavour, but also for its traditional use in natural medicine as it has a high content of active therapeutic compounds. Mulberries are considered as valuable materials for pharmaceutical use because of bioactive compounds. In order to find the optimal conditions for the fermentation process, the juice is fermented naturally in various conditions to get its alcoholic beverage. The results are as follows: initial mulberry juice with 24^{o}Bx and pH 3.5, fermentation temperature 18÷20^{o}C. Fermentation is carried out for 96h and the ethanol content of product was 5 %v/v. This product is suitable for Vietnamese customers. This process can be applied to industrial scale.

Keywords: Mulberry juice, valuable materials, various conditions, natural fermentation, ethanol, alcoholic beverage, Vietnamese customers

1. INTRODUCTION

Classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Supdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Dilleniidae
Order: Urticales
Family: Moraceae
Genus: Morus
Species: Morus alba L.

Common names: *White mulberry* (English); *murbai, walot, besaran [jawa], kerta, kitau [sumatera], babasaran [sunda]* (Indonesian); *may mon, dau tam* (Vietnamese); *morera* (Filipino); *sangye* (Chinese). The mulberry fruit is a multiple fruit, 2–3 cm (0.79–1.2 in) long. Immature fruits are white, green, or pale yellow. In most species, the fruits turn pink then red while ripening, then dark purple or black and have a sweet flavor when fully ripe. The fruits of the white-fruited cultivar are white when ripe; the fruit in this cultivar is also sweet but has a very mild flavor compared with the darker variety. Mulberries ripen over an extended period of time unlike many other fruits which seem to come all at once. As a rule, the fast growing mulberry trees bear few berries in the first year The fruit have the richness of sweet cherries White and red mulberries and hybrid fruits generally are ready for harvest in spring. The fruits of black mulberries ripen in summer to late summer depending upon the locality and the climate.

The mulberry fruits are often harvested by spreading a sheet on the ground and shaking the branches. In some of the genotypes, the fruits are so ripe that just picking them brea the fragile skin of the fruit and stain the fingers purple with juice [5, 6] Mulberry fruit has pigment named anthocyanins. Anthocyanins are pigments which hold potential use as dietary modulators of mechanisms for various diseases and as natural food colorants. Due to increasing demand for natural food colorants, their significance in the food industry is increasing. Anthocyanins are responsible for the attractive colors of fresh plant foods, producing colors such as orange, red, purple, black, and blue. They are water-soluble and easily extractable [5]. A cheap and industrially feasible method to purify anthocyanins from mulberry fruit which could be used as a fabric tanning agent or food colorant of high color value (of above 100) has been established. Total sugars, total acids, and vitamins remained intact in the residual juice after removal of anthocyanins and, the residual juice could be fermented to produce products such as juice, wine, and sauce [6] Jacinto Darias-Martín et al. (2003) presented some characteristics of black mulberry juice (TSS, pH, titratable acidity, citric acid, lactic acid, polyphenols, anthocyanins, the potassiumetc.) and alcoholic beverages (alcoholic grade, pH, total acidity, volatile acidity, tannins, phenolsetc.) obtained from black mulberry. Moreover, they have studied the quality of liquors obtained from black mulberry [7].

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Table 1. Mean value of the quality parameters measured in black mulberry juice obtained between 1996 and 1999 [7]

Parameters measured	Mean value 1996–1999	Range values	Standard deviation 1996–1999
pH	3.28	3.10–3.36	0.08
Titratable acidity (g/L citric acid)	21.07	16.21–28.16	3.20
TSS (°Brix)	15.63	13.00–17.50	1.68
Malic acid (g/L)	1.44	0.90–2.30	0.34
Lactic acid (g/L)	0.19	0.17–0.26	0.05
Citric acid (g/L)	25.20	20.00–34.00	5.93
AU 280 nm	35.30	30.00–45.00	5.10
Anthocyanins (mg/L)	2120.00	2000–2600	268.33
Potassium (ppm)	3361.36	3075–4000	307.07
Sodium (ppm)	98.37	72.45–114.32	22.64
Volumic mass (g/cm^3)	1.067	1.06–1.07	0.004
Probable alcoholic grade (%)	9.18	8.41–9.73	0.69

