ORIGINAL ARTICLE

Estimated Bioaccessibility to 5-hydroxymethylfurfural from Frequently Consumed Dried Fruits in Iran

Nooshin Rahimzadeh, Mohammad Alizadeh*, Seyed Jamal Ghaemmaghami Hezaveh

Department of Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT: We sought to determine levels of oral bioaccessibility of hydroxymethylfurfural (HMF) from frequently consumed dried fruits in Iran. Fifty samples from frequently consumed types of dried fruits were analyzed for moisture, acidity and HMF content before and after in vitro digestion. Besides, bioaccessibility of HMF in dried fruits using an in vitro gastrointestinal digestive model and HMF intake from dried fruits based on consumption of each dried fruit groups was determined. The mean estimated intake of HMF was 72.90 mg/kg and the maximum intake was 240.23 mg/kg for fruit bread. The mean bioaccessibility was 60.26%. There was a correlation between HMF and acid content of fruit bread (r= 0.98, P<0.05). In conclusion, the HMF levels in dried fruits remains high even after the in vitro digestion.

KEYWORDS
HMF
Bioaccessibility dried
Fruits
Iran

INTRODUCTION

Heat treatment such as baking, toasting, frying, roasting, sterilization and drying are frequently used in food manufacturing and preparation to acquire production of foods with a longer shelf-life or better consumer acceptance. This thermal process has both desired and undesired effects on the final quality of foods due to various chemical reactions being Maillard reaction (MR), caramelization and lipid oxidation are the most prominent ones. During the thermal process of foods in both production lines or food handling facilities the reaction between amino acids and reducing sugars, known as non-enzymatic browning or the MR, may result in development of undesired tastes, colors and off-flavors together with loss in thermo labile nutritional components in various food items [1]. Thus, evaluation of these detrimental effects is compulsory to ensure better food quality and to avoid adverse health consequences. Hydroxymethylfurfural (HMF) is one of the most important heat-induced contaminants occurring in many foods including cereals, bakery product, milk, fruit juices, spirits and honey [2]. This yellow, low-melting solid, is highly water-soluble. The molecule consists of a furan ring, containing both aldehyde and alcohol functional groups, also formed by the caramelization of sugars [3]. HMF is regarded a quality measure to predict degree of heating or duration of storage in a wide range of foods.

*Corresponding author: m dalizadeh@tbzmed.ac.ir (M. Alizadeh).
containing carbohydrates. Its content is remarkable low in fresh unprocessed foods. [4]. Levels of this compound is also used to determine the most useful method for assessing the effectiveness of heat treatment [5]. In vegetable products, such as tomato pastes, the extent of damage can be determined by the HMF content [6]. In addition, the level of HMF has been used as indicator of long-term storage quality in apple sauce and grape jelly [7]. Formation of HMF occurs into two pathways [8], including transformation of fructofuranose ring and development of an acyclic intermediate. The main source for HMF production is carbohydrates such as fructose or glucose [9]. It is suggested that the dehydration of fructose is easier than that of glucose [10, 11]. A more detailed illustration of HMF formation is summarized in Figure 1.

Figure1. HMF formation mechanism: A– main mechanism of fructose dehydration, B – inversion of sucrose, C– side path of glucose transformation

Thermal processing is a critical part of dried fruits manufacturing. Moreover, these products are usually stored for long periods of time. These conditions may trigger MR and consequently production of variable amounts of HMF [12]. Although there are some studies on the presence of HMF in fruit products [13], fewercases on dried fruits have been reported.

Up to now, little is known to address bioaccessibility of this compound and its kinetics during food digestion. Accordingly, it is not clear to which extend exposure to HMF elicits health risks to human. Harmful effects may be a direct result of HMF impact or the result of HMF transition to the 5-sulphoxoymethylfurfural (5-SMF) and other metabolites [14]. Evaluation of oral bioavailability, defined as the fraction of an orally administered dose that reaches the systemic circulation, is the first step to address this issue. Hence, in vitro digestion models are simple simulation tools for assessing kinetic and fate of many food contaminants. The aim as current work was to (1) assess levels of oral bioaccessibility to HMF among frequently consumed dried fruits in Iran and (2) to estimate levels of dietary exposure to this food contaminant from the selected food items.

