UNIVERSITI PUTRA MALAYSIA

GENERATION OF MEATY FLAVORING USING CHICKEN FEATHER KERATIN HYDROLYSATE-GLUCOSE INTERACTION MODEL

LASEKAN ADESEYE OPEYEMI

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By

LASEKAN ADESEYE OPEYEMI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chair: Prof. Fatimah Abu Bakar, PhD

Faculty: Food Science and Technology

Some of the major products of the flavour industry are flavour ingredients which are used to enhance the sensory attributes (especially taste and aroma) of different foods which also ensure consumer's acceptability of the food products. Flavourists use the understanding of the Maillard reaction between reducing sugars and nitrogen source (amino acids and peptides) to create "process flavours" which are natural flavour ingredients that can enhance or impart certain aroma and taste to foods.

Hydrolysed proteins derived mostly from plant sources are commonly used to create process flavours especially those that can impart the meaty character. However, occurrence of certain carcinogens and high salt contents of these hydrolysed vegetable or plant proteins are some of the factors affecting their acceptance by consumers. Moreover, the potential of animal proteins for the creation of process flavour has not been extensively studied eventhough they are also good sources of process flavour precursors. More so, if these proteins can be sourced from animal by-

products and hydrolysed to amino acids and peptides, this might help reduce the cost of producing process flavours. Chicken feather is a major by-product of the poultry processing industry which is largely made up of a protein known as keratin which is rich in cysteine, an important precursor for the development of meat flavour. Thus, the potential of the hydrolysate of feather keratin for the generation of meaty flavouring was tested in this study.

Chemical composition of chicken feather revealed that it has crude protein content of about 88% and high content of glycine, serine, proline, glutamic, valine, leucine and alanine was observed. Chicken feather protein hydrolysate was produced via mild thermochemical pretreatment with sodium hydroxide to solubilise and denature the protein followed by enzymatic hydrolysis of the soluble and denatured protein with the protease from *Bacillus licheniformis*. Using Response Surface Methodology as a statistical optimisation tool as well as pH Stat method to quantify the extent of hydrolysis, a keratin hydrolysate with a degree of hydrolysis (DH) of about 12% was obtained using the thermochemical pretreatment conditions of 0.08M sodium hydroxide, temperature 81ºC and pretreatment time of 54 minutes followed by enzymatic hydrolysis using 2.5% enzyme-substrate concentration, reaction pH of 9.5, at 60ºC for 300 minutes. The hydrolysate is rich in glutamic acid, glycine and proline.

Two aqueous solutions of the freeze dried hydrolysate and glucose were prepared such that the pH of one was left unaltered while the pH of the other sample was adjusted to about 9.5. These solutions were heated at 155ºC for 2 hours in an oil bath in order to undergo Maillard reaction which is responsible for the formation flavour

volatiles. The extraction of volatiles was done by the headspace sampling of vials containing the solution with different solid phase microextraction (SPME) fibers followed by injection into a gas chromatography system coupled to a flame ionisation detector (FID) and mass spectrometer (MS). Polydimethylsiloxane/ divinylbenzene (PDMS/DVB, 65 µm) fiber was found to give the highest extraction efficiency among other fibers tested and the sample with the adjusted pH gave the highest number of volatiles, about 77 peaks from the GC-FID chromatogram. Tentative identification of the volatiles was done by computing their retention index and comparing same with those found in the literature or database and also by comparing their mass spectra data with those of National Institute of Standard and Technology (NIST) database.

Ten categories of volatiles were identified namely aldehydes, hydrocarbons, acids, ketones, thiophenes, furans, pyrazines, alcohols, phenols and esters. All of them had been shown to be important in the development of heated meat aroma. The participation of some amino acids such as phenylalanine, cysteine and isoleucine as well as lipid composition of chicken feather including oleic and linoleic acid in the formation of these aroma compounds was established. Potent meaty aroma compounds that were tentatively identified in this study include 3-methylbutanal, hexanal, 2, 4-decadienal, γ-dodecalactone, 2-methyl-3-furanthiol, 2-ethyl-3, 5-dimethylpyrazine, 3-hydroxy-4,5-dimethyl2(5H)-furanone, bis(2-methyl-3-furyl)disulphide, 2-methylthiophene and 2-thiophenecarboxladehyde. The formation of these compounds was influenced by the pH of the reaction medium. Increasing the time of heating and altering the water content of the solution do not lead to the formation new of compounds that gives meaty odour.

