



UNIVERSITI PUTRA MALAYSIA

**DETECTION AND REDUCTION OF AFLATOXIN AND
OCHRATOXIN A IN BLACK AND WHITE PEPPER**

MARYAM JALILI

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OCHRATOXIN A IN BLACK AND WHITE PEPPER**

By

MARYAM JALILI

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

October 2010



Specially dedicated:

***To the memory of my late father, my beloved mother,
My brothers and my sisters***



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

**DETECTION AND REDUCTION OF AFLATOXINS AND
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MARYAM JALILI

October 2010

Chairman : Professor Jinap Selamat, PhD

Faculty : Food Science and Technology

Despite the serious side effects of aflatoxins (AFs) and ochratoxin A (OTA) on human health, there is no published method for the reduction of AFs and OTA in black and white peppers. In light of this, this thesis describes methods that can be used to reduce AFs and OTA in black and white peppers. The separation and quantification of OTA by high performance liquid chromatography (HPLC) in black and white peppers was optimized. The influence of three variables: type of solvent, solvent-volume-to-sample-size (v/w), and amount of sodium chloride (NaCl) (g) on recovery of OTA was evaluated. The response surface methodology (RSM) was applied to determine the optimum amount of NaCl and solvent-volume-to-sample-size on the extraction of OTA from black and white peppers. This optimized method proved to be reliable with recovery values ranging from 94.3 to 102.0%. In order to assess occurrence of AFs and OTA in pepper



commercialized in Malaysia, samples of black and white pepper were randomly collected from supermarkets and wholesales located in Peninsular Malaysia. Results showed that 58.3 and 67.5% of samples were contaminated with AFs and OTA, with concentrations ranging from 0.1- 25.8 and 0.30-33.0 ng/g, respectively. In order to reduce AFs and OTA in black and white pepper, a method was established by using gamma ray at doses within the range 5 to 30 kGy at 12 and 18% pepper moisture contents. Results showed that the method could not completely reduce OTA and AFs even at dose of 30 kGy and 18% moisture content. The second method proposed applied chemical compounds including acids, alkalis, salts and oxidizing agents for reduction of AFs and OTA. The highest reduction (41.9 – 57.3%) was obtained by using alkaline compounds. This study also optimized a method for reducing AFs and OTA in white pepper. The RSM was used to evaluate the effect of four variables, i.e., time (20-60 min), temperature (30-70 °C), calcium hydroxide (0-1%) and hydrogen peroxide (1-3%), on reducing AFs and OTA during soaking. A treatment time of 57.8 min, temperature of 62 °C, calcium hydroxide concentration of 0.6% (w/v), and hydrogen peroxide concentration of 2.8% (v/v) were found to be the optimum conditions for achieving the highest value of mycotoxin reduction and the best value for color. Maximum reduction at the optimum conditions ranged from 70.7 for AFB₂ to 100% for AFG₁, respectively. Since hydrogen peroxide caused damage to the surfaces of black pepper, this method was not applicable for black pepper. Therefore, the effect of sodium hydrosulfite at different concentrations (0.25, 0.5, 1, 1.5 and 2%) during boiling and sterilization was investigated. The boiling was done at 100 °C and atmospheric pressure for 30 min while sterilization



was done under high pressure (1.5 bar) and 121 °C for 15 min. The maximum reductions were obtained by applying sterilization at 2% of Na₂S₂O₄ and corresponded to 96.0, 96.1, 77.7, 100% and 100% for OTA, AFB₁, AFB₂, AFG₁ and AFG₂, respectively. By applying these methods to black and white peppers, more than 95% reduction of OTA and the aflatoxins B₁, and G₁, which are the most dangerous mycotoxins, was obtained.