Table 2. Mean value of the quality parameters measured in the beverage obtained from Black mulberry juice fermentation withSaccharomyces cerevisiaebetween 1996 and 1999 [7]

Parameters measured	Mean value 1996–1999	Range values	DS 1996–1999
Alcoholic grade	7.40	5.4–9.4	1.63
pH	3.47	3.46–3.50	0.02
Total acidity (g/L citric acid)	19.62	15.58–23.17	3.11
Volatile acidity (g/L acetic acid)	0.58	0.56–0.59	0.02
Tannins (g/L)	3.70	3.12–4.27	0.58
AU 280 nm	52.80	44.6–61.0	8.20
Potassium	3326.32	3040–3825	325.30
Sodium	114.32	94.78–133.96	18.34

Mulberry fruit is a traditional Chinese edible fruit that is used effectively in folk medicines to treat fever, protect liver from damage, strengthen the joints, facilitate discharge of urine and lower blood pressure. Recently, it has gained an important position in the local soft drink market, although it has biological and pharmacological effects that are still poorly defined. Studies showed that mulberry fruit has significant effects in anti oxidation, reducing LDL (low density lipoprotein) level, delaying ageing and beautifying skin (Halliwell, 1992; Tomoyuki et al., 2006; Wang et al., 2011). In some European countries, mulberry alba and other mulberries are grown for fruit productions that have certain application in some traditional foodstuffs (Ercisli, 2004). At present, various

food-grade mulberry fruit products (that is, juice, jam, and dried fruit) have been developed commercially as functional foods in some traditional sericulture countries, such as China, Japan and Korea. However, mulberry alba fresh fruit is hardly commercialized. Due to its fragile structure and low stability in storage, it is usually processed as jelly or juice. Another possibility is to commercialize its fermented product. It has been widely reported that cardio protective effect can be achieved by moderate consumption of some alcoholic fermented beverages (such as wine or beer) as the consequence of their content of phenolic compounds (Gorinstein et al., 2000) and ethanol [8]. In order to diversify the products from mulberry on the Vietnam market, the scope of this research is to conduct the juice fermentation naturally in various conditions to get its alcoholic beverage.

2. MATERIAL AND METHODS

2.1 Raw material

Mulberry fruits are purchased from Da Lat (Lam Dong) at normal ripen stage, soft, intact, specific flavor. Reagent Pectinex Ultra SP-L in liquid status is provided from Novo-Nordisk (Denmark), its activity is 26000 PG/mL.

Figure 1. Variety of mulberry fruit

2.2 Researching method

Protocol of preparation

Mulberry → separation → washing → grinding → incubation with enzyme → extraction → mulberry extract.

Incubation is conducted at 43^{o}C in 4h and added enzyme pectinase with ratio 0.04 mL/100 g pulp.

Protocol of fermentation

Mulberry extract → fitration → mixing → fermentation → stability → fine filtration → bottle → heating → alcoholic beverage.

Fermentation period is carried out 2÷5 days.

2.3 Analytical method

Determine total yeast by direct counting
Determine ethanol by distillation
Determin total sugar by ferricyanur (TCVN 4074 : 2009)
Determine total acid by titration (TCVN 5483 : 2007)
Sensory evaluation by giving mark [1].

3. RESULTS AND DISCUSSION

3.1 Effect to initial dry matter in fermentation

Mulberry extract has Brix quite poor, in range 4.0÷4.5^{o}Bx so there is not enough sucrose as substrate for yeast during fermentation. We examine three levels of initial dry matter 22^{o}Bx, 24^{o}Bx, 26^{o}Bx so we can have basis to supplement sucrose into mulberry extract.

