International Food Research Journal 20(4): 1889-1894 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Fermentation of *Morinda citrifolia* extract by Saccharomyces cerevisiae as affected by substrate concentration, inoculum size, temperature and fermentation time

Nur Hafiza, Z., *Maskat, M.Y., Liew, S.L. and Mamot, S.

Food Science Program, School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor

Article history

Received: 19 October 2012
Received in revised form: 19 January 2013
Accepted: 31 January 2013

Keywords

Morinda citrifolia *L.*
Fermentation
Saccharomyces cerevisiae
Response Surface Methodology (RSM)

Abstract

A study was carried out to observe the fermentation process for noni (*Morinda citrifolia* L.) extract by *Saccharomyces cerevisiae*. The experiment was based on a central composite rotatable design (CCRD) employing 5 center points with augmented axial and factorial points resulting in 30 runs. The M. citrifolia extract was fermented with different combination of substrate concentration (40, 50, 60, 70 and 80%) (w/v), inoculum size (0, 1.5, 3, 4.5 and 6%) (v/v), temperature (30, 33.5, 37, 40.5 and 44°C) and fermentation time (0, 1.5, 3, 4.5 and 6 days). Five physico-chemical characteristics which include pH, titratable acidity, turbidity, total soluble solids and total polyphenol content were measured. Results showed that all the responses could be well represented using statistical models. For pH, only fermentation time was found to be not significant, while for titratable acidity and total polyphenol content, the effects of substrate concentration and fermentation time were significant. The effects of inoculum size and temperature level were found to be significant for turbidity. For total soluble solids, only the effect of substrate concentration and inoculum size were found to be significant.

Introduction

Noni or *Morinda citrifolia* is a small evergreen tree or shrub in the *Rubiaceae* family. Extract of *M. citrifolia* has been used for generations in traditional therapy (Goh *et al*., 2000). The use of this fruit in traditional therapy is carried out in several countries throughout the world (Singh *et al.*, 1984). Although *M. citrifolia* is widely used in traditional therapy, many people avoid consuming *M. citrifolia* because of its odor (Nur Hafiza *et al*., 2010). The unpleasant odor of *M. citrifolia* extract was reported to have been contributed by medium chain fatty acids such as capric, caproic and caprylic acids (Norma *et al.*, 2004). Farine *et al.* (1996) reported that volatile components of *M. citrifolia* extract consist of carboxylic acid (83%), alcohol (5%) and ester (3%). Octanoic acid has aroma descriptors that include "rancid" and "harsh" (Siebert *et al*., 2005). Norma *et al.*, (2004) suggested that the use of activated charcoal powder was able to reduce the levels of caproic (hexanoic), caprylic (octanoic) and capric (decanoic) acid in M. citrifolia extract. Deacidification is a process that has been used to reduce the level of acid in food systems using several methods such as microbiological and also chemical (Devatine *et al.*, 2002). *M. citrifolia* contains varying proportions of glucose, fructose and sucrose (European Commission 2002). Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process (Duarte *et al*., 2010). The production of fermented foods is one of the oldest food processing technologies known to man (Caplicec and Fitsgeralda, 1999). *Saccharomyces cerevisiae* is one of the most important microorganisms in industrial fermentations (Kalathenos *et al*., 1995). Since the beginning of the 1980s, the use of *S. cerevisiae* yeast starters has been extensively applied in the industrial and homemade beverage production processes (Duarte *et al*., 2010). Many of these foods are manufactured because their unique flavor, aroma and texture attributes are much appreciated by the consumer (Caplicec and Fitsgeralda 1999). Coe *et al.* (1999) believed that *S. cerevisiae* have short and long chain of acyl-coA synthetase that can degraded octanoic acid. Optimization studies have been carried out using response surface methodology (RSM). RSM has been widely applied for optimizing processes in the food industry (Luciane *et al*., 2001; Shieh *et al*., 1996). The aim of this paper is therefore to analyze the physico-chemical characteristics of the *M. citrifolia* extracts after the fermentation by *S. cerevisiae.*

