

STUDIES ON STERIGMATOCYSTIN IN SOME WHEAT BASED FOODS

BY

TAREK AHMED ELDOSOKY

B. Sc. Agric. Sc. (Food Science), Tanta University, 2001

**A thesis submitted in partial fulfillment
of
the requirements for the degree of
MASTER OF SCIENCE**

**In
Agricultural Science
(Food Science and Technology)**

**Dep. of Food Science-Faculty of Agriculture
Ain Shams University**

2008

STUDIES ON STERIGMATOCYSTIN IN SOME WHEAT BASED FOODS

BY

TAREK AHMED ELDOSOKY

B.Sc. Agric. Sc. (Food Science), Tanta University 2001

Under the Supervision of:

Prof. Dr. Neverty Fathy Mahmoud Gamal

Prof. of Food Science and Technology, Dep. of Food Science, Faculty of
Agriculture, Ain Shams University (Principal Supervisor)

Prof. Dr. Khayria Mahmoud Naguib

Prof. Food Toxicology and Contaminants, National Research Center

Dr. Hanan Mohamed Abdo Al-sayed

Lecturer of Food Science and Technology, Dep. of Food Science, Faculty
of Agriculture, Ain Shams University

ABSTRACT

Tarek Ahmed El dosoky. "Studies On Sterigmatocystin In Some Wheat Based Foods ", Unpublished M.Sc Thesis, Dept. of Food Science, Faculty of Agriculture, Ain Shams University, 2008

Sterigmatocystin (Stg) is a mycotoxin produced by *Aspergillus versicolor* and *Aspergillus nidulans*. Stg has been shown to be carcinogenic, causes cancer in number of animal species and classified as class 2B carcinogen (as possibly carcinogenic to humans) by IARC.

The results indicated that the developing system Benzene: acetic acid (9: 1 v/v) showed the best separation for small concentrations of Stg when used TLC technique. Moreover the data showed also that the mobile phase methanol: water (8: 2 v/v) succeeded to separate Stg at 5.4 min retention time when the flow rate was 1ml/ min. When comparison between different methods for Stg determination by HPLC in spiked wheat samples. The results clearly indicated that the percentages of loss in AOAC (2000) method were 10.44, 24.55, 17.07 and 28.95% for the tested concentration levels (5, 10, 50 and 100 μ g) respectively. Moreover the percentage of toxin loss ranged from 93.47 and 96.64% with average of 94.895 in TLC determination and however, it ranged from 10.52 and 27.95% with average of 19.68% in HPLC method.

Determination of naturally occurrence of Stg in Wheat, Bread (balady and Fino) and Cerelac. The results indicated that 16, 8, 12 and 6% for wheat, Balady bread, Fino bread and Cerelac respectively.

Studies on naturally sterigmatocystin-contaminated wheat have shown that highest concentrations of Stg were found in wheat flour with a reduction percentage ranged from 87.109 to 59.943% while the lowest concentrations were detected in bran with a reduction percentage ranged from 40.057 to 12.839%. The reduction percentage of Stg after baking was only 16.949% while it was 43.412% after fermentation.

The current results indicated that the fermentation process significantly reduced Stg to 3.380, 6.417 and 20.755 $\mu\text{g/g}$ in dough made from flour artificially contaminated with 5, 10 and 50 $\mu\text{g/g}$, respectively. The residual amount of Stg was 2.329, 4.240 and 12.625 $\mu\text{g/g}$ after baking dough artificially contaminated with different concentration of Stg i. e, 5, 10 and 50 $\mu\text{g/g}$, respectively.

A higher percent of reduction of Stg could be occurred during fermentation of lokmet el-khady made with 5 $\mu\text{g/g}$ Stg, being 42.766% while it reached 63.324% when 50 $\mu\text{g/g}$ Stg was used. The average percentage of Stg reduction during frying of lokmet el-khady was 63.26%, 61.23% and 72.28% for the level of 5, 10 and 50 $\mu\text{g/g}$, respectively.

Key Words: sterigmatocystin, HPLC, TLC, Wheat, processing

Acknowledgement

Praiseworthy and gratitude to **ALLAH** who gave me the ability to finish my thesis.

I would like to pray for **Prof. Dr. Mohamed A. Abdulla**, who was the supervisor of this thesis, suggesting the points of research, putting the plan of this work and for his valuable directions before his departure.

