DETECTION OF 5-HYDROXYMETHYL-2-FURFURALDEHYDE IN FERMENTED NONI JUICES

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ABSTRACT

Fermented noni juice is the popular beverage juice in Thailand. Noni fruit is renowned as the richest storehouse of enzymes, phytonutrients, vitamins, and trace minerals. However, after noni juice was produced and stored for a long time , it became darken color. 5-Hydroxymethyl-2-furfuraldehyde (HMF) is one of an important product from Maillard reaction and used to indicate the quality of many products. Thus in this work , HMF contents were analysed in noni juices which were prepared in the laboratory for 7 formulas and also analysed in storage noni juices for 180 days by using HPLC technique. The results were revealed that HMF could not be detected in all noni juices after the preparative noni juice. Noni juices which were stored for 7, 30, 60, 90 ,120 ,150 and 180 days contained HMF in the range of 0.5090 –0.6630 ppm. From this research showed that HMF contents in those juice depend on storaging time. Since after noni juices were stored at room temperature, HMF content in each noni formula showed the hightest level at 6 months. There are not the regulation about HMF content in fermented noni juice in Thailand. Fermented noni juice can compare with wine since both of them are an alcoholic beaverage. Total alcohol and furfural compounds were the regulation control in wine about 2500 ppm. HMF content found in those fermented noni juices preparative in the laboratory not exceed the regulation content in wine.

KEYWORDS : Fermented noni juice , 5-Hydroxymethyl-2- furfuraldehyde , HMF , Maillard reaction, HPLC

1. INTRODUCTION

It has long been known that fruit juice darken during storage. Nonenzymatic browning reaction is one of the reason for darkness color [1]. The reaction also well known as Maillard reaction which is an important for many kind of food products such as bakery , wine , honey , tomatoes source etc.[2]. Maillard reaction is the reaction between the carbonyl group of sugar and amino group from protein [1]. This reaction forms in many steps and goes on to many products.
5-Hydroxymethyl-2- furfuraldehyde (HMF) is one of an important product which can be an indicator for the nonenzymatic browning in many food products[2]. HMF is a reaction product of the transformation of hexose sugar in acid medium too [1]. Noni or Indian Mulberry has an scientific name as Morinda Citrifolia [3]. Noni fruit grows best in the rich, volcanic ash of the Hawaiian Islands. Hawaiian Noni fruit is renowned as the richest storehouse of enzymes, phytonutrients, vitamins, and trace minerals that give its legendary powers to rejuvenate and revitalize. Noni is widely used to support the body's ability to fight the effects of stress and modern life that can lead to immune deficiencies and diseases [4]. Now, the noni juice is one of popular drinking juice in Thailand , but they do not have details about theirs quality such as furfural content.Since Maillard reaction cause the darken of fermented juice , thus our work interested in analyzing HMF content and may indicate the quality of fermented noni juice. HMF was analysed in many food products by many methods such as GC[5], Spectrophotometry [6] and HPLC method [7]. In this work , HMF was analysed in preparative noni juice after preparation and also study the effect of storage on HMF content by reverse phase HPLC[8].

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2. MATERIALS AND METHODS

All chemicals used were Analytical grade. The 5-Hydroxymethyl-2- furfuraldehyde (HMF) was purchased from Fluka. Acetonitrile was purchased from Merck. Potassium ferrocyanide, zinc acetate and methanol were purchased from Carlo erba.

2.1 Preparation of Fermented Noni Juices

The fermented Noni juice were collected from Chiengmai University Plant for 7 formulas which contain the composition and pretreatment as the following.

Formula 1 noni fruit 3 parts : water 10 parts : red sugar 1 part add *lactobacillus Casei*
Formula 2 noni fruit 3 parts : water 10 parts : honey 1 part add *lactobacillus Casei*
Formula 3 noni fruit 3 parts : water 10 parts : red sugar 1 part
Formula 4 noni fruit 3 parts : water 10 parts : honey 1 part
Formula 5 noni fruit 3 parts : water 10 parts : red sugar 1 part . Ferment dry component for 15 days and addition 10 parts of water.
Formula 6 noni fruit 3 parts : water 10 parts : red sugar ½ part : honey ½ part then *lactobacillus Casei* was added .
Formula 7 noni fruit 3 parts : water 10 parts : red sugar 1 part . Mix all components and heating at 72 °C for 15 sec. then *lactobacillus Casei* was added after cooling.