*Corresponding author.
Email: *maskatmy@yahoo.com*

Table 1. Treatment combination and responses

Run	Actual and coded ( ) variable level				pH (y_1)	Titratable acidity (y_2)	Turbidity (y_3)	Total Soluble Solid (y_4)	Total Phenolic Content (y_5)
1	0 (60)	0 (3)	0 (37)	-2 (0)	3.85	2.24	2.37	5	1092
2	1 (70)	-1 (1.5)	-1 (33.5)	-1 (1.5)	3.87	3.2	4.47	6	1112
3	1 (70)	1 (4.5)	1 (40.5)	-1 (1.5)	3.89	3.2	3.85	5.5	1068
4	-1 (50)	-1 (1.5)	-1 (33.5)	1 (4.5)	3.82	2.56	3.9	4.5	480
5	0 (60)	2 (6)	0 (37)	0 (3)	3.91	2.88	4.96	6	680
6	-1 (50)	1 (4.5)	-1 (33.5)	1 (4.5)	3.9	2.56	4.06	4	308
7	1 (70)	-1 (1.5)	1 (40.5)	1 (4.5)	3.87	3.52	5.4	6	732
8	0 (60)	0 (3)	2 (44)	0 (3)	3.88	2.88	4.91	5	436
9	-2 (40)	0 (3)	0 (37)	0 (3)	3.91	1.92	3.03	4	206
10	-1 (50)	-1 (1.5)	1 (40.5)	-1 (1.5)	3.86	2.56	4.47	4	922
11	-1 (50)	-1 (1.5)	1 (40.5)	1 (4.5)	3.88	2.56	4.03	5	436
12	0 (60)	0 (3)	0 (37)	0 (3)	3.88	2.88	4.08	5	682
13	-1 (50)	1 (4.5)	1 (40.5)	-1 (1.5)	3.91	2.24	4.15	4	784
14	2 (80)	0 (3)	0 (37)	0 (3)	3.88	3.52	7.49	7	954
15	-1 (50)	-1 (1.5)	-1 (33.5)	-1 (1.5)	3.85	2.56	3.72	4.5	464
16	0 (60)	0 (3)	0 (37)	0 (3)	3.85	2.56	4.77	5	498
17	0 (60)	0 (3)	-2 (30)	0 (3)	3.86	2.88	4.95	5.5	516
18	1 (70)	-1 (1.5)	1 (40.5)	-1 (1.5)	3.84	3.2	5.87	5.5	844
19	0 (60)	0 (3)	0 (37)	0 (3)	3.87	2.88	4.41	5.5	546
20	0 (60)	0 (3)	0 (37)	2 (6)	3.85	2.88	0.5	6.5	918
21	1 (70)	1 (4.5)	-1 (33.5)	1 (4.5)	3.87	3.2	3.68	6	954
22	1 (70)	-1 (1.5)	-1 (33.5)	1 (4.5)	3.81	3.2	4.9	6	884
23	0 (60)	0 (3)	0 (37)	0 (3)	3.86	2.88	4.47	5	716
24	1 (70)	1 (4.5)	-1 (33.5)	-1 (1.5)	3.91	3.2	3.34	6	826
25	-1 (50)	1 (4.5)	1 (40.5)	1 (4.5)	3.92	2.56	3.66	4	158
26	1 (70)	1 (4.5)	1 (40.5)	1 (4.5)	3.89	3.2	6.15	6	610
27	0 (60)	0 (3)	0 (37)	0 (3)	3.89	2.88	4.45	5.5	656
28	0 (60)	-2 (0)	0 (37)	0 (3)	3.85	2.88	5.64	5	742
29	-1 (50)	1 (4.5)	-1 (33.5)	-1 (1.5)	3.93	2.24	3.62	4	436
30	0 (60)	0 (3)	0 (37)	0 (3)	3.86	2.88	5.6	5	838

a: X_1 = substrate concentration (%) w/v; X_2 = *inoculum size (%) v/v;* X_3 = temperature (°C); and X_4 = fermentation time (days)

Materials and Methods

Juice extraction

Fresh *M. citrifolia* were blended at a ratio of 1:1 (water: fruit). The blend was filtered using a cotton cloth and centrifuged at 4000 rpm for 20 min. Then the extract was sterilized by autoclaving at 121°C for 15 min and was stored at 4°C.