Table 1. Effect of initial dry matter (^{o}Bx) to change of pH, total acidity (g/L) and total sugar (g/L) during fermentation period.

Time (h)	Initial dry matter (^{o}Bx)								
	22	24	26	22	24	26	22	24	26
	pH			Total acidity (g/L)			Total sugar (g/L)		
24	3.97	3.96	3.83	6.51	5.44	7.04	77.04	90.40	122.35
48	3.76	3.74	3.88	7.89	8.10	7.46	73.00	80.00	104.00
72	3.68	3.61	3.75	9.92	11.09	8.32	65.00	71.72	94.54
96	3.63	3.51	3.69	10.08	12.26	9.88	61.18	71.72	74.29
120	3.60	3.42	3.56	10.66	13.06	10.82	59.43	63.03	77.04

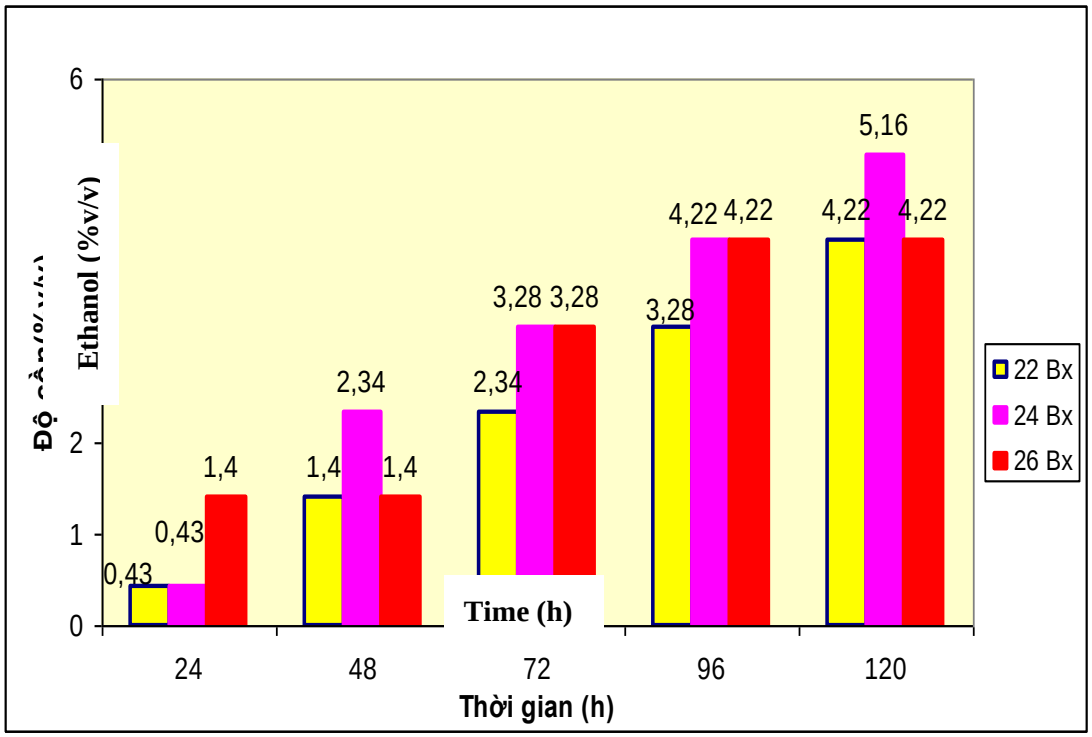

Figure 2. Effect of initial dry matter to ethanol formation in fermentation

After 5 days of fermentation for sample 24°Bx, it gets 5.16 %v/v ethanol, while sample 22°Bx and 26°Bx they haven't reached the desired ethanol concentration. Combine the main parameters of fermentation and the primary sensory evaluation for finish product, we acknowledge sample 24°Bx, the fermentation proceed smoothly and efficiently. So we choose mulberry sample 24°Bx for further experiments.