Deep appreciation and gratitude are extended to **Prof Dr. Neverty F. M. Gamal** Prof. of Food Science and Technology, Faculty of Agriculture, Ain-shams University for her kind supervision, editing and revision of the thesis.

I wish to express my deepest appreciation to **Prof. Dr. Khayria M. Naguib** Prof. of Food Toxicology and Contaminants Department, National Research Center, for her keen supervision, continuous guidance and encouragement as well as scientific revision of this work

Grateful appreciation to **Dr. Hanan, M. A. Al-Sayed**, Lecturer of Food Science and Technology, Faculty of Agriculture, Ain-shams University for co-supervising of this thesis.

Grateful appreciation and sincere gratitude to **Dr. Mona M. Abdel Galil** prof. of Food Contaminants, National Research Center for her keen supervision of this work

Grateful appreciation and sincere gratitude to **Dr. Mosaad Attia Abdel-Wahhab** Prof. of Toxicology, National Research Center for his keen supervision, able guidance as well as editing and valuable criticism during all stages of this work.

Grateful appreciation is also due to Prof , **Dr. Abdel-Ghany El-Gendy** former Dean of the Faculty of Agriculture, Ain-shams University for his great support during the course of this study. Deep thanks are also due to the staff members at the Arid Land Agricultural Research Services Center Faculty of Agriculture, Ain-shams University for their valuable help in the third part of this study

I wish to express my great thanks for all the staff members at the Food Science and Technology Dept., Faculty of Agriculture, Ain-Shams Univ. and Department of Food Toxicology & Contaminants, National Research Center for their help and encouragement during the course of this study.

CONTENTS

| | |
|---|-----|
| LIST OF TABLES | vi |
| LIST OF FIGURES | vii |
| LIST OF ABBREVIATION | ix |
| 1. INTRODUCTION | 1 |
| 2. REVIEW OF LITERATURE | 3 |
| 2.1. History and Identification of Sterigmatocystin..... | 3 |
| 2.2. Chemical Structure and Physical Properties of Stg..... | 3 |
| 2.3. Biosynthetic Pathways | 4 |
| 2.4. Natural Occurrence of Sterigmatocystin..... | 6 |
| 2.5. Conditions favoring the development of mycotoxins in grains with particular reference to sterigmatocystin..... | 10 |
| 2.6. Toxicological effect of Sterigmatocystin..... | 17 |
| 2.6.1. Toxicity to Animals..... | 17 |
| 2.6.2 Toxicity to human..... | 19 |
| 2.7. Diminution effect on aflatoxins during processing of some food products..... | 21 |
| 2.8. General Principals for the determination of mycotoxins..... | 23 |
| 2.8.1. Determination of Stg by TLC and HPLC techniques..... | 25 |
| 2.8.1.1. Determination of Stg by using Thin Layer Chromatography (TLC) technique..... | 25 |
| 2.8.1.2. Determination of Stg by using High Performance Liquid Chromatography (HPLC) technique..... | 28 |
| 3. MATERIALS AND METHODS | 32 |
| Part 1: Comparative study for the detection and determination of Sterigmatocystin in wheat..... | 32 |
| 3.1. Materials..... | 32 |
| 3.1.1. Solvent&Chemical..... | 32 |
| 3.1.2. Sterigmatocystin and Aflatoxins standards | 32 |

| | | |
|----------|---|----|
| 3.1.3. | Wheat samples | 32 |
| 3.1.4. | Apparatus | 32 |
| 3.1.5. | Thin Layer Chromatography (TLC) Plates. | 32 |
| 3.1.6. | Sep-pack silica cartridge C ₁₈ column | 33 |
| 3.2. | Methods | 33 |
| 3.2.1. | Preparation of spiked wheat samples..... | 33 |
| 3.2.2. | Extraction and determination of Stg according to AOAC (2000)..... | 33 |
| 3.2.2.1. | Extraction | 33 |
| 3.2.2.2. | Determination using Thin Layer Chromatography (TLC) technique..... | 33 |
| 3.2.2.3. | Calculation of Stg..... | 34 |
| 3.2.3. | Extraction and determination of Stg according to Athnasios and Kuhn (1977)..... | 34 |
| 3.2.3.1. | Extraction | 34 |
| 3.2.3.2. | Determination using TLC technique..... | 35 |
| 3.2.3.3. | Calculation of Stg | 35 |
| 3.2.4. | Extraction and determination of Stg according to Shannon and Shotwell (1976)..... | 35 |
| 3.2.4.1. | Extraction | 35 |
| 3.2.4.2. | Column cleanup | 35 |
| 3.2.4.3. | Determination by using TLC technique | 36 |
| 3.2.5. | Extraction and determination of Stg according to Schmidt <i>et al</i> (1980)..... | 36 |
| 3.2.5.1. | Extraction..... | 36 |
| 3.2.5.2. | Sep-pack silica cartridge C ₁₈ column..... | 37 |
| | Part 2: Natural occurrence of Stg in wheat, Bread (fino& balady) and cerelac..... | 37 |
| 3.3.1. | Materials..... | 37 |
| 3.3.1.1. | Collected samples | 37 |
| 3.3.2. | Method..... | 37 |
| 3.3.2.1. | Preparation of samples..... | 37 |