Yeast strains

S. cerevisiae ATCC 62418 was purchased from the American Type Culture Collection (ATCC 62418). Yeast cultures were routinely grown at 30°C in Yeast Peptone Dextrose (YPD) medium (Suutari *et al*., 1996).The yeast was stored in 10% (v/v) glycerol at -18°C as a culture stock (Sherman *et al.*, 1991).

Fermentation process

The yeast inoculum size used for fermenting the extract were 0, 1.5, 3, 4.5 and 6% v/v. Fermentation was carried out, in capped sterile flasks containing 100 ml of autoclaved *M. citrifolia* extract. The inoculated extracts were incubated at different temperatures: 30, 33.5, 37, 40.5 and 44°C; substrate concentration: 40, 50, 60, 70 and 80% w/v; and fermentation time: 0, 1.5, 3, 4.5 and 6 days. The treatment combinations were based on the Central Composite Rotatable Design as shown in Table 1. The fermented *M. citrifolia* extract was subsequently analyzed for physico-chemical characteristics.

Total sugar content

Total sugar content was determined by phenol - sulphuric methods (Dubois *et al.*, 1956). A standard curve was prepared to quantify the total sugar content by glucose.

pH

Measurement of pH value was done in room temperature using a pH meter (Model PHM 210, Radiometer Analytical). For each measurement, 10 ml of samples were used (AOAC 1990).

Titratable acidity

Acidity was expressed as g of citric acid per 100 g of extract. Tests were done using 5 ml of sample and titrated with 0.1N NaOH with phenolphthalein as indicator (Cheng *et al.*, 2007).

Turbidity

Turbidity was determined using Spectrophotometer (Model Spectronic 20 PRIM, Secomam) by measuring the absorbance at 580 nm. Distilled water was used as a reference (Sin *et al*., 2006).

Total soluble solid

Total soluble solid was measured using a hand-held refractometer (Model ATAGO, Japan) with a range of 0-50% Brix.

Total polyphenol content

Total polyphenol content was determined using the Folin-Ciocalteu reagent (Shahidi and Naczk, 1995), which contains sodium phosphomolibdate and sodium tungstate. The epicatechin (EAE) (µg /

Table 2. Statistical analysis of models representing the response surface of pH, titratable acidity, turbidity, total soluble solids and total polyphenol content during the fermentation process of *M. citrifolia* extract by *S. cerevisiae*

Response	Model P value	R^2	Lack-of-fit Test P value
pH	<0.0001*	0.8839	0.8206
Titratable acidity	<0.0001*	0.9213	0.6226
Turbidity	<0.0001*	0.9220	0.5915
Total Soluble Solids	<0.0001*	0.9095	0.3268
Total Polyphenol Content	<0.0001*	0.8739	0.6706

* Significant at level $p < 0.05$

ml) stock solution was prepared prior to use.

Experimental design

A central composite rotatable design (CCRD) was used to allocate treatment combinations in this experiment (see Table 2) using Design Expert Version 6.0.10 (Stat-ease, Inc) software. The independent variables were substrate concentration, X_1 (40, 50, 60, 70 and 80%) (w/v), inoculum size, X_2 (0, 1.5, 3, 4.5 and 6%) (v/v), temperature, X_3 (30, 33.5, 37, 40.5 and 44°C) and fermentation time, X_4 (0, 1.5, 3, 4.5 and 6 days). Each independent variable had five levels: -2, -1, 0, +1 and +2. A total of thirty combinations (including five replicates at the center point with each value coded as 0) were chosen in random order. The actual and coded values (x) of the four independent variables together with the responses are shown in Table 1. The responses functions (y) were pH, titratable acidity, turbidity, total soluble solid and total polyphenol content.

The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of all terms in the polynomial was judged statistically by computing the F-value at a probability (p) of 0.05. The regression coefficients were then used to make statistical calculation to generate contour maps from the regression models.