3.2 Effect of pH in fermentation

pH strongly affect to fermentatioin. pH can inhibit harmful bacteria and create acidity for final product. [2]. To survey the effect of pH to fermentation, we use acid citric and Na_2CO_3 to adjust pH value in solution. pH is adjusted at various levels 3.0; 3.5; 4.0.

Table 2. Effect of initial pH to change of pH, total acidity (g/L) and total sugar (g/L) during fermentation period.

Time (h)	Initial pH								
	3.0	3.5	4.0	3.0	3.5	4.0	3.0	3.5	4.0
	pH			Total acidity (g/L)			Total sugar (g/L)		
24	2.90	3.43	3.86	9.32	8.26	7.04	88.13	85.25	92.86
48	2.83	3.23	3.70	10.67	9.17	8.32	77.61	76.47	83.20
72	2.87	3.13	3.59	11.16	10.38	9.52	66.67	64.19	69.30
96	2.62	3.08	3.51	12.73	11.73	10.24	61.18	59.09	73.23

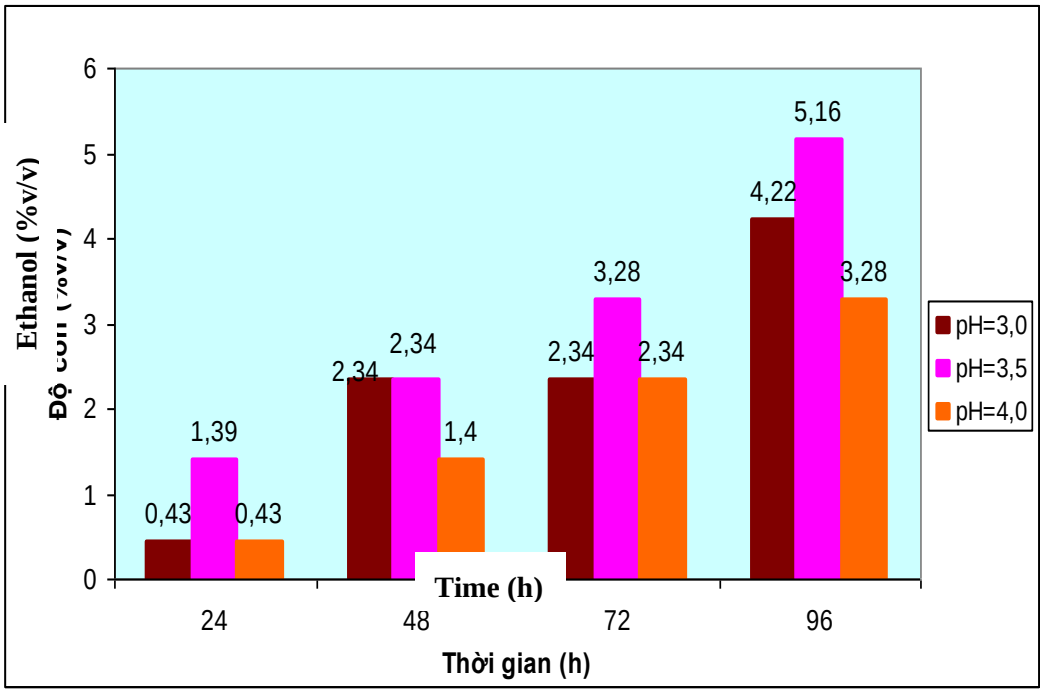

Figure 3. Effect of initial pH to ethanol formation in fermentation

We conclude that ethanol formation in sanple having pH 3.5 increased dramatically and even higher than two other samples so this demonstrates pH 3.5 is an adequate environment for yeast growing.

3. 3 Effect of temperature in fermentation

Temperature of fermentation plays an important role to fermenting speed and product quality. In this experiment, we survey two ranges of temperature: 18÷20^{o}C, cool temperature in Lam Dong province and 28÷30^{o}C normal temperature in the South of Vietnam.