After all those noni juices were processed , each noni juice was collected some part of samples for initial analysis of HMF content after preparation. Then ,they were fermented and collected the noni juice samples for 7 , 30 , 60, 90, 120, 150 and 180 days to analyse HMF content.

2.2 Sample Treatment

The noni juice samples at each period were measured pH by Methrom pH meter (model PH 713) before clarification process. Then noni juices were pipetted 5 ml and tranfered into 50ml of conical flask to clarification as in AOAC method [9]. The carrez Solution I ($K_4Fe(CN)_6.3\ H_2O$ 15 g / 100 ml water) 0.5 ml and carrez solution II($Zn(OAc)_2.2\ H_2O$ 10g /100 ml water) 0.5 ml were added to those juices. The solutions were mixed throughly and the precipitates were filtered by Whatman filter paper no.1. The clarified fermented noni juices were collected in brown bottle and storage at 4 °C until the analysis.

2.3 HPLC analysis

The clarified fermented noni juices from 2.2 were filtered again by SPE technique through millipore membrane cellulose filter 0.45 micron before the injection to HPLC spectrometer (HP 1100 series) which composed of reverse phase C-18 column (Bondclone) and eluted by the eluent of acetonitrile : water (5 : 95) , controlled flow rate at 1 ml/min.then signal was detected with DAD at 280 nm [10].

2.4 Efficiency Test method

2.4.1 Percent Recovery

The standard HMF 4 microgram /ml was injected to HPLC for 10 times. Then recorded the area of HMF peak in each time to calculate the average percent recovery of this experiment.

2.4.2. Limit of Dection (LOD)

The standard HMF 0.5 , 1.0 , 2.0 and 5.0 microgram /ml were injected to HPLC and recorded the area of the peak in each concentration and plotted the graph to calculate LOD.

3 RESULTS AND DISCUSSION

After preparation fermented noni juice in the laboratory, the noni juice of each formula was measured pH before clarifying and measured the HMF content. The pH of each noni juice formula was recorded between 7 – 180 days which were presented as in fig.1.