Results and Discussion

The models and analysis of variance for five responses, namely pH, titratable acidity, turbidity, total soluble solids and total polyphenol content are presented in Table 2. The high R^2 values (> 0.80) for all responses indicate a good agreement between the experimental results and the theoretical values predicted by the model (Weisberg, 1985). The lack-of-fit was not significant ($p > 0.05$) for all the ten responses. Based on the results in Table 2, models of the parameters showed that they fit the model well and thus represented the experimental data adequately. The coded and actual models for all responses are presented in Table 3.

Table 3. Regression models representing the effect of pH, titratable acidity, turbidity, total soluble solids and total polyphenol content of *M. citrifolia* extract, fermented by *S. cerevisiae*.

Responses	Equation[a]
pH	Coded: $y_1 = 3.87 - 7.5\text{x}10^{-3} X_1 + 0.023 X_2 + 5.833\text{x}10^{-3} X_3 - 4.167\text{x}10^{-3} X_4 + 6.964\text{x}10^{-3} X_1^2 +$
Titratable acidity	$3.214\text{x}10^{-3} X_2^2 - 4.286\text{x}10^{-3} X_4^2 - 5\text{x}10^{-3} X_{12} - 6.25\text{x}10^{-3} X_{23} + 0.014 X_{34}$
Turbidity	Coded: $y_2 = 2.82 + 0.39 X_1 - 0.040 X_2 + 0.013 X_3 + 0.093 X_4 + 0.031 X_2^2 + 0.031 X_3^2 - 0.049 X_4^2$
	Coded: $y_3 = 4.63 + 0.13 X_1 - 0.23 X_2 + 0.49 X_3 + 0.35 X_4 + 0.17 X_1^2 + 0.18 X_2^2 + 0.091 X_3^2 - 0.78$
	$X_4^2 - 0.19 X_{12} + 0.24 X_{13} + 0.18 X_{14} + 0.18 X_{24} + 0.25 X_1^3 - 0.13 X_3^3 - 0.20 X_4^3$
Total Soluble Solids	Coded: $y_4 = 5.13 + 0.79 X_1 - 0.25 X_2 - 0.083 X_3 + 0.042 X_4 + 0.016 X_2^2 + 0.078 X_4^2 + 0.13 X_{12} +$
	$0.13 X_{34} + 0.13 X_2^3 + 0.083 X_4^3$
Total Polyphenol Content	Coded: $y_5 = 649.29 + 189.08 X_1 - 35.58 X_2 - 2.92 X_3 - 93.42 X_4 - 44.89 X_3^2 + 87.36 X_4^2 + 31.37 X_{12}$
	$- 70.88 X_{13} + 34.63 X_{14} - 91.88 X_{34}$

a: X_1 = substrate concentration (%) w/v; X_2 = inoculum size (%) v/v; X_3 = temperature (°C); and X_4 = fermentation time (days)

Table 4. Coefficient estimate for regression models involving pH, titratable acidity, turbidity, total soluble solids and total polyphenol content during the fermentation process of *M. citrifolia* extract by *S. cerevisiae*

Coefficient Estimate[c]	pH	Titratable acidity (g/mL)	Turbidity (absorbance at 580 nm)	Total Soluble Solid (°Brix)	Total Polyphenol Content (ppm)
b_0	3.87*	2.82*	4.63*	5.13*	649.29*
b_1	$-7.5\text{x}10^{-3}$*	0.39*	0.13	0.79*	189.08*
b_2	0.023*	-0.04	-0.23*	-0.25*	-35.58
b_3	$5.833\text{x}10^{-3}$*	0.013	0.49*	-0.083	-2.92
b_4	$-4.167\text{x}10^{-3}$	0.093*	0.35	0.042	-93.42*
b_1^2	$6.964\text{x}10^{-3}$*	-	0.17	-	-
b_2^2	$3.214\text{x}10^{-3}$	0.031	0.18	0.016	-
b_3^2	-	0.031	0.091	-	-44.89*
b_4^2	$-4.286\text{x}10^{-3}$	-0.049	-0.78*	0.078	87.36*
b_{12}	$-5\text{x}10^{-3}$	-	-0.19	0.13	31.37
b_{13}	-	-	0.24	-	-70.88*
b_{14}	-	-	0.18	-	34.63
b_{23}	$-6.25\text{x}10^{-3}$	-	-	-	-
b_{24}	-	-	0.18	-	-
b_{34}	0.014*	-	-	0.13	-91.88*
b_1^3	-	-	0.25*	-	-
b_2^3	-	-	-	0.13*	-
b_3^3	-	-	-0.13	-	-
b_4^3	-	-	-0.2*	0.083	-

