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Research Article

Detection of non-enzymatic browning reaction in Thai herbal medicine

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Abstract

Herbal drugs have been used as a popular medicine in Thailand for a long time. Some drugs contain honey as binder substance. However, after storage for a long time, they develop dark brown colour in their composition. The dark brown colour sometimes occurs from nonenzymatic browning reaction known as Maillard reaction. The aim of this work is to detect nonenzymatic browning reaction by analysing the content of hydroxymethyl furfuraldehyde (HMF) in Thai herbal drugs. The experiment was planned to analyse the hydroxymethylfurfuraldehyde content in pure honey and heated honey at various temperatures (40–100^{o}C) and time (20–120 minutes) by High Performance Liquid Chromatography and to detect with a diode array. Then the hydroxymethylfurfuraldehyde was analysed in some Thai herbal drugs which were collected from the herbal store in Bangkok. The results showed that the hydroxymethylfurfuraldehyde content in pure honey was 0.45 ± 0.02 ppm, their value increased after the honey was heated to 100^{o}C .The highest value of hydroxymethylfurfuraldehyde content in honey was increased to 4.50 ± 0.03 ppm after heating the honey for 120 minutes. The LOD of the analysis method was 0.05 ppm. The hydroxymethylfurfuraldehyde content in Thai herbal drugs (30 samples) that contained honey were 0.05–44.95 ppm. This work revealed that the occurrence of browning colour due to Maillard reaction gave the indicator product as hydroxymethyl furfuraldehyde, which is generally accepted to be a good indicator for honey in Europe. However, the new honey should have hydroxymethylfurfuraldehyde content not exceeding 1 ppm. The brown colour in Thai herbal drugs may be the bolus form and showed hydroxymethylfurfuraldehyde content at a high level. Thus, consumers should be aware of the possibility that these medicines are not safe if they contain hydroxymethylfurfuraldehyde compounds.

Keywords: nonenzymatic browning, Maillard reaction, hydroxymethylfurfuraldehyde, herbal medicine, honey, HPLC

Introduction

Thai herbal drugs are an alternative medicine for many Thai people. A lot of people still prefer to use herbal drugs rather than using the new drugs that sold in general drugstore. There are many Thai herbals drug from Thai wisdom knowledge, some herbal drug made in bolus shape or spherical shape form, some form is a flat plate, some form is a liquid solution [1]. All or some Thai herbal drugs may contain honey in their composition especially bolus form. Honey from natural has a good properties act as human drug, it was also used as binder for bind other herbs to form shape. The honey which stored for a long time showed the darken brown to almost black color, since the occurrence on browning reaction which was known as Maillard reaction [2]. Maillard reaction is a nonenzymatic browning reaction, which occur from the reaction between a carbonyl group of reducing sugar and amino group from amino acid [3]. There are many products from Maillard reaction, the one product that used to be an indicator for nonenzymatic reaction is 5-hydroxymethylfurfuraldehyde(HMF). HMF has been identified in a wide variety of heat-processed foods including milk, fruit juices, spirits, honey, etc. [4]. HMF is used as an indicator of heat and storage changes in honey. HMF is formed by the breakdown of fructose in the presence of an acid. Heat increases the speed of this reaction. The increase in speed is exponential with increasing heat with the age and heat treatment on honey [5]. However, new honey should not contain HMF and permission not exceed 1 ppm [6]. There has the regulation to control HMF in honey in could not exceed 15 ppm in many countries such as Germany, Belgium, Italy, Austria and Spain [7]. There has been no reports about the HMF content in Thai herbal drugs, but there was found only the report of HMF in pharmaceutical drug [8]. So, the aim of this work is to analyse the HMF content in Thai herbal drugs. The HMF in honey and heated honey were also studied.

