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FORMATION AND REDUCTION OF 5-HYDROXYMETHYLFURFURAL DURING DEEP-FAT FRYING OF BANANAS

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FORMATION AND REDUCTION OF 5-HYDROXYMETHYLFURFURAL DURING DEEP-FAT FRYING OF BANANAS

By

PARVIZ KAVOUSI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

November 2014

DEDICATION

To my beloved parents

To My beloved children

Pouneh and Farshad Kavousi
Thank you for your patience, support and understanding

In memory of my beloved wife

Parvin Aslanbeigui, M.D. 1959-2007

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

FORMATION AND REDUCTION OF 5-HYDROXYMETHYLFURFURAL DURING DEEP-FAT FRYING OF BANANAS

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November 2014

Chairman: **Associate Professor Abdul Azis Ariffin, PhD**
Faculty: **Food Science and Technology**

5-hydroxymethylfurfural (HMF) is a heat-induced furanic compound that is formed through Maillard reaction or direct dehydration of hexoses under acidic conditions in carbohydrate-containing food products. Human exposure to HMF mainly takes place through diet. 5-sulfoxymethylfurfural (SMF), as a metabolite of HMF, can react with DNA and other macromolecules, and acts as a toxic and mutagenic compound. This gives rise to concern for reducing the HMF content in thermally processed foods especially fried food products. Up to now the formation and reduction of HMF during deep-fat frying is not clear. This study is conducted to investigate the formation and reduction of HMF during deep-fat frying of banana. The effects of amino acids (15 amino acids) and sugars (fructose, glucose and sucrose) as precursors on the formation and reduction of HMF are evaluated in model systems. The HMF is extracted and separated using an optimized extraction procedure and a validated HPLC method. The results reveal that glutamine, glutamic and aspartic acids enhance the formation of HMF. Conversly, lysine, arginine and histidine reduce the HMF content to non-detectable level. The HMF content in control samples containing glucose, fructose or sucrose alone was 167.99, 232.79 and 18.54 m/kg, respectively. While, in binary mixtures of these sugars with glutamine, aspartic acid and glutamic acid, the formation of HMF was significantly ($P<0.05$) increased and reached to 1084.60, 2020.30 and 3816.90 mg/kg, 1115.40, 751.00 and 1232.60 and 1653.30, 1556.60 and 3132.60 mg/kg, respectively ($P< 0.05$). The formation of HMF is significantly ($P<0.05$) reduced by 73.23% and 90.71% in Abu variety and 76.33% and 82.84% in Tanduk variety, respectively after pre-soaking of bananas in water containing lysine or arginine (0.06 M) for 2 h compared to control samples. The current study reveals that the food compositions have had a more significant effect than frying oil in the formation of HMF ($P<0.05$). The effect of frying time on HMF formation is significantly ($P< 0.05$) higher than frying temperature. Microwave frying causes the higher HMF formation than conventional deep-fat frying and boiling process. The HMF content in bananas after 6 min cooking was 2168.02,

31.92 and 0.17 mg/kg, respectively. In addition, the effects of the banana variety (7 varieties), ripening stage (ripe and unripe), surface area-to-volume ratio and blanching process on the reduction of HMF are studied. The results show that HMF forms at significantly lower amount in unripe fruits ($P<0.05$). HMF content in fried Tanduk is lower than Mas, Abu, Berangan, Raja, Ambon and Awak varieties. Moreover, HMF formation is significantly ($P<0.05$) increased from 26.81 to 124.83 mg/kg by increasing the surface area-to-volume ration from 0.02 mm^{-1} to 0.80 mm^{-1}($P<0.05$) The results reveal that the HMF formation is significantly reduced after blanching of bananas at 70 ºC for 45 min by 80.00% and 85.35% in Abu and Tanduk varieties, respectively ($P<0.05$).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBENTUKAN DAN PENGURANGAN 5-HIDROKSIMETHILFURFURAL SEMASA PENGGORENGAN PISANG MINYAK PENUHS

