Original Article

Determination and Comparision of Hydroxymethylfurfural in Industrial and Traditional Date Syrup Products

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ABSTRACT

Background: Hydroxymethylfurfural (HMF) is a common Maillard reaction product directly formed from dehydration of sugars under acidic conditions during heating and storage in carbohydrate rich foods. The aim of the present study was to detect and quantify the amount of HMF in date syrup by HPLC method. In addition, the amount of HMF in date syrup produced by traditional and industrial methods were compared.

Methods: A HPLC method for determination of HMF in date syrup was developed and validated. The amount of HMF in date syrup products produced by the traditional and industrial methods was compared. In addition, to determine whether HMF was produced during storage in date syrup, its amount was measured in fresh and old samples.

Results: The HMF content of fresh traditional products varied between 1000 to 2675 mg/kg and in the old products varied between 2580 to 6450 mg/kg. The HMF concentration of the fresh industrial products varied between 12 to 456 mg/kg and 611 to 943 mg/kg in the old ones. The HMF concentration of the traditionally produced products was significantly higher than industrial products (P<0.001). Moreover, the HMF content in old products significantly was more than fresh ones (*P*<0.001). Wide variation was found in HMF content of the traditionally produced products.

Conclusion: HMF was produced in date syrup during preparation and storage. Because HMF is recognized as an indicator of quality deterioration in a wide range of foods and is still under investigation for possible toxic effects, it is recommended that the amount of HMF is measured in date syrup and considered as an indicator of the quality control of this product.

Keywords: 5-hydroxymethylfurfural (HMF), Date syrup, HPLC, Industrial product, Traditional product.

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INTRODUCTION

Date syrup is one of the most common date fruit products. It is a tick-dark liquid extracted by water from date fruit [1]. The main component of date syrup is carbohydrate (about 70%). Glucose and fructose are the major sugar in the date syrup, besides, it contains some micronutrients such as iron, sodium, potassium, calcium, vitamin etc [2-4]. It is a rich nourishment and suitable source for energy. The date syrup is commonly produced in date producing countries such as Iran. It is both directly consumed as a food and used for producing beverages, biscuits, ice cream, baby food and cakes as a sweetening and flavouring agent [1]. Date syrup is mostly produced in traditional methods in Iran. However, as consumption of this product is increasing, it also has been produced by industrial units. In the traditional method for producing date syrup, date fruit and water are mixed and heated to boiling, filtered and then heated again to boiling until the desire concentration is obtained, but in industrial method the filtrate is concentrated under controlled heating and the temperature is not exceeded above 70˚C.

During heating of carbohydrate-rich foods at high temperature, sucrose is decomposed into furfural compounds by two possible pathways: the caramelization and the Maillard reaction. 5-Hydroxymethyl-2-furfuraldehyde (HMF) is a common product of these reactions [5, 6].

HMF has been identified in a wide variety of heat-processed foods. Depending of production technology and storage, HMF content in foods varies considerably. While HMF is practically not

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present in fresh and untreated foods, high levels can be found in dried fruits, coffee and caramel products and in some foods the level of HMF exceed 1g/kg [7, 8]. Spontaneous production of HMF during the storage has been reported for some foods such as honey and jam [9, 10]. HMF is a good indicator for quality deterioration following overheating and storage of carbohydrate containing foods. For example, the Codex Alimentarius standard sets a maximum limit of 40 mg/kg for HMF in honey as a way of assuring that the product has not undergone heating during processing [11].

The presence of HMF in food has raised concern in relation to human health. This compound and its metabolite (5-sulfidemethylfurfural) have genotoxic, mutagenic and carcinogenic effects in cell based and animal studies [7, 11-14]. The date syrup is a carbohydrate-rich food product and is a suitable food matrix for production of HMF during production and storage.

The aim of the present study was to detect and quantify the amount of HMF in date syrup by HPLC method. In addition, the amount of HMF in date syrup produced by traditional and industrial methods were compared.

MATERIALS AND METHODS

Chemical and Reagents

HMF, (Sigma, U.S.A.), Acetonitril (analytical grade, Merck,Germany), ultra-pure water (resistivity higher than 18MΩ) and 0.2 µm membrane filter (Millipore)

Samples

The samples were collected from different local markets in Jahrom City, Fars Province, Iran. These samples consisted of traditionally produced date syrup (n=40) and industrially produced date syrup (n=12). The industrial products were from two food production companies which produce date syrup in Iran. Besides, samples included old products with production date of more than 6 month and fresh products with production date of less than 1 month.