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

**PENENTUAN DAN PENGURANGAN AFLATOKSIN DAN
OCHRATOKSIN A DALAM LADA HITAM DAN PUTIH**

Oleh

MARYAM JALILI

Oktober 2010

Pengerusi: Profesor Jinap Selamat, PhD

Fakulti: Sains Makanan dan Teknologi

Walaupun terdapat kesan sampingan yang serius dari aflatoksin (AF_S) dan ochratoxin A (OTA) pada kesihatan manusia, tidak ada kaedah yang diterbitkan untuk pengurangan AF_S dan OTA pada lada hitam dan putih. Oleh itu, tesis ini menjelaskan kaedah yang boleh digunakan untuk mengurangkan AF_S dan OTA pada lada hitam dan putih. Pemisahan dan kuantifikasi OTA dengan kromatografi cecair berprestasi tinggi (KCKT) pada lada hitam dan putih telah dioptimumkan. Pengaruh dari tiga pembolehubah: jenis pelarut, isipadu pelarut terhadap saiz sampel (v/w), dan jumlah natrium klorida (NaCl) (g) atas hasil dapatan semula OTA dinilai. Kaedah respon permukaan (RSM) diaplikasikan untuk menentukan jumlah optimum NaCl dan isipadu pelarut terhadap saiz sampel pada pengekstrakan OTA dari lada hitam dan putih. Kaedah optimum ini terbukti boleh dipercayai dengan nilai hasil dapatan semula dalam lingkungan 94.3-102.0%. Untuk menguji kehadiran AF_S dan OTA pada tanaman lada komersial di Malaysia,



sampel lada hitam dan putih diambil secara rawak dari pasaraya dan pasar borong yang terletak di Semenanjung Malaysia. Keputusan kajian menunjukkan bahawa 58.3 dan 80% dari sampel yang telah tercemar dengan AF_S dan OTA, dengan kepekatan dalam lingkungan 0.1-25.8 dan 0.30-33.0 ng/ g, masing-masing. Untuk mengurangkan AF_S dan OTA dalam lada hitam dan putih, suatu kaedah telah dibangunkan dengan menggunakan sinar gamma pada dos dalam lingkungan 5 hingga 30 kGy pada kandungan air sebanyak 12 dan 18%. Keputusan kajian menunjukkan bahawa kaedah tersebut tidak dapat mengurangkan OTA dan AF_S sepenuhnya bahkan pada dos 30 kGy dan kandungan air sebanyak 18%. Kaedah kedua yang dicadangkan telah menggunakan sebatian kimia termasuk asid, alkali, garam dan agen pengoksidaan untuk mengurangkan AF_S dan OTA. Penurunan tertinggi (41.9-57.3%) diperolehi dengan menggunakan sebatian beralkali. Penyelidikan ini juga mengoptimumkan suatu kaedah untuk mengurangkan AF_S dan OTA dalam lada putih. RSM juga digunakan untuk menilai kesan empat pembolehubah, iaitu, masa (20-60 minit), suhu (30-70 °C), kalsium hidroksida (0-1%) dan hidrogen peroksida (1-3%), dalam mengurangkan AF_S dan OTA selama perendaman. Masa rawatan 57.8 minit, suhu 62 °C, kepekatan kalsium hidroksida 0.6% (b/v), dan kepekatan hidrogen peroksida sebanyak 2.8% (v/v) didapati sebagai keadaan optimum untuk mencapai nilai tertinggi penurunan mikotoksin dan nilai terbaik untuk warna. pengurangan maksimum pada keadaan optimum adalah antara 70.7 untuk AFB₂ hingga 100% untuk AFG₁, masing-masing. Sejak hydrogen peroksida menyebabkan kerosakan pada permukaan lada hitam, kaedah ini tidak boleh digunakan untuk lada hitam. Oleh kerana itu, pengaruh natrium hidrosulfit pada kepekatan yang berbeza (0.25, 0.5, 1, 1.5 dan 2%) semasa

perebusan dan sterilisasi telah diteliti. Perebusan tersebut telah dijalankan pada 100 °C dan tekanan atmosfera selama 30 menit manakala sterilisasi dilakukan dibawah tekanan tinggi (1.5 bar) dan 121 °C selama 15 menit. Pengurangan maksimum diperolehi dengan menggunakan sterilisasi sebanyak 2% dari Na₂S₂O₄ dan hasil berkurang sebanyak 96.0, 96.1, 77.7, 100% dan 100% untuk OTA, AFB₁, AFB₂, AFG₁ dan AFG₂, masing-masing. Dengan menerapkan kaedah ini untuk lada hitam dan putih, lebih daripada penurunan 95% dari OTA dan aflatoksin B₁, dan G₁, yang merupakan mikotoksin yang paling berbahaya, diperolehi.

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I certify that an Examination Committee met on - / - / - to conduct the final examination of Maryam Jalili on her PhD degree of Food Science thesis entitled “Detection and reduction of aflatoxins and ochratoxin A in black and white pepper” in accordance with Universiti Pertanian Malaysia (higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

(Chairman)

(Internal Examiner I)

(Internal Examiner II)

(External Examiner)

HASANAH MOHD. GHAZALI, Ph.D.
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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

Jinap Selamat, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Son Radu, PhD

Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Noranizan Mohd Adzahan, PhD

Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Deputy 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 institutions.

MARYAM JALILI

Date:



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