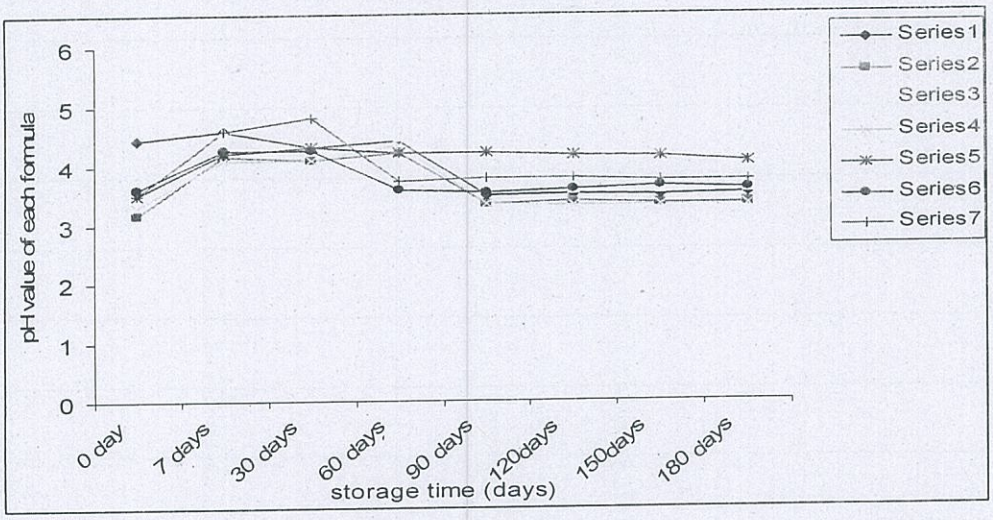

Fig 1. pH of each fermented noni juice between 0-180 days

Table 1 HMF content in fermented noni juices

time (days)	HMF content (ppm) F 1	F2	F3	F4	F 5	F6	F 7
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.11±0.001	0.11±0.001	0.15±0.001	0.00	0.00	0.00	0.00
30	0.13±0.003	0.13±0.002	0.16±0.001	0.11±0.002	0.16±0.001	0.00	0.00
60	0.14±0.002	0.15±0.001	0.18±0.003	0.11±0.003	0.16±0.002	0.11±0.001	0.12±0.001
90	0.15±0.001	0.16±0.003	0.18±0.002	0.11±0.001	0.17±0.002	0.12±0.001	0.13±0.003
120	0.16±0.002	0.15±0.001	0.19±0.001	0.11±0.002	0.18±0.003	0.12±0.001	0.13±0.002
150	0.17±0.001	0.18±0.002	0.22 ±0.002	0.13±0.001	0.21±0.001	0.17±0.002	0.14±0.001
180	0.60±0.001	0.53±0.001	0.75±0.001	0.51±0.002	0.62±0.001	0.66±0.001	0.55±0.002

Note: number of replications = 5, the value in the table represent mean ± SD

From fig. 1, the pH of each juice rapidly change between 0 – 7 days then it gradually change to pH 3 - 4. This means that all noni juices were acid juices. The HMF contents were measured and showed the results as in table 1. From the table 1 , HMF content in each noni juice could not be detected at the initial of fermentation , but after 7 days the HMF contents were found in noni juice formula 1 ,2 and 3 average 0.11 -0.15 ppm. At 180 days of storage , HMF content of each formula showed between 0.51 – 0.72 ppm. The formula 3 contained the highest HMF content since in this formula the red sugar was one of the component which contained sucrose as a main sugar. Sucrose is the most reactive sugar in forming HMF in Maillard reactions[2]. However the HMF in each juice also increase as the increasing of time and also depend on treatment process

in noni juice preparation too. In this experiment , the color of fermented noni juices were also recorded as presented in table 2.

From table 2 , we recorded the color of each noni formula trend to pale brown color to dark brown. In formula 3 , the color of noni form pale brown to turbid very dark brown at 180 days of storage which compromise with the value of HMF content in noni juice formula 3. From the regression plot between HMF contents in all formula versus time as in fig 2. showed that HMF contents correlated with time of storage as exponential form with r^2 between 0.71-0.80 at $p \leq 0.05$. So that , there are high probability to find HMF from Maillard reaction in fermented noni juice which was stored at room temperature for a long time such as 1 year or more than this and also confirmed the experiment of Babsky et.al [7] Now, there are not any regulation about HMF content in this juice. In wine industry , total aldehyde and alcohol are controlled at 2500 ppm ,so fermented noni juice is an beaverage like as wine thus HMF is an aldehyde compound which can be compare with aldehyde in wine . HMF contents in fermented noni juice not exceed than the specification of wine.

Table 2. The color of fermented noni juices.

days	Formula1	Formula2	Formula3	Formula4	Formula5	Formula6	Formula7
0	pB	C	pB	pB	pB	pB	pB
7	TpB	pB	pB	pB	pB	pB	pB
30	TpB	pB	DB	B	B	B	B
60	TpB	TpB	DB	B	TB	TB	TB
90	TpB	TpB	TDB	TB	TDB	TDB	TDB
120	TpB	TpB	TDB	TB	TDB	TDB	TDB
150	TpB	TpB	TvDB	TB	TDB	TDB	TDB
180	TB	TB	TvDB	TB	TDB	TDB	TDB

Note: B = brown color, pB = pale brown, DB = dark brown, TpB = turbid pale brown, TB =turbid brown, TDB = turbid dark brown, TvDB = turbid very dark brown, C = colorless

The method used in this experiment has 97.92 percent recovery and the limit of detection 0.0299 ppm. Thus this method has very good efficiency for detection of HMF.