Methods and Materials

Study of the appropriate conditions for HMF analysis

Standard HMF (HPLC grade purchase from Fluka) was prepared for 100 ppm in milli Q water, then it was filtered through 0.45 µm cellulose acetate membrane filter by SPE technique. The standard purified filtrate was injected through RP C_{18} column (Bondeclone 10 µ, 300 x 390 mm.) 100 µl to HPLC Chromatography (HP1100). The eluate was eluted by 3 mobile phase systems as per the following;

System 1: water : acetonitrile = 90 : 10 (as reference [5])

System 2: water : acetonitrile = 97 : 3 (modified from reference [5])

System 3: water : methanol = 90 : 10 (as reference [9])

The elution system was isocratic system in each system and control rate of flow at 1 ml/min. The signal in each system was detected by diode array detector at 280 nm. The chromatogram of the standard was recorded for the inspection of the appropriate condition. Honey 5.00 g was treated by addition 25 ml. of water and 0.5 ml. of carrez I solution. The mixing solution was mixed with 0.5 ml. of carrez II solution and diluted again to 50 ml. solution by milli Q water [9]. The clearance solution of honey sample was filtered through 0.45 µm cellulose acetate membrane filter and injected to RP C_{18}column.Then the solute was eluated by those 3 systems. The chromatogram of the honey was recorded for the inspection of the appropriate condition.

Study of the factors effecting heated honey

Effect of heating temperature on honey

The honey (Chitralada Brand) was weighed for 5.000 g and heated on hot plate at 40^0C for 30 min. The heated honey was treated by addition 25 ml. of water and 0.5 ml. of carrez I solution. The mixing solution was mixed with 0.5 ml. of carrez II solution and diluted again to 50 ml. solution by milli Q water [9]. The clearance solution of honey sample was filtered through 0.45 µm cellulose acetate membrane filter and injected to RP C_{18}column. Then the solute was eluated by the system from part 1 and recorded by chromatogram. The heating systems were studied with the new honey as above by heating the honey at 60^oC, 80^oC and 100^oC. All systems were trialed for 5 replications. Carrez I was the solution of 15% of $K_4Fe(CN)_6$, while carrez II was the solution of 30% of $Zn(OAc)_2$.

Effect of heating time on honey

The honey was weighed for 5.000 g and heated on hot plate at 100^0C for 20, 40, 60, 80, 100 and 120 min. and treated as per above**.**

Analysis of HMF in Thai herbal drug samples

Thai herbal drugs were purchased from Thai herb store at Tha Phra Chan in Bangkok and general drug store that sold Thai herbs. Those drugs were collected 25 examples as liquid form, bolus and pellet form. The drugs were weighed 5.0000 g and dissolved in 50 ml. of water. The drug solutions were treated with carrez I and carrez II as per the experiment described above. The chromatogram of samples was recorded and calculated the content of HMF in drugs. All experiments were carried out in 3 replicates.

Results and Discussion

The standard HMF was prepared and injected to RP C_{18} column using the 3 eluent systems. The chromatogram of each condition was recorded and considered the retention time of standard peak as presented in Table 1.

Table 1. R_T of standard HMF in each condition.

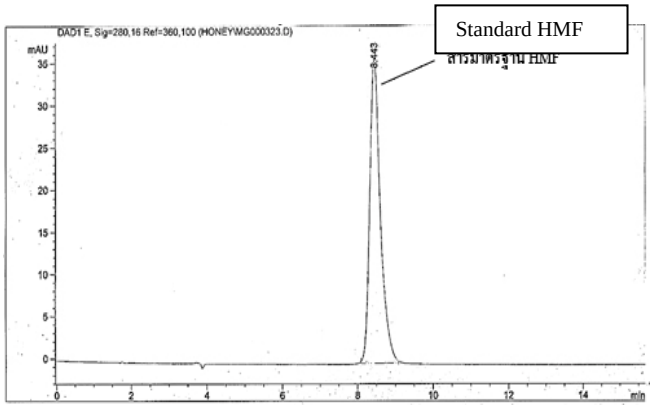
Mobile phase system	R_T (min)
System 1	5.16 $\pm$ 0.01
System 2	8.53 $\pm$ 0.05
System 3	7.21 $\pm$ 0.01

Following this treatment, the R_T was recorded as shown in Table 2.

Table 2. Characteristics peak of HMF in honey.