| | | |
|--|---|----|
| 3.3.2.2. | Extraction and determination of Stg..... | 37 |
| 3.3.2.2.1. | Extraction of Stg..... | 37 |
| 3.3.2.2.2. | Determination of Stg by HPLC..... | 38 |
| 3.3.3. | Detection and determination of aflatoxins..... | 38 |
| 3.3.3.1. | Preparation of working solution of aflatoxins..... | 38 |
| 3.3.3.2. | Determination of Aflatoxins (for food grains)–CB (Contaminants Branch) Method..... | 38 |
| 3.3.3.2.1. | Extraction..... | 38 |
| 3.3.3.2.2. | Clean-up procedures..... | 39 |
| 3.3.3.2.3. | Preliminary TLC..... | 39 |
| 3.3.3.2.4. | Determination of aflatoxins by HPLC technique..... | 40 |
| 3.3.3.2.5. | HPLC conditions..... | 40 |
| 3.4. | Determination of moisture content in wheat samples | 40 |
| Part 3: Diminution effect on sterigmatocystin during processing of wheat into balady bread and lokmet –el-khady | | 41 |
| 3.5. 1. | Materials..... | 41 |
| 3. 5. 1. 1. | Wheat grain samples..... | 41 |
| 3. 5. 1. 2 | Dough ingredients | 41 |
| 3. 5. 2. | Methods..... | 41 |
| 3. 5. 2. 1. | Preparation of wheat flour (82%extraction) and bran..... | 41 |
| 3.5.2.1.a. | Natural contaminated wheat flour..... | 41 |
| 3.5.2.1.b. | Artificial contaminated wheat flour..... | 41 |
| 3. 5. 3. | Preparation of balady bread..... | 41 |
| 3. 5. 4. | Preparation of lokmet el- khady..... | 42 |
| 3.5.5. | Extraction and determination of sterigmatocystin..... | 42 |
| 3.5.5.1. | Extraction of sterigmatocystin..... | 42 |
| 3.5.5.2. | Determination of sterigmatocystin..... | 42 |
| 4. RESULTS AND DISCUSSION..... | | 43 |
| Part I: Comparative study for the extraction and determination of Stg in wheat..... | | 43 |

| | | |
|--------------------|--|----|
| 4.1. | Applying of TLC technique for extraction and determination of Stg in wheat..... | 44 |
| 4.1.1. | Comparison between different developing system..... | 44 |
| 4.2. | Applying of HPLC technique for extraction and determination of Stg in wheat..... | 50 |
| 4.2.1. | Comparison between different mobile phases..... | 50 |
| 4.3. | Comparison between different selected methods for Stg extraction and determination by HPLC in spiked wheat samples..... | 52 |
| 4.4. | Determination of Stg in Spiked Wheat Samples extracted by AOAC (2000) and determined by TLC and HPLC techniques..... | 62 |
| 4.5. | Part II: Natural occurrence of Stg in Wheat, Bread (fino & balady) and cerelac using the current established method..... | 68 |
| 4.5.1 | Wheat..... | 69 |
| 4.5.2. | Bread (balady and fino)..... | 72 |
| 4.5.3. | Cerelac (Infant formula)..... | 76 |
| 4.5.4. | Co-occurrence of Aflatoxins in Stg contaminated samples..... | 78 |
| 4.6. | Part III:4. 6. Diminution effect on Stg during processing of balady bread and lokmet el-khady..... | 82 |
| 4. 6. 1. | Effect of bread making process on Stg reduction using naturally contaminated wheat..... | 82 |
| 4. 6. 1. 1 | Effect of milling | 82 |
| 4. 6. 1. 2. | Effect of fermentation (active dry yeast)..... | 84 |
| 4.6.1.3. | Effect of baking..... | 84 |
| 4. 6. 2. | Effect of bread making process in Stg reduction using artificially contaminated wheat flour..... | 86 |