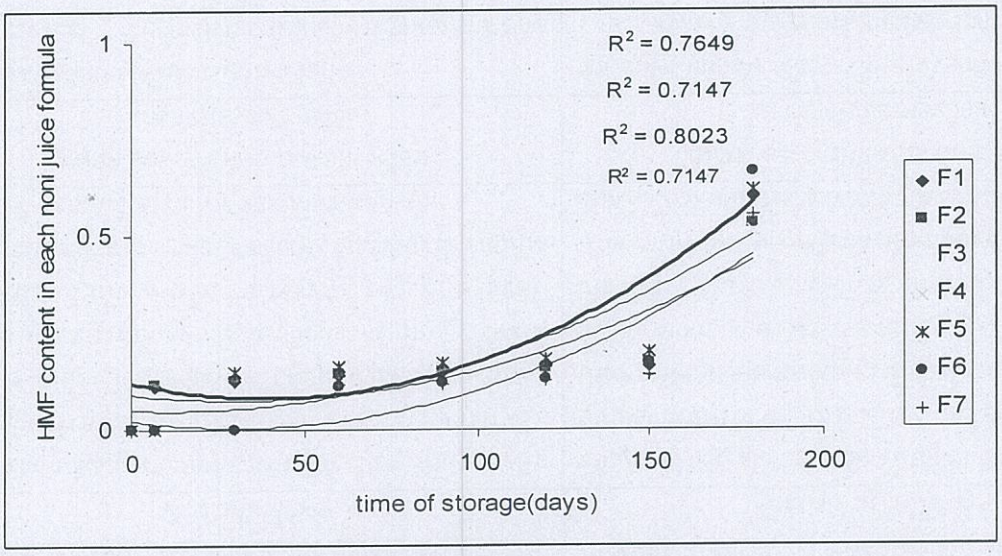

Fig 2. The regression between HMF content in noni juice and storaging time.

4. CONCLUSIONS

The advantage of this work is to quantitative analysis of HMF in fermented noni juice by reverse phase HPLC. The analysis method has LOD 0.0299 ppm and percent recovery is 97.92 , which showed that this method has good efficiency to detect the low level of HMF. The result from this work also presented that HMF content depend on storage time and the difference treatment or difference composition of noni juice contained the difference HMF content too. Finally , we have proved that the fermented noni juice safe for consumer.

5. ACKNOWLEDGEMENTS

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REFERENCES

[1] Belitz, H.D. and Grosch, W. **2000** *Food Chemistry*. 2 nd ed. Heidelberg. Springer Verlag.
[2] Boekel, V. **1998** Effect of heating on Maillard reactions in milk, *Food Chemistry,*62(4), 403-414.
[3] www.http://www.sarinoni.com/medical.htlm
[4] Horace,D.G.**1980**. *Safety of Foods*, 2 nd.ed.NewYork, AVI Publishing Company.,Inc.
[5] Burdulu, a and Karadeniz,F.**2003**. Kinetic model of nonenzymatic browning in apple puree.*Journal of Food Science.* 80(1).91-97.
[6] Akkan.A.**2001**.Derivative Spectrophotometric determination of 5- Hydroxymethyl-2-Furfuraldehyde and furfural in locust bean extract. *Journal of Article,* 45(1). 43-46.
[7] Babsky,N.E, Toribio,J.L.and Lozano,J.E. **1986**. Influence of storage on the composition of clarified apple juice concentrate. *Journal of Food Science,*51: 564-567.
[8] Hurtado, S.A, Veciana, M.T., Pulide, M.I. and Vidal, M.C.**1997**. Determination of Free and Total furfural compounds in infant milk formulas by Highperformance Liquid Chromatography. *Journal of Agricultural and Food Chemistry*.80(1): 91-97.
[9] AOAC.**1990**. Official Methods of Analysis of the Association of Official Analytical Chemists. 15 th ed. Vol 2.USA.The Association of Official Analytical Chemistry.Inc.
[10] Wunderlin, D.A., Desoc, S.F, Ame, M.V. and Faye, P.F. **1998**.Decomposition of Hydroxymethylfurfural in solution and protective effect of fructose. *Journal of Agricultural and Food Chemistry*.46 : 1855- 1863.