Oleh

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Pengerusi : Professor Madya Abdul Azis Ariffin, PhD
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5- hidroksimetilfurfural (HMF) adalah sebatian furanik teraruh haba yang terbentuk melalui tindak balas Maillard atau dehidrasi langsung heksosa di bawah keadaan berasid dalam produk makanan yang mengandungi karbohidrat. Pendedahan manusia kepada HMF berlaku terutamanya melalui pemakanan. 5- sulfoksimetilfurfural (SMF) merupakan metabolit daripada HMF, boleh bertindak balas dengan DNA dan makromolekul lain, dan bertindak sebagai sebatian toksik dan mutagen . Ini menimbulkan kebimbangan untuk mengurangkan kandungan HMF dalam makanan diproses haba terutamanya produk makanan bergoreng.Sehingga kini pembentukan dan pengurangan HMF sewaktu penggorengan minyak penuh adalah tidak jelas.Kajian ini dijalankan untuk mengkaji pembentukan dan pengurangan HMF sewaktu penggorengan pisang minyak penuh.Kesan asid amino (15 asid amino) dan gula (fruktosa, glukosa dan sukrosa) sebagai prekursor bagi pembentukan dan pengurangan HMF dinilai dalam sistem model. HMF diekstrak dan diasingkan menggunakan proses pengekstrakan optima dan kaedah HPLC yang diperakui. Dapatan kajian menunjukkan bahawa glutamina, asid aspartik dan asid glutamik meningkatkan pembentukan HMF. Lysina, arginina dan histidina sebaliknya mengurangkan kandungan HMF ke paras yang tidak dapat diukur. Kandungan HMF dalam sampel kawalan mengandungi glukosa, fruktosa dan sukrosa masing-masing adalah 167.99, 232.79 dan 18.54 m/kg. Manakala, dalam campuran dedua gula berkenaan dengan glutamina, asid aspartik dan asid glutamik, pembentukan HMF meningkat dengan ($P<0.05$) nyata sekali dan masing-masing mencapai 1084.60, 2020.30 dan 3816.90 mg/kg, 1115.40, 751.00 dan 1232.60 mg/kg serta 1653.30, 1556.60 dan 3132.60 mg/kg. Pembentukan HMF menurun ($P<0.05$) dengan nyata sekali ke 73.23% and 90.71% dalam variasi Abu dan 76.33% dan 82.84% dalam variasi Tanduk, masing-masing selepas pra rendaman pisang di dalam air mengandungi lysina atau arginina (0.06M) selama 2 jam dibandingkan dengan sampel kawalan. Kajian semasa menunjukkan bahawa komposisi makanan mempunyai kesan yang nyata berbanding minyak penggorengan dalam pembentukan HMF ($P<0.05$).Kesan masa penggorengan terhadap pembentukan HMF adalah nyata sekali ($P<0.05$) lebih tinggi berbanding suhu penggorengan. Penggorengan gelombang mikro menyebabkan pembentukan HMF lebih tinggi berbanding penggorengan minyak penuh konvensional dan proses pendidihan. Kandungan HMF dalam pisang selepas memasak selama 6 min

adalah masing-masing 2168.02, 31.92 dan 0.17 mg/kg. Di samping itu, kesan variasi pisang (7 variasi), tahap keranuman (ranum atau mengkal), nisbah luas permukaan ke isipadu dan proses celuran ke atas pengurangan HMF turut dikaji. Keputusan menunjukkan bahawa HMF terbentuk pada amaun yang nyata sekali ($P<0.05$) lebih rendah dalam buah mengkal.Kandungan HMF dalam pisang Tanduk goreng adalah lebih rendah dari variasi Mas, Abu, Berangan, Raja, Ambon dan Awak. Lagi pula, pembentukan HMF menigkat secara nyata sekali ($P<0.05$) dari 26.81 ke 124.83 mg/kg dengan meningkatkan nisbah luas permukaan ke isipadu dari 0.02 mm^{-1} ke 0.80 mm-1 ($P<0.05$). Hasil dapatan menunjukan bahawa pembentukan HMF adalah berkurangan dengan nyata ($P<0.05$) selepas penceluran pisang pada 70^{o}C selama 45 min masing-masing 80.00% dan 85.35% dalam variasi Abu dan Tanduk.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
/	Per
<	Less than
>	Higher than
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
AOCS	American Oil Chemists' Society
dw	Dried weight
et al.	And others
g	Gram
h	Hour
H^+	Hydrogen ion
i.d.	Internal diameter
IOOC	International Olive Oil Council
L	Liter
M	Molar
mg	Milligram
min	Minute
mL	Milliliter
ºC	Degrees Celsius
p	Probability
pH	Hydrogen ion concentration
ppm	Parts per million
rpm	Rotation per minute
s	Second
SD	Standard Deviation
SPP.	species
UV	Ultra Violet ray
v/v	Volume per volume
α	Alpha
β	Beta
µL	Micro liter

CHAPTER 1

INTRODUCTION

Deep-fat frying, as a popular method for food preparation, is widely used in homes, restaurants and food industries. It is well-known for producing the desirable flavour, colour and texture in fried food products. In deep-fat frying, food is immersed in frying oil at high temperature (150 to 190ºC) for a specified time, and the oil acts as a heat transfer medium (Choe & Min, 2007). Although, using high temperature leads to the formation of desirable surface colour and textural characteristics of fried foods, heat-induced toxicants such as HMF are also produced in carbohydrate-containing foods during deep-fat frying (Capuano & Fogliano, 2011). Deep-fat fried snack foods such as French fries, fried seafood, egg rolls, doughnuts, fried chicken and chips (potato, corn and tortilla) are very popular worldwide.