c: b_1 = substrate concentration (%) w/v; b_2 = inoculum size (%) v/v; b_3 = temperature (°C); and b_4 = fermentation time (days)

* Significant at level $p < 0.05$

Total sugar content

Total sugar content was determined in the *M. citrifolia* extract where the value is 49.9 g/L. According to the Chunghieng (2003), the glucose and fructose content of the fruit were 11.9 ± 0.2 g/L and 8.2 ± 0.2 g/L respectively. While European Commission (2002), the glucose and fructose content of the fruit were 3 – 4 g/100 g and 3 – 4 g/100 g.

pH

From Table 2, b_1, b_2, b_3, b_{11}, and b_{34} were significant ($p < 0.05$) for pH value. Substrate

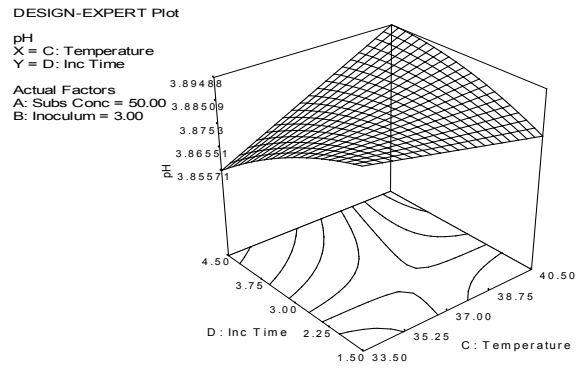

Figure 1. pH value of fermented *Morinda citrifolia* as a function of temperature and fermentation time for 50% substrate using 3% inoculum concentration

concentration showed a negative linear effect on pH where pH decreased with increasing substrate concentration. Higher substrate concentration produced lower pH due to the higher acid content in the extract. Farine *et al.* (1996) reported that 83% of the volatile components in M. citrifolia consist of carboxylic acid. At the same time, the significant ($p < 0.05$) quadratic coefficient (b_{11}) suggests that there is a maximum point for this model. Figure 1 shows that pH value of fermented M. citrifolia extract as a function of temperature and fermentation time for 50% substrate using 3% inoculum size. At fixed substrate concentration, pH of fermented *M. citrifolia* extract increased with increasing temperature. Within the experimental region, the effect of temperature showed a clear relationship with pH (significant pH and temperature interaction) (Arroyo-Lopez *et al.,* 2009). The interaction effect between fermentation time and temperature showed a significant positive ($p < 0.05$) suggesting that the effect of temperature during fermentation *S. cerevisiae* depended on relationship fermentation time during the specific temperature.

Titratable acidity

From Table 2, it may be observed that only substrate concentration (b_1) and fermentation time (b_4) have a significant effect on titratable acidity were a positive linear effect was observed. This shows that titratable acidity increased with increasing substrate concentration. Increase substrate concentration means increased concentration of fruit acids, hence of higher pH. The positive linear effect of substrate concentration on titratable acidity was in agreement with values of pH. Laopaiboon *et al.* (2009) reported that pH of the sweet sorghum juice at all condition was slightly decreased to 4.5 after 12 hours fermentation and relatively constant afterward. It showed that the titratable acidity increased as the pH decreased.