MATERIALS AND METHODS

Samples

Fifty samples (5 samples of each group) from frequently consumed types of dried fruits were randomly selected from local markets of Tabriz, East Azerbaijan Province, Iran. The fruits selected were fig, berry, apricot, golden
raisin, currant, date, plum, dark raisin and two types of commercially available fruit breads. A local basket survey was conducted to determine estimated intake of the selected dried fruits among healthy adult residents.

Measurement of physicochemical parameters

Chemical properties of the samples including moisture and acidity were measured. Moisture percentage was calculated following the standard AOAC method number 934.06 by an air-oven drying at 70±1°C for 6 h after reaching the temperature to the adjusted amount [15]. The acid content was determined following the national standard of Iran, number 672 [16].

In vitro digestion

Samples (1g) were placed in a 15 ml flask, one ml each of carrez I and II reagents were added with stirring and the volume made up with Milli-Q water. After standing for 30 minutes the samples were centrifuged at 3200 RPM and the supernatants were separated and filtered through a 0.45 µm filter.

The in vitro digestion method used following the method described by Delgado-Andrade [17]. It was comprised of two stages: gastric digestion and intestinal digestion. Shortly before use, 0.4g of pepsin was dissolved in 0.1 NHCl. For intestinal digestion 0.1g of pancreatin was dissolved in 25 ml of 0.1 N NaHCO₃. 1g of each sample was suspended in 10ml of Milli-Q water. pH was adjusted to 2 with HCl6N. Three hundred µl of pepsin solution was added and samples were incubated at 37°C in a shaking water bath for 2 h for the gastric digestion. For the intestinal stage, the pH of the solution was raised to 6 with 1N NaHCO₃ drop wise and 2.5 ml of pancreatin was added. Then, the pH was adjusted to pH 7.5 with 1N NaHCO₃ and samples were incubated at 37°C for 2 h.

After gastrointestinal digestion the digestive enzymes were inactivated by heat treatment for 4 min at 100°C in a hot water bath. The samples were then cooled by immersion in an ice bath. As a next step, 1 ml each of carrez I and II reagents were added with stirring to each samples and centrifuged at 3200 RPM to separate soluble and non-soluble fractions. The supernatants were carefully separated and filtered through a 0.45 µm filter and then injected in to the HPLC system.

Determination of Hydroxymethylfurfural

The analysis of HMF was carried out by HPLC before and also after in vitro digestion following the method of Bin Zhao et al. [18], using a CE-1100 pump, a CE-1100 ultraviolet-visible detector (Cecil instruments, Cambridge, England, United Kingdom) with a 50 µl injection loop chromatograph, a SpherisorbS5 ODS1 (250 mm x 4.6 mm id) column (Sugelabor, Madrid, Spain) set at ambient temperature. The mobile phase consisted of a mixture of acetonitrile and methanol in water. The flow rate was 0.8 ml/min and injection volume 50 µl and the UV detector was set at 283nm. Quantitation was carried using a commercial standard of HMF. HMF in different concentrations, within the range 5-100 mg/liter, was used for calibration. All analyses were done five times for each group and the mean values expressed as mg/kg.

Calculation of oral bioaccessibility

The bioaccessibility of HMF content for dried fruits is defined as the proportion of HMF in dried fruits available for absorption [19], and it was calculated as:

\[
\text{Bioaccessibility} = \frac{\text{HMF in bioaccessible fraction}}{\text{HMF in dried fruits}}
\]

STATISTICAL ANALYSIS

Pairwise comparisons were conducted before and after digestion for each of traditional breads using paired sample t-test. The correlation between the selected physicochemical parameters and after in vitro digestion content was determined using Pearson test. \( P \)-value less than 0.05 was considered as significant. SPSS software, version 16 (SPSS, Chicago, Illinois, USA) was used to perform statistical analyses.
RESULTS

The typical chromatogram of a sample of dried fruits before and after in vitro digestion are shown in Figure 2.