5-Hydroxymethylfurfural (HMF) as a heat-induced furanic compound is formed by dehydration of hexoses through Maillard reaction, caramelization and direct dehydration of hexoses under acidic conditions (Capuano & Fogliano, 2011). Although, hexoses are the forerunner of HMF, sugars such as sucrose that release hexoses after hydrolysis can convert into HMF. The hydrolysis of sucrose during processing of food products leads to formation of fructofuranosyl cation and glucose. This reactive cation can directly convert into HMF in dry conditions (Perez Locas & Yaylayan, 2008) or convert into fructose in the presence of water or contribute in formation of HMF through the reaction with a free amino group duringMaillard reaction. Fructose and glucose are the main precursors of HMF. They can react with amino acids in the early stage of Maillard reaction and form corresponding N-substituted amine (Hodge, 1953). After the formation of Amadori rearrangement products, the degradation pathway is pH-dependent. HMF forms from dehydration of sugar through 1, 2-enolization pathway at pH values lower than 7 (Anese & Suman, 2013). Moreover, HMF can be formed by dehydration of sugars through 1, 2-enolization pathway under acidic condition.

The formation of HMF is affected by food composition and processingconditions. Generally, besides the sugars as the main precursor of HMF, amino acid composition, temperature, pH and heating time are influential factors (Anese & Suman, 2013). Thus, the concentration of HMF in food products varies as a function of the dominant factors. To reduce the formation of HMF during processing of foods such as deep-fat frying, two preventive and removal strategies can be applied(Anese & Suman, 2013). The changes in processing conditions such as temperature and heating time, and reduction of HMF precursors prior to processing by pre-treatment of the foods are regarded as preventive strategy.However, the removal strategy deals with reduction of HMF after formation.

HMF has been used as an indicator to determine the extent of heat load applied to foods during their processing or as a quality marker for many products such as honey (Khalil, Sulaiman, & Gan, 2010). The presence of HMF has been reported in fruit juices (Lee, Sakai, Manaf, Rodhi, & Saad, 2014), dried fruits (Murkovic & Pichler, 2006), coffee

(Bignardi, Cavazza, & Corradini, 2014), bakery products (Petisca, Henriques, Pérez-Palacios, Pinho, & Ferreira, 2014), fried foods (Göncüoğlu & Gökmen, 2013) and infant formulae (Chávez-Servín, de la Torre Carbot, García-Gasca, Castellote, & López-Sabater, 2015). Human exposure to HMF mainly takes place through the diet, and based on the data reported in literature, its dietary intake is often higher than other food toxicants such as acrylamide (Capuano & Fogliano, 2011).

The risk associated with HMF can be induced by HMF itself or its metabolites. Despite controversial reports that are derived from *in vitro* studies on the mutagenicity and genotoxicity of HMF (Severin, Dumont, Jondeau-Cabaton, Graillot, & Chagnon, 2012), the influence of HMF on human health is not clear(Capuano & Fogliano, 2011). The major concern for HMF is related to its bioconversion *in vitro* into 5-sulfoxymethyfurfural (SMF) through sulfonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases (SLUTs) (Abraham et al., 2011). The latter compound can react with DNA and other macromolecules, thereby resulting in toxic and mutagenic effects (Surh & Tannenbaum, 1994). The European Food Safety Authority (EFSA) has confirmed the mutagenicity of SMF (EFSA, 2005). A recent study showed that SMF could be detected in the blood of FVB/N mice after HMF intravenous administration (Monien, Frank, Seidel, & Glatt, 2009). Owing to the higher activity of human SLUTs compared to those expressed by rodents (Bauer-Marinovic, Taugner, Florian, & Glatt, 2012), the risk associated with HMF in human may be higher than in rodents. Thus, due to a potentially high HMF intake from the food, even a limited conversion can negatively affect the human health.

As mentioned earlier, the formation of HMF mainly depends on sugar composition. The foods containing hexose (such as banana) are more susceptible to have HMF after deep fat frying. Deep-fat fried banana is also a very popular snack food in many countries especially in Malaysia. Banana is a general term embracing a number of species or hybrids in the genus *Musa* of the family Musaceae (Happi Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007). Generally, there are two broad groups of banana namely dessert bananas, and cooking banana or plantain. The dessert bananas are sweeter than plantain and can be eaten raw, while, plantain is more starchy than sweet and is used for cooking purposes. There is a limited literature data on the formation and reduction of HMF in fried products. To reduce HMF content in fried products, the mechanism that regulates the formation of HMF, and the effects of factors on HMF formation during deep-fat frying should be elucidated.

The first objective of this study was:

- To validate HPLC method to extract and quantify HMF from frying oils and fried foods.

In this study, three hypotheses were tested in relation to investigate the formation and reduction of HMF during deep-fat frying of banana. These hypotheses and objectives were as follows.

Hypothesis 1: The formation of HMF from sugars at frying temperature can be affected by type of amino acids.

Objective:

- To determine the effect of different sugars and amino acids on formation and reduction of HMF in different model systems

Hypothesis 2: Formation of HMF during deep-fat frying is dependent on food composition, type of frying media and frying conditions.

Objective:

- To investigate the effect of different oils, food matrices and frying condition on the formation and reduction of HMF during deep-fat frying

Hypothesis 3: Banana variety, ripening stage and pre-treatment of banana can affect the formation of HMF during deep-fat frying.

Objective:

- To investigate the effects of variety, ripening stage and pre-treatments of banana on the formation and reduction of HMF during deep-fat frying

Studies were designed, conducted and results were analyzed to test these hypotheses.

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