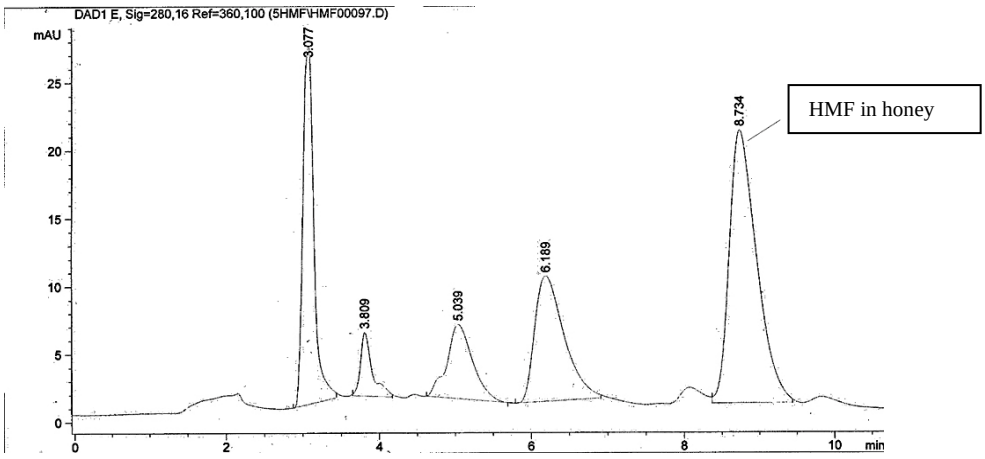
Mobile phase system	Peak resolution	Peak consistency
System 1	Not good	low
System 2	Good, sharp peak	high
System 3	Fair , board peak	medium

The eluent system 2 was selected to use in separation true honey analysis as showed standard peak in Figure 1 and honey sample in Figure 2.



Retention time (min)

Figure 1. Chromatogram of Standard HMF.



Retention time (min)

Figure 2. Chromatogram of pure honey.

The results in studied the effect of heating time at fixed temperature and effect of heating temperature at fixed time were compared the areas of HMF peak with the area of HMF peak from the standard calibration curve as shown in Figure 3.

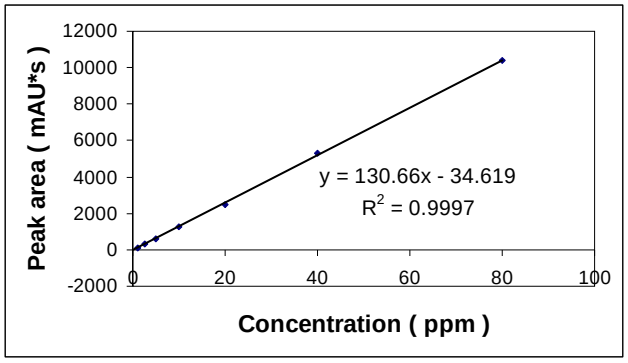


Figure 3. Standard calibration curve of standard HMF.

From the regression equation of the standard calibration curve of standard HMF was y = 130.66 x -34.62, with the R^2 = 0.9997. The areas of HMF peak from part 2.1 and 2.2 were calculated by the above regression to evaluate the HMF contents in heating honey shown in Tables 3 and 4.

Table 3. The HMF value of heated honey at various temperatures.

Heating temperature(^{o}C)	HMF content (ppm)	% RSD (n=6)
Room temperature	0.45 ± 0.02	3.90
40	0.47 ± 0.01	4.86
60	0.48 ± 0.01	5.41
80	0.49 ± 0.02	3.11
100	0.93 ± 0.03	2.43

Table 4. The HMF value of heated honey at various heating times.

Heating time (min)	HMF content (ppm)	% RSD (n=6)
20	0.59 ± 0.02	4.50
40	1.06 ± 0.05	6.66
60	1.46 ± 0.07	4.60
80	2.97 ± 0.15	4.88
100	4.20 ± 0.13	3.10

From Table 3, the HMF content remains constant from room temperature to heating at 80^{o}C and then doubles at 100^{o}C of heating. The relation between the heating temperature and HMF content as shown in Figure 4 was the polynomial relation with R^2 = 0.90.