![Figure 2. Typical HPLC chromatogram after (a) and before (b) in vitro in a selected sample of dried fruits](image)

Table 1 shows the content of HMF in dried fruits before and after in vitro digestion and also the bioaccessibility of HMF in dried fruits. The amount of HMF before and after in vitro digestion in the tested samples varied from 80.49±61.2 mg/kg to 8569.5±4.77 mg/kg and from 76.74±36.54 mg/kg to 5130.20±1.43 mg/kg, respectively.

<table>
<thead>
<tr>
<th>Dried fruits</th>
<th>HMF Before digestion (Mg/kg)</th>
<th>HMF After digestion (Mg/kg)</th>
<th>Bioaccessibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig</td>
<td>159.08±81.35</td>
<td>101.75±68.72</td>
<td>63.9</td>
</tr>
<tr>
<td>Berry</td>
<td>80.92±41.22</td>
<td>76.74±36.54</td>
<td>94.8</td>
</tr>
<tr>
<td>Apricot</td>
<td>838.52±3.10</td>
<td>644.70±4.44</td>
<td>76.8</td>
</tr>
<tr>
<td>Golden raisin</td>
<td>596.42±1.48</td>
<td>80.49±61.21</td>
<td>13.4</td>
</tr>
<tr>
<td>Currant</td>
<td>2349.37±2.34</td>
<td>1606.99±1.48</td>
<td>68.3</td>
</tr>
<tr>
<td>Date</td>
<td>107.79±28.09</td>
<td>92.02±27.82</td>
<td>85.4</td>
</tr>
<tr>
<td>Plum</td>
<td>1288.30±3.14</td>
<td>108.87±3.68</td>
<td>8.4</td>
</tr>
<tr>
<td>Fruit bread1*</td>
<td>8506.00±2.48</td>
<td>4177.54±9.18</td>
<td>49.1</td>
</tr>
<tr>
<td>Dark raisin</td>
<td>545.89±1.30</td>
<td>451.61±1.01</td>
<td>82.7</td>
</tr>
<tr>
<td>Fruit bread2*</td>
<td>8569.85±4.77</td>
<td>5130.20±1.43</td>
<td>59.8</td>
</tr>
</tbody>
</table>

*Fruit bread 1 was combination of apricot and apple, and fruit bread 2 was made of pomegranate, apple and cherry.
Table 2. Correlation between HMF and Acidity or Moisture in selected dried fruits

<table>
<thead>
<tr>
<th>Item</th>
<th>Acidity</th>
<th></th>
<th>Moisture</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Fig</td>
<td>0.84</td>
<td>0.02</td>
<td>0.73</td>
<td>0.08</td>
</tr>
<tr>
<td>Berry</td>
<td>0.12</td>
<td>0.42</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>Apricot</td>
<td>0.79</td>
<td>0.09</td>
<td>0.03</td>
<td>0.49</td>
</tr>
<tr>
<td>Golden raisin</td>
<td>-0.49</td>
<td>0.20</td>
<td>-0.73</td>
<td>0.08</td>
</tr>
<tr>
<td>Currant</td>
<td>0.09</td>
<td>0.43</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>date</td>
<td>0.22</td>
<td>0.08</td>
<td>0.76</td>
<td>0.08</td>
</tr>
<tr>
<td>Plum</td>
<td>-0.38</td>
<td>0.26</td>
<td>-0.47</td>
<td>0.20</td>
</tr>
<tr>
<td>Fruit bread1*</td>
<td>0.98</td>
<td>0.002</td>
<td>-0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>Dark raisin</td>
<td>0.70</td>
<td>0.09</td>
<td>-0.75</td>
<td>0.09</td>
</tr>
<tr>
<td>Fruit bread2*</td>
<td>0.86</td>
<td>0.04</td>
<td>0.23</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Fruit bread 1 was combination of apricot and apple, and fruit bread 2 was made of pomegranate, apple and cherry.