Turbidity

Turbidity in fruit juices can be a positive or a negative attribute depending on the expectation of the consumers (Hutchings, 1999). In the case of *M. citrifolia* juices as well as apple juices, the clear juices are more desirable and acceptable by the consumers. Inoculum size (b_2) and temperature (b_3) have significant ($p < 0.05$) linear effect on turbidity. Fermentation time (b_4) showed a significantly affected the turbidity in quadratic manner. Inoculums size showed a negative linear effect on turbidity. This shows that turbidity decreased with increasing inoculums size. *S. cerevisiae* has been reported as containing chitin and chitosan in their cell wall and septa (Gooday 1993), named fungal chitosan where proved highly effective in reducing the apple juice turbidity and gave lighter juices than the sample treated with shrimp chitosan (Rungsardthong *et al*., 2006). Fermentation time had a negative effect on turbidity at quadratic terms, showing a highly significant level of $p < 0.05$. Absorbance has been reported to decrease from initial in the fermentation of lingonberry juice by *S. cerevisiae* (Visti *et al.,* 2003). From Table 2, temperature showed a significant ($p < 0.05$) positive linear effect on turbidity which indicates that turbidity of fermented *M. citrifolia* extract increased with increasing the temperature during the fermentation. This observation maybe due to the formation of haze particles as the time of incubation increased (Visti *et al*., 2003).

Total soluble solid

From Table 2, only substrate concentration (b_1) and inoculum size (b_2) significantly affected total soluble solid. Substrate concentration had a positive linear effect on total soluble solid at a significant level of $p < 0.05$. Thus, total soluble solid of fermented *M. citrifolia* extract increased with increasing substrate concentration. As stated previously in titratable acidity, increasing the substrate concentration caused increased amount of total soluble solid in the extract. Total soluble solid was observed to decrease significantly with increasing inoculum size during fermentation. Visti *et al.* (2003) reported that citric acid and total sugar concentration in lingonberry juice decreased during fermentation due to increase of required total amount of yeast.

Total polyphenol content

From Table 2, it was observed that total polyphenol content was significantly ($p < 0.01$) affected by the linear effect of substrate concentration (b_1) and fermentation time (b_4). Increasing the

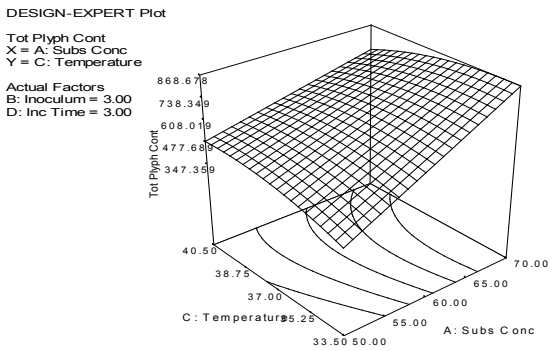

Figure 2. Total polyphenol content of fermented *Morinda citrifolia* as a function of substrate concentration and temperature using 3% of inoculum concentration for 3 days (72 hours)

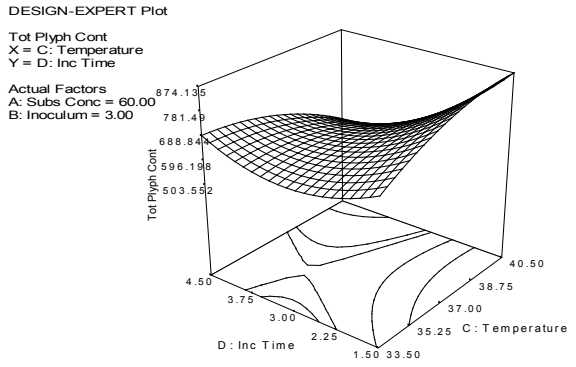

Figure 3. Total polyphenol content of fermented *Morinda citrifolia* as a function of temperature and fermentation time for 60% substrate using 3% of inoculum concentration