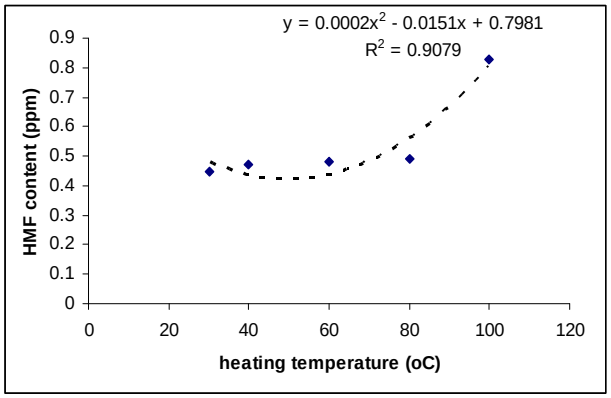


Figure 4. The relationship between heating temperature and HMF content in heating honey.

This experiment revealed that HMF in honey will increase as a polynomial form when the honey is heated at high temperature such as 100^{o}C, which had the same trend as referred to by Singh and Bathm [10]. Thus, if the honey is heated at high temperature, the HMF will increase too much, so Thai herbal drugs which contain honey as a component or binder also contain HMF as well.

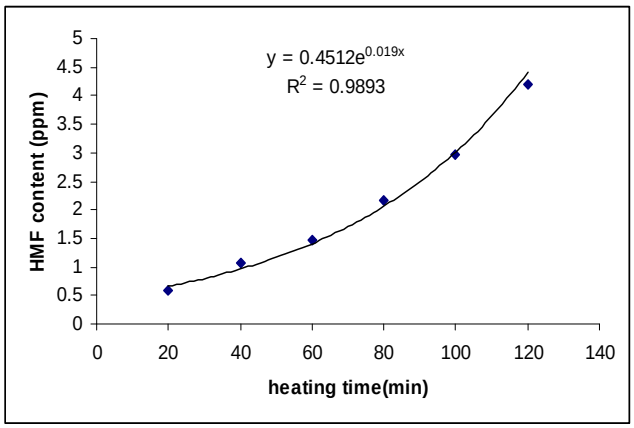


Figure 5. The relationship between heating time and HMF content in heating honey.

The relationship between heating time and HMF content in Figure 5 also shows the exponential relation as R^2 = 0.99. The heating time also effected on the HMF content in heating honey and had more effect than heating temperature on honey. This supports the results found by Coco *et al* [11] and Wen *et al* [12].

This part of the experiment revealed that the HMF occurred in honey and after using the heated honey in herbs as a component or binder, there is the likelihood of detecting HMF in high content. After applying method in analysis HMF content in honey with actual Thai herbal drug samples, the chromatogram of Thai herbal drugs was recorded as shown by way of example in Figure 6, while the results are shown in Table 5.