The highest level of HMF content in before in vitro digestion was obtained for fruit bread and the lowest one was for fig. After in vitro digestion, the highest amount of HMF was repeated for fruit bread and the lowest one was for berry. The bioaccessibility of HMF in dried fruits varied from 8.4% to 94.8%. The highest bioaccessibility was for berry and the lowest was for plum. As expected, the HMF level decreased after in vitro digestion in all samples. There was a significant difference in the HMF content between different dried fruit groups (P<0.05).

Estimated mean HMF intake from dried fruits based on consumption of each dried fruit groups is shown in Table 2. The highest and lowest dietary exposure to this compound was determined from fruit bread (46.83 mg/d) and berry (0.28 mg/d), respectively.

Table 3 shows correlation analysis of HMF with moisture and acidity of dried fruits. The highest and lowest amount of moisture was 24.04 ± 3.25 % and 7.48 ± 1.06 % which belonged to plum and dark raisin, respectively. The highest and lowest amount of acidity was 7.59 ± 1.51% and 0.30 ± 0.03% which belonged to fruit bread 1 and date, respectively.

Correlation analyses between chemical properties including moisture and acidity of dried fruits and HMF content after digestive process showed that only there was a strong straight relationship between acidity and HMF content of fruit bread, after in vitro digestion (r=0.98, P<0.05), while there was no statistically significant correlation between HMF content of dried fruits and acidity. Besides, there was no statistically significant correlation between HMF content of dried fruits and moisture in all groups.
DISCUSSION

The current study highlights the presence of relatively high amounts of HMF in frequently consumed dried fruits in Iran. The study also revealed a high bioaccessibility to the compound. HMF is practically not present in fresh food, but it is naturally generated in sugar-containing food during heat-treatments like drying or cooking. Along with many other flavor and color related substances, HMF is formed in the Maillard reaction as well as during caramelization. In these foods it is also slowly generated during storage [20].

Limited reports available on genotoxicity or mutagenicity of HMF are controversial. Experiences in rats at high HMF concentration have shown cytotoxic effects, causing irritation to eyes, respiratory tract, skin and mucos as with an oral LD50 of 3.1 g/kg of rat body weight [21]. Tumorigenic activities has also been associated with this compound, mainly attributed to preneoplastic lesions such as the colonic aberrant crypt [22] or skin papillmas [23]. Although the mechanisms of the toxic activity of HMF remain unclear, it has been proposed that it is metabolically activated through the sulfonation of its allylic hydroxyl functional group which can take place in the liver by activity of hepatic sulfotransferase. It is suggested that the electrophilic intermediate obtained, 5-sulfoxymethylfurfural (SMF), can interact with critical cellular nucleophiles (e.g. DNA, RNA and proteins) resulting in structural damage which can lead to toxicity and mutagenicity [23].

Various food items are known to contain remarkably low amounts of HMF. Thus, total daily intake of the compound is usually affected by individual feeding patterns [24]. This study was carried out to evaluate the HMF content of dried fruits and the bioaccessibility of HMF from the selected dried fruits. The in vitro gastro-