Table 3. Effect of fermenting temperature to change of pH, total acidity (g/L) and total sugar (g/L) during fermentation period.

Time (h)	Temperature (^{o}C)					
	18÷20	28÷30	18÷20	28÷30	18÷20	28÷30
	pH		Total acidity (g/L)		Total sugar (g/L)	
24	3,47	3,34	8,11	9,60	98,11	94,54
48	3,32	3,14	14,08	17,07	61,18	52,53

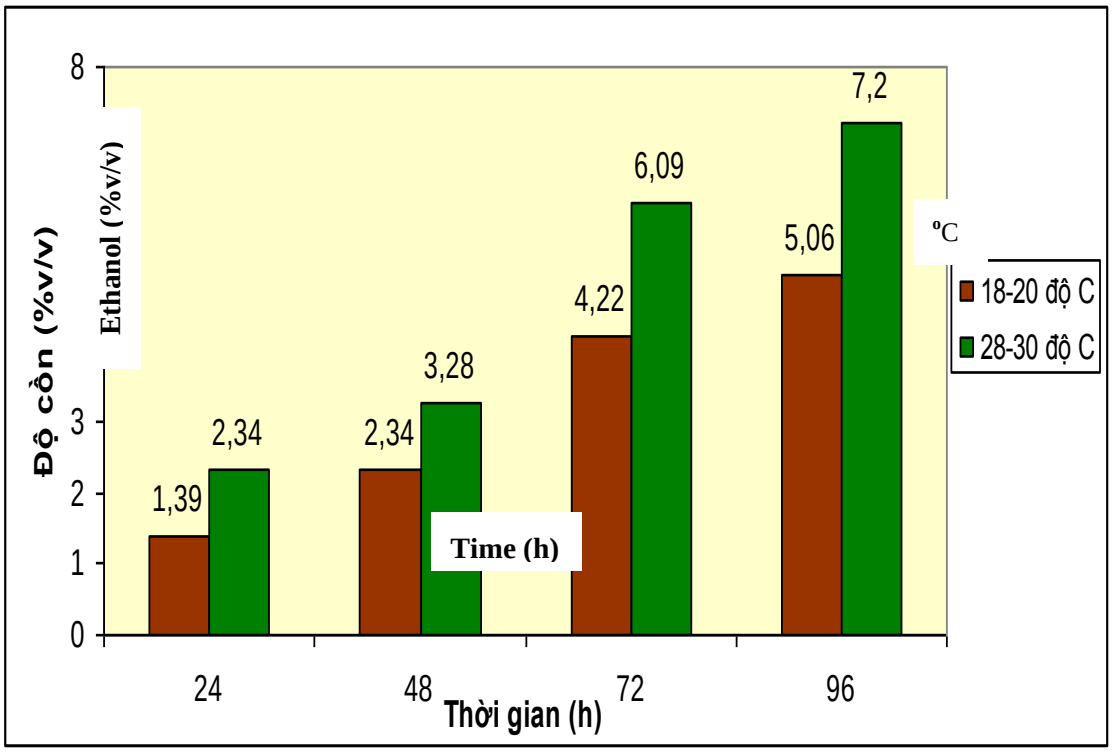

Figure 4. Effect of temprature to ethanol formation in fermentation

After 96 h fermentation, ethanol volume in final product fermented at 28÷30^{o}C is higher than ethanol volume in final product fermented at 18-20^{o}C; after 72h ethanol volume in 6.09 %v/v. Because at 28÷30^{o}C yeast uses substrate and metabolise more quickly [3,4]. Yeasts consume sugar to develop biomass and form ethanol and CO_2. At desired alcoholic level in final product 5%v/v, remained sugar in sample is still high (>50 g/L); and a mild acidity will balance the sweet-sour taste. We primarily evaluate the sensory of product fermented at low temperature (18÷20^{o}C), it shows better color, good flavor and taste.