Sample Preparation

0.5 gram of homogenised date syrup was dissolved in 500 ml ultra-pure water and filtered through a 0.2 µm membrane filter before injection into the HPLC column.

HPLC System and Condition

The HPLC system consisted of binary pump, degasser, sampling valve, 20 µl sample loop, column oven, photo-diode array detector and computing integrator connected to a PC, all from Shimadzu (Japan). The mobile phase was a mixture of acetonitrile in water (15% v/v) delivered at a flow rate of 1 ml/min under isocratic condition through the analytical column, Eurospher-100 C18 (5 µm, 4.6 × 250 mm). The chromatogram was monitored at 285 and the volume of injection was 20 µl. The column oven temperature was set on 30˚C.HMF was quantified using the external standard method.

Statistical Analysis

Statistical significance of data was tested by non-parametric Mann Whitney test between groups. Analyses were performed by Graph Pad Prism software version 5.

RESULTS

Method Development and Validation

Fig. 1 represents the chromatogram of date syrup sample spiked by 1 µg/ml HMF in optimized HPLC condition. The optimized condition achieved by acetonitril/water ratio of 15/85 and flow rate 1 ml/min. In this condition the sharp and pure peak for HMF was appeared in reasonable retention time (4.7 min).

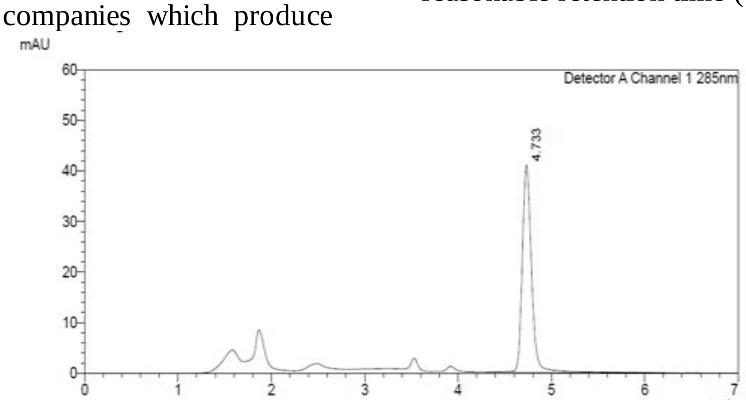

Figure 1. The chromatogram of date syrup sample which spiked by 1 µg/ml HMF.

The external calibration curve was used for quantification of HMF. For assessing of matrix effect, the calibration curve of spiked samples was compared with calibration curve of aqueous standard solution in concentration range of 0.2-2 μg/ml. The slope of the regression line for the calibration curve of spiked samples and aqueous standard solution was the same. Then the calibration curve of aqueous standard solution was used for the quantification of HMF in the samples. The validation parameters are represented in Table 1. Linearity was determined in concentration range of 0.2-2 μg/ml and correlation coefficient (R^2) of 0.99 was found indicating a good linear relationship between concentration and peak area. The limit of detection (LOD) and limit of quantification (LOQ) were determined by following formula: LOD= 3.3SD/m, LOQ= 10SD/m where SD is residual standard deviation of regression line and m is the slope of regression line. Intra-day precision was determined by replicate analysis (n = 6) of the spiked sample on the same day and inter-day precision was determined by replicate analysis of the spiked sample on six consecutive days. Assay precision was expressed as the relative standard deviation (RSD %). Intra-day and inter-day precisions for different concentration were between 1-3% and 1-6%, respectively. The recoveries for spiked samples at different concentrations (0.2-2μg/ml) were between 97-102%. These data are in acceptable ranges described in the AOAC manual for peer-verified methods.

Comparison of HMF Content in the Date Products

Comparison of average HMF contents of industrial and traditional products is shown in Fig. 2. Amount of HMF in traditional products significantly was more than industrial products ($P<0.05$). The amount of HMF in traditional products varies between concentrations of 1000 to 2675 mg⁄kg in the fresh and 2580 to 6450 mg⁄kg in the old products. The amount of HMF in the industrial products varies between concentration of 12 to 456 mg⁄kg in fresh, and 611 to 943 mg⁄kg in the old ones. The wide variation was observed between traditional products (Fig. 3). Fig. 4 shows comparison of average HMF content between old and fresh products. The amount of HMF was significantly higher in old products rather than fresh ones ($P<0.05$).