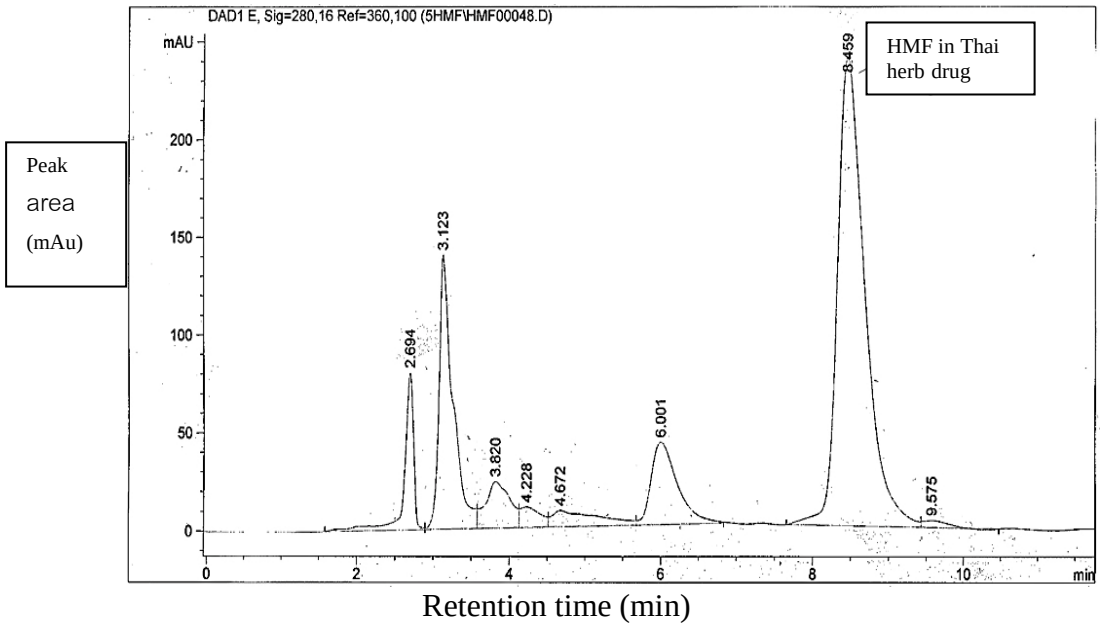


Figure 6. The chromatogram of HMF in Thai herbal drugs.

Table 5. The HMF content found in Thai herbal drug samples.

Sample number	Status of drugs/form	HMF content(ppm)
1	Dark brown liquid/liquid	1.51 ± 0.05
2	Dark brown solid/bolus	0.65 ± 0.01
3	Dark brown semisolid/bolus	1.85 ± 0.03
4	Dark brown semisolid/plate	1.47 ± 0.02
5	Brown semisolid/plate	0.87 ± 0.02
6	Brown semisolid/plate	0.71 ± 0.02
7	Brown semisolid/plate	0.67 ± 0.02
8	Brown semisolid/plate	0.24± 0.02
9	Dark brown semisolid/plate	3.12 ± 0.02
10	Dark brown semisolid/plate	1.07± 0.02
11	Dark brown solid/tablet	1.52 ± 0.15
12	Dark brown solid/tablet	3.02 ± 0.12
13	Dark brown solid/tablet	4.52 ± 0.23
14	Brown solid/tablet	0.65 ± 0.15
15	Black solid/tablet	20.85 ± 0.03
16	Brown solid/tablet	0.47 ± 0.18
17	Dark brown solid/tablet	5.58 ± 0.05
18	Pale brown solid/tablet	0.05 ± 0.02
19	Brown solid/tablet	0.56 ± 0.04
20	Pale brown solid/tablet	0.05 ± 0.01
21	Dark brown solid/tablet	8.89 ± 0.08
22	Dark brown solid/tablet	6.29 ± 0.14
23	Black solid/tablet	23.28 ± 0.27
24	Dark brown liquid/liquid	1.16 ± 0.11
25	Dark brown liquid/liquid	3.17 ± 0.20
26	Dark brown liquid/liquid	3.24 ± 0.05
27	Black solid/bolus	22.30 ± 0.14
28	Black solid/bolus	44.95 ± 0.50
29	Dark brown solid/bolus	5.08 ± 0.21
30	Dark brown solid/bolus	3.87 ± 0.09

The values of HMF content in 30 samples of Thai herbal drugs, which had pale brown colour to black colour, and were either in liquid or solid form, were 0.05 – 44.95 ppm. Those herbal drugs contained HMF from the production process, however it was noticed that the black or darker coloured drugs contained the higher HMF content than the paler colour. Since there is no regulation for controlling the quality of these Thai herbs and the effects from the producer using heated honey in the process, so the consumers are unaware of and exposed to toxins created by the HMF content in the drugs. HMF has been claimed to be a carcinogen in animals [11], it may dangerous for human health. Even though there is no regulation for controlling the HMF content, HMF content in honey is being increasingly controlled in developed countries [9].

Conclusion

This research demonstrated that all samples of the honey used in herbal drugs had some HMF content. Further study on the effects of heating time and heating temperature on honey revealed that the HMF content depends on both parameters. This showed that whenever the heated honey was used as a component in Thai herbal drugs or as a binder, there was a high probability of HMF content. After the analysis method was applied to actual samples of herbal drugs, in a variety of forms including liquid, bolus and pellet and also four different shades of colour such as pale brown, brown, dark brown and black, the results showed that these drugs contained HMF from low levels of about 0.05 ppm to the highest level recorded of 44.95 ppm. The results also indicated that the darker the colour of the herbal drugs, the higher the level of HMF value. However, the HMF content is the one parameter used to control the honey quality and there have been some reports about the toxicity of HMF, so the consumer should be cautious in taking drugs or herbal medicines which contain honey, due to the possibility of HMF and the potential for HMF to be toxic.

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