substrate concentration would produce higher total polyphenol content. However, increasing the fermentation time could result in reduction of total polyphenol content. Table 2 also indicated a significant ($p < 0.05$) interaction between substrate concentration and temperature. Figure 2 shows that total polyphenol content of fermented *M. citrifolia* extract as a function of substrate concentration and temperature using 3% of inoculum size for 3 days (72 hours) of fermentation. At fixed temperature, total polyphenol content of fermented M. citrifolia extract increased with increasing amount of substrate concentration of *M. citrifolia.* Increased substrate concentration corresponds with an increase in the amount of *M. citrifolia* fruits that were used in the samples. It has been described that *M. citrifolia* fruits contain some bioactive components such as phenolic compounds in particular coumarins and flavonoids and iridoids (Potterat *et al.,* 2007). Dang *et al.* (2010) also reported that a few other compounds such as scopoletin, quercetin and rutin occurring in significant, although much less quantities. Tavirini *et al.* (2008) told that fruits with high antioxidant activity generally contain large quantity of antioxidant substrate especially phenolic compounds and specifically flavonoids. Figure 3 shows that total polyphenol content of fermented *M. citrifolia* extract as a function of temperature and fermentation time for 60% substrate using 3% of inoculum size. At higher fermentation time approximately more than 3 days, total polyphenol content decreased with increasing the temperature. Despite the popularity of commercially available fermented *M. citrifolia* juice, fermentation process greatly decreased the radical scavenging activity (RSA) of the product within 2 weeks. The total phenolics of M. citrifolia juice during fermentation are stable at 1.95–2.41 mg ml^{-1} for 10 weeks then decreases by 30 – 40% from 10 to 12 weeks (Yang *et al.*, 2007). However, in this study, total polyphenol content of *M. citrifolia* gradually decreased during 6 days of the fermentation process by *S. cerevisiae*.

Conclusion

The methodology of the experimental design was shown to be very useful for the evaluation of four responses for fermentation of *M. citrifolia* extracts by *S. cerevisiae.* The different conditions (substrate concentration, inoculum size, temperature and fermentation time) for the fermentation showed that all these variables markedly affect the pH, titratable acidity, turbidity, total soluble solid and total polyphenol content. From the results, pH, titratable acidity, turbidity, total soluble solids and total polyphenol content were successfully fitted with mathematical models.

Acknowledgements

The authors would like to thank Universiti Malaysia Terengganu for the financial assistance and scholarship and Universiti Kebangsaan Malaysia (UKM) for their financial support under the INDUSTRI-2011-004 Research grant.

References

Arroyo-Lopez, F.N., Orlic, S., Querol, A. and Barrio, E. 2009. Effect of temperature, pH and sugar concentration on the growth parameters of Saccharomyces cerevisiae, S. kudriavzevii and their interspecific hybrid. International Journal of Food Microbiology 131: 120-127.

Association of Official Analytical Chemists (AOAC). 1990. Official methods of analysis. 15th Edition. USA:AOAC Inc.

Caplicec, E. and Fitzgeralda, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. International Journal of Food Microbiology 50: 131–149.

Cheng, L.H., Soh, C.Y. and Liew, S.C. 2007. The, F.F. Effects of sonication and carbonation on guava juice quality. Food Chemistry 104: 1396–1401.

Coe, N.R., Smith, A.J., Frohnert, B.I., Watkins, P.A. and Bernlohr, D.A. 1999. The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase. Journal of Biology Chemistry 274:36300–36304.

Dang, S., West, B.J., Palu, A.K. and Jensen, C.J. 2010. A quantitative comparison of phytochemical components in global noni fruits and their commercial products. Food Chemical 122: 267-270.

Duarte, W.F., Dragone, G., Dias, D.R., Oliveira, J.M., Teixeira, J.A., Almeida e Silva, J.B. and Schwan, R.F. 2010. Fermentative behavior of Saccharomyces strains during microvinification of raspberry juice (*Rubus idaeus* L.). International Journal of Food Microbiology 143:173–182.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350–356.

European Commission, Scientific Committee of Food. 2002. Opinion of the Scientific Committee on Food of Tahition Noni® Juice. SCF/CS/DOS/18 ADD 2 Final. Belgium. *http://europa.eu.int/comm/food/fs/sc/scf/out151_en.pdf*.

Farine, J.P., Legal, L., Moreteau, B. and Le Quere, J.L. 1996. Volatile components of ripe fruits of *Morinda citrifolia* and their effects on Drosophila. Phytochemistry 41:433–438.