Table 1. Validation parameters data for the HMF quantified in date syrup.

Linearity		sensitivity		Precision		(%)Recovery
Concentration range(μg/ml)	R^2	LOD (μg/ml)	LOQ (μg/ml)	Intra-day (RSD %)	Inter-day (RSD %)	
0.2-2	0.99	0.03	0.187	1-3	1-6	97-102

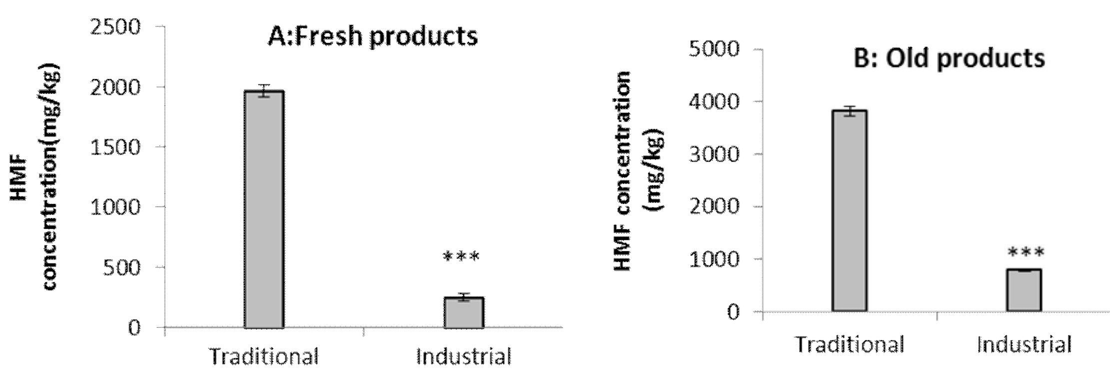

Figure 2. Comparison of HMF content of industrially and traditionally produced date syrup products. Fresh products (A), old products (B) ***$p<0.001$.