Consequently, this work has demonstrated that keratin hydrolysate can serve as a good precursor or can be blended with other precursors for the generation of meaty process flavour. It also has the potential of reducing the overall cost of production because the nitrogen source is obtained from a cheap and readily available by-product of the poultry industry. However, it is important to carry out further work on the hydrolysis of this protein solely by enzymes that can achieve a high degree of hydrolysis in a reasonable time. More so, further research that focuses on the reaction conditions that favour the development of meaty flavours as well as improved identification techniques to confirm the volatiles extracted need to be carried out.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN PERASA DAGING MENGGUNAKAN MODEL INTERAKSI GLUKOSA- KERATIN HIDROLISAT BULU AYAM

Oleh

LASEKAN ADESEYE OPEYEMI

Julai 2013

Pengerusi: Prof. Fatimah Abu Bakar, PhD

Fakulti: Teknologi dan Sains Pemakanan

Sesetengah hasil utama dalam industri makanan merupakan bahan makanan yang dapat digunakan untuk menambahkan lagi sensasi deria rasa (khususnya aroma dan rasa) untuk makanan berbeza yang dapat memastikan tahap penerimaan pelanggan terhadap produk terbabit. Pakar perasa menggunakan konsep tindek belas Maillard, diantara gula penurun dan sumber nitrogen (asid amino dan peptida) untuk menghasilkan "perisa sintetik" yang mana merupakan bahan makanan yang dapat meningkaikan rasa dan aroma makanan.

Terbitan hidrolisat protein yang kebanyakannya dari sumber tumbuhan biasanya digunakan dalam penghasilan "bahan perisa sintetik" khususnya yang dapat memberikan karakter impak rasa daging. Walaubagaimana pun, kehadiran bahan seperti yang bersifat karsinogen dan jumlah garam tinggi dalam hidroliset protein tumbuhan adalah merupakan sebahagian faktor mengganggu tahap penerimaan pelanggan terhadap produk terbabit. Selain itu, potensi protein haiwan untuk

penghasilwan bahan perasa tidak dikaji secara mendalam walaupun ianya merupakan sumber yang baik untuk menghasilkan bahan perisa sintetik. Tambahan lagi, sekiranya protein ini boleh didapati dari produk haiwan dan dihidrolisasikan kepada bentuk asid amino dan peptida, ianya dapat membantu mengurangkan kos penghasilan bahan perasa. Maka secara ringkasnya, potensi untuk protein bulu ayam (yang mana kaya dengan cystein-satu bahan penggalak untuk pembentukan perasa daging) digunakan dalam penghasilan bahan perasa telah diuji dalam kajian ini.

Hidrolisat protein bulu ayam ini telah dihasilkan melalui kaedah rawatan kimia termal dengan natrium hidroksida dan diikuti hidrolisis enzim protease *Bacillus licheniformis.* Dengan penggunaan "Response Surface Methadology" sebagai instrumen pengiraan dan pengukur seperti Kaedah pH Stat dalam penentuan tahap hidrolisis, sebanyak 12% darjah hidrolisis telah didapati dengan menggunakan kaedah rawatan kimiatermal optimum menggunakan 0.08 natrium hidroksida pada suhu 81ºC dengan masa rawatan 54 minit, diikuti oleh hidrolisis enzimatik menghasilkan 2.5% kepekatan enzim, pH 9.5, suhu 60ºC selama 300 minit.