Gooday, G.W. 1993. Cell envelope diversity and dynamics in yeasts and filamentous fungi. Journal Applied Bacteriology (Suppl) 74:12S–20S.

Goh, S.H., Chuah, C.H., Mok, J.S.L. and Soepadmo, E. 2000. Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases. Pelanduk Publications.

Hutchings, J.B. 1999. Food Color and Appearance. 2nd Edition. Aspen Publishers Gaithersburg, MD.

Laopaiboon, L., Nuan peng, S., Srinophakun, P., Klanrit, P. and Laopaiboon, P. 2009. Ethanol production from sweet sorghum juice using very high gravity technique: Effect of carbon and nitrogen supplementations. Bioresource Technology 100: 4176-4182.

Luciane, C.M., Hilary, C.M., Aparecida, M. and De Silva, A.P. 2001. Optimization of the roasting of robusta coffee (*C. Canephora* conillon) using acceptability tests and RSM. Food Quality and Preference 12: 153–162.

Norma, H., Normah, A., Ahmad, A.W., Rohani, M.Y., Muhammad Gawas, M. and Sharizan, A. 2004. Reducing the smelly compounds (caproic, caprylic and capric acids) in noni by treating the juice with activated charcoal powder. Proceeding of the National Food Technology Seminar p. 125-129.

Nur Hafiza, Z., Maskat, M.Y., Wan Aida, W.M. and Osman, H. 2010. Optimization of deacidification process for Morinda citrifolia extracts using packed column of calcium carbonate. International Food Research Journal 17:1051-1066.

Potterat, O., Von Felten, R., Dalsgaard, P.W. and Hamburger, M. 2007. Identification of TLC markers and quantification by HPLC–MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 55: 7489–7494.

Rungsardthong, V., Wongvuttanakul, N., Kongpien, N. and Chotiwaranon, P. 2006. Application of fungal chitosan for clarification of apple juice. Process Biochemistry 41:589–593.

Sherman, F., Fink, G.R. and Hicks, J. 1991. Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Shahidi, F. and Naczk, M. 1995. Food phenolics sources, chemistry, effects, and application. Lancaster Basel: Technomic Publishing Co. p. 128.

Shieh, C.J., Koehler, P.E. and Akoh, C.C. 1996. Optimization of sucrose polyester synthesis using response surface methodology. Journal of Food Science 61: 97–100.

Siebert, T.E., Smyth, H.E., Capone, D.L., Neuwöhoner, C., Pardon, K.H., Skouroumounis, G.K., Herderich, M.J., Sefton, M.A. and Pollnitz, A.P. 2005. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Analytical and Bioanalytical Chemistry 381:937–947.

Sin, H. N., Yusof, S., Sheikh Abdul Hamid, N. and Rahman, R. Abd. 2006. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. Journal of Food Engineering 73:313–319.

Singh, Y.N., Ikahihifo, T., Panuve, M. and Slatter, C. 1984. Folk medicine in Tonga – A study on the use of herbal medicines for obstetric and gynaecological conditions and disorders. Journal of Ethnoparmacology 12: 305 – 329.

Suutari, M., Liukkonen, K. and Laakso, S. 1996. Temperature gradient incubator for the study of alterations occurring with fatty acids of *Saccharomyces cerevisiae* when approaching extreme growth temperatures. Journal of Microbiological Methods 25:207-214.

Tavirini, S., D'Innocenti, E., Remorini, D., Massai, R. and Guidi, L. 2008. Antioxidants capacity ascorbic acid, total phenols & carotenoids changes during harvest and after storage of Hayward kiwifruit. Food Chemistry 107: 282-288.

Visti, A., Viljakainen, S. and Laakso, S. 2003. Preparation of fermentable lingonberry juice through removal of benzoic acid by *Saccharomyces cerevisiae* yeast. Food Research International 36: 597-602.

Weisberg, S. 1985. Applied linear regression. New York : Wiley.

Yang, J., Paulino, R., Janke-Stediosky, S. and Abawi, F. 2007. Free radical scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage. Food Chemistry 102: 302-308.