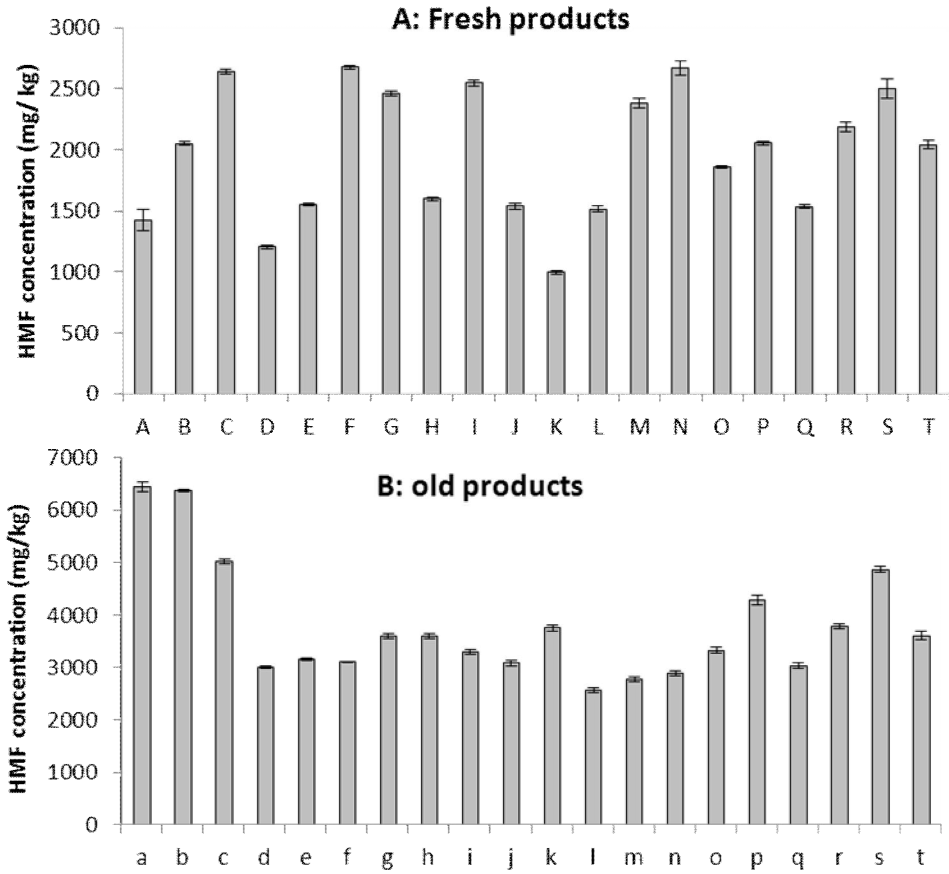

Figure 3. HMF content of traditionally produced date syrup products. Fresh products (A), Old products (B).

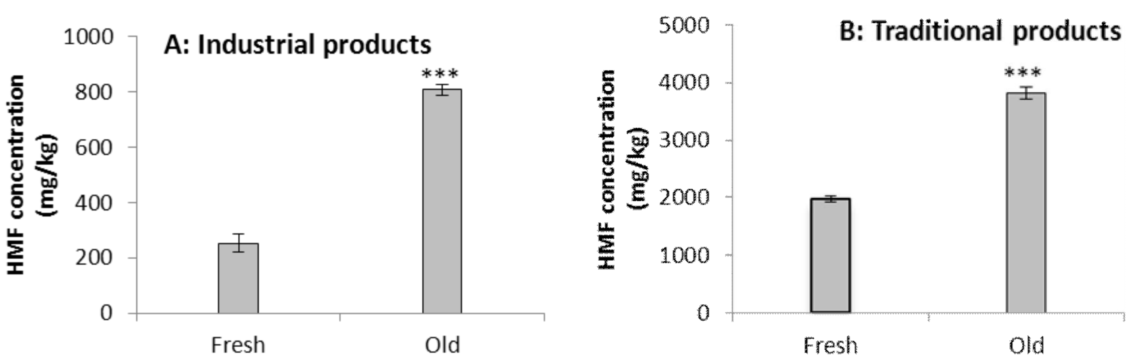

Figure 4. Comparison of HMF content of old and Fresh products, industrially produced date syrup products (A)and traditionally produced date syrup products (B) ****p*<0.001.

DISCUSSION

In the present study the amount of HMF in date syrup was quantified by HPLC method. HMF was determined and quantified in various food stuffs such as honey, bakery products, jams, dried fruits and fruit juices by HPLC method.

In the present study the amount of HMF in the 40 traditionally and 12 industrially produced date syrup was measured. The wide variation was observed between HMF content of traditional products. Moreover, the amount of HMF in traditional products was significantly greater than industrial products. These differences may be due to lack of control on temperature, boiling, and long duration of heating in traditional methods. In traditional method the date juice is boiled for a long time to meet desire concentration and a long time of evaporation process is necessary, but in manufacturing processes the date juice is evaporated under vacuum in controlled low temperature condition then the juice is concentrated during less time. Overheating of carbohydrate-containing foods produces more HMF. Higher amount of HMF was produced in palm sugar syrup when high temperature (110˚ C) processing method was used [15]. The same result was reported for other foods such as bakery product [16] and the formation of HMF is dependent on time of process in boiling juice. Highly variable amount of HMF, changing from 12.8 to 3500 μg/kg, were reported for the boiled juices. Among them HMF concentration of boiled pomegranate juice was the highest (514-3500 μg/kg).

Our result indicated that the amount of HMF was significantly higher in old product rather than fresh ones. This result implies that HMF is continuously produced during the storage of date syrup. The similar findings were reported for other food commodities. The amount of HMF significantly increases in honey [10], jams and fruit-based infant foods [9] one year after production. Dehydration of carbohydrate produces HMF during storage. The temperature of storage condition is an important factor for production of HMF in food product. Storage of food products at high temperatures causes to produce more HMF.