| | | |
|----------------------------|--|-----|
| 4. 6. 2. 1. | Effect of fermentation..... | 86 |
| 4. 6. 2. 2. | Effect of baking..... | 86 |
| 4.6.3. | Effect of lokmet el-khady process on Stg reduction using artificially contaminated wheat flour..... | 87 |
| 4.6.3.1. | Effect of fermentation..... | 88 |
| 4.6.3.2. | Effect of frying | 88 |
| 5. SUMMARY | | 91 |
| 6. REFERENCES..... | | 96 |
| ARABIC SUMMARY..... | | --- |

LIST OF TABLES

| No | Title | Page |
|----|---|------|
| 1 | Upper limit of safe moisture contents ^a for storing grain up to a year under Canadian Prairies climatic conditions..... | 13 |
| 2 | LD ₅₀ of Stg in different animal species..... | 17 |
| 3 | Recovery percentage of Stg in spiked wheat samples extracted by different extraction and determination methods..... | 43 |
| 4 | Composition of developing systems used in TLC technique..... | 45 |
| 5 | Retention time (min) of Stg in different mobile phase systems on Hyper clone 5 μ ODS C18 column..... | 51 |
| 6 | Concentration of Stg determined in spike wheat samples by HPLC using different selected methods..... | 53 |
| 7 | Analysis of variance for the difference between Stg concentrations in spiked wheat samples extracted by different methods and determined by HPLC..... | 58 |
| 8 | Estimated cost (LE) and estimated time (min) for the different exciting methods..... | 61 |
| 9 | Concentration of Stg detected in spiked wheat samples extracted with AOAC (2000) method and determined by either TLC or HPLC..... | 62 |
| 10 | Natural Occurrence of Stg in wheat grain samples..... | 70 |
| 11 | Moisture content in positive wheat samples..... | 72 |
| 12 | Natural occurrence of Stg in balady bread..... | 74 |
| 13 | Natural occurrence of Stg in fino bread..... | 74 |
| 14 | Natural occurrence of Stg in cerelac..... | 77 |
| 15 | Co-occurrence of AFs and Stg in different commodity tested..... | 80 |
| 16 | Distribution of Stg into commercially milled naturally contaminated wheat fractions..... | 82 |

| | | |
|----|--|----|
| 17 | Effect of balady bread making process on Stg reduction using naturally contaminated wheat..... | 84 |
| 18 | Effect of bread making process on Stg reduction using artificially contaminated wheat flour with different concentrations of Stg..... | 86 |
| 19 | Effect of lokmet el-khady process on Stg reduction using artificially contaminated wheat flour with different concentrations of Stg..... | 89 |

LIST OF FIGURES

| No | Title | Page |
|----|--|------|
| 1 | Chemical structures of (A) sterigmatocystin and (B) aflatoxin B1..... | 3 |
| 2 | Aflatoxin and sterigmatocystin biosynthetic pathway..... | 5 |
| 3 | Recovery percentage of Stg in spiked wheat samples extracted by different extraction and determination methods..... | 44 |
| 4 | TLC plate of Stg developed in the developing system benzene: acetic acid(9:1v/v) shows Stg in wheat samples spiked with 5 µg (1,2) ,10 µg (3,4), standard (5,6), 50 µg (7,8),100 µg(9,10)..... | 46 |
| 5 | Two dimension TLC plate of Stg developed in the developing system of benzene: acetic acid (9:1v/v) followed by toluene: ethyl acetate: formic acid (6:3:1v/v/v), (a) sample was spiked with 5µg and (b) sample was spiked with 10µg..... | 47 |
| 6 | TLC plate of Stg in spiked wheat samples developed in benzene: methanol: acetic acid (90:5:5 v/v/v). 5µg and 10 µg concentration could not be detected..... | 48 |
| 7 | TLC plate of Stg developed in the developing system of Shannon and Shotwell (1976); (a) Stg standard and (b) detection of Stg in wheat spiked with 5, 10, 50, 100µg concentration could not be detected. | 49 |
| 8 | HPLC chromatogram of Stg standard extracted by Schmidt et al (1980) method with mobile phase methanol: water (8:2)..... | 51 |
| 9 | HPLC chromatogram of standard Stg (5µg) with inject 20µ..... | 53 |
| 9a | HPLC chromatogram of Stg in the sample spiked with 5µg and extracted by AOAC (2000) method (Stg appeared at 5.33 min and inject 10 µl) | 54 |
| 9b | HPLC chromatogram of Stg in the sample spiked with 5µg and extracted by Athnasios and Kuhn (1977) method (Stg appeared at | |