Dua sebatian akueus hasil hidrolisat ini dan juga glukosa telah disediakan yang mana satu daripada sebatian ini telah dibiarkan pH awalnya dan satu lagi telah diubahsuai pHnya kepada 10. Untuk menjalani proses Maillard yang membolehkan pembentukan perisa meruap, dua sebatian ini telah dipanaskan pada suhu 155ºC selama 2 jam dalam rendaman minyak. Hasil ekstrasi yang meruap ini disampelkan dengan vial yang mengandungi sebatian yang berbeza fasa pepejal mikroekstraksi (SPME) fiber dan diikuti dengan suntikan ke dalam gas kromatografi yang menggunakan pengesan ion berapi (FID) sebagai detektor. Polidimetilsiloheksane

divinilbenzene (PDMS/DVB, 65 µm) fiber telah dijumpai di mana secara keseluruhannya merupakan hasil ekstraksi yang tertinggi jika dibandingkan dengan ujikaji fiber yang lain dan sampel yang diubahsuai pHnya menghasilkan bilangan sebatian meruap yang tertinggi iaitu 77 puncak dari GC-FID kromatografi. Pencirian hasil sebatian meruap ini telah dijalankan dengan program indeks retantasi dan perbandingan hasil data berdasarkan rujukan serta perbandingan data spektrum dari rujukan sumber NIST.

Sebanyak 10 kategori telah dicirikan iaitu aldehid, alkana, asid, ketones, thiophenes, furans, pyrazines, alkohols, fenols and esters yang mana kesemuanya memainkan peranan dalam penghasilan "aroma perisa daging sintetik". Kemasukan sesetengah asid amino seperti phenylalanine, cysteine dan isoleucine dan juga komposisi lemak bulu ayam termasuklah asid oleik dan asid linoleik dalam pembentukan sebatian beraroma telah distabilkan. Bahan aroma daging yang berpontensi yang dapat dikenalpasti dalam kajian ini ialah 3 metilbutanal, hexanal, 2,4-dekadienal, γ-dodecalactone, 2-metil-3-furantiol, 2-etil-3,5-dimetilpirazine, 3-hidroksi-4,5-dimetil2(5H)-furanon, bis(2-metil-3-furil)disulfida, 2-metilthiopene dan 2-thiopenekarboksildehyde. Proses pembentukan sebatian ini dipengaruhi oleh nilai pH medium tindak balas. Penaikan masa suhu pemanasan dan juga pengubahsuaian jumlah kandungan air dalam campuran tidak menjurus ke arah penghasilan sebatian baru yang dapat memberikan rasa atau bau daging

Maka, kajian ini telah menunjukkan bahawa hidrolisat keratin dapat digunakan sebagai bahan penggalak atau dapat dicampurkan dengan bahan penggalak lain dalam metebihi proses haba daging. Ia turut mempunyai potensi dalam mengurangkan kos penghasilan kerana sumber nitrogen boleh didapati dari sumber

yang murah- iaitu produk dari industri ayam. Walaubagaimanapun, ianya sangat penting untuk diteruskan lagi kajian ini pada pada tindak balas hidrolisasi protein dengan penggunaan enzim agar dapat menghasilkan tahap darjah yang tinggi untuk hidrolisis pada masa yang munasabah.

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Approval Sheet 1

I certify that a Thesis Examination Committee has met on 4th of July, 2013 to conduct the final examination of Lasekan Adeseye Opeyemi on his thesis entitled **"Generation Of Meaty Flavoring Using Chicken Feather Keratin Hydrolysate-Glucose Interaction Model"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

Jamilah Bakar, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Nazamid Saari, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Azis Ariffin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Mohd Khan Ayub, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 Sepetember 2013

This Thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Fatimah Abu Bakar, PhD
Professor
Faculty of Food science and Technology
Universiti Putra Malaysia
(Chairman)

Mr. Dzulkifly Mat Hashim
Lecturer
Halal Products Research Institute
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT,PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

LASEKAN ADESEYE OPEYEMI

Date: 4 July 2013

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