<table>
<thead>
<tr>
<th>Dried fruits</th>
<th>Dried fruits intake [g/d]</th>
<th>Estimated mean HMF [mg/d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig</td>
<td>13.31</td>
<td>1.35</td>
</tr>
<tr>
<td>Berry</td>
<td>3.75</td>
<td>0.28</td>
</tr>
<tr>
<td>Apricot</td>
<td>9.36</td>
<td>6.03</td>
</tr>
<tr>
<td>Golden raisin</td>
<td>7.25</td>
<td>0.58</td>
</tr>
<tr>
<td>Currant</td>
<td>7.31</td>
<td>11.74</td>
</tr>
<tr>
<td>Date</td>
<td>29.61</td>
<td>2.72</td>
</tr>
<tr>
<td>Plum</td>
<td>5.92</td>
<td>0.64</td>
</tr>
<tr>
<td>Fruit bread 1*</td>
<td>5.25</td>
<td>38.64</td>
</tr>
<tr>
<td>Dark raisin</td>
<td>8.95</td>
<td>4.04</td>
</tr>
<tr>
<td>Fruit bread 2*</td>
<td>5.13</td>
<td>46.83</td>
</tr>
</tbody>
</table>

*Fruit bread 1 was combination of apricot and apple, and fruit bread 2 was made of pomegranate, apple and cherry
intestinal (GI) digestive model was applied to examine the effect of GI tract enzymes on HMF degradation or absorption. Previous studies have already determined high quantities of HMF in coffee, breakfast cereals and fruit products. Several types of roasted coffee contained between 300 – 2900 mg/kg HMF [25]. Dried plums contained up to 2200 mg/kg HMF. In dark beer 13.3 mg/kg were found [26], bakery-products contained between 4.1 – 151 mg/kg HMF [24]. In the samples analyzed the mean content of HMF was 2304. 21 mg/kg before in vitro digestion and 1247.09 mg/kg after in vitro digestion. This wide variation in amount of HMF could be attributed to duration and intensity of heat treatments applied in food processing, as well as diverse aqueous activity of different food items.

The dried fruits used in the study are frequently consumed all over Iran and even all over the world. There was a significant difference between the HMF content of dried fruit groups in before and also after in vitro digested samples. Fruit bread 2 with (5130.20±1.43) mg/kg had the highest, and the berry with (76.74 ±36.54) mg/kg had the lowest amount of HMF between all after in vitro digested groups. The amounts of HMF in commercial jams and in fruit-based infant foods reported by MaiteRada-Mendoza et al. [13] were much lower than the HMF amount of the dried fruit samples tested. The observation could be explained with effect of the higher moisture which is naturally presented in jams and other fruit-based foods. As expected, there was an overall reduction in HMF content of all digested dried fruits. The finding was in agreement with similar study carried out by Delgado-Andrade et al. They reported that HMF content tended to decrease in soluble fraction after digestion of breakfast cereals [6].

In the present study the dietary intake of HMF from dried fruits were estimated on the basis of a local basket survey. Having regard to limited samples included in the survey; mean intake of HMF was estimated as 72.90 mg/d with maximum intake accounting for fruit bread (240.23 mg/d). It should be mentioned that although the consumption of fruit bread was not the most, maximum intake of HMF belonged to fruit bread due to very high amount of HMF in this particular food item. In Spain, dietary intake of HMF from coffee was above the threshold of concern (8.6 mg/d). The authors investigated the dietary exposure to HMF from coffee, because of its high consumption rate as compared with other food items [20]. Other food items that contained high level of HMF were prunes (237 mg/kg), dark beer (13.3 mg/kg), canned peaches (5.8 mg/kg) and raisins (5 mg/kg), [25].

As we see, although the consumption of dried fruits is not high but the dietary intake of HMF from this products may exceed the threshold of concern set by European Union. We also noticed a strong straight relationship between acidity and HMF content of fruit breads, after in vitro digestion while there was no statistically significant correlation between HMF content of other dried fruits and acidity. This is in agreement with study of Arribas-Lorenzo et al. who reported that acid conditions favor generation of HMF [20]. As we see the content of acids in fruit breads was higher than all other samples.

CONCLUSION

In vitro digestion process caused a significant decline in HMF content of the fruit breads, but its bioaccessibility remained still higher than the recommended upper limit. Further studies are needed to estimate in vivo bioaccessibility of HMF from both traditional and processed foods.

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