3.4 Sensory evaluation for final product

Figure 5 shows a huge difference among criteria, especially flavor to other one. In order to examine if they differ significantly or not, we use varian ANOVA with theory H_o - the difference among criteria is naturally. Through our varian analysis, we see $F_{calculated} > F_{table}$ (78.39 > 2.66). So we discard theory H_o, that means there is diversely different among criteria. Other meaning, one person can use one particular criteria to evaluate their acceptability.

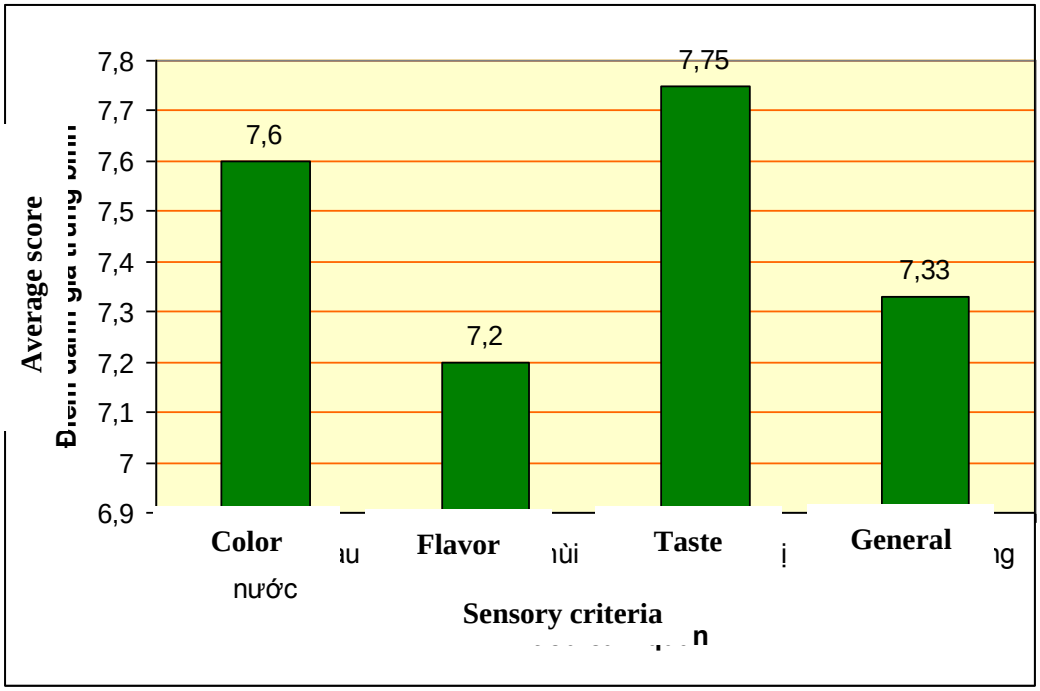

Figure 5. Evaluate the pleasure of sensory criteria in fermented mulberry beverage.

Correlaction between color, flavor, taste to general acceptability is higher than R_{table} (R_{table}= 0.25 with significant level at α = 0.05). So above correlation is significant, all criteria affect to the general product acceptability. Correlation between the general acceptability to falvor pleasure is the smallest. So we conclude flavor affects not too much among criteria. Meanwhile, color and taste pleasure is utmost so they have the highest effect to the general acceptability.

4. CONCLUSION

Alcoholic fermentation of mulberry fruit juice into wine is carried out by a complex and temporally dynamic interplay. Wine properties such as wine flavor, quality, consistency and economic value depend in part on the species and strains of yeasts that develop during the fermentation. Although mulberry alcoholic beverage naturally fermented has a long duration of fermentation (96h), sensory product quality is still appropriate with specific flavor, alcohol 5 %v/v so it's very suitable to Vietnamese style.

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