The various amount of HMF was reported in different food products. The amount of HMF was 4.1 -151.2 mg⁄kg in bakery product[16] ,10.4 - 54.88 mg⁄kg in honey [17], 6.9-240.5 mg⁄kg In breakfast cereal [18], 5.5-37.7 mg⁄kg in jam [19] and 2-22 mg⁄kg in fruit juices[20]. The highest amount of HMF has been reported in coffee 24-4023 mg⁄kg [21], dried plum1600-2200 mg⁄kg [8] and boiled pomegranate juice (514-3500 μg/kg). The results of our study indicate that the amount of HMF in old traditional products was 2580-6450 mg⁄kg that is the highest value ever reported.

CONCLUSION

There is no survey about HMF content of date syrup and our study is the first investigation. Date syrup is a suitable matrix for production of HMF and traditional method is not a proper method for preparation of this food product. HMF can be a good indicator of quality deterioration during production and storage of date syrup. Because the date syrup is –highly consumed in date-producing areas like south of Iran, high levels of HMF in traditional products can threaten the health of consumers of this product. Then it is recommended that amount of HMF in date syrup is regulated.

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REFERENCES

1. Tang ZX, Shi LE, Aleid SM. Date fruit: chemical composition, nutritional and medicinal values, products. J Sci Food Agric 2013;93(10):2351-61.
2. Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem 2007;104(3):943-7.
3. Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. Compositional and sensory characteristics of three native sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. J Agric Food Chem 2005;53(19):7586-91.
4. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: a review. Crit Rev Food Sci Nutr 2008;48(10):877-87.
5. Kroh LW. Caramelisation in food and beverages. Food Chem 1994;51(4):373-9.
6. Ames JM. The Maillard reaction. Biochemistry of food proteins: Springer; 1992. p. 99-153.
7. Capuano E, Fogliano V. Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT- Food Sci Technol 2011;44(4):793-810.
8. Murkovic M, Pichler N. Analysis of 5-hydroxymethylfurfual in coffee, dried fruits and urine. Mol Nutr Food Res 2006;50(9):842-6.
9. Rada-Mendoza M, Sanz MaL, Olano An, Villamiel M. Formation of hydroxymethylfurfural and furosine during the storage of jams and fruit-based infant foods. Food Chem 2004;85(4):605-9.
10. Spano N, Ciulu M, Floris I, Panzanelli A, Pilo MI, Piu PC, et al. A direct RP-HPLC method for the determination of furanic aldehydes and acids in honey. Talanta 2009;78(1):310-4.
11. Abraham K, Gürtler R, Berg K, Heinemeyer G, Lampen A, Appel KE. Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. Mol Nutr Food Res 2011;55(5):667-78.
12. Durling LJ, Busk L, Hellman BE. Evaluation of the DNA damaging effect of the heat-induced food

toxicant 5-hydroxymethylfurfural (HMF) in various cell lines with different activities of sulfotransferases. Food Chem Toxicol 2009;47(4):880-4.

13. Severin I, Dumont C, Jondeau-Cabaton A, Graillot V, Chagnon M-C. Genotoxic activities of the food contaminant 5-hydroxymethylfurfural using different in vitro bioassays. Toxicol Lett 2010;192(2):189-94.
14. Janzowski C, Glaab V, Samimi E, Schlatter J, Eisenbrand G. 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem Toxicol 2000;38(9):801-9.
15. Naknean P, MeenuneM, Roudaut G. Change in physical and chemical properties during of palm sugar syrup by open pan and vacuum evaporator. Asian J. Food Agro-Industry 2009:2(4):448-456.
16. Ramírez-Jiménez A, García-Villanova B, Guerra-Hernández E. Hydroxymethylfurfural and methylfurfural content of selected bakery products. Food Res Int 2000;33(10):833-8.
17. Zappala M, Fallico B, Arena E, Verzera A. Methods for the determination of HMF in honey: a comparison. Food control 2005;16(3):273-7.
18. Rufian-Henares J, De la Cueva S. Assessment of hydroxymethylfurfural intake in the Spanish diet. Food Addit Contam 2008;25(11):1306-12.
19. Rada-Mendoza M, Olano A, Villamiel M. Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chem 2002;79(4):513-6.
20. Yuan J-P, Chen F. Separation and identification of furanic compounds in fruit juices and drinks by high-performance liquid chromatography photodiode array detection. J Agric Food Chem 1998;46(4):1286-91.
21. Arribas-Lorenzo G, Morales FJ. Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. Food Chem Toxicol 2010;48(2):644-9.