| | |
|---|----|
| 5.38 min and inject 10 µl)..... | 54 |
| 9c HPLC chromatogram of Stg in the sample spiked with 5µg and extracted by Shannon and Shotwell (1976) method (Stg appeared at 5.38 min and inject 10 µl)..... | 55 |
| 9d HPLC chromatogram of Stg in the sample spiked with 5µg and extracted by Schmidt <i>et al.</i> (1980) method (Stg appeared at 5.35 min and inject 10 µl)..... | 55 |
| 10a HPLC chromatogram of Stg in the sample spiked with 100µg and extracted by AOAC (2000) method (Stg appeared at 5.33 min with inject 10µl) | 56 |
| 10b HPLC chromatogram of Stg in the sample spiked with 100µg extracted by Athnasios and Kuhn (1977) method (Stg appeared at 5.55 min with inject 10µl)..... | 56 |
| 10c HPLC chromatogram of Stg in the sample spiked with 100µg and extracted by Shannon and Shotwell (1976) method (Stg appeared at 5.4 min with inject 10µl)..... | 57 |
| 10d HPLC chromatogram of Stg in the samples spiked with 100 µg and extracted by Schmidt <i>et al.</i> (1980) method (Stg appeared at 5.332 min with inject 10µl)..... | 57 |
| 11 Percentage of Stg loss during the determination of spiked samples by HPLC using the different methods of extraction..... | 58 |
| 12a Estimated cost (LE) for the different extracting methods..... | 60 |
| 12b Estimated time (min) for the different extracting methods..... | 60 |
| 13a Densitometer chromatogram of standard Stg with 20 µl spots on TLC plate..... | 63 |
| 13b Densitometer chromatogram of Stg in spiked wheat sample with 5µg and extracted by AOAC (2000) method and 20 µl spots on TLC plate | 64 |
| 13c Densitometer chromatogram of Stg in spiked wheat sample with | |

| | | |
|-----|---|----|
| | 50µg and extracted by AOAC(2000) method and 20 µl spots on TLC plate..... | 65 |
| 13d | Densitometer chromatogram of Stg in the spiked wheat sample with 100 µg and extracted by AOAC(2000) method and 20 µl spots on TLC plate..... | 66 |
| 14 | The percentage of Stg losses during extraction by AOAC(2000) method and determination by TLC and HPLC for spiked wheat samples..... | 67 |
| 15 | HPLC chromatogram of positive wheat grains sample contaminated with 2.3µg/g of Stg (Stg appeared at 5.63 min) with inject 20µl of sample | 71 |
| 16 | HPLC chromatogram for the Stg in balady bread (Stg appeared at 5.21 min with inject 20µl of sample | 75 |
| 17 | HPLC chromatogram for the Stg in the fino bread (Stg appeared at 5.26 min with inject 20µl of bread sample..... | 75 |
| 18 | HPLC chromatogram for the Stg in cerelac (Stg appeared at 5.29 min with inject 20µl of cerelac sample | 77 |
| 19 | The incidence % of Stg in wheat, balady bread, fino bread, cerelac..... | 78 |
| 20 | Distribution of Stg into commercially milled naturally contaminated wheat fractions..... | 82 |
| 21 | Effect of bread making process on Stg reduction using naturally contaminated wheat..... | 84 |
| 22 | Effect of bread making process on Sterigmatocystin residual using artificially contaminated wheat flour with different concentration of Stg (a) 5µg/g (b) 10µg/g (c) 50µg/g..... | 87 |
| 23 | Effect of lokmet el-khadi process on Sterigmatocystin residual using artificially contaminated wheat flour with different concentration of Stg (a) 5µg/g (b) 10µg/g (c) 50µg/g..... | 90 |